



haematologica

the hematology journal

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ISSN 0390-6078

Official Organ of the European Hematology Association
Published by the Ferrata-Storti Foundation, Pavia, Italy
Volume 90, supplement no. 2, June 2005

www.haematologica-thj.org
www.ehaweb.org
cme.haematologica.org

**10th Congress of
the European Hematology Association
Stockholm, Sweden, June 2-5, 2005**

ABSTRACT BOOK



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The origin of a name that reflects Europe's cultural roots.

Ancient Greek

αἷμα [haima] = blood
αἵματος [haimatos] = of blood
λόγος [logos] = reasoning

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

haematologica (adjective, plural and neuter,
used as a noun) = hematological subjects

Modern English

the hematology journal
2003 JCR® Impact Factor = 3.453

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Direttore responsabile: Prof. Edoardo Ascari; Autorizzazione del Tribunale di Pavia n. 63 del 5 marzo 1955.

Editing: Mikimos - Medical Editions, via A. Fogazzaro 5, Voghera, Italy

Printing: Tipografia PI-ME, via Vigentina 136, Pavia, Italy. Printed in May 2005.

Image of blood cells on cover: Giorgio Lambertenghi Deliliers, University of Milan, Italy.



EUROPEAN HEMATOLOGY ASSOCIATION

EHA Fellowships & Grants Program



Fellowships & Grants Program

Program

In 2004 EHA established a Fellowships and Grants Program, including a number of EHA Research Fellowships and EHA Clinical Research Grants. This program also includes the long established EHA - José Carreras Young Investigator Fellowships. The aim of the program is to encourage European hematological researchers to become established investigators. Each grant covers a two-year period and mainly provides support for salaries, but may also be used for supplies, small equipment and travel necessary for the project. The fellowships and grants selection committee, chaired by Irene Roberts reviews all applications.

Eligibility

Candidates may apply EITHER for a fellowship OR for a Clinical Research Grant. Research fellowships and clinical research grants are specially intended to support young investigators (aged 38 or younger). All applicants must be a member of EHA, at least one month before application. The program is also for members from non-European countries.

For more information, please visit www.ehaweb.org or send a message to fellowships.grants@ehaweb.org

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10TH CONGRESS
JUNE 2 - 5, 2005
STOCKHOLM

10TH CONGRESS OF THE
EUROPEAN HEMATOLOGY
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STOCKHOLM, SWEDEN,
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JUNE 2 - 5, 2005
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10th CONGRESS
JUNE 2 - 5, 2005
STOCKHOLM

Word of welcome

The congress of the European Hematology Association (EHA) has now been reserved in the yearly calendars of many European and international hematologists. The organizers of this years' meeting have sought to put together a first-rate European hematology congress of the highest international standards, presenting state-of-the-art advances and attracting clinicians and researchers from all parts of the globe.

This year, EHA welcomes delegates from all fields of hematology including clinical and laboratory/experimental hematology, hemostasis, transfusion medicine and pediatric hematology as well as specialists from related disciplines. The Scientific and Education Committee has reviewed over 1500 abstracts and put together what we believe to be an outstanding scientific and education program developed to attract young scientists and to focus on hematology-related science.

The program of this years' congress will comprise a variety of meeting platforms. The first day (Thursday, June 2nd, 2005) is allocated for our corporate sponsors who will present a series of Satellite Symposia. The following day is dedicated to Education Sessions which, will clarify current requirements for up-to-date treatment. Both Saturday and Sunday are devoted to recent updates in hematology related science. These sessions are set up in the now well known EHA composition including hematology-in-focus, meet-the-expert and plenary sessions and will run parallel to oral communications, poster sessions, lunch debates, and clinical trial updates.

In an ongoing effort to attract young scientists the organizers of the 10th Congress have included Science in Progress Sessions in which leading scientist, will present novel basic science developments.

In addition, the best scientific submissions will be presented during the Presidential Symposium. The European School of Haematology in collaboration with EHA will present a special session on training in communication skills. European Working Groups have also been offered a platform to meet at EHA; this year six of the eight groups will meet. European Networks will also be holding special sessions.

On behalf of the EHA Board and its Scientific and Education Committee, we would like to welcome you to Stockholm for the 10th anniversary Congress. With your active participation, the 10th Congress will be an outstanding congress and the highlight of the year in European hematology.

Yours sincerely,

Magnus Björkholm
Congress President

Willem Fibbe
*Chair Scientific and
Education Committee*

Abstract Book

10th Congress of the European Hematology Association, Stockholm, Sweden, June 2-5, 2005

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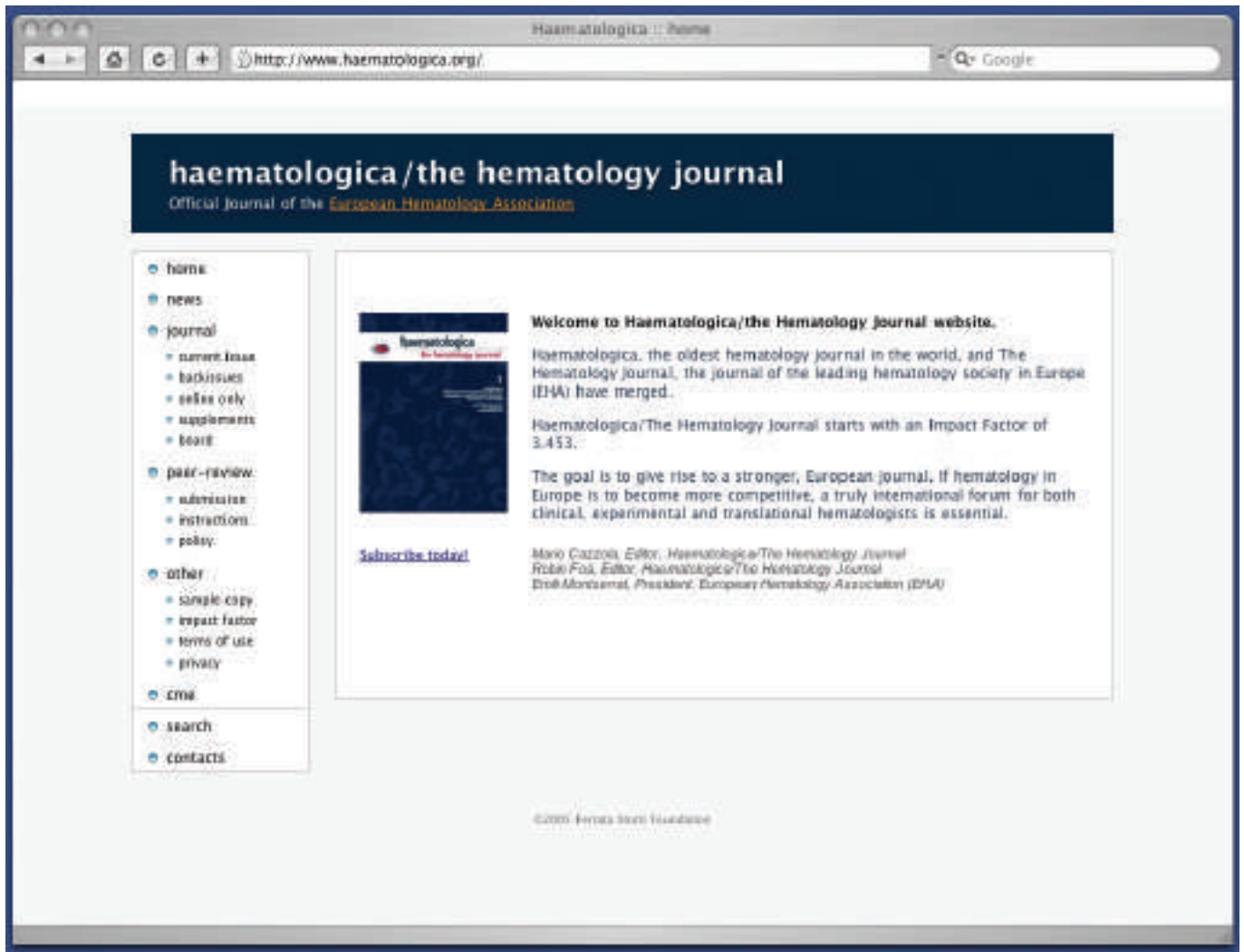
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The **European Hematology Association (EHA)** has promoted the creation of an interactive on-line integrated platform to improve, harmonize and develop skills and competences in hematological knowledge through out Europe. This integrated platform supports on-line education, training and accreditation of professional competences in the field of hematology using an interactive system, allowing users to take part in a global high standard hematological educative process.



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POSTER SESSION I

Acute leukemia - Prognostic factors

0001

CYTOGENETICS, IMMUNOPHENOTYPE, TUMOR BURDEN, AND AGE AS AN INDEPENDENT PROGNOSTIC FACTORS IN ACUTE MYELOID LEUKEMIA. ANALYSIS OF PATIENTS TREATED WITHIN PALG 1999 PROSPECTIVE, RANDOMIZED STUDY

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Acute myeloid leukemia (AML) refers to a group of distinct diseases that differ with regard to their genetic characteristics, clinical features, response to therapy, and prognosis. Patients require precise initial evaluation of risk factors including cytogenetics and immunophenotype to plan an optimal treatment program. In this study we analyzed impact of cytogenetics, cell surface marker expression and clinical features on complete remission (CR) rate, leukemia-free survival (LFS) and overall survival (OS). Four-hundred-forty-five AML patients, aged 18-60 years, treated within a multicentre study by the Polish Adult Leukemia Group (PALG 1999 Study) between 1999-2002 were included in the analysis. Induction therapy consisted of daunorubicine+cytarabine+/-cladribine, consolidation included two courses containing high-dose cytarabine (HAM, HD-AraC+/-cladribine). In multivariate analysis the following factors were found to be associated with poor outcome: 1. For CR rate: unfavorable cytogenetics according to CALGB criteria (RR=5,5, $p=0,00001$), $WBC \geq 50 \times 10^9/L$ at diagnosis (RR=3,3, $p=0,00007$), and age ≥ 50 (RR=1,9, $p=0,021$). 2. For LFS: unfavorable cytogenetics (RR=1,7, $p=0,026$), and lack of CD117 expression on leukemic cells (RR=1,8, $p=0,026$). 3. For OS: unfavorable cytogenetics (RR=1,8, $p=0,00002$), $WBC \geq 50 \times 10^9/L$ at diagnosis (RR=1,1, $p=0,0007$), age ≥ 50 (RR=1,6, $p=0,002$), and lack of CD33 expression on leukemic cells (RR=1,7, $p=0,014$). In this study we demonstrated that besides cytogenetics, other factors including particular phenotypic features, as well as initial tumor burden, and age independently influence outcome of AML patients. Results of the analysis provide practical information that could be taken into account when planning individualized treatment including indications for high-dose therapy followed by bone marrow transplantation.

0002

HIGH EFFICIENCY OF FLOW CYTOMETRY IN IDENTIFYING RESIDUAL LEUKEMIC CELLS IN ADULT B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA

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Flow cytometry is a powerful tool to assess the persistence of minimal residual disease (MRD) in childhood B-lineage acute lymphoblastic leukemia (ALL). On the other hand, the value of

this method in adult ALL is still debated. *Aims.* We have used a multi-parametric flow cytometry approach to assess the frequency of some leukemia-associated immunophenotypes (LAIP) and the sensitivity of flow cytometry in detecting residual leukemic cells. Then, we have evaluated the stability of LAIP in leukemic cells after co-culture with a BM mesenchymal stem cell (MSC) monolayer. Finally, we have estimated the prognostic value of immunophenotypic MRD detection in 10 adult patients. *Methods.* We have analyzed 20 normal or regenerating bone marrow (BM) samples to quantify the expression by normal CD19+ B-cell precursors of different antigens (CD34, CD10, CD38, CD45, CD58, CD22, CD21, CD13, CD33, CD15, CD65, CD66c, CD56, NG2, TdT). We have identified the normal distribution of B-cell precursors inside the BM mononuclear cells (MNC) and we have drawn some 'normality templates'; we have assessed the expression of the same markers by leukemic cells and thus identified the presence of LAIP. The sensitivity of immunophenotypic MRD detection has been tested by scalar dilutions of leukemic blasts with normal BM MNC. BM blasts obtained from 5 cases of adult B-lineage ALL with suitable LAIP have been diluted with MNC from healthy BM donors (ratio 1:10) and co-cultured with a MSC monolayer. After 14 and 21 days of culture, immunophenotypic analysis has been carried out to verify the stability of LAIP. Finally, from January 2003 to January 2005 we performed the immunophenotypic MRD monitoring in 10 adult B-lineage ALL patients and correlated the flow-cytometric analysis with the clinical outcome. *Results.* Of 64 patients analyzed, 61 (95.3%) had at least one LAIP. Of them, 26 (40.6%) had only one marker suitable for MRD monitoring; the remaining 35 cases (54.7%) had at least two LAIP. We could identify at least 1/1,000 leukemic cells (10-3 sensitivity) in all cases and 1/10,000 leukemic cells (10-4 sensitivity) in 72% of cases. However when only the LAIP with over- or down-expression of normal markers (such as CD38 or CD45) were considered, the frequency of false negatives at the 10-4 dilution was only 11%, as compared to 44% with LAIP based on aberrant antigens (such as CD13 or CD33). The LAIP were stable, as they were still detectable at the end of the co-culture of blasts with MSC. *Clinical follow-up:* 4/10 patients are in complete remission (CR) without MRD. Four patients have relapsed following positive MRD controls. Two patients are in CR with a positive MRD detection (median follow-up: 39 weeks, range 23-91). *Conclusions.* Immunophenotypic MRD detection can be considered a potential tool to detect residual leukemic cells in adult B-lineage ALL, with the same efficiency observed in childhood B-lineage ALL. However, a larger number of patients is necessary to better assess the prognostic value of immunophenotypic MRD detection.

0003

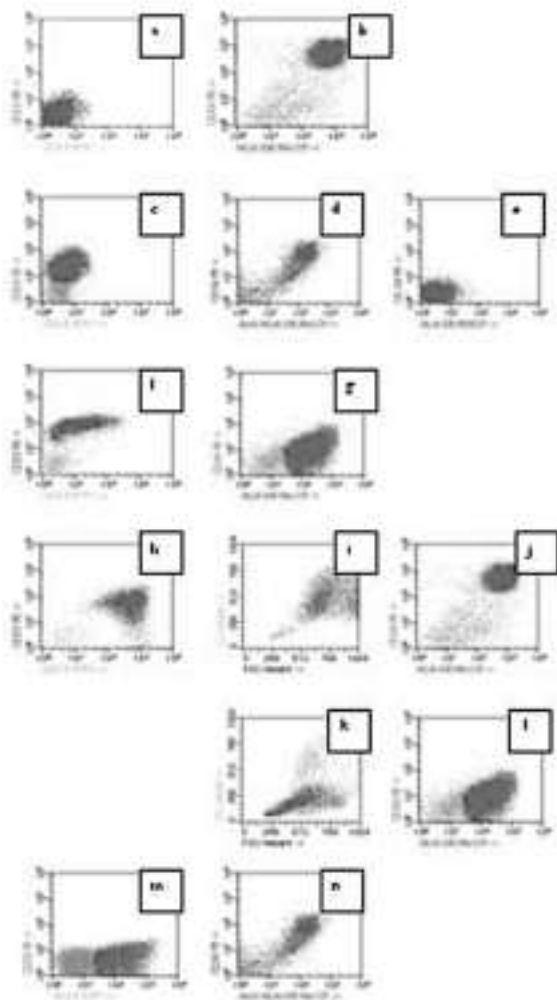
PATTERNS OF EXPRESSION OF CD34, CD33, AND CD15 PREDICT PROGNOSIS IN PATIENTS WITH AML

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Cytogenetic analysis is the most important diagnostic tool for determining prognosis in AML. The need for additional predictors of prognosis is obvious, since chromosomal aberrations

are found in only 50% of patients. No single antigen expression of leukemic cells has been proven to reliably predict prognosis in AML. Expression of CD34, CD33 and CD15 defines various stages of normal myeloid differentiation. We have grouped AML patients according to patterns of these antigen expressions and prognostic significance of this new classification was investigated. *Methods.* Expression patterns of CD34, CD33 and CD15 were determined by flow cytometry in leukemic blasts from 129 previously untreated consecutive patients with non-APL AML diagnosed 1994-2001. The median age was 64 years (range 19-85). Median follow-up in 33 surviving patients was 44 months (range 20-105). Bone marrow samples from 20 patients with reactive bone marrow were also investigated. The Cox proportional hazard model was used in survival analysis. *Results.* Based on the expression of CD15 and CD33 five distinct groups were identified: [I: CD33-/CD15- (n=18), II: CD33⁺/CD15- (n=43), III: CD33⁺/CD15 heterogeneous (n=10), IV: CD33⁺/CD15⁺ (n=50), V: CD33-/CD15⁺ (n=8)].



These groups were subdivided according to CD34 expression (Figure 1) corresponding to maturation stages of neutrophil differentiation. Complete remission (CR) was achieved in 88 patients (69%) and 51 patients (58%) eventually relapsed. The median overall survival (OS) for all patients and CR patients was 15 and 32 months, respectively. Cytogenetic results were available in 112 patients. Only 3 patients (2%) had favorable cytogenetic changes (all in group IV). 17 patients (13%) had

unfavorable chromosomal changes that were more frequent in groups IIA and IV. The shortest median OS (8 months) was observed in patients in group II (median age 73 years). Patients <60 years in this group also had a short OS (9 months). Patients with pattern V showed short OS (14 months) despite a median age of 57 years and high CR rate (88%). Group I and III patients (median age 63 years) had a median OS of 28 and 29 months, respectively. The longest median OS was found in patients within group IVC (33 months). In univariate survival analysis, age ($p<0.0001$), cytogenetics ($p=0.005$), and our immunophenotypic classification ($p<0.0001$) were found to be significant for OS. In multivariate analysis these factors remained independent of each other. The prognostic significance of our immunophenotypic classification was retained ($p=0.0235$) when patients younger than 60 years were analyzed separately. *Conclusions.* Immunophenotype patterns using CD34/CD33/CD15 antigen expression define distinct clinical subgroups of AML and seem to add useful prognostic information. Figure 1 FC plots showing the expression of CD33/CD15 and CD34/HLA-DR for each category. (a,b) pattern I, (c) pattern II, (d) and (e) subgroups IIA and IIB with differences in expression of CD34 and HLA-DR, (f,g) pattern III, (h) pattern IV, (i,j) IVA with a myeloblastic scatter and positivity for CD34 and HLA-DR, (k) IVB with monoblastic scatter, (l) IVC with CD34-/HLA-DR+ pattern, (m,n) pattern V.

0004

PREVALENCE AND PROGNOSTIC VALUE OF MYELOID ANTIGEN (MYAG) EXPRESSION IN HIGH-RISK (HR) ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL). STUDY OF 222 PATIENTS TREATED WITH THE PETHEMA ALL-93 TRIAL

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The prognostic value of MyAg expression in ALL is controversial. Initial studies seemed to suggest a worse prognosis for ALL with MyAg expression, but recent studies with high doses of chemotherapy do not confirm this observation. *Aims.* To evaluate the frequency and prognostic value of MyAg expression in adults with HR-ALL. *Methods.* Between June 1993 and July 2002, 222 adult patients with HR-ALL (age ≤ 30 years, WBC count $\geq 25 \times 10^9/L$ or presence of t(9;22), t(1;19) or t(4;11)) were treated according to the PETHEMA ALL 93 protocol. My+ALL was considered when CD13, CD14 or CD33 were coexpressed with T or B-cell antigens in $\geq 20\%$ of blast cells. The frequency of MyAg expression, its relation with other clinical and biologic variables and their prognostic significance for complete remission (CR), event free survival (EFS) and overall survival (OS) were analyzed. Mean (SD) age was 29 (10) yr. and 131 patients (59%) were males. The mean (SD) values for Hb, WBC and platelets were 97 (2.8) g/L, 60 (98) $\times 10^9/L$ and 66 (36) $\times 10^9/L$, respectively. Immunological phenotype: early pre-B 43 (19%), common+pre-B 113 (51%) and T 66 (30%). Cytogenetics (161 evaluable patients after revision): normal 67 (41%), t(9;22) 37 (23%), t(4;11) 6 (4%), t(1;19) 2 (1%) and other rearrangements 49 (31%). MyAg expression was present in 96 out of 222 patients (43%). No correlation was found between MyAg expression and the main clinical and biologic characteristics of ALL. Response to induction treatment was slower in patients expressing MyAg ($>10\%$ of blast cells in BM at day 14 in 45/93 My+ALL vs 42/123 My-ALL, $p=0.03$), but no

differences were found in CR achievement (82% in each group), EFS or OS. The probability (95% CI) of EFS at 10 yr. for My+ALL patients was 35% (26-44) vs. 34% (26-42) for My-ALL. The probability of OS at 10 yr. was 30% (22-38) vs. 33% (25-41). This lack of differences in EFS and OS probabilities was also observed when only slow responding patients were analyzed. In this study, HR-ALL patients with My+ALL did not show more adverse clinical and biologic features. MyAg expression did not have prognostic significance in adult patients with HR-ALL.

Supported in part by Grant 97/1049 from Fondo de Investigaciones Sanitarias and FIJC P-EF/03, from Fundación Internacional José Carreras para la lucha contra la Leucemia.

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PROGNOSTIC IMPLICATION OF CD56 ANTIGEN EXPRESSION IN ACUTE MYELOID LEUKEMIA

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The prognostic significance of immunophenotype in acute leukemia is well established. CD56 antigen expression has been reported in 10-25% of acute myeloid leukemia (AML) cases and its role remains uncertain. CD56 has been associated with an unfavorable outcome in AML, especially the M2 and M3 subtypes. We investigated CD56 expression in AML and assessed its prognostic relevance as well as possible associations with other disease characteristics. Immunophenotypic data were available for 137 AML patients (pts) (75 male, 62 female) with a median age of 48 (8-74) years. A sample of fresh bone marrow or peripheral blood cells taken at diagnosis was recorded as positive for CD56 when $\geq 20\%$ of myeloid or monocytoid blasts stained with specific anti-CD56 monoclonal antibody. Nineteen out of 137 pts were CD56 positive (14%), with a median positivity percentage of blastic cells of 42% (21-99); CD56 positivity was more frequent in the M2 and M5 subtypes, while M3 and secondary AML cases were CD56-negative. Statistical analysis revealed a significant correlation between CD56 positivity and increased LDH ($p=0.023$), abnormal karyotype ($p=0.006$) and myeloperoxidase positivity ($p=0.0011$). No correlation was found with age, sex, WBC at diagnosis, the presence of FLT3-ITD or extramedullary leukemia. CD56 expression did not influence the rates of complete remission [79% for CD56 (+) pts vs 66% for CD56 (-) pts., $p=$ non significant (ns)], relapse (85% vs 58%, $p=$ ns), 5-year disease free survival (DFS) (14% vs 39%, $p=$ ns) and overall survival (OS) (32% vs 37%, $p=$ ns). However, CD56 expression was associated with increased relapse rate in the subgroup of 10 AML- M2 pts with t(8;21) karyotype, with 6/7 CD56 (+) patients eventually relapsing. On Cox regression analysis, DFS was statistically related with karyotype and white cell blood count at diagnosis while age, karyotype and performance status were the only variables independently associated with OS. In conclusion, CD56 antigen expression does not seem to represent an independent prognostic factor in AML, with the possible exception of t(8;21) cases.

0006

MIXED CLINICO-CYTOGENETIC RISK MODEL TO IDENTIFY ACUTE MYELOBLASTIC LEUKAEMIA (AML) PATIENTS AT HIGH RISK OF FAILURE AFTER STANDARD INDUCTION THERAPY

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Background/Aims. In adult AML, resistance to standard '3+7'-like induction confers lower survival chances and correlates with disease and host-related features. Thus high risk patients could be considered for early therapeutic switch (high-dose ara-C, fludarabine, Pgp-inhibitors, gemtuzumab etc.). To emphasize this point, we analyzed the cumulative effects of cytogenetics plus selected clinical characteristics on outcome following standard 'ICE' regimen. **Methods.** Three-hundred and twenty unselected adult patients with AML, aged up to 66 years (median 52), were enrolled into risk-adapted NILG-AML study 01/00. ICE consisted of IDR 12 mg/m²/d on days 1-3, Ara-C 100 mg/m²/bd on days 1-7, VP16 100 mg/m²/d on days 1-5, and G-CSF from d 8. Cytogenetic risk classes were: F (favourable) with t(8;21) and inv(16); I, intermediate with aberrations other than F/U plus not known; normal (N); and U (unfavourable) with -5/del(5q), -7, t(11q23), t(9;22), abn 3q, 9q, 11q, 20q, 21q, 17p, iso(17q), t(3;5), t(6;9), complex with >3 unrelated clonal anomalies.

	F-SR	F-HR	N-SR	N-HR	I-SR	I-HR	U-SR	U-HR
N	14	9	51	94	30	54	26	42
CR (n)	12	9	42	70	23	37	16	20
NR (n)	2	0	7	20	7	14	10	18
(%)	(14)	(0)	(14)	(21)	(23)	(26)	(38.5)	(43)

Associated high risk (HR) factors were any of the following: WBC $>50 \times 10^9/L$ ($n=74$), MDS/secondary-AML ($n=47$), FLT3 mutation ($n=47/185$), hepato/splenomegaly ($n=45/37$), FAB M0/6/7 ($n=20/11/3$) and granulocytic sarcoma ($n=2$). NR, i.e. non-response rates excluding early deaths were determined in each cytogenetic risk group also by concurrent HR factors (SR, none present i.e. standard risk). **Results.** NR rate following ICE ranged from $<10\%$ in F group (cumulative) to $>40\%$ in U-HR group, with a clear relationship between worse NR results and HR features in each cytogenetic risk group. Interestingly, the N cytogenetic group had lower incidence of NR than the I group (Table). Altogether three prognostic subsets were identified, with NR $<20\%$, 20%-30%, and $>30\%$ (arbitrarily defining an increasing risk of failure): low-risk (F-SR/F-HR/N-SR, $n=74$, NR 12%) intermediate risk (N-HR/I-SR/I-HR, $n=178$, NR 23%) and high-risk (U-SR/U-HR, $n=68$, NR 41%; $p=0.005$). **Conclusions.** Cytogenetics alone cannot predict with accuracy the risk of refractory AML after conventional treatment, requiring integration with clinico-laboratory data, particularly in the larger I and N cytogenetic risk groups. While the search for prognostic indicators outside cytogenetics may lead to a more refined risk model, ICE induction appears insufficient for cases at greater risk of failure ($>20\%$).

0007**CLINICAL IMPLICATIONS OF ABERRANT DNA METHYLATION PATTERNS IN ACUTE MYELOGENOUS LEUKEMIA**O. Galm¹, S. Wilop¹, C. Lueders¹, E. Jost¹, G. Gehbauer¹, J.G. Herman², R. Osieka¹¹Universitätsklinikum Aachen, AACHEN, Germany²Johns Hopkins University, BALTIMORE, USA

Background. Hypermethylation of CpG islands within gene promoter regions is associated with transcriptional inactivation and represents an important mechanism of gene silencing in the pathogenesis of human cancer. This epigenetic phenomenon acts as an alternative to mutations and deletions to disrupt tumor suppressor gene function. **Aims.** In this study, we examined the methylation status of eleven well-characterized cancer-related genes in samples of 60 adult patients with acute myelogenous leukemia (AML) at diagnosis and explored possible correlations between methylation patterns and clinical parameters. **Methods.** The methylation status of the eleven candidate genes was analyzed by methylation-specific polymerase chain reaction. **Results.** The frequency of aberrant methylation among the patient samples was 45.0% (27/60) for SOCS-1, 31.7% (19/60) for p15, 20.0% (12/60) for RAR α , 13.3% (8/60) for p73 and E-cadherin, 5.0% (3/60) for MGMT, 3.3% (2/60) for DAP kinase 1 and hMLH1, 1.7% (1/60) for p16, and 0% (0/60) for TIMP-3 and RASSF1A. We detected at least one hypermethylated gene promoter region in 70% (42/60) of the primary patient samples. Aberrant CpG island methylation was found in all AML FAB subtypes and throughout all cytogenetic risk groups. There was a trend towards a higher methylation frequency in AML patients with an unfavorable karyotype. **Conclusions:** Our data indicate that hypermethylation of multiple genes is a common event in AML. The accumulation of epigenetic events affecting genes regulating cell cycle inhibition, cell adhesion, growth factor signaling and apoptosis may, in addition to genetic aberrations, contribute to the malignant AML phenotype. Our growing knowledge about epigenetic aberrations provides a rationale and molecular basis for targeted therapeutic approaches with demethylating agents in AML.

0008**PRETREATMENT CYTOGENETIC ABNORMALITIES ARE PREDICTIVE OF INDUCTION SUCCESS, CUMULATIVE INCIDENCE OF RELAPSE AND OVERALL SURVIVAL IN PATIENTS >60 YEARS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA**R.F. Schlenk¹, K. Döhner¹, M. Morhardt¹, B. Addamo¹, S. Fröhling¹, H. Döhner¹, For the AMLSG²¹University of Ulm, Ulm, Germany, Hannover/Ulm, Germany

Purpose. Karyotype at diagnosis provides the most important prognostic information in younger adults with acute myeloid leukemia (AML). However, there are few data available looking in particular at patients above 60 years of age. **Methods.** and Patients: We analyzed prospectively 346 adults with newly diagnosed acute myeloid leukemia (AML). All patients were treated within the AMLHD98B treatment trial and received intensive induction and consolidation therapy. Median follow up for survival was 37 months. The median age was 67 years (range 61-85 years). **Results.** Analyses were normalized to the complete remission (CR) rate (59%), cumulative incidence of relapse (CIR) after 3 years (77%) and overall survival (OS) after 3 years (17%) of patients with normal karyotype (n=138). Nonprioritized cytogenetics did not distinguish t(8;21) (n=11) and inv(16)/t(16;16) (n=15) from patients with normal karyotype. On the one hand, there were aberrations frequently associated with a complex karyotype and an inferior prognosis (abn(3q), -5/5q-, t(6;9), -7/7q-, del(9q), abn(12p), +13, del(13q), +14, -17/17p-, -18, -20/20q-, +21), whereas on the other hand, there were aberrations appearing in more than half of the cases in no-complex karyotypes and which are associated with a prognosis comparable to that of patients exhibiting a normal karyotype (+8, inv

(16)/t(16;16), t(8;21), -Y, t(9;11), +11). A complex karyotype was associated with a dismal prognosis and there was no difference in outcome whether it was defined by +3 aberrations or +5 aberrations in absence of a balanced translocation [t(8;21), inv(16), t(9;11)]. Based on outcome for specific cytogenetic abnormalities and karyotype complexity, patients were divided into three risk groups: t(15;17) (n=10, CR 80%, CIR 29%, OS 57%), favorable (n=192, CR 60%, CIR 79%, OS 19%) and unfavorable (n=142, CR 25%, CIR 80%, OS 4%). One aim of our study was to evaluate the prognostic value of the MRC (Grimwade *et al. Blood* 2004) and the CALGB (Byrd *et al. Blood* 2002) classification systems in our independent dataset excluding patients exhibiting a t(15;17) at diagnosis: MRC favorable (n=26, CR 77%, CIR 100%, OS 11%) intermediate (n=266, CR 49%, CIR 78%, OS 14%) unfavorable (n=42, CR 7%, CIR 100%, OS 3%), CALGB favorable (n=27, CR 74%, CIR 100%, OS 11%) intermediate (n=210, CR 53%, CIR 77%, OS 15%) unfavorable (n=97, CR 22%, CIR 86%, OS 11%). Multivariate analyses revealed age ($p<0.0001$), LDH ($p<0.0001$) and our classification ($p<0.0001$) as significant predictors for survival. **Conclusions.** Karyotype at diagnosis provides important prognostic information also in elderly adults with AML. However, the MRC and the CALGB classification systems seems not adequately predict prognosis in our patient population aged >60 years.

0009**MINIMAL RESIDUAL DISEASE (MRD) EVALUATION IN CHILDHOOD B LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) - CORRELATION BETWEEN RQ-PCR AND FLOW CYTOMETRY ANALYSIS**P.G. Gameiro¹, M.J. Martins², T. Faria³, M.R.G. Silva⁴, A. Parreira⁴, P. Lucio³¹Instituto Português de Oncologia Lisboa, Lisboa; ²CIPM-IPOFG, Lisboa; ³Flow Cytometry Dept., IPOFG, Lisboa; ⁴Hematology Department - IPOFG, Lisboa, Portugal

Background. MRD detection at end of induction treatment is essential for patient risk-stratification in childhood ALL. Currently, two methodologies can be used to quantify MRD during follow-up of ALL: detection of leukaemia-associated immunophenotypes by flow cytometry (FC) and real-time quantitative (RQ)-PCR amplification of fusion transcripts or antigen-receptor gene rearrangements. Although both approaches are already validated, the correlation between them is not clearly defined. In order to address this issue, we performed MRD evaluation by both assays in 19 childhood B-lineage ALL cases enrolled in the DFCI 01/91 clinical protocol. **Methodology:** Thirty-eight bone marrow samples were collected at time of diagnosis (n=19) and after the induction treatment (n=19); additionally, 15 samples were collected at different time points after day 90. In 14/19 patients MRD was monitored by RQ-PCR amplification of the t(12;21) (TEL-AML1) fusion transcript and in the remaining 5 patients an ASO to the IGH junctional region was used for MRD quantification. The RQ-PCR of the TEL-AML1 was based on the EAC protocol (Gabert *et al., Leukemia* 2003). The ASO-JH RQ-PCR was performed according to Verhagen OJHM *et al. (Leukemia* 2000). Multiparameter FC was performed according to previously standardized methodologies (Lucio *et al., Leukemia* 2004). **Results.** Overall, we obtained concordant results between RQ-PCR and FC in 27 samples (80%). In what concerns discrepant results (7 out of 34) we found 5 FC+/RQ-PCR- and 2 FC-/RQ-PCR+ samples. The latter are probably due to the limited sensitivity of FC (within the range of 0.01-0.001%). The former may be attributable to the long storage of the RNA samples (>18 months) or to sampling effect. Moreover, false positive results might have been obtained by FC. In 18 follow-up samples with MRD levels quantified by FC and RQ-PCR (results within the quantitative range), we found a positive correlation between the two techniques: $p=0.001$, Pearson correlation $r=0.734$. **Conclusions.** MRD measurement by RQ-PCR and FC correlates well, but discrepant results were still identified. Prospective follow-up studies might clarify this issue. Until then, both techniques should be used together in order to facilitate clinical decisions concerning risk stratification in ALL patients.

0010**DETECTION OF MINIMAL RESIDUAL DISEASE IN CHILDHOOD ACUTE MYELOID LEUKEMIA**

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The kinetics of tumor cell reduction, as determined by measuring minimal residual disease (MRD), may be of prognostic relevance in childhood acute myeloid leukemia (AML). In this study we aimed to: 1, establish the frequency of leukemia-associated immunophenotypes (LAIPs) in over 150 pediatric AML patients enrolled in the British MRC AML12 and the identical Dutch DCOG ANLL97 protocol; 2, establish the frequency of the three most common fusion gene transcripts and FLT3-ITD in these patients; 3, determine the stability of these MRD targets between diagnosis and relapse; 4, determine whether MRD is of prognostic relevance in the MRC-AML12/DCOG ANLL97 protocol; and 5, analyse MRD in paired bone marrow (BM) and peripheral blood (PB) samples. In 96% of patients an aberrant immunophenotype could be detected; in 69% these aberrant immunophenotypes allowed a sensitivity of 10e-4, whereas a sensitivity of 10e-3 was reached in all cases. Fusion gene transcripts and FLT3-ITD were detected in 25% and 22% of patients at diagnosis, respectively. Overall, an MRD-PCR target was detected in 43% of patients. Comparison of paired diagnosis and relapse samples (n=23) showed that immunophenotypic shifts at relapse were observed in 91% of cases, but this did not significantly hamper MRD analysis. Fusion gene transcripts were always stable between diagnosis and relapse, but FLT3-ITD were lost at relapse in four out of seven patients (57%). Preliminary data (from 47 patients) indicate that an MRD level >0.4% in the post-induction sample (as determined by flowcytometry) is associated with high chance of relapse (76% versus 17% for patients with MRD levels equal to or lower than 0.4%; $p < 0.001$ (log rank)). The number of (relapsed) patients in the molecular subgroups was too small for reliable statistical analysis. Finally, analysis of paired bone marrow and peripheral blood samples (25 paired samples) showed that MRD levels in BM were significantly and variably higher than in corresponding PB samples. Our data show that MRD analysis in pediatric AML can be of prognostic relevance. However, MRD data obtained by different methods are not yet easily exchangeable and more work is required to further optimize the flowcytometric MRD analysis. Finally, additional studies are required in order to determine the most optimal time point(s) and cut-off level for MRD analysis in childhood AML.

0011**IDENTIFICATION OF MOLECULAR PREDICTORS OF RESPONSE TO TIPIFARNIB (ZARNESTRA®, R115777) IN RELAPSED AND REFRACTORY ACUTE MYELOID LEUKEMIA**

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Background. Tipifarnib (ZARNESTRA®, R115777) is an orally bioavailable selective farnesyl transferase inhibitor (FTI) that is currently under clinical development as monotherapy and in combination for a variety of cancers. While it is clear that FTIs function by inhibiting protein farnesylation, it is still unknown which genes are implicated in the antitumor effects of tipifarnib

in hematopoietic malignancies. Microarray technology allows for the measurement of the steady-state mRNA levels of thousands of genes simultaneously, thereby representing a powerful tool for identifying genes and gene pathways that correlate with farnesyl transferase inhibition. **Aims.** In an effort to identify patients with a higher likelihood of response, we have employed microarray technology to identify gene expression markers that predict response to tipifarnib in patients with acute myeloid leukemia (AML). The current study was part of an open label, multicenter, non-comparative phase 2 clinical study in which patients with relapsed or refractory AML were treated with tipifarnib at a starting dose of 600 mg BID administered orally for the first 21 consecutive days of each 28-day cycle. **Methods.** Gene expression profiles from 80 bone marrow samples, collected from a cohort of relapsed and refractory AML patients before drug treatment, were analyzed on the Affymetrix U133A gene chip that contains approximately 22,000 genes. Statistical analyses were performed to identify genes that predict patient outcome following treatment with tipifarnib. For the purpose of gene expression profiling, response to tipifarnib was defined as patients who had an objective response (complete response, complete response with incomplete platelet recovery, or partial response), a hematological response (decrease of >50% of leukemic blast cells in bone marrow), or stable disease (no hematological response but no progression of the disease). Response had to be confirmed for at least 4 weeks after initial documentation. **Results.** Supervised analysis identified a combination of 3 gene expression markers that together may be used to predict patient response to tipifarnib with an overall accuracy of 74%. This signature provided a negative predictive value of 94% and a positive predictive value of 48% in the leave-one-out cross validation. One of these genes, the lymphoid blast crisis oncogene (oncoLBC or AKAP13), was over-expressed in patients who were resistant to tipifarnib. When over-expressed in the HL60 cell line, AKAP13 increased the resistance to tipifarnib by approximately 20 fold. **Summary/Conclusions.** These data indicate that diagnostic gene expression signatures may be used to select a group of patients for whom the response rate to tipifarnib, as defined by stable disease or better, doubles from 24% to 48%. The identification of these gene expression markers is an important step towards defining diagnostic signatures that could be used to identify AML patients who are likely to respond to tipifarnib. The identification of genes differentially expressed by responders compared to non-responders may provide a better understanding of the anti-tumorigenic effects of tipifarnib in patients with AML.

0012**UNFAVORABLE PROGNOSTIC ROLE OF BCRP/MRP1 CO-EXPRESSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS**

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The Breast Cancer Resistance Protein (BCRP), the most recent member of ABC drug efflux membrane transporters, is considered as one of three major transporters causing drug resistance in mammalian cells. Prognostic role of BCRP in adult acute lymphoblastic leukemia (ALL) is presently not well defined. **Aims.** Here we investigated in 93 untreated adult ALL patients enrolled in the GIMEMA protocols LAL 0496 and LAL 2000 the frequency of BCRP expression, its correlation with other MDR-related proteins and the prognostic role. BCRP protein expression was detected by flow cytometry using the monoclonal antibody BXP-34 (Kamiya, Seattle, WA) and the analysis was performed by the Kolmogorov-Smirnov (KS) statistic test (D-value). **Results.** Detection of BCRP in the cell lines MCF7 pcDNA3 and MDA231 pcDNA3 showed a D-value of 0.12±0.11 and 0.09±0.06, respectively (negative controls). In contrast, the cell lines MCF7 pcDNA3 clone 8 and MDA231 pcDNA3 clone 23 overexpressed BCRP with a D-value of 0.44±0.21 and 0.33±0.11, respectively (positive controls). Analysis of primary

ALL samples showed a BCRP expression (D-value >0.20) in 70/93 (75.3%) cases, with a mean value of 0.33 ± 0.19 (range 0.00-0.87, median 0.33) in the overall population analyzed. BCRP expression resulted higher in samples from patients with WBC counts $\geq 100 \times 10^9/L$ compared to those with lower WBC counts (mean 0.34 ± 0.03 vs. 0.26 ± 0.13 , $p=0.06$). No statistically significant differences were found between BCRP expression and patients' clinical characteristics. The analysis was then extended to the Multidrug Resistance Associated protein (MRP1) and to the MDR1/P-glycoprotein-170 (MDR1). A D-value ≥ 0.20 and ≥ 0.05 was found in 53.4% (39/73) and 30.2% (26/86) of cases, respectively. Samples analyzed for both BCRP and MRP1 expression (73/93) showed a significant correlation ($R^2 = 0.25$; $p=0.0001$): 12.3% of samples were negative for both proteins, while 43.8% expressed both BCRP and MRP1 proteins. In addition, MRP1 negative samples showed lower BCRP levels (mean 0.30, range 0-0.60) compared to MRP1 positive cases (mean 0.38, range 0-0.87) ($p=0.017$). BCRP expression did not correlate with MDR1 expression. None of these MDR markers correlated separately with achievement of complete remission (CR). In contrast, BCRP/MRP1 co-expression significantly correlated ($p=0.034$) with failure to respond to induction treatment: 47.8% (11/23) of BCRP+/MRP1+ patients failed to achieve CR, while 78.4% (29/37) of cases negative for one (BCRP-/MRP1+ or BCRP+/MRP1-) or for both proteins (BCRP-/MRP1-) responded to induction treatment. Multivariate analysis (Backward method) confirmed the unfavorable prognostic role on CR of these two proteins concomitantly expressed ($p=0.029$; OR 0.27, 95% CI, 0.081-0.87). In conclusion, our study shows that BCRP is expressed in a significant proportion of adult ALL. The co-expression of BCRP and MRP1 plays an unfavorable prognostic role on achievement of CR in ALL. These data suggest that the resistance phenotype may be the result of the combined effects of several transporter proteins involved in the MDR process and that the detection of all these proteins may better predict impact of resistance mechanisms in the prognosis of adult ALL.

0013

MONITORING OF MINIMAL RESIDUAL DISEASE (MRD) IN ACUTE MYELOID LEUKEMIA USING PERIPHERAL BLOOD (PB) AS AN ALTERNATIVE SOURCE TO BONE MARROW (BM)

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In acute myeloid leukemia (AML), the level of MRD as determined by multiparametric flow cytometry (MPFC) has been shown to impact on remission duration and survival. BM is the most common source to perform MRD assessment. We have previously showed that MRD negativity, as defined by a number of BM residual leukemic cells (BMRLC) $< 3.5 \times 10^{-4}$ after the consolidation cycle, was associated with a significant longer disease free survival (DFS) and overall survival (OS). However, it has recently reported that PB could be used as an alternative source to BM, for MRD studies in children with T acute lymphoid leukemia. Based on this, we investigated whether PB can be used instead of BM to monitor MRD in AML patients. Thirty-four adult patients with AML were entered into the EORTC/GIMEMA protocols AML10/AML12 (age <61 yrs) or AML13/AML15 (age >61 yrs), all consisting in intensive induction and consolidation cycles, and, for patients aged <61 years, autologous or allogeneic stem cell transplantation. Median age was 50 years (range 21-73), all FAB subtypes were represented with the exception of M3 cases. We used MPFC to compare MRD measurements in 34 and 32 pairs of BM and PB after induction and consolidation, respectively. Findings in BM e PB were completely concordant after induction and consolidation therapy. Median value of BMRLC and PB residual leukemic cells (PBRLC) after induction, were 5.1×10^{-3} (range 1×10^{-4} - 1.64×10^{-1}) and 4.7×10^{-3} (range 3×10^{-4} - 1.14×10^{-1}), respectively ($r=0.84$,

$p<0.001$). After consolidation, the median value of BMRLC and PBRLC were 6.8×10^{-3} (range 1×10^{-4} - 6.6×10^{-2}) and 7.7×10^{-3} (range 3.5×10^{-5} - 1.34×10^{-1}), respectively ($r=0.83$, $p<0.001$). The cut-off value of PBRLC which correlated with the clinical outcome was 1×10^{-4} ; in fact, 22 of 31 (71%) patients with PBRLC $> 1 \times 10^{-4}$ after induction (MRDInd+) had a relapse, whereas, the 3 patients with $< 1 \times 10^{-4}$ did not ($p=0.036$). After consolidation, using the same threshold, 27 patients were considered MRD-Cons+, and 78% (21/27) experienced a relapse; the remaining 5 patients, who were MRDCons-, are still in complete remission ($p=0.0022$). The duration of DFS was significantly longer in the MRDCons- patients as compared to those MRDCons+ (median 52.6 mos, range 20-163 versus median 28 mos, range 4.4-140.6, respectively) ($p=0.035$); the multivariate analysis confirmed the independent prognostic role of the PB MRD status at the end of consolidation ($p=0.009$). In conclusion: 1) PB may be used to monitor MRD in patients with AML, allowing closer monitoring of leukemia while sparing patients the discomfort of BM aspirations; 2) the level of MRD in the PB after consolidation therapy, may provide strong prognostic informations. Our results warrant further studies in a larger group of patients recruited to different treatment protocols and monitored at different intervals.

0014

CORRELATION OF S100A10 GENE EXPRESSION LEVEL WITH CLINICAL AND BIOLOGICAL FEATURES OF ACUTE PROMYELOCYTIC LEUKEMIA

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Background. Using unsupervised analysis with a cDNA microarray (OncoChip[®]), especially designed for analyzing genes involved in cancer, we have previously described a specific gene expression profile for acute promyelocytic leukemia (APL) (Garcia-Casado et al., *Blood* 104;2004). **Aims.** To identify genes differentially expressed in APL which could be related to specific clinical and biological features (white blood cell (WBC) count, presence of f1t3 mutation, FAB subtype and risk group). **Patients and Methods.** Fifty-three APL patients (27 men and 26 women; 39 M3/14 M3v) enrolled in two consecutive PETHEMA trials were included in the present study. The median age was 41 years (range: 9-80 years) with a median WBC count and a median platelet count of $2.67 \times 10^9/L$ (range: 0.5-128) and $19 \times 10^9/L$ (range: 4.4-135), respectively. Supervised analyses were performed using the Pomelo-Tool multitest software (<http://pomelo.bioinfo.cnio.es/>). Expression data were validated by quantitative real-time PCR. **Results.** The expression of annexin-II ligand gene (S100A10) was found to be the only common significant factor to all the clinical and biological variables analyzed. According to this result, patients were classified as high or low expression level using the median value of S100A10 expression. Patients with a high expression level had a higher median WBC count compared with patients with a low expression level (16.1 vs. 1.4×10^9 ; $p<0.001$). As expected, patients with S100A10 high expression level had a higher frequency of ITD- or D835-f1t3 mutations (63 vs. 26%; $p=0.02$); M3v subtype (52 vs. 0%; $p<0.001$) and high risk group (59 vs. 4%; $p<0.001$) compared with patients with S100A10 low expression level. **Conclusions.** Our results suggest that S100A10 high expression level correlates with adverse clinical and biological features of APL.

0015

PROAPOPTOTIC HIGH BAK/BID PROFILE OF LEUKEMIC BLASTS AS AN INDICATOR OF FAVORABLE RESPONSE TO INDUCTION CHEMOTHERAPY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Apoptosis is an important mechanism of anti-tumor activity of chemotherapeutic agents. It is likely, therefore, that spontaneous apoptosis and/or expression of apoptosis-regulating proteins in tumor cells may serve as indicators of clinical outcome in patients with hematological malignancies. There are several contradictory reports concerning this problem in acute leukemias. **Aims.** Assessment of potential prognostic significance of the spontaneous apoptosis level and the pretreatment expression of proteins regulating this process in leukemic cells from patients with acute myeloid leukemia (AML). **Methods.** The study was performed in 26 AML patients. The percentage of spontaneous apoptosis (apoptotic index, AI) was measured at the diagnosis using phosphatidylserine externalization and caspase activation detection flow cytometry assays. Expression of the panel of apoptosis-regulating proteins was also assessed, including those poorly investigated in this regard or not examined yet, such as Mcl-1, XIAP, Bak, Bid and FLIP. The endpoints of the study were response to the induction treatment and the relapse free survival time (RFS). **Results.** All 26 patients were eligible to receive classical '3+7' induction treatment daunorubicine (60-100mg/m²/d) on days 1-3 and cytarabine (200mg/m²/d) on days 1-7. Eighteen patients (69.2%) achieved complete remission (CR) after induction chemotherapy (1-3 courses). Eight patients (30.7%) did not achieved CR, including one (3.8%) partial remission (PR) and seven (26.9%) non responded (NR) patients. The median time of follow up in the examined group was 1 year (range 3-23 months). Ten (46.1%) patients still remain in CR, with the median RFS 9 months (range 5-11 months). Eight patients relapsed with the median RFS 5 months. Seven (26.9%) patients died during observation from the disease progression. Surprisingly, AI of leukemic cells did not correlate with neither response to treatment nor RFS. Significantly higher pretreatment expression of proapoptotic Bak and Bid proteins was found in patients with durable CR, continuous during observation, when compared to those who did not respond or relapsed early after initial CR ($p=0.011$ and $p=0.032$, respectively). High expression of Bak protein correlated also with RFS ($p=0.013$). Simultaneous coincidence of highly expressed Bak and Bid proteins ('high Bak/Bid' profile) was the strongest favorable prognostic factor, significantly correlating with longer RFS ($p=0.008$) (Figure 1).

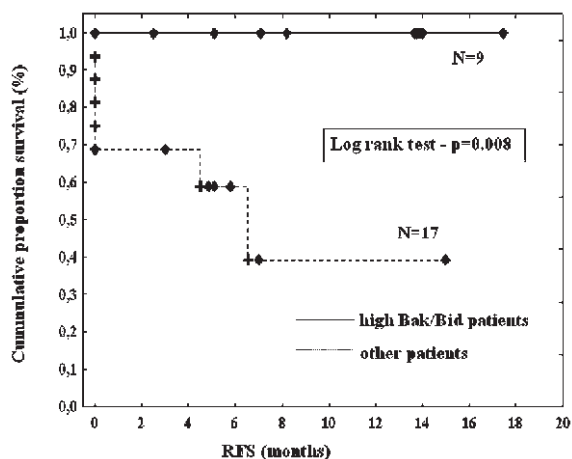


Figure 1. High Bak/Bid profile of AML cells and RFS.

Expression of other proteins did not show any prognostic impact in examined patients. There was no significant correlation between protein expression and routinely used prognostic factors, such as age at the diagnosis, cytogenetics, pretreatment leukocyte count or the number of chemotherapy cycles needed to achieve CR. **Conclusions.** These results indicate that the proapoptotic high Bak/Bid profile of leukemic blasts at the diagnosis can serve as an indicator of good response to induction chemotherapy in AML patients. Interestingly, the rate of spontaneous apoptosis of leukemic cells has no statistical strength for predicting outcome in this disease. The follow up of examined group to assess a relationship between those factors and overall survival is continuing in our center.

0016

HUMORAL IMMUNE RESPONSES AGAINST THE INHIBITOR OF APOPTOSIS PROTEIN SURVIVIN IN PATIENTS WITH HEMATOPOIETIC MALIGNANCIESM. Zeis¹, B. Friedrichs¹, R. Stuhlmann¹, S. Siegel¹, M. Bornhäuser², M. Schmitz², N. Schmitz¹¹General Hospital St. Georg, Hamburg, Germany; ²University of Dresden, Dresden, Germany

Background. The inhibitor of apoptosis protein survivin is expressed at high levels in hematopoietic malignancies, especially in acute myeloid and lymphoid leukemias (AML, ALL) and myelodysplastic syndromes (MDS). **Aim:** In the current study, we determined the prevalence of anti-survivin antibodies in sera of leukemia patients. **Methods.** Sera obtained from patients with survivin-expressing leukemia were analyzed in enzyme-linked immunosorbent assay (ELISA). **Results.** IgG and IgM survivin antibodies were measured in 112 patients (82 AML, 10 ALL, 20 MDS) and 40 healthy individuals. Immunoglobulin IgG and IgM survivin antibodies were detected in 18 (22%), 4 (40%), and 4 (20%), respectively, of the 112 patients, whereas none of the healthy volunteers had IgG, IgM or IgG/IgM survivin antibodies. To determine epitope specificity of the antibody response patient samples reacting with survivin protein were tested against 22 survivin-derived peptides. Of the 26 patient samples that were positive for antibodies to survivin protein, 20 (76%) were also positive for at least one survivin peptide. **Summary.** The data demonstrate that spontaneous humoral immune responses against survivin protein could be detected in a significant proportion of patients with survivin-expressing hematopoietic malignancies. Studies are currently underway to analyze CD4 T cell-mediated anti-survivin immune responses in patients reacting with survivin protein.

0017

TELOMERE LENGTH IN PATIENT WITH ACUTE PROMYELOCYTIC LEUKEMIA (APL) REFLECTS RESPONSE TO TREATMENT WITH ARSENICS.H. Ghaffari¹, N. Shayan-Asl², S.H. Ghaffari¹, A.H. Jamialahmadi³, A. Ghavamzadeh¹¹Tehran University Medical Sciences, Tehran, Iran²Azad University, Tehran, Iran ³Khatam University, Tehran, Iran

Introduction. The telomeric DNA together with its associated proteins protects the chromosome ends from degradation or aberrant recombination. The length of telomere in cancer cells depends on a balance between the telomere shortening at each cell cycle and the telomere elongation resulting from telomerase activity. In leukemias and in some solid tumors, a correlation between decreasing telomere length and an increasing severity of disease has been described. Telomere reduction was previously demonstrated in acute and chronic leukemia. Acute promyelocytic leukemia (APL) characterized by a specific chromosomal translocation t(15;17) that form a PML-RAR fusion gene. Arsenic trioxide (As₂O₃) is able to induce complete remission in t(15;17)-positive APLs. Arsenic trioxide treatment promotes telomere shortening and apoptosis. **Methods.** 300 peripheral blood samples were taken from 30 APL patients before,

during and after therapy with Arsenic Trioxide. Leukemic blasts were isolated by ficoll- gradient, and then genomic DNA extracted by salting out protocol from those samples, and NB4 cells. Genomic DNA was digested with Rsa1 and Hinf1 restriction enzymes; electrophoresis was performed in 0.8% agarose gels. Finally telomere length was determined by southern analysis. **Results.** We studied telomeric DNA in APL leukemic cells from patients as well as NB4 cell line as a human APL model. Marked differences were observed in the sizes of the telomeric repeats in the normal blood cells and APL leukemic cells. The leukemic cells of 30 patients with APL showed a variable reduction in the length of telomeric DNA, ranging from 2.0 to 7.0 kb, while the telomere length in PB mononuclear cells obtained from the same patients during complete remission was 9.0 to 10 kb. **Conclusions.** Arsenic therapy leads to telomere shortening, growth arrest, and leukemic cell death (by apoptosis). Longer telomeres were found in APL patients after induction by arsenic treatment compared with those found in diagnostic specimens. Most likely this was due to the loss of the leukemic clone (with shorter telomeres) and the emergence of normal hematopoietic cells (with longer telomeres) after induction therapy. These data indicate that telomere length shortening in APL patient treated with arsenic can be used as a marker to monitor disease condition and response to therapy.

0018

PERSISTENCE OF IGH REARRANGEMENTS AND ABSENCE OF DETECTABLE BCR-ABL POSITIVE CELLS DURING REMISSION OF PH+ ALL

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Detection of BCR-ABL mRNA by reverse transcriptase-polymerase chain reaction (RT-PCR) is generally used to assess minimal residual disease in patient with Ph chromosome positive acute leukaemia. Many studies have shown that qualitative and/or quantitative analysis of bcr-abl levels after allogeneic stem cell transplantation, chemotherapy and more recently, imatinib, has a strong predictive value for relapse. However, some patients without detectable level of MRD eventually relapse and MRD positive patients with long term clinical remission has been reported. In order to better clarify the clinical significance of MRD, 7 patients with Ph+ ALL in first complete remission after BMT and 2 patients treated with a chemotherapy/imatinib based therapy, were investigated with the simultaneous use of qualitative/quantitative RT-PCR for p190 and p210 and IgH gene rearrangement. MRD assessment was performed every three months after BMT or consolidation therapy. After a median follow up of 24 months 7 patients are alive in first complete remission (CR), 2 patients died with MRD but in CR. At the last follow up, MRD by both of markers was not detectable in only 3 out of 9 cases studied. All these patients had been treated with BMT. In 6 out of 9 patients we found that a clonal proliferation, marked by a unique IgH rearrangement, persisted in absence of detectable level of bcr/abl during hematologic remission. These observations are consistent with the hypothesis that a pre/leukemic clone bcr/abl negative could precede the appearance of t(9;22). Alternatively, IgH rearrangement might identify a *silent* pre/leukemic clone, carrying the t(9;22), with reduced capacity to express the leukemia specific fusion gene. In both circumstances the pre/leukemic clone have a poor response to the therapy probably due to a slower cell cycle than the fully leukemic clone. So, *true molecular* remission needed an undetectable level for both markers but at the same time persistence of MRD by Jh evaluation is not a marker of impending relapse. In this scenario we tested the significance of WT1 gene expression. This marker is still under investigation but our preliminary data, in according to the hypothesis of Saglio and co., show that Wt1 expression could reflect a 'functional state' of the pre/leukemic clone representing a marker suitable to identify the persistence/reappearance of leukemic blast and to stratify the patients in different prognostic / therapeutic (donor lymphocyte infusion, withdrawal of immuno-

suppression etc.) subgroups. In conclusion, our data shown that a pre/leukemic clone might survive after TMO/Imatinib therapy during complete remission of ph+all and perhaps spawn later relapse. If so, this work could have implications for PCR based monitoring of minimal residual disease and for our understanding of the biological basis of cure in this subset of patients.

0019

MDM2 OVEREXPRESSION PREDICTS WORSE PROGNOSIS IN ACUTE MYELOID LEUKEMIA

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Tumor progress is a multistep process in which clones of cells become abnormal by accumulating growth alterations until they are transformed and/or immortalized. In order to identify potential causative molecular alterations implicated in leukemogenesis, we investigated the expression of Mdm2, P53, and Ras genes in a cohort of 19 patients with acute myeloid leukemia (AML) at diagnosis. Bone marrow levels of Mdm2 mRNA were measured by quantitative reverse transcriptase polymerase reaction (RT-PCR) technique, while P53 and p21ras protein expression was assessed by alkaline phosphatase anti-alkaline phosphatase procedure in bone marrow cytopspins. The mutational status of Ras and P53 genes was also performed at the time of diagnosis. Mdm2 mRNA level was overexpressed (> 0.35) in 12 out of 19 cases of acute myeloid leukemia and conferred worse overall survival to this group ($p=0.01$ by Kaplan Meyer analysis) when compared to Mdm2mRNA expression of a group with 6 normal bone marrow donors which exhibited a maximum Mdm2/b-actin ratio of 0.35. Patients who exhibited Mdm2 mRNA >0.6 revealed much worse prognosis ($p=0.0096$ by Kaplan Meyer analysis) with no survivors in this subgroup, when compared to those with Mdm2mRNA expression <0.6. After age, leucocyte and bone marrow blast count adjustment by the stochastic model, p values remained significant in both comparisons (0.05 and 0.03 respectively), with a minimum follow up of 4.5 years. Additionally, 7 out 10 samples which overexpressed Mdm2 showed loss of cytoplasmic wild type p53 and p21ras proteins, while 7 out 9 cases with Mdm2 overexpression by RT-PCR exhibited loss of cytoplasmic Mdm2. The correlation of loss of cytoplasmic expression of p21ras, p53 and mdm2 proteins may indicate that this signalling pathway may be activated in AML. We demonstrated that 64% of the AML patients in our series exhibited Mdm2mRNA overexpression and this independent variable confers long-term worse prognosis to this subcategory of AML patients as shown by multivariate analysis. Our data strongly suggest that Mdm2 overexpression is a long term marker of poor prognosis in AML and that Mdm2 gene plays a pivotal role in the pathogenesis of this disease.

0020

PROTEIN KINASE CK2 ACTIVITY IS ASSOCIATED WITH PTEN PHOSPHORYLATION, AKT/PKB ACTIVATION, AND POOR CLINICAL OUTCOME IN ACUTE MYELOID LEUKEMIA

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We previously demonstrated that Akt/PKB activation and the phosphorylation of c-terminal regulatory domain of phosphatase and tensin homologue (PTEN) protein are frequently observed and significantly associated with poor clinical outcome in acute myeloid leukemia (AML). Phosphorylation of PTEN stabilizes the PTEN protein, but makes it less active towards its substrate, phosphatidylinositol 3,4,5-triphosphate, resulting in an antagonizing of the growth-promoting and anti-apoptotic pathways. However, until now, the regulatory mech-

anism for the PTEN phosphorylation has not been elucidated in AML. In this study, we evaluate the PTEN phosphorylation and protein kinase CK2 activity simultaneously in leukemic blasts obtained from 59 patients with AML. Western blot analysis revealed that PTEN phosphorylation was observed in 30 (50.9%) cases. PTEN phosphorylation was not associated with clinical parameters, including white blood cell count, patient age, and cytogenetic abnormality. Levels of CK2 activity were significantly higher in AML cases with PTEN phosphorylation compared with those without PTEN phosphorylation ($p < 0.05$). The complete remission rate was not different according to the CK2 activity. However, the disease-free survival was significantly shorter in cases with high CK2 activity ($p < 0.05$). Further laboratory experiments demonstrated that the treatment of U937, KG1 cells and primary AML cells with CK2 inhibitor apigenin or emodin for 24 hours resulted in a decrease in the levels of PTEN phosphorylation, Akt/PKB activation and increase in the extent of apoptotic cell death in a dose-dependent manner. In contrast, the induced CK2 overexpression resulted in an increase in the levels of PTEN phosphorylation and Akt/PKB activation in AML cells. Taken together, these results suggest that the phosphorylation of PTEN protein and Akt/PKB protein was directly regulated by protein kinase CK2 in AML cells. These findings additionally extend our understanding of the role of CK2 in AML, and buttress the case of CK2 activity as a prognostic indicator and CK2/PTEN phosphorylation pathway as a ready target for antileukemia therapy.

Acute myeloid leukemia - Clinical trials I

0021

EVALUATION OF ANTILEUKEMIC EFFECTS OF RAPAMYCIN, RAD001, AND CCI-779: IDENTIFICATION OF MTOR AS A NOVEL TARGET OF THERAPY IN AML BLASTS

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Background. The mammalian target of rapamycin (mTOR), a key-regulator of cell cycle progression, has recently been implicated in growth of leukemic cells and leukemia-associated angiogenesis. In addition, mTOR has been described as a regulator of expression of vascular endothelial growth factor (VEGF) in leukemic cells. **Aims.** We asked whether mTOR can be employed as a new therapeutic target in acute myeloid leukemia (AML) using the mTOR-targeting drug rapamycin and its derivatives RAD001 (everolimus) and CCI-779. **Methods.** The effects of mTOR inhibitors on growth of AML blasts were determined by ³H-thymidine incorporation and evaluation of apoptosis. Expression of VEGF in AML cells was examined by Northern blotting, RT-PCR, and ELISA. **Results.** As assessed by ³H-thymidine uptake, rapamycin was found to counteract growth of AML cells *in vitro* in 14/18 patients examined as well as in the AML-derived cell lines U937, HL60, and KG1a. The effects of rapamycin on growth of AML cells were dose-dependent (primary AML cells: IC₅₀: 10 pM-1 nM; inhibition of ³H-thymidine uptake at 1 nM of rapamycin: 51±16% of control; $p < 0.05$) and associated with G1 cell cycle arrest as well as apoptosis. The same results were obtained with RAD001 and CCI-779. In all responsive cells analyzed, rapamycin and its derivatives decreased expression of VEGF in AML cells at the mRNA- and protein level. In a next step, we examined the role of VEGF as an autocrine regulator of cell growth in AML cells. As assessed by RT-PCR, primary AML cells were found to express VEGF-, VEGFR1-, VEGFR2- and mTOR mRNA. In addition, VEGF was

found to promote the growth of HL60 cells and to revert the rapamycin-induced inhibition of proliferation in this cell line. By contrast, VEGF did not revert the rapamycin-induced inhibition of growth of KG1a cells or U937 cells. **Conclusions.** rapamycin and its derivatives act antileukemic in AML cells by two distinct mechanisms, namely direct inhibition of cell growth and suppression of proangiogenic VEGF. mTOR may be a new interesting target in AML.

0022

ADDITION OF PURINE ANALOGUES TO INDUCTION/CONSOLIDATION REGIMEN DOES NOT IMPAIR PERIPHERAL BLOOD AND BONE MARROW STEM CELL HARVEST FOR AUTOTRANSPLANTATION IN ACUTE MYELOID LEUKEMIA

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In the previous study by the Polish Adult Leukemia Group (PALG 1999 Study) we demonstrated that addition of cladribine to standard daunorubicine + cytarabine (DA-7) induction potentiates antileukemic activity of the regimen in acute myeloid leukemia (AML). However, there is a concern that the treatment with purine analogues may affect a successful collection of hematopoietic cells for transplantation (autoHCT). The goal of this study was to compare the efficacy of peripheral blood and bone marrow hematopoietic CD34+ cell harvest in patients who both in induction and consolidation were treated with or without purine analogues and who were intended for autoHCT. Sixty-seven AML patients, aged 41 (17-58) years, were included in this study; 33 patients received cladribine-containing regimen (DAC-7), one patient-fludarabine-containing regimen (DAF-7), 33 patients received standard treatment (DA-7, HAM, HD cytarabine). In the DAC-7 treated patients cladribine was also given as an adjunct to second HD cytarabine consolidation. AutoHCT using bone marrow (autoBMT) or peripheral blood (autoBCT) as a source of hematopoietic cells was performed in first complete remission after completion of consolidation therapy. An additional course of AraC 200 mg/m² on days 1, 3, 5 + G-CSF 10 µg/kg since day 7 was used as mobilization in case of autoBCT. The number of collected CD34+ cells/kg was similar for patients pre-treated with purine analogues and those not receiving purine analogues: 2.55 (0.79-9.25) × 10⁶/kg vs 2.5 (1.41-23.5) × 10⁶/kg ($p = NS$) for peripheral blood and 1.62 (0.32-3.01) × 10⁶/kg vs 1.55 (0.5-2.45) × 10⁶/kg ($p = NS$) for bone marrow, respectively. In 90% and 95% of patients for both subgroups sufficient number of hematopoietic cells for transplantation could not be collected ($p = NS$). The proportion of unsuccessful bone marrow harvest was significantly lower for patients pre-treated purine analogues with purine analogues compared to those not receiving purine analogues (90% vs 56%, $p = 0.04$), whereas no difference was found with respect to peripheral blood cell collection (95% vs 62%, $p = NS$). All patients who received autoHCT engrafted. The time to neutrophil and platelet recovery was similar for both study subgroups.

We conclude that treatment with purine analogues in course of induction/consolidation therapy does not impair the hematopoietic cell harvest and does not decrease a chance to perform autoBCT or autoBMT in AML patients.

0023

CHARACTERISTIC PATTERN OF NATURAL KILLER CELL RECEPTORS IN PATIENTS WITH AML AND THEIR HLA-MATCHED ALLOGENEIC DONORS

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Background. NK cells have been implicated in alloreaactions following hematopoietic stem cell transplantation (HSCT), and they play an important part in a reorganization of patient's immunity post-transplant. NK cells express the killer cell immunoglobuline-like receptors (KIRs) and C-type lectin-like receptors that contained the inhibitory and activating signals. The occurrence of GVHD after allogeneic HSCT in AML patients seemed to be, at least in part, related to the expression patterns of KIR including activating signal receptors. **Aims.** The object of this study is to search the distribution of KIR genes in AML patients. So, this clue will provide for an important idea to understand and even modulate the graft-versus-leukemia effect by selection of the most appropriate NK alloreactive donor in the future. In addition, we would understand genetic differences in association with post-transplant complications. **Methods.** PBMCs were separated by density gradient centrifugation method and NK cells were purified through a negative magnetic sorting kit(Miltenyi Biotech) from AML patients (n=46) and their transplantation donors (n=46). After the isolation of NK cells, DNA extracted from isolated NK cells according to the manufacturer's guideline (iNtron Biotechnology, G-Dex™). The PCR executed for 19 different kinds of KIR genes. PCR data representing KIR genotypes and each of HLA type from patients were compared.

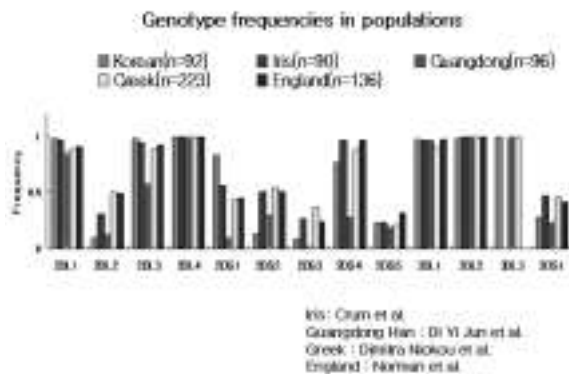


Figure. Genotype differences

Results. Generally, the pattern of KIR gene expressions showed a similarity in patients and donors. Most have KIR2DL1, KIR2DL3, KIR3DL1, KIR3DL3, KIR3DP1, and KIR3DP2 (*00301-00302) gene of KIR dominantly. Compared with Caucasians, Greeks, Guangdong Han population by the review of literature, the frequency of KIR2DL3, KIR2DS1 gene expressions displayed higher in this study population. In contrast, KIR2DL2, KIR2DS2, KIR2DS3 expressions were relatively low. Also, KIR2DL2 and KIR2DS2 co-expression association was very high. Interestingly, the KIR2DL2, KIR2DL5B, KIR3DL1 expressions are rather higher in patients than in donors. The matching rate between KIR genotypes and their own HLA typing results was high in 2DL1, 2DL3, 3DL1, and 3DL2. **Conclusions.** Findings from this study concerning the polymorphism of KIR genes will be used to indicate the ethnical differences of the immunological response such as GvHD after allogeneic HSCT.

0024

CONSISTENT SALVAGE RATE WITH A SEQUENTIAL HIGH-DOSE REGIMEN IN PRIMARY REFRACTORY ACUTE MYELOGENOUS LEUKAEMIA (AML) AT DIFFERENT CYTOGENETIC AND CLINICAL RISK

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Background/Aims. After conventional remission induction for adult AML approximately 20% of the patients have resistant disease. Salvage regimens could be used upfront once confirmed active in poor prognosis subsets, defined mainly by cytogenetics and additional risk factors. The information concerning AML salvage by cytogenetic risk class is limited. Hence we reviewed the response to a high-dose regimen in a series of 'ICE'-refractory patients, according to cytogenetics and other clinical characteristics. **Methods.** In NILG-AML trial 01/00 patients refractory to conventional-dose ICE induction were eligible to receive high-dose sequential Ara-C 3 g/m²/bd on days 1,2 and 8,9 plus Idarubicin 17.5 mg/m² on days 3 and 10. G-CSF was given from d 11. Cytogenetic risk class was F (favourable) with t(8;21) and inv (16); I, intermediate with aberrations other than F/U plus not known; normal (N); and U (unfavourable) with -5/del (5q), -7, t(11q23), t(9;22), abn 3q, 9q, 11q, 20q, 21q, 17p, iso (17q), t(3;5), t(6;9), complex with >3 unrelated clonal anomalies. Associated high risk (HR) factors were WBC >50x10³/L, MDS/secondary, FLT3 mutation, hepato/splenomegaly, FAB M0/6/7 and granulocytic sarcoma. Outcome was analyzed in different cytogenetic groups also according to these additional risk factors. **Results.** Forty-seven ICE-refractory patients with AML received the rescue regimen illustrated above.

	F-SR	N-SR	N-HR	I-SR	I-HR	U-SR	U-HR	Total
N	2	5	8	5	11	5	10	46
CR (n)	2	3	3	3	5	3	5	24
(%)	100	60	37.5	60	45.5	60	50	52
NR	0	2	4	0	4	1	5	16
ED	0	0	1	2	2	1	0	6

Forty-six were fully evaluable for response (CR, complete remission; NR, non-response; ED, early death). The results are summarized in the Table, showing consistent CR rates of about 50% in all identified prognostic subsets, regardless cytogenetic classification and additional SR/HR characteristics (SR, standard risk i.e. no HR feature present). Responsive patients were offered postremission therapy as planned, including allogeneic SCT as primary goal. **Conclusions.** The current salvage regimen is highly active in 'ICE'-resistant AML, independently of the clinical and cytogenetic risk class. On the contrary primary refractoriness to 'ICE' increases progressively from <20% in F/N-SR group to >40% in U group (prior analysis). As survival is positively influenced by the achievement of an early remission, an upfront use of this regimen could improve results by allowing more intermediate/high-risk patients to enter CR, and by an earlier transfer of residual NR cases to other experimental salvage therapy.

0025**TREATMENT OF NEW CASES OF ACUTE PROMYELOCYTIC LEUKEMIA WITH ARSENIC TRIOXIDE**

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Purpose. Arsenic Trioxide is effective and approved for treatment of relapsed or refractory APL cases to ATRA. We studied used Arsenic Trioxide as a first line treatment of new cases of APL and studied their long term clinical and molecular follow up. **Material and Methods.** we studied 109 cases of APL (94 new case and 15 relapsed) diagnosed by morphologic criteria and confirmed by cytogenetic, RT-PCR for PML/RARA and/or FISH. Arsenic Trioxide infused as 0.15 mg/kg/day doses, until complete remission by morphologic criteria or till day +60. After 28 days rest, in case of complete remission Arsenic Trioxide 0.15 mg/kg/days infused for additional 28 days as consolidation. Also we studied presence of minimal residual disease by nested real time PCR after complete remission. **Results.** Complete remission observed in 82 of new patients (87.2%) and median time to complete remission was 30 days. During the induction phase, the most common cause of toxicity and mortality was APL differentiation syndrome (in 10 cases or 14.1%). Other toxicities were serosistis (8.3%) and hepatotoxicity (26.1%). With a median follow up of 16.5 (1-57) months for patients in complete remission, mean overall survival was 47.7 months and one and two year disease free survival (DFS) were 90.9% and 67.6% respectively. We observed 14 relapses and 10 achieved to second complete remission by Arsenic Trioxide. We observed a third remission for some patients, who relapsed, again by Arsenic Trioxide. By intention to treat analysis median survival were 41.31+/-2.13 months and one year and 2 year survival were 87.9% and 81.7% respectively. Minimal residual disease was positive in 4 (7.8%) out of 51 new cases after remission induction and 3 of these patients clinically relapsed. **Conclusions.** The result of Arsenic Trioxide is acceptable as first line treatment of APL. It seems that it is applicable for relapsed patients and drug resistance is an unusual event.

0026**PROLONGATION OF REMISSION DURATION WITH LOW DOSE GEMTUZUMAB OZOGAMICIN MAINTENANCE IN A SERIES OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA AND HIGH RISK MYELODYSPLASIA**

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Fifty-three consecutive elderly (mean age 69.3 y; range 65-79) patients with Acute Myeloid Leukemia *de novo* (AML = 36) or secondary to antecedent hematological disorder (sAML=11) or high risk Myelodysplasia (RAEB=6) received an induction chemotherapy with Fludarabine 30 mg/m² and Cytarabine 1 g/sqm for four days plus Idarubicin 10 mg/m² delivered as a continuous iv perfusion on days 1,3 and 5. G-CSF was given from day 7 to granulocyte recovery. After the induction course 30 patients (56.6%) entered complete remission (CR), 3 patients died during the induction phase and 20 (37.7%) were resistant. One patient died while in CR for invasive fungal infection, one patient was lost to follow-up after CR and one patient discontinued chemotherapy because of toxicity. Twenty-seven patients proceeded to an outpatient consolidation with Fludarabine 30 mg/m² and Cytarabine 1 g/m² for two days plus Idarubicin 10 mg/m² on day 2. Four patients relapsed after consolidation. In 2 patients the therapy was stopped because of toxicity. Maintenance therapy with Cytarabine 100 mg/m² once a week and Thioguanine 50 mg/m² for five days a week was given to 12 patients. In may 2003 the protocol was amended and, instead of chemotherapy, low dose Gemtuzumab Ozogamicin (GO) (3 mg/m² iv every three months for a maximum of three

doses) was allowed in patients with CD33 positive leukemia. Nine patients have so far received a minimum of one GO course. This preliminary remission duration analysis shows a better DFS in patients treated with GO compared to patients treated with chemotherapy (16 months vs 7 months; $p=0.02$). Toxicity of GO was mild and minimum transfusion support was required. No infectious complication was observed. Infusion reactions were generally mild. However in one patient an anaphylactic shock occurred during the first infusion. All infusion were given on an outpatient setting. The results are encouraging and the study is ongoing. Updated results will be presented.

0027**A MULTICENTER, PHASE II STUDY OF A COMBINATION OF CLADRIBINE, CYTARABINE, MITOXANTRONE AND G-CSF (CLAG-M) AS INDUCTION THERAPY IN REFRACTORY AML - A REPORT OF POLISH ADULT LEUKEMIA GROUP (PALG)**

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Background. Primary resistant and relapsed AML have poor prognosis. We have published recently the promising results of a study of the combination of 2-CdA, Ara-C and G-CSF (CLAG) in refractory AML (Eur J Haematol 2003;71:155-162). 29 (50%) out of 58 patients achieved CR, its median duration was 17 weeks. Median OS for all patients and for patients in CR was 34 and 59 weeks respectively. **Aims.** The aim of this study was to assess, in a multicenter phase II study, the efficacy and toxicity of the addition of mitoxantrone to the CLAG regimen (CLAG-M) in primary resistant and relapsed AML patients. **Methods.** Induction chemotherapy consisted of 2-CdA 5 mg/m² in 2h infusion, Ara-C 2 g/m² in 4h infusion 2 h after 2-CdA (days 1-5), mitoxantrone 10 mg/m² (days 1-3) and G-CSF 300 µg sc (days 0-5). In case of PR second CLAG-M was administered. Patients in CR received 2 consolidation courses based on HD Ara-C, mitoxantrone with or without 2-CdA. Refractory AML was defined according to following criteria: 1) primary resistance to initial induction therapy 2) first early relapse with CR1 of less than 6 months 3) second or subsequent relapse 4) recurrence after stem cell transplantation. **Results.** 43 patients from 5 centers were registered, 25 primary resistant and 18 relapsed. After CLAG-M regimen CR was achieved in 21 (49%) of the whole group of patients, 20 (47%) were refractory and 2 (5%) died early. Hematological toxicity was the most prominent toxicity of this regimen. The OS (1 year) for the 42 patients as a whole and the 20 patients in CR were 43% and 73%, respectively. DFS (1 year) was 68.6%. None of the analyzed prognostic factors influenced the CR and OS probability significantly. Only SCT performed after CLAG-M significantly influenced DFS probability. **Conclusions:** We conclude that CLAG-M regimen has significant anti-leukemia activity in refractory AML, which seems to be better than activity of many other regimens. The toxicity of the treatment is acceptable.

0028**COMPARISON OF FREE AND LIPOSOMAL DAUNORUBICIN IN BLAST CELLS FROM PATIENTS WITH AML AND IN SENSITIVE AND ANTHRACYCLINE-RESISTANT HL-60 CELLS**

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Background. The anthracycline daunorubicin (DNR) is a major drug in the treatment of acute leukemia. Liposomal encapsulated DNR (daunoxome) has been produced in an attempt to get higher tumor activity and less toxic side effects than that of free DNR. It has also been proposed that liposomal daunorubicin can partly escape the pump activity of the multidrug resistance

(MDR) membrane pump P-glycoprotein (P-gp). *Aims.* To study differences in toxicity of free DNR compared to liposomal DNR on leukemic blast cells from patients with acute myelocytic leukemia and in the human leukemia cell line, HL-60, and its anthracycline resistant P-gp expressing sub line (HL-60-R). *Methods.* Leukemic blast cells from 66 patients with AML, were isolated from bone marrow or peripheral blood and incubated in cell culture media either for 1h at 0.2 μ M and cultured for 4 days or at 0.05 μ M as continuous incubation for 4 days. Cell viability was assayed by bioluminescence where the intracellular level of ATP was determined. The cytotoxicity was expressed as the ratio of the ATP content in drug-exposed to unexposed cells. Patient cells were also incubated with the cyclosporin D analogue, PSC833, in combination with DNR as a functional method to identify P-gp activity. A synergistic or additiv effect was regarded as P-gp positivity. To study the activity of the two formulations in MDR resistant cells we also performed incubations of the leukemia cell line (HL-60), consisting of the parental drug-sensitive cell line and a MDR variant(HL-60-R) over-expressing P-gp, where cytotoxicity were studied by bioluminescence. *Results.* After 1h incubation, the mean cytotoxic effect on patient cells was higher for free compared to liposomal DNR (50.8% versus 67.8% surviving cells $p < 0.001$). In contrast, after continuous incubation liposomal DNR was more cytotoxic (53.3% compared to 63.9% surviving cells $p < 0.01$). In the patient cells with functional P-gp activity the IC50 concentration for DXM was 0,90 μ M compared to 0,44 μ M ($p < 0.001$) indicating that DXM is a substrate for the pump activity of P-gp. The latter finding was confirmed by incubation of the P-gp expressing HL-60-R cells. *Conclusions.* By short time incubation liposomal DNR was less toxic compared to free DNR but continuous incubation abolishes this difference making DXM more effective than DNR against leukemic cells from AML patients. We could not support that liposomal formulation made DNR more effective against multidrugresistant P-gp expressing leukemic cells.

0029

COOPERATIVE ANALYSIS OF PATIENT WITH ACUTE MYELOID LEUKEMIA EXHIBITING A NORMAL KARYOTYPE TREATED WITHIN THE GERMAN MULTICENTER TREATMENT TRIALS AML-2/95, AML-1/99, AMLHD93 AND AMLHD98A

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Purpose. Karyotype at diagnosis provides the most important prognostic information in adult acute myeloid leukemia (AML). However, approximately 50% of patients lack clonal chromosomal aberrations. The value of different postremission strategies such as intensive chemotherapy, autologous or allogeneic transplantation in these patients remains open and, in particular, if molecular markers such as MLL-partial tandem duplications (MLL-PTD), activating FLT3-mutations (FLT3-ITD, FLT3-D835) and CEBPA-mutations are included into the analysis. Therefore, we performed a cooperative analysis of patients exhibiting a normal karyotype at diagnosis treated within the prospective treatment trials AML-2/95, AML-1/99, AMLHD93 and AMLHD98A. *Methods.* and Patients: All patients (age 16-60 years) received two cycles of induction therapy with standard dose cytarabine (ARAC) combined with etoposide and idarubicin. Patients with good response to first induction therapy and complete remission (CR) after second induction therapy received a first consolidation. For second consolidation therapy these patients were assigned to an allogeneic transplantation if an HLA-identical sibling donor was available in all four trials (DONOR-group). In the AML-2/95 and AMLHD93 trials all other patients were assigned to a high-dose ARAC-based regimen whereas in the AML-1/99 and AMLHD98A trials patients were randomly assigned to autologous transplantation (AUTO-group) or a high-

dose ARAC-based regimen (CHEMO-group). Patients with no response to first induction therapy and/or no CR after second induction therapy were assigned to salvage therapies. *Results.* Between 1993 and 2003 a total of n=795 patients exhibiting a normal karyotype had been registered within one of the four trials (AML-2/95 n=154, AML-1/99 n=203, AMLHD93 n=101, AMLHD98A n=337). The response to induction therapy was CR 78%, refractory disease 13%, early/hypoblastic death (9%); good response to first induction therapy and CR after second induction therapy was achieved in 524 of 761 evaluable patients (69%). In these 524 patients the intention-to-treat analysis revealed no difference between the AUTO-group (n=91) and CHEMO-group (n=220). Donor versus no-donor analysis revealed a significantly better relapse-free survival (RFS) ($p=0.02$) in favour for the DONOR-group (n=123). However, there was no significant difference in overall-survival ($p=0.09$). A differential effect of FLT3-ITD mutations by postremission therapy on prognosis was observed. RFS in patients with FLT3-ITD was significantly inferior in the AUTO-group ($p=0.002$) and CHEMO-group ($p=0.006$) but not in the DONOR-group ($p=0.82$). Multi-variable analysis on RFS revealed as significant prognostic markers an HLA-identical family-donor (hazard ratio [HR] 0.6), FLT3-ITD (HR 1.8), MLL-PTD (HR 1.6) and age (HR for a difference of 10 years 1.2). Analyses of CEBPA-mutations are available for the AML 2/95 and AML 1/99 trials. Therefore, they are so far not included into the analyses. *Conclusions.* Allogeneic transplantation from an HLA-identical sibling donor in first CR in AML exhibiting a normal karyotype seems to be beneficial in respect to RFS, especially, in cases with FLT3-ITD.

0030

COMPARISON OF EFFICACY BETWEEN CALICHEAMICIN CONJUGATE ANTI-CD33 MONOCLONAL ANTIBODY AND FREE CALICHEAMICIN ON LEUKEMIA CELLS AND THEIR DRUG RESISTANT CELLS

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Background. Recently, gemtuzumab ozogamicin (GO, Mylotarg), a calicheamicin-conjugated humanized anti-CD33 monoclonal antibody (mAb), was introduced for the treatment of AML. The cytotoxic agent is N-acetyl \square calicheamicin dimethyl hydrazide (NAC \square calicheamicin DMH), a derivative of the calicheamicin antitumor antibiotics. After binding to CD33, GO is internalized in cells and modified to active calicheamicin derivatives. In our previous *in vitro* studies, the efficacy of GO was positively related to the amount of CD33 and negatively related to amount and function of P-glycoprotein (P-gp) on the cell. *Aims.* To understand the efficacy of GO via CD33 antigen and P-gp, it is important to compare anti-proliferative and cytotoxic effects of GO with those of free calicheamicin. (Materials and Methods) The cell lines used were K562, NOMO-1 and NB4, and respective doxorubicin-resistant sublines, K562/ADR, NOMO-1/ADR and NB4/MDR. Other CD33-positive (HL60 and NKM-1) and negative (Daudi) cell lines were also used. GO, humanized non-conjugated anti-CD33 mAb (hP67.6) and free NAC \square calicheamicin DMH were kindly provided by Wyeth Pharmaceuticals Inc. (Philadelphia, PA, USA). The amount of GO used in an experiment was determined based on the concentration of calicheamicin bound to the antibody. For evaluation of CD33 expression, cells were stained with phycoerythrin-conjugated anti-CD33 mAb. For P-gp analysis, cells were incubated with biotinylated MRK16 (Fab') mouse mAb or a subclass-matched control mAb, and stained with streptavidin-Per CP. The level of 3H-TdR incorporation obtained upon incubation of cells with GO was compared with that obtained upon incubation with several concentrations of free calicheamicin. Viable cells were quantified by dye exclusion test. (Results) Upon 72-hour incubation with GO containing 10 ng/mL calicheamicin, 3H-TdR incorporation into CD33-positive cells

decreased in a time-dependent manner. There were significant differences in the level of ^3H -TdR incorporation between P-gp-negative and -positive cells at 72 hours ($p < 0.01$ each). Similar results were obtained in cells that had been incubated with 1,000 ng/mL free calicheamicin, which corresponded to a concentration of calicheamicin that was approximately 100 times greater than that in GO. The rate did not change between P-gp-negative and -positive cells. Similar results were obtained in the experiments of viable cell count. GO had a limited effect on CD33 negative cells while 1,000 ng/mL of free calicheamicin had a significant effect. (Conclusion) GO significantly reduced the amount of calicheamicin needed for cytotoxicity in CD33-positive cells. However, conjugation of calicheamicin to antibody did not significantly overcome P-gp-related drug resistance to free calicheamicin.

0031

SYNERGISTIC EFFECTS OF ATRA OR/AND ARSENIC TRIOXIDE WITH DOXORUBICIN ON ACUTE PROMYELOCYTIC LEUKEMIA CELLS THAT ACQUIRED DIFFERENT DRUG RESISTANT MECHANISMS

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Background. The combination of all-trans retinoic acid (ATRA) or arsenic acid (ATO) with anthracyclines has been reported in acute promyelocytic leukemia (APL). However, the synergistic efficacy of these drugs for APL cells that acquired several different resistant mechanisms has been elucidated. It is also important to determine the optimal dosage of these drugs as well as the optimal timing of their administration. **Aims.** In this study, using multidrug (MDR)-, ATRA-, and/or ATO-resistant APL cells, we clarified the efficacy of concomitant usage of these drugs, and intended to provide information on establishment of the combination methods for resistant APL. (Materials and Methods) The cell lines used were a human APL cell line, NB4, ATRA-resistant NB4 (NB4/RA) cells; NB4 and NB4/RA cells transfected with MDR-1 cDNA (NB4/MDR and NB4/RA/MDR, respectively); and ATO-resistant NB4 (NB4/ATO) cells. The NB4/RA and NB4/As cells were obtained by culturing NB4 cells with gradually increasing concentrations of ATRA and ATO, respectively. NB4/MDR and NB4/RA/MDR cells had detectable *mdr-1* mRNA. Cells were incubated with ATRA (10-6M), ATO (10-6M) and/or several concentrations of doxorubicin (DOX). After incubation, viable cells were determined by dye exclusion test. Early and late stage apoptosis were determined by the Annexin V-Azami-Green and PI staining. Each sample was analyzed by flow cytometry, and viable cells, apoptotic cells in early stage and those in late stage were evaluated by the scatter gram. (Results) DOX decreased the number of NB4 and NB4/RA cells by a similar degree. It also decreased the number of NB4/ATO cells. The combination of ATRA and DOX reduced the number of NB4 cells by a greater degree than ATRA or DOX alone, but did not reduce the number of NB4/RA cells by a significantly greater degree than ATRA or DOX alone. Upon incubation with ATO and DOX, the viable cell counts of NB4 and NB4/RA cells were less than those upon incubation with ATO or DOX alone. The addition of ATRA to DOX further increased the percentage of early and late apoptotic cells in NB4 cells and NB4/MDR cells, but not in NB4/RA cells. Upon incubation with DOX and ATO, the percentage of apoptotic cells in NB4 and NB4/RA cells were higher than those upon incubation with DOX alone. One day incubation with ATRA following incubation with ATRA and ADR increased early-stage apoptotic cells upon simultaneous incubation with ATRA and ADR. (Conclusions) Combination of ATRA and anthracycline is still effective on MDR-ATRA-sensitive APL cells, but does not provide any synergistic effect on ATRA-resistant cells. Combination of ATO and anthracycline is significantly effective on ATRA-resistant APL cells, and moderately in MDR-APL cells, and by a limited degree in ATO-resistant cells.

0032

TIPIFARNIB (ZARNESTRA®, R115777) THERAPY FOR ELDERLY PATIENTS WITH AML RESULTED IN LOW HOSPITALIZATION RATES AND ALLOWED OUTPATIENT THERAPY IN MOST PATIENTS: COMBINED RESULTS FROM PHASE 2 STUDIES

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Background. Acute myeloid leukemia (AML) is a heterogeneous disease that is most prevalent among the elderly. Treatment for elderly patients with AML continues to be a significant clinical challenge. Chemotherapies such as the '7+3' regimen that consists of cytarabine and daunorubicin or idarubicin are not well tolerated by elderly patients with AML, and are associated with serious morbidity in these patients. Tipifarnib (ZARNESTRA®, R115777) is a selective, orally available farnesyl transferase inhibitor that has demonstrated complete remissions in hematologic malignancies. Notably, hospitalization is not required for the administration of tipifarnib because of its oral availability. **Aims.** To analyze hospitalization data for tipifarnib monotherapy in the treatment of elderly patients with poor-risk AML. **Methods.** The rate of hospitalization during tipifarnib therapy was based on integrated safety data from 409 adult patients who were enrolled in 2 different phase 2, open-label, uncontrolled studies in either newly diagnosed (Study 1; N=157) or relapsed/refractory (Study 2; N=252) AML. **Results.** The majority of patients (60%) were 65 years or older. The initial dosage of tipifarnib in both studies was 600 mg twice daily for the first 21 days of a 28-day cycle. A total of 197 patients (48%) received 1 cycle, 162 patients (40%) received 2 or 3 cycles, 40 (10%) received 4 to 6 cycles, and 10 (2%) received tipifarnib for more than 6 cycles. The majority of patients were hospitalized once or twice during the treatment period for an average of 22.3 days. The most common reasons for hospitalization were disease- or treatment-related adverse events. Transfusion was the most common reason for hospitalization in Study 2 (23%), but only 2 patients (1%) were hospitalized in Study 1. The most common drug-related, nonhematologic serious adverse events that led to hospitalization were increased creatinine blood levels, fever, and sepsis. **Summary/conclusions:** Based on the integrated hospitalization data from two independent phase 2 studies, tipifarnib therapy resulted in low hospitalization rates and allowed outpatient therapy for most elderly patients with poor-risk AML. The low rate of hospitalization observed in this analysis suggests that there was a low incidence of nonhematologic adverse events associated with tipifarnib therapy. Additionally, if hospitalization rate is a practical albeit indirect measure of quality of life, this combined analysis suggests that tipifarnib compares favorably with currently available regimens in this patient population.

0033**SAFETY PROFILE OF TIPIFARNIB (ZARNESTRA®, R115777) IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA: COMBINED RESULTS FROM PHASE 2 STUDIES**

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Background. Elderly patients with poor-risk acute myeloid leukemia (AML) have lower survival rates due to, at least in part, early treatment-related mortality. Treatment-related mortality may be as high as 25% in elderly patients with poor-risk AML. Therefore, elderly patients with poor-risk AML are frequently offered palliative treatments or supportive care only. These patients might benefit from a novel therapy that is better tolerated and effective. Tipifarnib (ZARNESTRA®, R115777) is a selective, orally available farnesyl transferase inhibitor that has demonstrated complete remissions in hematologic malignancies. **Aims.** To analyze safety data for tipifarnib monotherapy in the treatment of elderly patients with poor-risk AML. **Methods.** The safety profile of tipifarnib was based on integrated safety data from 409 adult patients who were enrolled in 2 phase II, open-label studies in newly diagnosed (Study-1; N = 157) or relapsed/refractory (Study 2; N = 252) AML. **Results.** Most patients (60%) were 65 years or older. The initial dosage of tipifarnib was 600 mg twice daily for the first 21 days of a 28-day cycle. A total of 197 patients (48%) received 1 cycle, 162 patients (40%) received 2 or 3 cycles, 40 (10%) received 4 to 6 cycles, and 10 (2%) received tipifarnib >6 cycles. The overall adverse event profile of tipifarnib in AML patients was comparable between the 2 studies; however, the rate of withdrawals and deaths was lower in Study 1 (11% and 7%, respectively), the first-line trial, as compared to Study 2 (21% and 24%, respectively), the relapsed/refractory trial. Thrombocytopenia (42%), neutropenia (17%), and anemia (39%) were common adverse events. The majority of hematologic adverse events were grade 3 or 4. Most patients (93%) had grade 3 or 4 platelet counts during treatment with tipifarnib; however, 80% of these patients had ≥grade 2 thrombocytopenia at study entry. Grade 3 or 4 neutrophil counts were reported in 83% of patients; 80% of these patients had ≥grade 2 neutropenia at study entry. Grade 3 or 4 nonhematologic adverse events were reported in 82% of patients, of which 46% were considered drug related. Hypokalemia (14%), pneumonia (10%), fatigue (18%), fever (40%), and bacterial infection (9%) were the most common grade 3 nonhematologic adverse events. Sepsis (9%) was the most common grade 4 nonhematologic adverse event. Severe mucositis was rare (1% grade 3; <1% grade 4). There were 167 deaths (41%) during treatment or within 30 days after the last dose of tipifarnib; 4% of deaths were associated with drug-related adverse events. No death was due to aplasia (grade 4 absolute neutrophil count, grade 4 platelet count, and 0% blasts). The number and causes of death for patients older than 75 years were comparable to those in the younger age groups. **Summary/conclusions:** Based on the integrated safety data from two independent phase 2 studies, outpatient administration of tipifarnib offers a relatively safe treatment for elderly patients with poor-risk AML. The low incidence of mucositis during tipifarnib therapy may explain, at least in part, the low hospitalization rate in these patients.

0034**WT1-PEPTIDE VACCINATION OF PATIENTS WITH ACUTE MYELOID LEUKEMIA SHOWS HIGH IMMUNOGENICITY AND CLINICAL EFFICACY: RESULTS FROM A PILOT STUDY**

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Background. The transcription factor Wilms tumor protein 1 (WT1) is a highly interesting antigen for specific immunotherapy in leukemia and carcinomas. WT1 is strongly expressed in the majority of myeloid blasts and is a key molecule for tumor cell proliferation. **Aims.** We initiated a phase I/II study in patients with AML to analyze immunogenicity and toxicity of vaccination with the HLA-A2-binding WT1 126-134 peptide, and the adjuvants GM-CSF and keyhole limpet hemocyanin. **Methods.** WT1-specific T cell responses have been characterized by tetramer staining, intracellular cytokine cytometry, proliferation and flow-cytometric assays detecting cytolysis. WT1 transcripts were monitored by quantitative PCR. **Results.** We report here on the first 10 patients who have received between 4 and 16 vaccinations (median 7). Detailed T cell response analysis has been performed in the first 8 patients showing induction of WT1-specific T cells in 6 of 8 patients ranging from 0.42% to 1.95% (median 0.55%) both in peripheral blood and bone marrow. In two patients with high frequency marrow blasts at study onset and rapid disease progression we failed to induce specific T cells. Peptide immunization was able to generate a fully functional T cell response against WT1 characterized by production of IFN γ and TNF α , proliferation, cytolytic activity and differentiation into effector and memory T cells of both central and effector phenotype. Ten patients are evaluable for clinical response. Patient 1 who had 2nd PR progressed during the initial 4 weeks of vaccination with an increase of marrow blasts to 30%, but subsequently achieved a CR after 6 vaccinations lasting for 12 months. Patient 2 who had a formal CR following chemotherapy for secondary AML with persistence of residual marrow blasts and unfavourable 11q23 abnormality is in continuous CR for 30 months. In 3 additional patients disease stabilization was achieved, accompanied by a decrease of the elevated LDH in one patient, and a 50% reduction of marrow blasts in a patients with AML after MDS, and in 2 of these patients vaccination is ongoing. One further patient has been vaccinated in 2nd CR and is still in CR. Three patients had disease progression after 4 and one after 9 vaccinations. Real-time RT-PCR showed a 50-, and 2000-fold reduction of marrow WT1 transcripts in patients 1 and 2, respectively, to levels as low as in healthy controls, and a stabilization or reduction in patients with stable disease, while WT1 levels increased in patients with progressive disease. **Conclusions.** Taken together, we observed high immunogenicity of the vaccine associated with clinical efficacy in the absence of significant toxicity. Accrual into a phase II study, including patients with high-risk myelodysplastic syndrome, is ongoing.

0035**LONG-TERM MOLECULAR COMPLETE REMISSION WITH PULSED ATRA AS SINGLE AGENT IN PML-RAR α -POSITIVE ACUTE PROMYELOCYTIC LEUKEMIA**

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All-trans retinoic acid (ATRA), alone or combined with chemotherapy (CHT) is widely used in the treatment of acute promyelocytic leukemia (APL). If used alone, ATRA results in a substantial proportion of complete remissions (CR). However,

the continuous administration of ATRA as single therapy almost invariably leads to relapse in a short period of time (months). Thus, conventional chemotherapy often followed by autologous or allogeneic stem cell transplant(SCT) are used to maintain long term remission. Basing on pharmacokinetic evidence that that acquired resistance to ATRA is frequently suppressed by the intermittent use of the drug, we treated with 'pulsed' ATRA seven APL patients who were either molecularly refractory after combined ATRA/CHT treatment, or relapsed, or at diagnosis, but not eligible for the combination treatment. They were treated with ATRA (45 mg/m²/day) for 15 days. After 2 weeks, the treatment was then prolonged continuously for 1 week every 2 weeks. Molecular analysis was performed by qualitative and quantitative reverse transcription-polymerase chain reaction (RT-PCR). All but one patients (87%) obtained molecular CR, as assessed by qualitative RT-PCR. Quantitative RT-PCR confirmed these results, showing a progressive reduction to a negligible quantity of PML-RAR fusion transcript (ratio PML-RAR/ABL $\times 10^4$ ABL $< 10^{-3}$) in all but one patient treated with pulsed ATRA therapy. One patient achieved a molecular CR by receiving a combination of arsenic trioxide and pulsed ATRA. The median progression free survival was 24 months (13-60 months). After a median follow-up of 30 months, 5/7 patients (71%) are in continuous molecular CR, in one case after allogeneic SCT. We report on very long molecular CRs obtained with intermittent ATRA alone (without chemotherapy), confirming our previous experience. This approach, if validated in larger studies, could therefore be effective in relapsed/refractory or high risk frail patients unsuitable for high-dose therapy and SCT. Furthermore, it may be proposed as induction therapy for selected older APL patients if considered not to be eligible for combined ATRA/CHT due to inadequate performance status or concurrent disease.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L. grants.

0036

COMBINED TREATMENT WITH SODIUM VALPROATE, A HISTONE DEACETYLASE INHIBITOR, AND ALL-TRANS RETINOIC ACID SHOWS CLINICAL AND BIOLOGICAL ACTIVITY IN HIGH RISK ACUTE MYELOID LEUKEMIA

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There is accumulating evidence that the abnormal patterns of gene expression documented in AML are associated with perturbations in chromatin modelling, raising the possibility that modulators of chromatin structure may be of therapeutic benefit. Sodium valproate (SV) is a potent histone deacetylase inhibitor (HDI) and, in conjunction with ATRA, induces apoptosis in primary AML blasts *in vitro*. We report the results of a Phase I/II study examining the tolerability and efficacy of SV and ATRA in patients with high risk AML ineligible for intensive chemotherapy. In light of evidence that modulating cAMP levels may increase retinoid responsiveness theophylline was administered in addition to SV and ATRA in patients who failed to respond to 28 days treatment with SV/ATRA. Clinical and biological markers of responsiveness were assessed 28 and 56 days after commencement of therapy. These included quantitation of levels of histone acetylation and methylation and induction of p21, p15 and p16 gene expression. Fourteen patients with AML (8 relapsed, 4 *de novo* and 2 refractory) with

a median age of 71 yrs (51-82) have been treated according to this protocol. Seven patients completed treatment with SV/ATRA/theophylline for at least 8 weeks. Treatment was discontinued in seven patients because of disease progression or toxicity. The most commonly noted side-effects were fatigue and drowsiness which occurred in all evaluable patients. Three patients (all with relapsed AML) demonstrated a clinical response. One patient achieved a morphological and immunophenotypic complete remission after three months therapy with SV/ATRA/theophylline whilst two demonstrated normalisation of peripheral blood counts with either no or a partial response in the bone marrow after treatment with SV/ATRA alone. Of the 3 patients that did respond, one had a normal karyotype and the other two had abnormalities of chromosome 20q. Blasts from all responding patients demonstrated marked sensitivity *in vitro* to combined SV and ATRA treatment. In four of six patients studied increased levels of histone acetylation were observed in peripheral blood mononuclear cells 28 or 56 days after commencement of therapy. The patient who achieved a CR demonstrated increased levels of histone methylation at H3 lysine4 in addition to histone hyperacetylation at H3 and H4. Increased expression of p15, p16 and p21 was documented in primary blasts from patients, after 28 days therapy with SV/ATRA. Induction of p21 expression was most marked in responding patients. In addition, specific induction of HDAC 11 was documented in all patients. This study demonstrates that combination therapy including an HDI in the form of SV results in modulation of gene expression patterns in leukaemic blasts *in vivo*. In addition, this treatment strategy was shown to have significant clinical activity in patients with relapsed AML lacking a defined transcriptionally repressive fusion protein.

Acute myeloid leukemia - Molecular

0037

THE EFFECTS ON REVERSE ATRA RESISTANCE BY MCL-1 GENE KNOCK DOWN IN ATRA-RESISTANT LEUKEMIA CELL LINE HL-60/ATRA

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Background. ATRA is widely used to treat AML in humans, but often becomes ineffective because of increased resistance to the drug. **2. Aims.** To investigate the effects on reverse the resistance of all-trans retinoic acid (ATRA) in leukemia cells by Mcl-1 gene knockdown. **3. Methods.** A long-term, intermittent, inefficient all-trans retinoic acid (ATRA) was used to induce human Acute Myeloid leukemia cell line cells (HL-60) and try to establish a multidrug-resistance cell line (HL-60/ATRA). HL-60/ATRA cells were transfected using Mcl-1 siRNA formulated with Lipofectamine 2000; Western blot was used to detect the expression of Mcl-1. the cells' proliferation, the apoptosis and the state of the differentiation were evaluated by MTT assay, In situ nick end-labeling (TUNEL) and NBT assay. **4. Results.** 1) The HL-60/ATRA could keep its undifferentiated states, intrinsic proliferation and cell function at a high acceptable concentration (100nMol/L ATRA) with high-expressed Mcl-1 protein. 2) Mcl-1 gene knock down could reverse the resistance of ATRA in HL-60/ATRA by inhibiting the proliferation, inducing its differentiation and apoptosis. **5. Summary/conclusions.** Mcl-1 gene might be related to ATRA resistance in leukemia and thus inhibiting its expression could be a new method to reverse the resistance of ATRA.

0038**EVALUATION OF MITOGENIC AND AUTOCRINE ACTIVITY OF TRYPTASE DERIVED FROM BLAST CELLS IN AML**K. Sonneck¹, S. Florian¹, A. Boehm¹, M.T. Krauth¹, K. Gleixner¹, K.J. Aichberger¹, S. Derdak¹, W. Pickl¹, L.B. Schwartz², W.R. Sperr¹, P. Valent¹¹Medical University Vienna, Wien, Austria; ²Virginia Commonwealth University, Richmond, VA, USA

Background. A number of autocrine and paracrine growth regulators are considered to be involved in survival and proliferation of blast cells in acute myeloid leukemia (AML). We have recently shown that in a group of patients with AML (roughly 30-40%), blast cells produce (and secrete) pro- α -tryptase and to a lesser degree, the beta-form of this serine protease. In these patients, serum tryptase levels are highly elevated. Mast cell-derived beta-tryptase is a well known mitogen for fibroblasts. **Aims.** In the present study, we examined the effects of blast cell-derived tryptase and of heparin-complexed recombinant human (rh) beta-tryptase on growth of bone marrow and lung fibroblasts as well as growth of AML blasts (autocrine growth). **Methods.** Primary AML blasts (peripheral blood or bone marrow aspirates) were isolated from 10 patients with AML. Fibroblasts were cultured from bone marrow of patients with AML or from normal bone marrow. Proliferative responses to tryptase were determined by ³H-thymidine uptake experiments. Total tryptase levels were quantified in the patients' sera as well as in supernatants of cultured blast cells by a fluoroenzyme-immunoassay. Expression of the protease-activated receptor PAR-2, a putative target for tryptases, on AML blasts was assessed by multicolor flow cytometry. **Results.** In all patients with tryptase-positive AML (n=5), tryptase was constantly secreted from isolated blast cells resulting in a time-dependent accumulation of the enzyme in cell-free supernatants. As assessed by ³H-thymidine uptake, tryptase-containing sera from patients with AML as well as rh tryptase were found to promote the proliferation of cultured bone marrow- and lung fibroblasts in a dose-dependent manner (EC50: 10-20 ng/mL). A neutralizing antibody against human beta-tryptase (B12) was found to abrogate the growth-stimulatory effects of both rh tryptase as well as tryptase in sera derived from patients with AML. The tryptase-target-receptor PAR-2 was found to be expressed on blast cells in most patients with monoclonal leukemia (FAB M5) irrespective of the growth characteristics of AML blasts or their tryptase content. Neither rh beta-tryptase nor AML blast-derived tryptase showed an effect on growth of leukemic blasts (obtained from patients with tryptase-negative AML) irrespective of the FAB type of AML or expression of PAR-2. **Summary.** Tryptase is secreted from AML blasts as a biologically active molecule that exhibits growth stimulatory effects on bone marrow fibroblasts, but not autocrine growth effects on blast cells in AML.

0039**DEREGULATED WNT PATHWAY GOVERNS CLONOGENIC GROWTH AND PREDICTS POOR PROGNOSTIC IN ADULT ACUTE MYELOID LEUKEMIA (AML)**

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Background. Wnt signaling pathway governs crucial processes at the hematopoietic stem cell level (i.e. self-renewal or differentiation cues), and is also activated in AML, resulting in beta-catenin accumulation in blasts. No clinical data is available with view to its role (s) in leukemia, due to the lack of large cohort study. **Aims.** We provide insights into the implication of Wnt/beta-catenin in the pathophysiology of AML, in a non-selected population of 82 patients uniformly treated in a single institution. We also propose a mechanism by which beta-catenin accumulation is achieved in blasts. **Methods.** Beta-catenin

being mainly regulated at the post-translational level by a multi-protein degradation complex (i.e. Axin, Adenomatous Polyposis Coli, and Glycogen Synthase Kinase 3b), we assessed its accumulation by Western blotting, and tried to correlate results with biochemical parameters. **Results.** In normal CD34⁺ progenitor cells, beta-catenin is readily expressed, but rapidly lost upon myeloid differentiation. Of note, nor Wnt3a neither Wnt5a stimulation could up-regulate beta-catenin levels since it was down-regulated. At earlier differentiation stages, Wnt5a stimulated beta-catenin more efficiently than Wnt3a ligand. We then assessed beta-catenin expression in 82 patients with AML. Among these, 39% were classified β -catenin- and 61% β -catenin⁺. Compared to their normal counterparts, higher β -catenin levels were preferentially found in AML M4 and M5. Importantly, β -catenin levels were up-regulated in negative patients upon Wnt3a, but not Wnt5a, stimulation. Moreover, Wnt5a could not antagonize the effects of Wnt3a on β -catenin, and RT-PCR analyses showed differential Wnt transcripts in blasts, thus suggesting a biological basis for the differences in beta-catenin expression among patients. In a retrospective analysis, beta-catenin level was not correlated to leukocytosis, Flt3-ITD mutation, complete response rate, prior myelodysplasia or karyotype, but rather to enhanced clonogenic growth response to cytokine stimulation, and replating efficiency (that mirrors self-renewing capacities) of leukemic cells. Expression of beta-catenin was statistically linked to shorter relapse-free and overall survivals, and is a new independent prognostic factor in our cohort. **Conclusions:** Wnt deregulation seems important in driving the clonogenic growth of AML-CFU, leading to adverse outcome, and thus appears as an attractive new therapeutic target in AML.

0040**ROLE OF IL-8 AND GRO- α IN THE MIGRATION OF ALL-TRANS RETINOIC ACID-ACTIVATED NB4 PROMYELOCYTIC LEUKEMIC CELLS TOWARD A549 LUNG CELLS**H.-Ch. Hsu¹, W.H. Tsai², C.C. Lin³, C.K. Ho³, Y.R. Kou²¹Taipei City Hospital, Taipei, Taiwan (China); ²National Yang-Ming University, Taipei, Taiwan (China); ³Taipei-Veterans General Hospital, Taipei, Taiwan (China)

Background. All-trans retinoic acid (ATRA) can induce high complete remission in acute promyelocytic leukemia (APL) by granulocyte differentiation mechanism. However, ATRA can cause retinoic acid syndrome in some patient with clinical picture of adult respiratory distress syndrome. **Aims.** We determine the role of IL-8 and GRO- α in the cross-talk between APL cells (NB4) and alveolar type II cells (A549), and their relative contribution in the ATRA-induced migration of APL cells into alveolar space. **Methods. and Results.** ATRA can induce transmigration of NB4 cells toward A549 cells in a dose-dependent manner by transmigration assay. IL-8 and GRO- α itself has chemotactic activity in the transmigration of ATRA-treated NB4 cells. Increased amount of IL-8 and GRO- α were produced by A549 cells after ATRA treatment, which can in turn cause transmigration of ATRA-treated NB4 cells toward them. The latter was evidenced by increased amount of IL-8 bound to the differentiated NB4 cells in the chemokine binding assay, and the transmigration activity can be inhibited upto 60% by IL-8 and GRO- α receptor specific antibodies. Our data also showed that the therapeutic effect of dexamethasone in the retinoic acid syndrome is by suppressing the IL-8 production in A549 cells and inhibiting the transmigration of differentiated NB4 cells toward A549 cells. **Conclusions.** IL-8 and GRO- α act as important chemokines between ATRA-treated APL cells and alveolar type II cells, which can in turn cause in the transmigration of differentiated APL cells into the alveolar space and contribute to the development of RA syndrome.

0041**A NEW ROLE FOR THE DEATH ASSOCIATED PROTEIN KINASE 2 (DAPK2) DURING NEUTROPHIL DIFFERENTIATION**

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Myeloid differentiation is a complex process governed by orchestrated regulation of many different genes. We were interested in identifying new genes involved in both normal and leukaemic myeloid differentiation. Using Affymetrix oligonucleotide microarrays we identified DAPK2 as a gene product distinctly induced during myeloid differentiation of leukaemic cell lines. DAPK2 is a 42-kDa Ca²⁺/Calmodulin-regulated serine/threonine kinase acting as a positive mediator of apoptosis but its role in myeloid development has so far never been described. In our microarray analysis, elevated DAPK2 mRNA levels were seen in the acute promyelocytic (APL) cell line NB-4 differentiated towards granulocytes with all-trans retinoic acid (ATRA). This observation was confirmed by Real-time Quantitative RT-PCR (RQ-PCR) and increased DAPK2 message was paralleled by increased DAPK2 protein as shown in Western blotting experiments. A similar induction of DAPK2 mRNA was seen in the myelomonocytic cell line U937 treated with ATRA. In contrast, when U937 cells were differentiated with phorbol myristate acetate (PMA) towards monocytes/macrophages no significant changes in DAPK2 mRNA expression were observed. Interestingly, when DAPK2 expression was measured in primary cells, fresh primary granulocytes (n=6) showed significantly higher DAPK2 mRNA levels as compared to primary monocytes (n=10, One Way ANOVA $p < 0.001$), undifferentiated CD34⁺ myeloid progenitor cells (n=5, One Way ANOVA $p < 0.001$) and acute myeloid leukemia (AML) (n=102, One Way ANOVA $p < 0.001$) samples. The protein level was seen to correlate with the mRNA expression. Furthermore, in *ex vivo* differentiation experiments of cord blood CD34⁺ myeloid progenitor cells towards granulocytes with granulocyte colony stimulating factor (G-CSF), or toward monocytes/macrophages with monocyte colony stimulating factor (M-CSF), up-regulation of DAPK2 mRNA was only seen during neutrophil development. This further emphasizes a specific role for DAPK2 in granulopoiesis. In summary, our data clearly show that high DAPK2 expression is associated with differentiated granulocytes whilst monocytes, CD34⁺ progenitor cells and AML samples show low DAPK2 expression. This supports a possible functional role for DAPK2 in normal granulocyte maturation, and lack or low expression of DAPK2 may contribute to the maturation block observed in AML. Furthermore, this report describes for the first time an involvement of DAPK2 in normal or leukaemic myeloid differentiation. Currently a lentiviral small interfering (si) RNA system is used to study a DAPK2 knock-down phenotype during myeloid differentiation.

0042**EXPRESSION OF WT-1 IN HUMAN ACUTE LEUKEMIA AND MYELODYSPLASTIC SYNDROMES**

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Despite the great effort, the majority of acute myeloid leukaemia (AML) patients don't have a suitable specific molecular marker for monitoring minimal residual disease (MRD). The results of some studies suggest the Wilms' tumour gene (Wt-1) as a useful and possible marker of MRD. Additionally, the Wt-1 gene is suggested as a useful marker for monitoring the progression of myelodysplastic syndromes (MDS). In our study we have studied the expression of Wt-1 at diagnosis during treatment of AML and MDS patients. The expression of Wt-1 was measured by the quantitative real-time PCR with the spe-

cific TaqMan probe; the Wt-1 expression was related to expression of the control gene ABL. The cDNA level of Wt-1 was detected in peripheral leukocytes from 58 AML and 65 MDS patients at diagnosis. The Wt-1 cDNA level was significantly elevated (3 orders of magnitude) in peripheral blood samples (PB) of AML patients at diagnosis compared to PB samples of healthy donors $p = 0.0001$. The level of Wt-1 expression in MDS depends on FAB MDS subtype (or subtype according to the new WHO classification) with the highest being found in MDS transformed to leukemia and/or in MDS RAEB II. Further, sequence samples of nine long-term followed AML patients treated by chemotherapy and/or by allogeneic bone marrow transplantation were tested for the Wt-1 expression concomitantly with the expression of specific fusion genes (3 patients with AML1/ETO, 3 patients with PML/RAR α , 2 patients with CBFb/MYH11 and one patient with the presence of FLT3/ITD). The levels of Wt-1 gene expression and the above mentioned specific fusion genes were found significantly correlated: r^2 from 0.67 to 0.99. The haematological relapses were associated with the rise of Wt-1 gene expression and with expression of the specific fusion genes. The progression of MDS toward acute leukaemia is associated with the increasing level of Wt-1 expression. Our results (in concordance with the previously published ones) confirm that the monitoring Wt-1 expression is a potential reliable marker of minimal residual disease in AML patients and a marker of MDS progression.

The study was financially supported by the IGA MZCR grant: NC/7560-3. E-mail: jarošlav.polak@uhkt.cz

0043**THE STUDY OF MUTATION OF TYROSINE KINASES AND THEIR DOWNSTREAM EFFECTORS IN ACUTE MYELOID LEUKEMIA**

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Background. Recently there is hypothesis of multistep pathogenesis of Acute Myeloid Leukemia (AML). This model hypothesizes that AML is the consequence of a collaboration between at least two broad classes of mutations. Class I mutations, exemplified by constitutively activated tyrosine kinases or their downstream effectors, such as BCR/ABL, TEL/PDGFB, N-RAS, or K-RAS mutants, or constitutively activated FLT3, confer a proliferative and/or survival advantage to hematopoietic cells. Class II mutations result in loss of function of transcription factors that are important for normal hematopoietic differentiation and include the AML1/ETO, CBFb/SMMHC, PML/RAR α , and NUP98/HOXA9 fusions as well as point mutations in hematopoietic transcription factors such as AML1 and C/EBP α . These mutations would also be predicted to impair subsequent apoptosis in cells that do not undergo terminal differentiation. **Aims.** To search the Class I mutation of tyrosine kinases as well as their downstream effectors including FLT3, PDGFbR, KDR, CSF2Rb, SOCS1, PIAS3, SHIP and explore the novel leukemogenesis mechanisms of AML. **Methods.** A total of 200 successive cases of newly diagnosed AML were included in the current study. Mononucleated cells were collected from BM samples of all AML patients and genomic DNA, RNA was isolated according to the manufacturer's protocol. In addition, blood samples from 200 normal individuals were also used as control. Coding sequence of these genes was analyzed by RT-PCR in a small size of 30 AML patients. The new mutation was validated by genomic DNA PCR and genotyped by MALDI-TOF-MS in a total of 200 AML patients and 200 normal individuals. **Results.** 1) 27 patients (13.5%) were found to have FLT3-ITD mutation and 16 (8%) patients have Asp835 point mutation. FLT3-ITD was associated with leukocytosis and a high percentage of bone marrow blast cells ($P < 0.001$ for both) and with poor prognosis of AML patients. Remarkably, FLT3 aberrations (including FLT3-ITD and Asp835 point mutation) were also associated with AML patients following MDS or MDS/MPD ($P < 0.05$). 2) A new mutation (A>T,Q1153L) of SHIP was identified in a AML patients following MDS (1/200,0.5%). 3) We also identified a new mutation (R685C) of PDGFbR in two AML patients

(2/200,1%). One is AML-M3 patient and the other is AML-M0 patient. 4) We found no mutation of KDR, CSF2Rb, SOCS1, PIAS3 in 30 AML patients. **Conclusions.** Class tyrosine kinases such as FLT3, PDGFR and their downstream effectors such as SHIP may play an important role in the leukemogenesis mechanism of AML. Furthermore, the mutation of FLT3 and SHIP may emerge as a second hit in the progression of MDS transformation to AML.

0044

PRIMA-1 RESTORES P53 FUNCTIONS IN ACUTE MYELOID LEUKAEMIA WITH DELETED 17P

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The tumor suppressor gene p53 is the most commonly mutated gene in solid tumors. Although less common in hematologic malignancies, 5-15% of AML cases carry a p53 mutation. Recently, the compound PRIMA-1 has been shown to induce cytotoxic effects and apoptosis in human tumor cells by restoration of the transcriptional activity of mutated p53. This is believed to be mediated by a change in the conformation of mutated p53 protein, restoring DNA binding and subsequent activation of p53 target genes. We studied the effects of PRIMA-1 and commonly used antileukemic drugs on leukemic cells from 59 patients with *de novo* AML. Chromosome analysis showed abnormalities in chromosome 17 (abn 17) in seven patients, patients with t(15;17) were excluded. Myeloblasts were obtained from the bone marrow and the cells were exposed to 5, 10 and 20 μ M of PRIMA-1; 0.2 μ M of daunorubicin; 2 μ M of fludarabine, 50nM of CdA and 1 μ M Ara-C. Cell viability was assessed by bioluminescence method measuring cellular ATP and apoptosis was analyzed by flowcytometry for Annexin V binding and PI uptake. PRIMA-1 induced dose dependent cytotoxic effects in AML cells. For all 52 patients without abnormalities in chromosome 17, the mean viability compared to unexposed controls after four days of incubation was 65% at 5 μ M, 47% at 10 μ M and 26% at 20 μ M. In the patients with abnormal chromosome 17, the cell viability mean values were 24%, 11% and 1%, respectively. At the same concentrations as PRIMA-1 induced the cytotoxic effects, apoptosis was induced in AML cells. For the cytostatic drugs used in this study, the mean viability in patient samples without abn 17 was 48% at 1 μ M Ara-c, 42% at 50nM Cda, 61% at 0.2 μ M daunorubicin, 35% at 2 μ M fludarabine while in patient samples with abn 17 it was 76, 57, 47 and 42%, samples with abn 17 were significantly more resistant against Ara-C and CdA. In conclusion, PRIMA-1 induces apoptosis in AML cells. In AML samples with abn 17 PRIMA-1 result in a significantly higher cytotoxic effect (at all concentrations) compared to samples without abn 17.

0045

UNIPARENTAL DISOMY CAN BE ACQUIRED DURING THE RELAPSE OF ACUTE MYELOID LEUKAEMIA RESULTING IN HOMOZYGOSITY FOR A PRE-EXISTING MUTATION

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Background. It has been demonstrated that acquired uniparental disomy, an abnormality cryptic to conventional cytogenetic methods, occurs in approximately 20% of acute myeloid leukemias (Raghavan et al, Cancer Res 2005 65: 375-378). This abnormality can lead to homozygosity for known mutations in the region of UPD. One may hypothesize that the mutation precedes the UPD event and that the latter leads to a 'second hit', replacing the wild type gene with its mutated coun-

terpart. **Aims.** To look for UPD acquired only at relapse and any associated single gene mutations. **Methods.** DNA was extracted from patients with AML using bone marrow or blood samples taken at presentation and relapse. Germ line samples were taken by buccal smear or from remission bone marrow. Using high-resolution SNP genotype analysis performed on the DNA using the 10K SNP array (Affymetrix, Inc., Santa Clara) more than 10,000 SNP genotype calls were obtained per sample. **Results.** UPD13q was observed in three of five relapse samples where no such abnormalities were seen at diagnosis. All AMLs had two normal copies of chromosome 13 on karyotype analysis at diagnosis. Each diagnostic sample also had heterozygous internal tandem duplications (ITD) of the FLT3 gene (located at 13q12), detected by PCR sequencing. At relapse, all three AMLs retained the same karyotype but SNP genotype analysis demonstrated homozygosity across chromosome 13, consistent with an acquired UPD. PCR for the FLT3 ITD mutations showed loss of the wild type allele, and homozygosity of the mutation. **Conclusions.** These findings show that acquired UPD, which could occur by homologous recombination or a non-disjunction event, can be the *second hit* required for clonal evolution of the leukemia and lead to relapse. As relapsed disease tends to be more resistant to treatment, it suggests UPD leading to a homozygous mutation confers a more resistant phenotype. Further investigations into the prognostic value of UPD at diagnosis and relapse are warranted.

0046

DHPLC ANALYSIS OF NPM1 MUTATIONS IN ADULT PRIMARY AML

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Background. Nucleophosmin (NPM1, B23, numatrin) is a nucleolar phosphoprotein which regulates the ARF-p53 tumor suppressor pathway and it is the translocation partner of MLF1 (Myeloid Leukemia Factor 1) in AML with t(3;5), of RARA (Retinoic Acid Receptor α) in t(5;17) of acute promyelocytic leukemia, and of ALK (Anaplastic Lymphoma Kinase) in t(2;5) of large anaplastic cell lymphoma. By immunohistochemistry, we recently identified NPM1 mutations as the most frequent lesion in 60% of adult AML with normal karyotype and absence of major fusion genes (BCR/ABL1, PML/RARA, AML1/ETO, CBF β /MYH11, DEK/CAN). We also observed a good response to induction therapy in this subgroup.¹ Denaturing high-performance liquid chromatography (DHPLC) has emerged as one of the most powerful technologies for the screening of mutations.² **Aims.** We develop a DHPLC screening protocol to detect NPM1 mutations. **Materials and Methods.** 65 adult AML were selected on the basis of reaction to an anti-NPM monoclonal antibody (25 NPMc+; 40 NPMc-). Genomic DNA was extracted from blood and/or bone marrow aspirate as previously described.³ Polymerase chain reaction (PCR) to amplify NPM1 exons 12 was carried out as in (1), using primers pairs NPM1-F (5'TTAACCTCTGGTGGTAGAATGAA3') NPM1R (5'CAAGACTATTTGCCATTCCTAAC3'). RNA extracted by TRIzol (Invitrogen Life Technologies, Inc., Paisley, UK) was retro-transcribed with use of the ThermoScript RT-PCR System (Invitrogen). Sequencing was done using primers NPM1_25F (5'GGTTGTTCTCTGGAGCAGCGTTC3') and NPM1_1112R (5'CCTGGACAACATTTATCAAACACGGTA3'). DHPLC analysis was conducted on the automated WAVETM nucleic acid fragment analysis system (Transgenomic, Omaha, Nebraska, USA). The elution temperatures was obtained from the dHPLC Melt Program (<http://insertion.stanford.edu/melt.html>) and then optimized by studying alterations in the elution profiles of the samples. The DNA samples were typed by analysis of dHPLC distinct profiles using Navigator 1.5.4 software (Transgenomic). Electrophoregrams from patients were first superposed to those of a normal control. **Results.** 40/65 NPMc- AML gave a wild type chromatogram. 25/60 NPMc+ leukemias shared ele-

ctrophoregram profiles different from wild type. All 25 corresponded to cases with a NPM mutation according to sequencing. Six different elution profiles were obtained considering specific time of retention between homo- and hetero-duplex, shape and number of peaks. Sequencing allowed us to categorize 9/25, 5/25, and 5/25, as mutations A, B and D respectively. (1) In 6/25 three other variants were found. No changes on work conditions (melting temperature and elution gradient) were necessary to unraveling the 6 types of mutations. Summary/conclusion. Our preliminary results indicate dHPLC is comparable to direct sequencing to detect NPM1 mutations. We showed that dHPLC is a sensitive, rapid, and cost-effective method helpful for routine molecular diagnosis of NPM1 mutations in AML. New mutations were found in combination with sequencing. A number of additional variants are predicted in larger series of cases.

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Supported by: CNR-MIUR, FIRB and Fondazione Cassa di Risparmio, Perugia, Italy

0047

THE INHIBITION OF IKK KINASE IS ABLE TO INDUCE GROWTH ARREST AND APOPTOSIS IN AML BLAST CELLS

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Background. The therapeutic results for many patients affected by Acute Myeloid Leukemia (AML) are at present largely unsatisfactory. The overall failure of current treatments is even more disappointing in older patients who cannot be enrolled in clinical trials with conventional chemotherapy. It is therefore of great interest to identify specific molecular targets to design new therapeutic approaches. An increased NF- κ B activity has been demonstrated in blast cells from AML patients. PS1145 (Millennium) is a compound which acts as an inhibitor of NF- κ B through the inhibition of the IKK kinase. **Aims.** The aim of the study was to evaluate the *in vitro* effects of PS1145 in AML blast cells and cell lines collected from AML patients at diagnosis. **Methods.** BM cells collected from 15 AML patients were studied. Purified blast cells were obtained from BM samples by MACS separation in 8 out of 15 cases, in the remaining 7 cases unfractionated BM cells had been analyzed. Moreover HL60 and U937 cell lines had been analyzed. As control we analyzed 5 BM samples from healthy volunteers. Cells were incubated with 20 microM PS1145 for 24-48 and 72 hrs. The inhibition of NF- κ B DNA binding activity was evaluated using an ELISA method. Immunofluorescence technique with an antibody against NF- κ B for the detection of nuclear-cytosol localization has been performed. The inhibition of I κ B phosphorylation has been demonstrated by Western Blot. The proliferation rate was evaluated by MTT assay and the percentage of apoptotic cells by the detection of annexin V positive cells. After incubation, colony growth inhibition has been evaluated. **Results.** In HL60 and U937 the incubation with PS1145 resulted in an inhibition of NF- κ B activity of 70% and 85%. The proliferation rate was reduced of 63% and 72% respectively and the apoptotic cells increased to a value of 55% and 58%. In all these experiments, western blot detected the absence of the phosphorylated form of I κ B. Similar results were obtained in BM MNC cells and sorted blast cells from AML patients. After incubation western blot assay demonstrated the block of I κ B phosphorylation, and immunofluorescence analysis was able to detect the localization of NF- κ B mainly in the cytoplasm. ELISA assay performed after incubation with PS1145 20 microM was able to detect a reduction of the NF- κ B activity of 85% as a mean value (range 70-92%). After 48 hrs of incubation with PS1145 we detected a decrease of proliferation of 62% (range 35-75%) and an increased percentage of apoptosis to a mean value of 75%. (range 54-89%).

In addition, colony growth was suppressed of 62% as a mean value (range 46%-70%). BY contrast no significant effects were noted in normal samples, neither in terms of proliferation nor of apoptosis or colony growth inhibition. **Conclusions.** These data demonstrated that the *in vitro* treatment with the NF- κ B inhibitors PS1145 is able to block the proliferation and to induce apoptosis in AML blast cells. IKK may therefore be considered an attractive target for a molecular therapy in AML patients not candidate for conventional chemotherapy.

0048

THE ONCOGENIC POTENTIAL OF THE CDX2 IS DEPENDENT ON THE FUNCTIONAL INTEGRITY OF DISTINCT DOMAINS AND CAN BE ANTAGONIZED BY INHIBITION OF THE MAPK PATHWAY IN A MOUSE MODEL OF T (12;13) POSITIVE AML

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Background. In AML the translocation t(12;13) (p13;q12) results in the ectopic expression of the homeobox gene Cdx2 and the expression of the ETV6-CDX2 fusion. We have recently shown that in a murine model of t(12;13) (p13;q12) AML the ectopic expression of the homeobox gene CDX2, induced by the chromosomal translocation, is the key event in myeloid leukemogenesis and not the expression of the ETV6-CDX2 fusion gene, generated by the translocation. **Aim.** To characterize the contribution of different Cdx2 domains for the hematopoietic activity of the proto-oncogene and to test whether CDX2 induced transformation can be antagonized by the inhibition of signal transduction pathways responsible for CDX2 activity. **Methods.** We generated different Cdx2 mutants, inactivating the DNA binding homeodomain (N51S), or the PBX1-interacting motif (W167A), or deleting the N-terminal portion of Cdx2 (N-Del). Expression of Cdx2 and the different mutants were induced in primary murine BM cells by retroviral gene transfer, using an MSCV based retroviral construct with an IRES-YFP cassette. **Results.** Expression of Cdx2 and the different mutants were induced in primary murine bone marrow cells by retroviral gene transfer. Inactivation of the PBX-interacting domain did not abrogate the *in vitro* hematopoietic activity of Cdx2 with an increase of primary colony formation (3-fold) (n=3; $p < 0.001$) and a higher number of CFU-G/GM colonies ($p < 0.015$) compared to the GFP control. As the wild-type CDX2 the W167A-Cdx2 construct enhanced the replating capacity of clonogenic progenitors with an 80-100fold increase in secondary colonies ($p < 0.005$) compared to the control and induced the outgrowth of blast colonies (2700fold; $p < 0.02$). In contrast, inactivation of the homeodomain or loss of the N-terminal transactivation domain resulted in a complete loss of hematopoietic activity *in vitro*. *In vivo* all mice transplanted with cells expressing Cdx2 or the W167A-Cdx2 mutant developed transplantable AML. Of note, the N51S mutant induced a distinct leukemia phenotype compared to Cdx2 wild type (GR1+/MAC-1-/c-Kit+ versus GR1+/Mac-1+/c-Kitlow). We extended structure-function analyses, inactivating the phosphorylation site (S60) in the Cdx2 transactivation domain, previously shown to be regulated by the MAPK family. S60 position mutation did not reduce the hematopoietic activity of Cdx2. However, incubation with the MEK1 inhibitor PD98059 decreased the frequency of CFU-S 8fold (n=7; $p < 0.001$) and blocked growth of leukemic Cdx2 transfected blasts *in vitro*, indicating that other phosphorylation sites are relevant for leukemic activity. In contrast, the p38 inhibitor SB2059 did not prevent phosphorylation and was unable to antagonize Cdx2 induced transformation. **Summary.** These data demonstrate that the transforming activity of Cdx2 and the phenotype of Cdx2 induced leukemias is depending on the functional integrity of distinct Cdx2 domains. Furthermore, our data link the oncogenic potential of Cdx2 directly to the MAPK signaling, opening the possibility to counteract Cdx2 associated leukemogenesis by kinase inhibitors.

0049

QUANTITATIVE MEASUREMENT OF WILMS' TUMOR 1 GENE (WT1) IN ACUTE MYELOID LEUKEMIA PATIENTS USING THE WT1 PROFILEQUANT KIT

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Background. WT1 is identified as a tumor suppressor gene encoding a transcriptional regulator and playing a role in the development of Wilms Tumor. WT1 overexpression is described in several oncological diseases including leukemias. Quantification of WT1 in blood cells is reported to be useful as a biomarker for minimal residual disease (MRD) and predicting relapse in the majority of acute myeloid leukemia (AML) cases. **Aims.** We wished to evaluate the suitability of 'WT1 Profile-Quant™ kit' for monitoring of MRD in AML patients. Firstly, by comparing the WT1 expression level in diagnostic AML samples to the background expression level in normal peripheral blood (PB) and bone marrow (BM). Secondly, by performing longitudinal MRD studies of AML patients, including parallel analysis of fusion gene transcript levels (CBF β -MYH11, AML1-ETO, PML-RARA, and BCR-ABL). **Methods.** Quantification of WT1 expression level in normal and AML samples was performed by using the WT1 ProfileQuant™ kit (Ipsogen) and the ABI7900 Detection System. Precise measurement of the copy numbers of WT1 and the Abelson (ABL) control gene were determined by generating standard curves based on known concentrations of plasmid transcripts. The ratio of WT1 to ABL provides a normalized copy number of WT1 (WT1 NCN) which is independent of the cell count and reverse transcription efficiency in each sample. Evaluation of the fusion gene transcripts was performed according to the Europe Against Cancer program. **Results.** We determined WT1 levels in normal BM (n = 7) and PB samples (n = 11), and found a higher median number of WT1 copies for 104 ABL copies in BM as compared to PB (155 versus 23 respectively). Twenty-two PB and 28 BM diagnostic AML samples were measured for WT1 expression. The values were similar between these 2 sample types, allowing us to pool the 50 measurements. The median WT1 NCN was 16181, confirming WT1 overexpression in diagnostic AML. Parallel quantification of WT1 and fusion genes in follow-up AML samples, showed a strong correlation between these two molecular markers. Both showed a significant increase several months before clinical relapse. In some cases WT1 predicted the relapse earlier than the fusion gene. Also in patients lacking a specific fusion gene, WT1 levels reflected the clinical evolution of the patient. **Conclusions.** Our results show that the 'WT1 Profile-Quant™ kit' is an easy and reliable assay for standardized detection and quantification of WT1 levels in human PB and BM cells. The new WT1-assay is shown to be useful in the monitoring of MRD in AML patients.

0050

PU.1 AND C/EBP β EXPRESSION IN ACUTE MYELOID LEUKAEMIA

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Background. The myeloid transcription factors C/EBP β and PU.1 play a pivotal role in normal hematopoiesis. PU.1 has been shown to be essential for monocytic development, while C/EBP β is necessary for granulocytic differentiation of hematopoietic precursors. In particular, their reciprocal expression is essential for lineage differentiation. Alterations of their function are involved in leukaemogenesis: mutations of C/EBP β and PU.1 have been described in about 8% and 7% of AML, respectively, and other mechanisms affecting gene transcription, mRNA translation and protein function appear to be important in some AML subsets. **Aims.** We have investigated C/EBP β and PU.1 expression levels, and their reciprocal ratio, in different subsets of AML and correlated these data to mor-

phology, FLT3 mutations and cytogenetics. **Methods.** Bone marrow mononuclear cells were isolated from 80 patients with AML at the time of diagnosis, and from 20 normal bone marrows using Ficoll gradient. AML diagnosis was made according to WHO criteria. Cytogenetic data were available for 48 patients. C/EBP β and PU.1 levels, quantified by real time RT-PCR, were quantified using 18S as reference gene. FLT3 mutations were studied using current protocols. **Results.** We found higher PU.1 levels in AML when compared to normal bone marrows (average level 11.96 versus 3.04, $p=0.007$). Looking for possible associations to patients' characteristics, PU.1 levels showed a trend towards down-regulation in monoblastic leukaemias (FAB M5) compared to other subtypes (2.54 versus 12.58, $p=0.13$). Heterogeneous expression of PU.1 and C/EBP β was observed in other AML subsets. When analysing the distribution of PU.1/C/EBP β ratio, AMLs with granulocytic maturation (FAB M2) showed the highest ratio (170.71), while monoblastic leukemias had the lowest ratio (11.39). In particular, when comparing AML with maturation to all other AMLs, the differences in PU.1/C/EBP β ratio reached statistical significance ($p=0.003$). Since down-regulation of C/EBP β and PU.1 has been described in cell lines expressing FLT3-ITD, we correlated their expression to FLT3 mutations. FLT3-ITD were present in 12 of 80 patients (15%) while FLT3 D835 occurred in 5 of 80 patients (6.25%). Lower C/EBP β levels were observed in FLT3-mutated patients, although this difference was not statistically significant (0.51 versus 2.24). No differences were observed for PU.1 levels. **Summary/conclusions.** Abnormal C/EBP β and PU.1 expression and alteration of their reciprocal ratio may play a role in the pathogenesis of specific subsets of AML, such as AML with maturation and acute monoblastic leukaemia. The increased levels of these transcription factors in some AMLs warrant further investigation since other mechanisms, mutations or post-transcriptional events, can disrupt their function and deregulated expression could be a consequence of an ineffective transcriptional activity.

0051

HISTONE DEACETYLASES IN ACUTE MYELOID LEUKAEMIA SHOW A DISTINCTIVE PATTERN OF EXPRESSION THAT CHANGES SELECTIVELY IN RESPONSE TO DEACETYLASE INHIBITORSC.A. Bradbury¹, F. Khanim², D. White¹, M. Drayson¹, P. Mehta³, C.M. Bunce², C. Craddock⁴, B.M. Turner¹¹University of Birmingham, Birmingham, UK;²Birmingham University, Birmingham, UK;³Department of Haematology, QEHS, Birmingham, UK; ⁴Dept Haematology, QEHS, Birmingham, UK

Post-translational modifications of chromatin structure are recognised as having a potential role in the pathogenesis of acute myeloid leukaemia (AML). Histone deacetylases play a central role in determining the acetylation status of histones and are emerging as novel targets in AML therapy. Histone deacetylase inhibitors (HDIs) inhibit growth of primary AML blasts *in vitro* and demonstrate clinical activity in patients with relapsed AML. To date, three major classes of HDACs have been characterised which differ in their susceptibility to HDIs. However, little is known of the pattern of HDAC expression in AML and this limits the rational use of HDIs in this disease. To explore a possible mechanism for the disease-specific, pro-apoptotic activity of histone deacetylase inhibitors (HDI), we have used Real Time Quantitative PCR to assay the expression of their target enzymes in primary AML blasts and CD34+ progenitors harvested from adult donors or umbilical cord blood. We find a characteristic pattern of HDAC gene expression in AML, with over-expression (>2 fold) of SIRT1 in most patients, irrespective of which of the four control cell types they were compared against. HDAC-2 and HDAC-6 were also commonly over-expressed. Conversely, HDAC5 and SIRT4 were under-expressed (<0.5 fold) in all or most patients. HDAC expression patterns in primary AML and leukaemic cell lines, were altered

by exposure to HDI (sodium valproate, butyrate, TSA and SAHA), with a selective, several-fold induction of HDAC11, confirmed by western blotting. This increase occurred in all myeloid cell lines tested but not in HeLa cells. SET7, a histone methyltransferase was also induced in some cell lines. In light of this data, we examined the effect of the HDI sodium valproate (SV), with or without concurrent ATRA, on chromatin structure and survival of primary AML blasts. Significant *in vitro* killing of primary AML blasts was observed at 1 mM SV. SV treatment resulted in time and dose dependent increases in histone acetylation and specific methylation at H3K4, in both AML cell lines and primary AML cells. These data which identify a specific pattern of HDAC expression in AML may be of value in determining choice of HDIs in Phase I/II studies in AML. In addition they identify alterations in histone methylation status as an additional mechanism by which HDIs mediate apoptosis of AML blasts.

0052

GATA2 AND WT1 OVEREXPRESSION, AND ITD MUTATIONS OF FLT3, COULD DEFINE A SUBGROUP OF PATIENTS WITH AML AND NORMAL KARYOTYPE

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The presence or absence of recurrent cytogenetic aberrations in patients with acute myeloid leukemia (AML) has significant prognostic impact. However, the current classification system does not fully reflect the molecular heterogeneity of the disease, and treatment stratification is difficult, especially for patients with intermediate-risk AML and a normal karyotype. The presence of FLT3 mutations, or the overexpression of WT1, EVI1 or GATA2, has been reported to discriminate in patients with normal karyotype those with a worse prognosis. Our aim was to characterize genetically samples with AML in order to contribute to define a genetic profile of this disease. We analyzed 5 cell lines and 208 samples of AML patients at diagnosis: 38 included in the favorable prognosis group, 98 in the intermediate (81 with normal karyotype) and 77 in the unfavorable. Expression of GATA2, MDS1/EVI1, EVI1 and WT1 was measured by a quantitative real-time RT-PCR (Taqman) assay and standardized to endogenous GAPD mRNA levels (Applied Biosystems). Mutations of FLT3 (ITD and D835) were also analyzed. FISH analysis was performed using six BACs on 3q21, and two on 3q26. We have detected a different expression profile among the three prognosis groups. None of the patients included in the favorable group (FG) had overexpression of MDS1/EVI1 or EVI1, and this event allowed to distinguish between the intermediate (IG) and the unfavorable groups (UG) ($p < 0.001$) (Table 1).

Table 1. Incidence of the overexpression of GATA2 and MDS1/EVI1 and/or EVI1 in 213 patients with AML

Table 1. Incidence of the overex

FG: favorable group; IG: intermediate group; UG: unfavorable group; n.s.: no significative; 3g: samples with 3g rearrangements.

This result confirms the unfavorable prognosis of EVI1 overexpression. The number of cases with GATA2 overexpression was significantly higher in the unfavorable group if comparing with the favorable ($p < 0.01$) and the intermediate ($p < 0.004$). However, 10 cases included in the FG had GATA2 overexpression (10/38, 26%), indicating that this analysis could discriminate two groups with different prognosis in the FG, in the same way that happens in the IG. In the group with normal karyotype, GATA2 overexpression was more frequent in samples with FLT3-ITD mutations (69,2% vs 29,2%; $p = 0.001$), and these cases overexpressed WT1 as well. Moreover, seven of the patients with this pattern had AML-M1 (7/18, 39%). In the unfavorable group, overexpression of GATA2 and MDS1/EVI1 and/or EVI1, was significantly higher in patients with 3q rearrangements than in patients with complex karyotypes ($p < 0.05$). Moreover, in patients with 3q21q26 rearrangements more cases overexpressed GATA2 than MDS1/EVI1 and/or EVI1 (85% vs 60%), confirming that not all cases with 3q21q26 rearrangements express EVI1 transcripts, and suggesting a more complex mechanism involving GATA2. FISH analysis confirmed the heterogeneity of the breakpoints in these cases. In conclusion, we have detected a statistically significant different expression profile of the genes analyzed among the three prognosis groups, confirming their prognosis value. Our results show that GATA2 overexpression could discriminate a worse prognostic subgroup in the favorable group, in the same way that it has been reported in patients with normal karyotype. The mutational pattern FLT3-ITD/GATA2 and WT1 overexpression could define a subgroup of patients with normal karyotype. We have confirmed that not all cases with 3q21q26 rearrangements express EVI1 transcripts, suggesting a more complex mechanism involving GATA2.

0053

WNT SIGNALING IN PATHOGENESIS AND LEUKEMOGENESIS IN SEVERE CONGENITAL NEUTROPENIA

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Background. Severe congenital neutropenia (CN; Kostmann's syndrome) is characterized by the onset of recurrent life-threatening infections in early childhood due to a „maturation arrest“ of myeloid progenitors at the promyelocytic stage with a few or no mature neutrophils in the bone marrow and blood. CN patients are at increased risk of developing AML or MDS (10-15% of patients). The pathogenesis of CN and the mechanisms underlying malignant transformation in CN patients are still unknown. Approximately 35% of them have acquired somatic mutations in the G-CSF receptor (G-CSFR) gene. G-CSFR gene mutations have been linked to the secondary leukemia in CN patients. Recent data provide new evidence for the involvement of Wnt/ β -catenin/ β -catenin pathway in the pathogenesis of AML and CML. **Aims.** In the present study, we aimed to evaluate whether there is an association between increased levels of oncogenes ‘beta’-catenin and β -catenin and leukemic signal transduction in CN. **Methods.** We investigated expression patterns of β -catenin, β -catenin, and other members of the Wnt signaling in CD33+ myeloid progenitor cells from 11 long-term G-CSF-treated CN patients, 4 patients with cyclic neutropenia (CyN) and 3 G-CSF treated healthy controls. Two of studied CN patients developed AML. **Results.** We found significantly up-regulated mRNA expression of ‘beta’-catenin in all CN patients, as compared to G-CSF-treated healthy controls and CyN patients. In line with high mRNA expression, FACS analysis and immunofluorescence staining with confocal microscopy revealed high expression of activated nuclear as well as cytoplasmic β -catenin in CN patients. Moreover, mRNA and protein expression levels of β -catenin were further increased in two CN patients, who developed AML. High β -catenin expression was substantiated by the fact that mRNA expression of Wnt target genes fra-1, c-jun, and PPAR δ were also up-regulated. Intrigu-

ingly, the expression level of LEF-1, a β -catenin binding partner, was significantly lower or even absent in these patients. Abolished LEF-1 expression was correlated with low mRNA levels of β -catenin/LEF-1 target genes cyclin D1, c-myc, and survivin. Interestingly, we also found elevated mRNA expression of β -catenin in all CN patients. **Conclusions.** Our results demonstrated the dual role of Wnt signaling system in CN patients. Defective LEF-1 expression might have an impact in the *maturation arrest* of myeloid progenitors in these patients. On the other hand, high expression of β -catenin and β -catenin could contribute to the malignant transformation of hematopoiesis in CN.

0054**QUANTITATIVE ANALYSIS OF WT1 GENE FOR MINIMAL RESIDUAL DISEASE DETECTION IN LEUKEMIC PATIENTS**

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Introduction: WT1 gene encodes a transcription factor which is involved in differentiation and proliferation of Hemtopoeitic precursor cells as well as some other tissues like kidney, ovary, heart etc. It is also expressed in 80% of Acute Leukemia cases (AML, ALL) and in plastic crisis of CML as determined by various qualitative and quantitative RT-PCR methods. It is proposed to be a useful marker in minimal residual disease (MRD) detection and leukemia management. **Methods.** To assess the relevance of this gene, sequential peripheral blood samples from 83 leukemic patients (62 AML, 10 ALL, and 11 CML) were analyzed for the expression level of WT1 mRNA, using Real-Time Quantitative RT-PCR. Samples from patients obtained at the time of diagnosis, and during treatment (follow-up), in remission, relapse and after relapse. **Results.** Samples of diagnosis and relapse showed significantly higher WT1 expression levels (90%), compared to samples from patients in complete remission (CR) or healthy volunteers. No significant difference in expression levels was found between various AML subtypes. ALL patients showed lower levels of WT1 expression compared to AML ones. In CML patients WT1 expression levels were low during chronic phase (near to undetectable level) but begin to rise along with progression towards accelerated phase and plastic crisis. Our study revealed that rising of WT1 expression predicts a forthcoming relapse 2-4 months before overt hematologic or clinical relapse. A linear correlation between quantities of WT1 and PML-RARa fusion transcripts could be seen in APL patients treated with arsenic trioxide. **Conclusions.** There was a strong correlation between WT1 and specific fusion gene expression in leukemic patients, showing the significant potential of WT1 as a non-specific leukemia marker (NSLM) for monitoring of MRD and treatment approaches in leukemia.

Apoptosis and drug resistance I**0055****CYCLOOXYGENASE INHIBITION SUPPORTS REGENERATION OF HEMATOPOIESIS SUPPRESSED BY IONIZING RADIATION**

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Background. Cyclooxygenases catalyze production of prostaglandins from the arachidonic acid. Prostaglandins, particularly those of the E series, play a role in the negative feedback control of myeloid cell proliferation. Non-steroidal anti-inflammatory drugs (NSAIDs) act on the principle of inhibition

of cyclooxygenases. **Aims.** We have tested if NSAIDs might be used for supporting the regeneration of a mammalian organism from radiation damage. **Methods.** Our studies have been performed in mice irradiated from a cobalt source. Complex analysis of hematopoiesis and lethality studies have been done in sublethally and lethally irradiated animals. **Results.** Various common NSAIDs, namely indomethacin, diclofenac, and flurbiprofen, have been found in our experiments to support regeneration of hematopoiesis in mice irradiated with a sublethal dose evoking the bone marrow radiation syndrome. However, common NSAIDs are known to produce gastrointestinal side effects. These effects might prevent NSAIDs from being used in the treatment of radiation damage because higher, near-lethal or lethal radiation doses induce the gastrointestinal radiation syndrome which might be potentiated by NSAIDs. This assumption has been confirmed also in our studies. Therefore, our current efforts are aimed at finding out whether selective cyclooxygenase-2 inhibitors, which should be nearly devoid of gastrointestinal side effects, retain the stimulatory action of NSAIDs on hematopoiesis. **Conclusions.** Hematopoiesis-supporting action of cyclooxygenase inhibitors might find use in the treatment of radiation-induced myelosuppression, as well as in the situations of hematopoietic damage provoked by other agents, e.g. by cytotoxic drugs.

0056**PROTEIN PHOSPHATASE 2A AND PROTEIN KINASE C ζ UNDERPIN P-GLYCOPROTEIN FUNCTION IN PRIMARY AML**

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Reversible covalent modification of protein by phosphorylation and dephosphorylation are key events in the regulation of protein activity involved in cellular growth, differentiation and apoptosis pathways. Multidrug resistance is one of the major obstacles to the chemosensitivity of patients with acute myeloid leukemia (AML). **Aims.** To determine the contributions of protein kinase and phosphatase activities to the multidrug resistance phenotype in AML. **Method:** P-glycoprotein (pgp) activity, total phosphoserine, phosphoPKC α (ser657), phosphoPKC ζ (thr410) were measured by flowcytometry and PP2A activity was determined by using a cell-free system in blast samples from AML patients entered into MRC/NCRN clinical trials. **Results.** We found that Pgp activity was associated with high cellular total phosphoserine expression ($p=0.004$ by Mann-Whitney analysis), and with low PP2A activity ($p=0.003$). Pgp activity was found in 34/85 patients (40%), of whom activity was classified as high in 9 patients (11%). PhosphoPKC ζ (thr410) levels were elevated in all 9 of these patients ($p=0.006$). To confirm that PKC ζ and PP2A-mediated pathways contribute to pgp function in AML, pgp positive KG1a cells were treated with the PP2A inhibitor cantharidic acid, which increased total phosphoserine and PKC α and ζ phosphorylation as well as pgp function. However, pgp function may be at least partially independent of PKC α and ζ , because the protein phosphatase activator 8-Bromo-cAMP increased PP2A activity and decreased pgp function with no effect on PKC ζ and PKC α phosphorylation. The PKC inhibitors PKC412 and Ro32-0432 decreased pgp function at doses where PKC ζ but not PKC α phosphorylation was inhibited. **Summary.** In conclusion, moderate and high pgp function in AML is underpinned by low PP2A activity and high pgp function is further associated with PKC ζ phosphorylation.

0057

IDENTIFICATION OF A NOVAL MECHANISM INVOLVED IN MULTIDRUG RESISTANCE OF ACUTE LEUKEMIA, CYTOCHROME P450, SUBFAMILY IIIA, POLYPEPTIDE 5 GENE MEDIATED LOCALIZING-DETOXIFICATION OF ANTICANCER DRUGS (PART I)

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Background. To some extent, the essence of drug resistance is a self-protective detoxification response performed by human body and tumor tissues against harmful substances. Cytochrome P450 A (CYP3A) subfamily is the most important metabolic enzyme and is well known for its large inter-individual variability and its inducibility by various drugs. Thus, we hypothesize it might be the molecular mechanism for individual variation in treatment responsiveness and might contribute to the development of primary drug resistance, secondary drug resistance and multidrug resistance. As we only detected CYP3A5 member of CYP3A subfamily in acute leukemia cell lines in preliminary experiments, so in the follow-up research we focused on the role of CYP3A5 gene. *Aims.* To investigate if CYP3A5 is one of the molecular mechanisms for MDR in acute leukemia. *Methods.* RT-PCR, immunohistochemistry and reverse-phase high-performance liquid chromatography HPLC assay were used to detect transcription, expression and activities of CYP3A5 gene in leukemia cells.

Table 1
Prognostic predictor value for CR ratio, early relapse rate and OS

Parameter	CR ratio	CR1 ratio	Early relapse rate	Overall survival
CYP3A5 positive (mean fold of expression)	4.7%	0.001001	0.0001	0.0001
CYP3A5 positive (mean fold of CR1)	1%	0.0001	0.0001	0.0001
CYP3A5 positive (mean fold of OS)	4.7%	0.0001	0.0001	0.0001
CYP3A5 positive (mean fold of CR1 and OS)	4.7%	0.0001	0.0001	0.0001
CR ratio	11.6%	0.0001	0.0001	0.0001
CR1 ratio	11.6%	0.0001	0.0001	0.0001
OS	11.6%	0.0001	0.0001	0.0001

Cell lines' sensitivities to daunorubicin (DNR) were observed through MTT assay. *Results.* Among leukemia cell line panels (K562, U937, HL-60, NB4 and Jurkat cells), CYP3A5 mRNA was only measured in K562 and U937 cells. Interestingly, K562 and U937 cells were statistically significant resistant to DNR than the other three cell lines. Furthermore, regulation of transcription and activities of CYP3A5 gene were observed. DNR increased CYP3A5 mRNA level in K562/A02 cells and activated its transcription in HL-60/ADR cells. Dexamethasone (Dex) and ATRA activated transcription of CYP3A5 gene in Jurkat cells and NB4 cells respectively. K562 cells treated with DNR, Jurkat cells with Dex and NB4 cells with ATRA had statistically significant increasing CYP3A5 activities, and each induction was time-dependent. We have now examined, for the first time, quantifiable CYP3A5 expression in leukemia patients to evaluate the prognostic impact of CYP3A5 expression in a multivariate setting including other possible prognostic factors. 53 acute leukemia patients presented a polymorphism of CYP3A5 expression in their bone marrow blasts at diagnosis. And CYP3A5 expression at diagnosis was statistically significant correlated with primary drug resistance. CYP3A5 expression at the time of CR / CR1 was statistically significant correlated with early relapse (Table 1). Logistic regression analysis and Cox regression analysis obtained predictive formula for initial induction chemotherapy response (ICR score) and prognosis index (W value) respectively, suggesting that CYP3A5 expression at

diagnosis is a strong independent prognostic factor both for CR ratio and overall survival. Transcription, expression and activities of CYP3A5 gene were correlated with drug resistance appearance in both leukemia cell lines and patients' blasts. Furthermore, anticancer drugs could induce transcription and activities of CYP3A5 gene in leukemia cells. CYP3A5 positive blasts percent at diagnosis could be regarded as one of the predictive indexes for chemotherapy response and prognosis in acute leukemia.

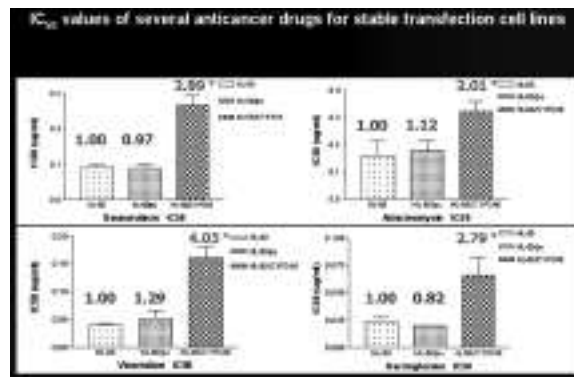
0058

IDENTIFICATION OF NOVAL MECHANISM INVOLVED IN MULTIDRUG RESISTANCE OF ACUTE LEUKEMIA, CYTOCHROME P450, SUBFAMILY IIIA, POLYPEPTIDE 5 GENE MEDIATED LOCALIZING-DETOXIFICATION OF ANTICANCER DRUGS (PART II)

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Background. Despite improvements in chemotherapy, the overall results of leukemia treatment remain unsatisfactory due to drug resistance. To some extent, the essence of drug resistance is a self-protective detoxification response performed by human body and tumor tissues against harmful substances. While recent studies corresponding to multidrug resistance (MDR) have focused mainly on drug transporters. In reality this process encompasses the full range of cellular responses to xenobiotics, including up-regulation of drug-metabolism processes. The most important family of enzymes involved in the metabolism of xenobiotics is cytochrome P450 (CYP). Consistent with a role in xenobiotic detoxification, CYP is mainly expressed in liver and intestine. However, there is a growing awareness of extrahepatic CYP, because the expression of CYP in neoplastic cells may confer a protective survival advantage by providing tumors with a metabolic mechanism for detoxification of harmful substances and drugs, and thus may play an important role in the development of MDR.



Of the various P450 isozymes, cytochrome P450 A (CYP3A) subfamily is the most abundant form, and is responsible for the metabolism of >60% drugs including anticancer drugs. Importantly, the activity of CYP3A is well known for its large inter-individual variability and its inducibility by various drugs. Thus, we hypothesize that CYP3A might be the molecular mechanism for individual variation in treatment responsiveness and might contribute to the development of MDR. *Aims.* To investigate if CYP3A5 is one of the molecular mechanisms for MDR in acute leukemia. *Methods.* The full-length cDNA of CYP3A5 gene was cloned. And the recombinant eukaryotic expression plasmid pcDNA3-CYP3A5 was constructed, confirmed through enzyme digestion, PCR and sequencing. Then the stable transfection cell line HL-60/CYP3A5 was established, determined from mRNA to protein levels and characterized their sensitivities to several kinds of anticancer drugs using RT-PCR, immunohistochemistry, flow cytometry and MTT cytotoxicity assay. Antisense oligonucleotides complementary to CYP3A5 were

used to reverse the drug-resistant phenotype of K562/A02 cells. **Results.** Transfecting HL-60 cells which didn't transcribe CYP3A5 gene with recombinant plasmid pcDNA3-CYP3A5 made HL-60/CYP3A5 lines, and transfecting HL-60 cells with the parental pcDNA3 vector made control HL-60/pc lines, both were identified by mRNA and protein level. There's no difference among cell survival curves of HL-60, HL-60/pc and HL-60/CYP3A5 cells. Daunorubicin 1 μ g/mL induced remarkable apoptosis peaks in HL-60 and HL-60/pc cells, while such appearance didn't occur in HL-60/CYP3A5 cells (Figure 6). Compared with HL-60 and HL-60/pc cells, HL-60/CYP3A5 cells were statistically significant resistant to daunorubicin, aclacinomycin A, vincristine and harringtonine (Figure 7), however the sensitivity to teniposide didn't change. Transfecting K562/A02 cells with antisense oligonucleotides complementary to CYP3A5 gene remarkably inhibited CYP3A5 gene transcription after 48 hours, and it also statistically significant reduced DNR IC50 values of K562/A02, while no significant differences were found in sense and mismatch oligonucleotides treatment groups. Transcription of CYP3A5 gene in leukemia cells directly conferred resistance to anthracyclines and alkaloids, thus confirmed a new mechanism of MDR. Antisense oligonucleotides complementary to CYP3A5 gene could reverse drug-resistance.

0059

THE CD40/CD40L INTERACTION ON HUMAN BONE MARROW CD34+ CELLS REPRESENTS A NOVEL MECHANISM FOR THE INDUCTION OF CD34+ CELL APOPTOSIS IN PATIENTS WITH TUMOR NECROSIS FACTOR α MEDIATED MARROW FAILURE

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The tumor necrosis factor receptor (TNFR) family members and their ligands (L) have been implicated in the apoptotic depletion of bone marrow (BM) haemopoietic stem/progenitor (CD34⁺) cells in certain BM failure syndromes. The CD40/CD40L molecules belong to TNFR/ligand family; however, their putative role on BM haemopoiesis has not been investigated so far. Interestingly, CD40/CD40L interactions have been reported to induce apoptosis in non-haemopoietic cells by up-regulating Fas/FasL expression. **Aims.** To evaluate the CD40 and CD40L expression in normal BM CD34⁺ cells and microenvironment cells, respectively, and investigate the possible involvement of CD40/CD40L interaction in the pathophysiology of BM failure associated with the TNFR/ligand-mediated apoptotic depletion of CD34⁺ cells. **Methods.** The expression of CD40 on normal BM CD34⁺ cells (n=15) was evaluated by flow-cytometry under steady-state conditions and following 48-h induction with recombinant-human (rh)TNF α (0.25-5.0ng/200microl). CD40 expression was also evaluated within BM CD34⁺ cells of patients with TNF-related BM failure including hypoplastic chronic idiopathic neutropenia (CIN, n=15) and myelodysplastic syndromes (MDS, n=10). The effect of CD40 activation in the survival of normal and patient BM CD34⁺ cells was evaluated (a) by flow-cytometry for the quantification of 7-amino-actinomycin-D and Fas expression in normal and patient CD34⁺ cells following 48-h incubation with rhCD40L (1microgr/200 μ L) and/or rhFasL (0.4microgr/200 μ L) (b) by clonogenic assays for the enumeration of the colony-forming-cells within normal and patient BM mononuclear cells following 14-day culture in methylcellulose in the presence of rhCD40L and/or rhFasL. CD40L expression was studied by RT-PCR in the adherent layer of normal and patient long-term BM cultures (LTBMCs). **Results.** CD40 was minimally expressed in normal CD34⁺ cells (5.37% \pm 2.84%). Following treatment with rhTNF α a significant dose-related increase in the proportion of CD40-expressing CD34⁺ cells was observed compared to baseline (p <0.001). The induction of CD40 by rhTNF α was confirmed by using the KG-1 CD34⁺/CD40- leukaemic cell line. In keeping with the above findings, the CD34⁺ cells from CIN and MDS patients showed elevated levels of CD40 expression compared to controls (10.42% \pm 6.05% and 29.12% \pm 21.84%, respec-

tively; P <0.001 and p <0.0001, respectively). Ligation of CD40 by rhCD40L on normal CD34⁺ cells following induction with rhTNF α , resulted in a significant dose-related increased of Fas expression compared to baseline (F184=7.61, P <0.001). In the patients, rhCD40L addition increased significantly the proportion of apoptotic CD34⁺ BMMCs compared to baseline and this increase was further augmented in the presence of rhFasL (p <0.01). Additionally, rhCD40L decreased patient colony-forming-cells (46 \pm 25) compared to baseline (69 \pm 44, p <0.01) and this decrease was further augmented in the presence of rhFasL (46 \pm 25, p <0.01). CD40L expression on BM CD34⁺ cells was not identified in any of the subjects studied. However, CD40L expression was identified in LTBM cell extracts of 66.7% of CIN and 50% of MDS patients but none of the controls (p <0.001 and p <0.001, respectively). **Conclusions.** CD40 is expressed at low levels on normal CD34⁺ cells but it is induced by TNF α . The TNF α -mediated induction of CD40 on haemopoietic stem/progenitor cells and its ligation by CD40L present in BM microenvironment represents probably a contributory mechanism to Fas/FasL-mediated CD34⁺ cell apoptosis in patients with BM failure.

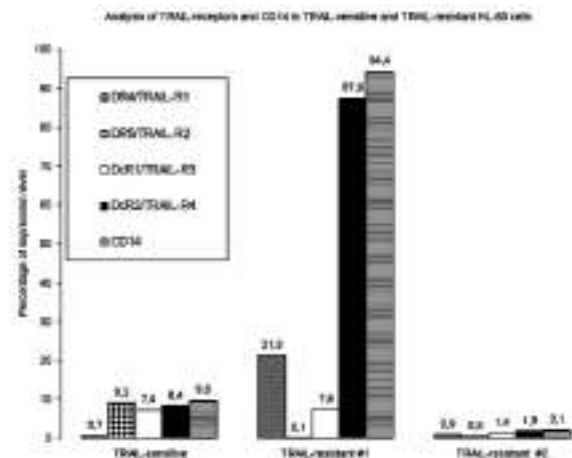
0060

MOLECULAR MECHANISMS OF RESISTANCE TO TRAIL-INDUCED APOPTOSIS IN THE MYELOID CELL LINE HL-60

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Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL/Apo-2L) is a potential anti-tumor agent because of its specific cytotoxic effect to a variety of transformed cell lines and primary tumor cells, including leukemia cells, but not to most normal cells. The TRAIL receptor family consists of death and decoy receptors. Stimulation of death receptors 4 and 5 (DR4/TRAIL-R1, DR5/TRAIL-R2) leads to activation of the extrinsic/receptor-mediated apoptotic pathway. Decoy receptors 1 and 2 (DcR1/TRAIL-R3, DcR2/TRAIL-R4) are unable to transduce apoptotic signals. To determine the molecular mechanisms by which leukemic cells might escape TRAIL-mediated apoptosis. TRAIL-resistant cells were selected from the original HL-60 population using selective pressure of recombinant TRAIL (200 ng/mL). The frequency of TRAIL-resistant cells in the HL-60 cell population was determined by limiting dilutions. The expression levels of the TRAIL-receptors and CD14 were measured by flow cytometry. Apoptosis was measured by flow cytometry using Annexin-V-PE apoptosis detection kit.



Finally, NF- κ B essays were performed by ELISA-based kit. **Results.** Using limiting dilutions we determined that about 1 of 1800 cells in the HL-60 cell population is TRAIL-resistant. By a long term cultivation of TRAIL-resistant HL-60 cells without TRAIL we established that the resistance to TRAIL was stable. TRAIL-sensitive HL-60 cells are characterized by a constitutive expression of DR5, Dcr1, Dcr2, and hardly detectable expression of DR4. Two distinct TRAIL-resistant phenotypes were detected in HL-60 cell population based on the expression of TRAIL-receptors and CD14. A phenotype with *switched off* expression of all TRAIL-receptors and low expression of CD14, and a phenotype with down-regulated expression of DR5, weakly up-regulated expression of DR4, and strongly up-regulated Dcr2 compared to TRAIL-sensitive HL-60 cells (Figure 1). We analyzed whether the resistance to TRAIL-induced apoptosis is associated with the resistance to other apoptosis-inducing agents. We found that the sensitivity of TRAIL-resistant cells to cytostatics (AraC and Idarubicin), as well as to apoptosis inducing ligand TNF- α , was not significantly different compared to control TRAIL-sensitive HL-60 cells. To identify factors possibly associated with TRAIL-resistance we measured intranuclear NF- κ B family proteins, RELA (p65) and p50. We did not find significant differences in the intranuclear concentration of RELA and p50 between TRAIL-sensitive and TRAIL-resistant HL-60 cells. The stimulation with TRAIL for 4 hours led to translocation of RELA and p50 to the nucleus. The increase of intranuclear RELA was to 241% in TRAIL-sensitive HL-60 population and to 277% and 138% in two analyzed TRAIL-resistant HL-60 cell populations compared to relevant unstimulated control cells. The translocation of p50 to the nucleus followed the similar pattern as the RELA translocation. **Conclusions.** HL-60 cells can escape TRAIL-induced apoptosis by at least two different molecular mechanisms, characterized by changes in the expressions of cell surface TRAIL receptors. The TRAIL-resistant phenotype is stable and TRAIL-specific. The concentration of intranuclear NF- κ B family proteins RELA (p65) and p50 is not different between TRAIL-sensitive and TRAIL-resistant HL-60 cells and increase after the stimulation with TRAIL in both TRAIL-sensitive and TRAIL-resistant phenotypes.

0061

ORALLY ADMINISTERED LENALIDOMIDE IS ANTI-ANGIOGENIC *IN VIVO* AND INHIBITS ENDOTHELIAL CELL MIGRATION, CADHERIN 5/CD31 INTERACTION, AND AKT PHOSPHORYLATION *IN VITRO*

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The immunomodulatory drug (IMiD®) lenalidomide (Revlimid™) is emerging as a useful treatment for a number of cancers including myelodysplastic syndromes and multiple myeloma. The anti-tumor effect of lenalidomide appears to be related in part to its anti-angiogenic activity. **Aims.** To investigate the anti-angiogenic effects of lenalidomide in a variety of experimental systems representing several key events of the angiogenic process. **Methods.** *In vivo* anti-angiogenic activity was assessed using the rat mesenteric window assay, and the effects on growth factor-induced migration and tubule formation of human umbilical vein endothelial cells were measured. Molecular characterizations included immunoprecipitation and immunoblot analyses. **Results.** Oral administration of lenalidomide attenuated growth factor-induced angiogenesis *in vivo* in the rat mesenteric window assay; lenalidomide significantly inhibited vascularisation (percent vascularisation, number of branch points, and microvessel length) in a dose-dependent manner. Lenalidomide inhibited the growth factor-induced migration and tubule formation of human umbilical vein endothelial cells. Proliferation of endothelial cells was not inhibited by lenalidomide at concentrations up to 100 micromol/L. Immunoprecipitation and immunoblot analyses indicated that

lenalidomide inhibited the interaction between cadherin 5 and CD31, which is important for the formation of adherens junction and subsequent capillary tube formation. Finally, lenalidomide had an inhibitory effect on growth factor-induced Akt phosphorylation at Ser473 and Thr308. Together, these data provide new mechanistic insights into the effect of lenalidomide on key events in the angiogenic process; namely, cadherin 5/CD31 interaction; growth factor-induced migration; tubule formation; and Akt phosphorylation in endothelial cells. These data confirm that lenalidomide is a promising drug for the treatment of angiogenesis-related pathologies and further support the use of lenalidomide as an orally administered drug for the effective treatment of angiogenesis-dependent diseases, such as cancer.

0062

EFFECT OF BUSULFAN ON ENDOTHELIAL CELLS IN CULTURE: TOWARDS UNDERSTANDING PATHOGENESIS OF HEPATIC VENO-OCCLUSIVE DISEASE

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Busulfan (Bu), a myeloablative agent, is commonly used in the conditioning regimen in haematopoietic stem cell transplantation (HSCT) in combination with cyclophosphamide (CP). Although clinical efficacy of this combination is well established, interindividual differences in susceptibility to drug-induced toxicities are significant. One among them manifests as hepatic veno-occlusive disease (HVD), a major complication of HSCT with incidence and mortality rate as high as 70%. The pathogenesis of HVD is complex. Although age, sex, pre-existing liver damage and type of transplant have been associated with this complication, high bioexposure to these drugs is considered to be the major determinant. Analysis of biological markers suggest the potential involvement of cytokines and haemostasis in mediating the drug-induced damage of sinusoidal endothelial cells (SEC) and adjacent centrilobular hepatocytes in HVD. *In vivo*, Bu is metabolised by conjugation with glutathione (GSH), catalysed by a family of Glutathion S-transferase (GST) enzymes. Of the 4 main subfamilies of GST (GST A1, GST M1, GST T1 and GST P1), GST M1 and GST T1 are highly polymorphic, with homozygous deletion of either or both genes found in significant frequencies in different ethnic groups. Although these isoforms are considered to be less efficient than GST A1 in the metabolism of Bu, we reported earlier that both Bu clearance and GST M1-null genotype were significantly associated with the incidence of HVD. In order to dissect the pathogenic and genetic mechanisms of HVD, we have studied the effect of Bu on one endothelial cell line (TrHB-MEC), several primary endothelial cells (HUVEC) and one hepatic cell line (HepG2). These cells had previously been genotyped for *GST A1*, *GST M1* and *GST T1*. We analysed the mRNA expression of GSTs, Tissue Factor (TF), Endothelin-1 (ET-1) and the surface adhesive marker ICAM-1 (Intercellular adhesive molecular-1) by real-time quantitative PCR (RQ-PCR). Protein expression was also carried out for ET-1 and *ICAM-1* by Elisa. We show that Bu: i) moderately up-regulates *GST A1*, a major Bu metabolising enzyme, in the hepatic cell line, ii) does not alter GST M1 and ICAM-1 expression but down-regulates GST T1 (2 fold), iii) down-regulates the expression of ET-1 (2 fold) and TF (2 fold) These data are not in support of involvement of TF and ET-1 up-regulation in the pathogenesis of HVD. Our study also demonstrated that the expression status of ET-1, ICAM-1 and TF is not related to GST genotype in endothelial cells. Ongoing studies of Bu/CP-induced functional interactions between EC and hepatic cells must provide further insights into the pathogenesis of HVD. The striking observation is the absence of expression of GST A1 in all endothelial cell types, regardless of GST genotypes and may provide an explanation for the Bu-induced endothelial desquamation that ensues conditioning in HSCT.

0063

IN VIVO AND IN VITRO TOXICITY OF ROSCOVITINE ON MURINE HEMATOPOIETIC STEM CELLS

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Background. Roscovitine is a purine analog and cyclin-dependent kinase inhibitor that has been considered for cancer treatment. The drug has been recently evaluated in clinical trials for different types of tumors. **Aims.** In the present work, the effect of roscovitine on hematopoiesis has been examined in mice model both *in vitro* and *in vivo*. **Methods.** Progenitor cells were tested by their ability to form *in vitro* colonies in soft gel cultures. For *in vivo* studies, a single dose of roscovitine (50, 100 and 250 mg/kg) was administered intraperitoneally, hematotoxicity, liver toxicity and immunosuppressive effect of the drug were investigated after 1, 3, 12 and 24 days post administration. For *in vitro* studies, bone marrow cells from untreated mice were treated for 0.5 to 24 hours to different concentrations of roscovitine (25, 50 and 100 microM). In both studies, the proliferative capacity of murine bone marrow progenitors was estimated by evaluation of the number of immature erythroid (BFU-E) and granulocyte-macrophage (CFU-GM) colony forming cells. Liver status was determined by monitoring liver enzymes (AST, ALT, and bilirubin). Immunological analyses were performed using spleen cells. **Results.** and **Conclusions.** No behavioral changes were observed in treated animals. Body weight was not affected by drug treatment through the evaluation period. No significant changes on peripheral blood and differential count were observed in treated animals compared to untreated mice during the time period studied. Biochemical measurements of liver enzymes showed that no significant hepatotoxicity was associated with drug administration since the values were within normal limits. No significant changes in murine erythroid and granulocyte-macrophage progenitors were observed in the time-period studied at any of the doses administered compared to the untreated animals. However, *in vitro* assay using normal mice bone marrow cells treated with roscovitine showed a significant reduction in the number of both BFU-E and CFU-GM in a time- and concentration-dependent manner. The present results may be explained by the short half-life shown previously in rat. This is an important issue to consider prior to clinical trials.

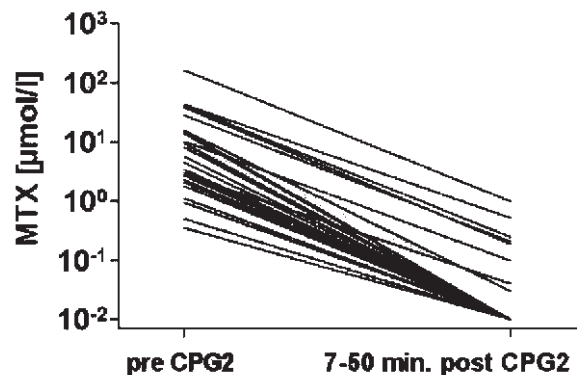
0064

EMERGENCY TREATMENT WITH CARBOXYPEPTIDASE G2 IN PATIENTS WITH DELAYED METHOTREXATE-CLEARANCE AND RENAL FAILURE AFTER HIGH-DOSE METHOTREXATE THERAPYS. Schwartz¹, K. Bomer¹, K. Müller¹, L. Fischer¹, A. Korfel¹, T.R. Auton², E. Thiel¹¹Charite Campus Benjamin Franklin, Berlin, Germany;²Protherics PLC, Runcorn, UK

Background. Life-threatening accumulation of methotrexate (MTX) caused by renal failure is a rare complication in patients (pts.) treated with high-dose MTX (HD-MTX). In this condition, standard supportive care measures (e.g. leucovorin rescue, hemodialysis) have limited effects.

Carboxypeptidase G2 (CPG2) rapidly cleaves MTX into 2,4-diamino-N-pterico acid, a non-toxic metabolite, and glutamate. **Aims.** In a nationwide emergency-use study, the clinical and pharmacological effects of CPG2 were evaluated in pts. with delayed MTX-clearance and renal failure after HD-MTX therapy. **Methods.** Pts. with renal insufficiency (creatinine >1.5 upper limit of normal) were eligible for treatment with CPG2 at <42 hours, or when the MTX serum levels (sMTX) remained >1 µmol/L at 42 hours, or >0.4 µmol/L at 48 hours after start of HD-MTX. In addition, pts. with sMTX >5 µmol/L at 42 hours (+/- renal insufficiency) were also eligible. Pts. were registered by

telephone and CPG2 was sent to the demanding center by a 24-hour courier service. Levels of MTX and metabolites were determined by high-performance-liquid-chromatography (HPLC) in pts. with available acidified serum samples. **Results.** Fifty-three pts. (age: 10-78 years) with lymphoma (30), acute lymphoblastic leukemia (17), germ cell tumor (1) or sarcoma (5) were registered 4-138 hours (median: 42) after start of HD-MTX. At registration, MTX serum levels ranged from 1.01-1187 µmol/L (median: 9.8). First treatment with CPG2 (median dosage: 50 units/kg i.v.) was given 27-176 hours (median: 55) after start of HD-MTX. Three pts. received additional CPG2 treatment, which was optional, 24-41 hours after first CPG2 administration. A skin reaction grade III and fever grade II, possibly related to CPG2, occurred in one pt. each. The remaining 51 pts. tolerated CPG2 treatment without any side-effects. Serial serum samples were available in 24 pts. and analysed by HPLC: MTX serum levels rapidly decreased from a median of 5.1 µmol/L (range: 0.35-166) before to <=1 µmol/L within 7-50 minutes after CPG2 administration (Figure 1).



In 3 pts. the serum creatinine levels remained normal. In another 31 pts. the renal function fully recovered with normalization of the serum creatinine by days 1-127 (median: 17). The remaining 19 pts. had median peak serum creatinine levels of 285 µmol/L with subsequent decline to a median of 139 µmol/L by days 1-56 after first CPG2 treatment. Summary CPG2 is safe and effective in pts. with delayed MTX-clearance and renal failure after HD-MTX therapy. Treatment with CPG2 produced a rapid and substantial reduction in circulating MTX levels. Pre-emptive therapy with CPG2 might also allow the use of HD-MTX in pts. with impaired renal function and further studies on this issue are clearly desirable.

0065

DOWN-REGULATION OF PKC-ETA SENSITIZES HODGKIN'S LYMPHOMA CELL LINES TO APOPTOSISS. Abu-Ghanem¹, J. Gopas², D. Benharroch², E. Livneh¹¹Ben-Gurion University, Beer Sheva, Israel; ²Soroka Medical Center, Beer Sheva, Israel

Background. The malignant cells in HL are called Hodgkin-Reed-Sternberg cells (HRS) and are believed to originate from germinal center B lymphocytes which did not undergo apoptosis. Protein Kinase C (PKC) is a family of serine/threonine kinases, which are key enzymes considered to play a crucial role in signal transduction leading to cell growth, differentiation and oncogenesis. **Aims.** In this work we studied the correlation between PKC-eta expression and apoptosis in Hodgkin's lymphoma (HL) derived cell lines. **Methods.** The research was conducted on two cell lines, KMH2 and L428, derived from HL patients in the late stages of the disease. Apoptosis was induced by camptothecin (CPT), doxorubicin and UV radiation treatments. Downregulation of PKC-eta expression in L428 cells was achieved by siRNA technique. PKC expression was determined

by western blot using specific antibodies. **Results.** First we examined the sensitivity of two cell lines to U.V radiation (L428: G1 arrest, KMH2: S phase arrest) and to the anti cancer drugs: doxorubicin (L428: G1 arrest; KMH2: G2/M arrest) and CPT (L428 and KMH2: G1 arrest). We found that in response to all three apoptotic treatments L428 cells were more resistant than KMH2 cells. Equivalent results were obtained using poly ADP ribose polymerase (PARP) cleavage as an apoptosis marker. Next we examined the role of PKC in the resistance of the cells to apoptosis. We determined the expression of several PKC family members in both cell lines. We found similar levels of PKC-delta and PKC- ζ in both cell lines; PKC-epsilon, PKC beta1 and PKC- η were not found in the cells. PKC η 2 was found only in L428 and PKC-eta was highly expressed in L428 as compared to KMH2, which was also confirmed by immunohistochemistry staining. Expression and proteolytic activation of PKC-eta play an important role in the regulation of cell division and apoptosis during early B- cell development; therefore we chose to examine the presence and importance of PKC- η in apoptosis. We found a decrease in PKC- η levels in a dose dependent manner after treatments with CPT, doxorubicin and UV. When we used siRNA technique to knockdown PKC-eta expression in the resistant cell line-L428 we observed that these cells were more sensitive to doxorubicin treatment than L428 cells expressing PKC- η . In conclusion: PKC-eta is present in HRS cells. High levels of PKC- η in HL cell lines correlate with resistance to apoptosis. The enzyme is partially depleted following pro-apoptotic treatment. Using siRNA technique we found that down-regulation of PKC- η induces sensitivity to apoptosis.

Acquired anemias

0066

SIXTEEN CASES OF PURE RED CELL APLASIA OVER A 8-YEAR PERIOD RELATED TO RECOMBINANT ERYTHROPOIETIN USE

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Since 2002, pure red cell aplasia (PRCA) associated with the use of recombinant erythropoietin (Epo) in chronic renal failure (CRF) has been recognised worldwide. The use of subcutaneous (SC) Epo to correct anemia in CRF patients became standard practice in Singapore in 1997. From 1997 to 2004, we identified 16 patients with recurrent severe transfusion-dependent anemia due to PRCA. The incidence and outcome of this disorder in relation to clinical practice are presented. All cases except one were related to the α -Epo product Eprex (Janssen-Ortho Incorporated). Eight were anti-Epo antibody positive. In the remaining patients, diagnosis was by exclusion as antibody testing was only available from 2002. Of the 16 cases, 3 developed severe anemia in 1997, none in 1998, 1 in 1999 and 2 in 2000. A surge in the number of cases was noted in 2001 and 2002 with 5 and 4 patients respectively. In 2003, only 1 patient who had received the SC beta-Epo product Neorecormon (Roche) for 8.5 months developed PRCA. No further cases have been reported since. The median age was 44 years (range, 41-75 years) while median time from initiating Epo to development of severe anemia was 9 months (3-29 months). The prognosis was generally good with resolution in 13 of 15 cases treated with immunosuppressive therapy. One patient declined treatment but had spontaneous resolution 9 months after stopping Epo. Time to resolution ranged from 2 to 29 months (median, 7 months). Median follow up period was 41.5 months (range, 9-92 months). The rise and fall in incidence of this disorder and its association with Eprex can be attributed to several factors. Inadequate handling and storage of the drug has been implicated. Also, SC administration is associated with increased immunogenicity compared with intravenous route. A change in Eprex formulation in 1998 to human serum albumin free prefilled syringe probably played an important role. Prior to 2000, Eprex was the only Epo prod-

uct registered locally and thus has been the predominant Epo used. In 2002, Neorecormon received registration also. Significant changes in clinical practice were instituted from end 2002. All CRF patients on hemodialysis were given Eprex intravenously. Where this was not feasible, SC Neorecormon was prescribed. Storage and handling conditions of the drug were also more stringently applied. Why this condition appears mainly in CRF patients remains unclear. With increasing use of Epo to treat a wide variety of disorders we need to be vigilant. It would be prudent to consider possibility of PRCA in all patients receiving Epo should they develop severe anemia.

0067

EXPRESSION OF GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEINS ON DIFFERENT SUBSETS OF NORMAL PERIPHERAL BLOOD: A FRAME OF REFERENCE FOR THE DIAGNOSIS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA.

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal hematopoietic stem cell disorder related to the occurrence of a somatic mutation in the PIG-A gene, which encodes for an enzyme required for the synthesis of glycosylphosphatidylinositol (GPI). This alteration translates into a deficient expression of GPI-anchored proteins (GPI-AP), the evaluation of this deficiency by flow cytometry being used for the diagnosis of PNH. **Aim.** In this study we analyzed the amount of expression of the CD14, CD16, CD24, CD48, CD52, CD55, CD58, CD59, CD66b, CD87, CD109 and CD157 GPI-AP in different cell subsets present in normal peripheral blood (PB), in order to establish their normal pattern of expression and define the best combination of surface proteins for each PB cell subsets which might be useful for the diagnosis and monitoring of PNH. **Methods.** Ten normal PB samples from an identical number of healthy donors were analyzed by flow cytometry, using 4-and-6-color stainings to quantify the expression of a large panel of GPI-AP in specific cell subsets. **Results.** Our results show that the amount of expression of GPI-AP was highly variable in the different cell subsets measured. Even if the combined use of CD55 and CD59 represented the most useful dual-marker combination, its utility remained suboptimal for several subsets of leukocytes and for platelets. According to our data, for some cell subsets such as the neutrophils additional useful markers could be selected from a relatively wide panel (CD16, CD24, CD55, CD59, CD66b, CD157) whereas for other cell subsets the number of useful antigens was either restricted (monocytes: CD14, CD55, CD157; B-cells: CD24, CD48, CD52, CD55; CD4+ T-cells: CD48, CD52, CD55; eosinophils: CD55, CD59; CD8+ T-cells: CD48, CD55) or limited to a single marker (CD48 on CD56low NK-cells, CD55 on BDCA3- dendritic cells and CD56high NK-cells and CD59 for red cells) from all antigens analyzed. **Conclusions.** The pattern of expression of GPI-AP varies significantly between different normal PB cell subsets, its quantitative analysis providing a frame of reference for the identification of PNH+ cells.

0068

THE USE OF FLUORESCENT INACTIVATED BACTERIAL AEROLYSIN AS A SINGLE STAINING AGENT IN THE DIAGNOSIS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background. Paroxysmal nocturnal haemoglobinuria (PNH) is a rare (oligo)clonal disorder of the HSC characterized by complement-mediated haemolysis, venous thrombosis and bone marrow failure. Acquired mutation (s) in the PIG-A gene of HSC

result in complete or partial block in the synthesis of glycosylphosphatidylinositol (GPI). The detection and accurate quantitation of a PNH clone in a patient is important for management decision-making, such as implementation of primary prophylactic anticoagulation. Traditionally, the detection of these clones has relied on the increased susceptibility of PNH red cells to complement mediated lysis by the Ham's or sucrose lysis tests. Flow cytometry has largely supplanted these tests in the diagnosis of PNH. Two or more mAb specific for different GPI-linked proteins must be used to confirm that the deficiency is indeed in GPI synthesis and not in the synthesis of the GPI-linked proteins themselves. Furthermore, different mAb are required for different cell lineages: anti-CD59 for RBC, granulocytes and monocytes, -CD24 and -CD66b for granulocytes and -CD48 for lymphocytes. Aerolysin is a bacterial toxin which binds to GPI directly. In its active form, it causes cell lysis by forming channels in the cell membrane. FLAER is a fluorescently labelled inactive form of aerolysin which can be used to detect the presence of GPI on white cell membranes by flow cytometry. Its main disadvantage is that it is not suitable for red cell analysis as it also binds glycoporphin. *Aims.* To investigate the suitability of FLAER as a single staining agent for the diagnosis of PNH we compared the staining properties of FLAER to those of mAb specific for GPI-linked proteins in samples from patients with PNH and from normal individuals. *Methods.* Fifty µl whole blood in EDTA from 5 patients with PNH and from 5 normal subjects were stained in parallel with anti-CD59, anti-CD24, anti-CD48 and FLAER. Following red cell lysis with formic acid, the white cells were resuspended in PBS and analysed by flow cytometry on a Becton Dickinson FACScalibur instrument. *Results.* In normal samples, granulocytes, lymphocytes and monocytes stained positively with FLAER showing well defined, unimodal populations. Staining with mAb specific to GPI-linked proteins showed identical patterns. In the samples from patients with PNH, staining with FLAER showed very clear bimodal populations in all white cell sublineages. Compared to the patterns seen with the mAb, FLAER produced clearer separation of the positive and negative cells, enabling more accurate quantitation of the size of the clone. This difference was more evident when compared to anti-CD59, the antibody most commonly used in the diagnosis of PNH. *Conclusions.* FLAER is sensitive and reliable as a single staining agent for the detection of PNH clones in white blood cells. It stains all white cell sublineages reliably and enables accurate estimation of the sizes of PNH clones.

0069**CA 15-3 A MARKER FOR THE MEGALOBlastic ANAEMIA?**V. Mettievier¹, L. Pezzullo², O. Finizio², L. Bene², S. Rocco², C. De Rosa²¹A.O.R.N. 'A. Cardarelli', Naples, Italy; ²A.O.R.N. 'A. Cardarelli', Naples, Italy

Ca 15-3 is a glycoprotein present in the cells of the mammary carcinoma and in some epithelial cells. Is a marker used for the monitoring of the breast and gastrointestinal carcinoma. The megaloblastic anaemia is an anaemia characterized by deficit of absorption of Vitamin B12 and is associated with gastritis atrophic and the target cell is the parietal gastric cell. In our institution, from June 2003 to December 2004, the level of Ca15-3 and of others tumour markers (CEA; Ca125; Ca19.9; alfa-FETO) they have been tested in the serum of 16 patients (9 male and 7 female with median age of 64,5 and range of 37-80 years) with *de novo* megaloblastic anaemia, 2 patients were gastrectomized. In all patients has been effected: esophagogastroduodenoscopy and control anti-parietal gastric cells antibody (APCA). Increase level in the serum of CA 15-3 with normal level of other tumour markers have been found in 14/16 patient with median value of 61 mm/ cc (range 35-100 mm/cc) in two patients (gastrectomized) the value of CA 15-3 was normal. Besides in 12/14 patients have shown positivity for the APCA, only in two patients has been diagnosed a gastritis atrophic, in the other patients has been observed a normal gastric mucous.

After a median observation of 24 months any patient has developed a mammary or gastro-intestinal carcinoma. These results indicate what the increased level of CA 15-3 antigen in patient with megaloblastic anaemia is positively correlated with APCA and with the presence of a normal gastric mucous. These clinical conditions make to suppose the destruction from the APCA of the parietal gastric cells with the liberation of this glycoprotein and this is shown in two patients gastrectomized with presence of APCA and not increased level of the CA 15-3. In conclusion the CA15-3 antigen is probably a specific marker for the diagnosis of megaloblastic anaemia and is probably associated with the destruction of parietal gastric cells.

0070**THE ESTABLISHMENT OF DIFFERENTIALLY EXPRESSED GENE LIBRARY OF CD4 POSITIVE T CELLS IN APLASTIC ANEMIA BY IMPROVED SUPPRESSION SUBSTRACTIVE HYBRIDIZATION**

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Background. Aplastic anemia (AA) remains an elusive disease, some research showed the CD4 positive T cells played a very important role in the immune pathophysiology of AA, but the mechanism has not been quite clear till now. Purpose: Try to investigate the molecular mechanisms of the over-proliferation, activation, infiltrating bone marrow and inhibition of hematopoietic cells in CD4+ T cells of aplastic anemia. *Methods.* CD4+ T cells were separated from bone marrow by density gradient centrifugation using Ficoll-Hypaque lymphocyte separating medium combined with magnetic bead sorting technique. An improved suppression subtractive hybridization technique were established based on the suppression subtractive hybridization established by Dabacho, using a microcentrifuged filtering column combined with three hybridization reactions. Then subtracted cDNA library of CD4+ T cells was generated with the CD4+ T cells 'cDNA of aplastic anemia as target and normal donor's as drivers. Approximately 8 of the resulting subtracted cDNA clones were partially sequenced and analyzed by blast. *Results.* 1) 110 Clones were selected and identified from the established subtractive library, which contained an inserted fragment with 250-700bp, the positive ratio was amount to 88%. 3) Among the 8 sequenced clones, 7 sequences were considered as part of the known genes, which containing the genes regulating the cell's proliferation, activation and related to the signal transduction. Also there was 1 sequences representing previously unknown genes was found. All these genes have not been reported to be related to mechanism of the hematopoietic damage mediated by CD4+T cells in the aplastic anemia. *Conclusions.* Many genes both known and unknown were found to relate to the function of CD4+T cells in aplastic anemia. Discovery of these gene provided a solid foundation to elucidate the mechanism of the over-proliferation, activation, infiltrating bone marrow and inhibition of hematopoietic cells of CD4+ T cells in aplastic anemia

0071**IMMUNOLOGICAL AND MOLECULAR CHARACTERIZATION OF A PNH PATIENT FOLLOWING ALLOGENIC BMT**W. Barcellini, F.G. Imperiali, A. Zaninoni, E. Fermo, C. Boschetti, D. Soligo, G. Lambertenghi Deliliers, A. Zanella
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Background. Paroxysmal Nocturnal Hemoglobinuria (PNH) is a clonal disorder of hematopoietic stem cell characterised by bone marrow failure and somatic mutations of the phosphatidylinositolglycan-class A (PIG-A) gene, resulting in the absence or decreased surface expression of GPI-anchored proteins (GPI-AP). *Aims.* We describe the clinical, immunological and molecular characteristics of a 39 yrs-old male with 8 yrs story of PNH who underwent BMT from identical sibling donor. The patient was pancytopenic and transfusion-dependent and

had suffered from Budd-Chiari syndrome. The conditioning regimen was busulfan and cyclophosphamide and the clinical course of BMT was uncomplicated. **Materials and Methods.** We investigated clonogenic activity of the patient before BMT and at one, six and twelve months after BMT and we performed the molecular analysis of the PIG-A gene on every single colony. We evaluated the production of IFN- γ , TNF- α and TGF- β by bone marrow mononuclear cells (BMMC). **Results.** At one year after the transplant the patient is well, with Hb levels of 16 g/dL, but with low WBC ($1.9 \times 10^9/L$) and platelets ($70 \times 10^9/L$). In addition, the patient shows persistent lymphocyte (CD3) mixed chimerism in PBMC (40% donor), in spite of 3 lymphocyte donor infusions (2 of 0.5×10^7 , and 1 of 1×10^7 CD3/kg). The number of CFU-GM and BFU-E in bone marrow were clearly reduced before BMT (see Table), and 95% of colonies displayed the Del T774 mutation, as detected by PCR and sequencing of the PIG-A gene on every single colony.

	CFU-GM n° of colonies	BFU n° of colonies	TNF- α (pg/mL)	IFN- γ (pg/mL)	TGF- β (pg/mL)
Patient before BMT	7	12	22	587	22927
1 month after BMT	10	23	nd	nd	nd
6 months after BMT	7	46	22	474	2803
12 months after BMT	10	108	488	1182	1780
Controls (mean \pm SE)	21 \pm 9	62 \pm 18	377 \pm 185	1207 \pm 400	1361 \pm 391

The number of BFU-E clearly increased after BMT, reaching normal ranges, and none of the CFU-GM and BFU-E showed the original PIG-A gene mutation. Likewise, studies on PB granulocytes showed that the PIG-A mutation was present in virtually all cells before BMT, and in none of them at 14 days and 1 year after BMT. As regards immunological studies, production of cytokines by BMMC before BMT showed reduced TNF- α and IFN- γ and increased TGF- β , compared with controls. After BMT, TNF- α and IFN- γ production increased and were comparable to controls, and TGF- β decreased, although did not reach normal values. Regarding PB cultures, cytokine production before BMT showed higher TNF- α , IFN- γ and TGF- β levels than controls. After BMT, TNF- α and TGF- β decreased reaching normal values, whereas IFN- γ production was unchanged (*data not shown*). **Conclusions:** These data suggest the existence of a compartmentalization of cytotoxic cells before BMT, possibly involved in PNH bone marrow failure. After BMT, cytokine production showed a trend towards normality both in BM and PB suggesting the correction of the hypothesized compartmentalization.

0072

GLYCOSYL-PHOSPHATIDYL-INOSITOL (GPI)-DEFECTIVE GRANULOCYTES IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS SHOW INCREASED BACTERIAL INGESTION AND REDUCED RESPIRATORY BURST INDUCTION

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Background. PNH is an acquired haematological disorder characterised by the emergence and expansion of a GPI-defective

clonal hemopoiesis. PNH main clinical features are haemolytic anemia, thrombosis and cytopenia with recurrent infections. Granulocytes play, together with monocytes, a critical role for effector functions in innate immunity. The majority of peripheral monocytes and granulocytes are usually GPI defective in PNH patients. **Aims.** and methods In this study we aim to investigate granulocyte functional effectiveness in PNH patients. We analysed bacterial dependent intracellular ingestion and the consequent activation of oxidative burst in GPI defective granulocytes from PNH patients. In comparison the functional behaviour of normal granulocytes was studied in a population of age-sex matched healthy controls. **Results.** Our study indicates that GPI defective granulocytes from PNH patients show a significant increase in their ability to ingest opsonised bacteria and an impaired respiratory burst effectiveness in response to two independent bacterial stimuli represented by the N formyl MetLeuPhe (fMLP) synthetic bacterial peptide and E. Coli. The latter alteration was maintained after triggering with phorbol 12 myristate 13 acetate (PMA), a pharmacological stimulus able to recruit and to extensively trigger intracellular Protein Kinase C. **Summary/ Conclusions.** In PNH the lack of GPI-linked molecules CD55 and CD59 on the cellular membrane produces an altered control of activated complement fractions. The consequent excess of C3 activated molecules could act as a continuous stimulus, probably depleting the intracellular concentration of PKC. This condition could be involved in the pathogenesis of both the increased phagocytic effectiveness and the impaired oxidative burst generation observed in GPI-defective granulocytes. The possible role of these alterations in the increased susceptibility to infections of PNH patients, mainly due to neutropenia, needs further investigations.

0073

ECAS AND MEWACAS: TWO REGIONAL, MULTINATIONAL, PROSPECTIVE SURVEYS DEFINING THE SEVERITY AND TREATMENT OF ANEMIA IN CANCER PATIENTS

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Background. European Cancer Anaemia Survey was the first survey to evaluate prospectively the prevalence, incidence, and management of anemia (Ludwig 2004), and define predictive risk factors for anemia (Van Belle 2004) in a large population of European cancer patients. Patients (n=15,367) with solid (78.9%) and hematologic (21.1%) malignancies from 24 countries in 824 centers were followed for up to 6 months. These data demonstrate a high prevalence of anemia in cancer patients and an adverse effect on performance status. **Aims.** Comparable data on anemia was needed from cancer patients in the Middle East / Western Asia (MEWA) region. The MEWA Cancer Anaemia Survey (MEWACAS) was instituted: a prospective, epidemiologic, observational survey conducted in 11 countries, including Cyprus, Egypt, Iran, Israel, Jordan, Kuwait, Lebanon, Libya, Malta, Saudi Arabia, and the United Arab Emirates. Data was collected for up to 6 months on patient characteristics, Hb levels, tumor types, and type and intensity of cancer treatments. **Results.** There were 954 patients enrolled in MEWACAS from community, academic, and private centers specializing in cancer treatment; 917 patients had data available for analysis after enrollment, including 83.2% with solid tumors and 16.8% with hematologic malignancies. Although the majority of patients (79.6%) had WHO Performance Scores of 0 or 1 at enrollment, there was an association of low Hb levels and poor WHO scores (2-4). For the 921 patients with Hb levels available at enrollment, 54.5% were anemic (Hb < 12 g/dL). Most patients (35.5%) had mild anemia (Hb 10.0 to 11.9 g/dL); moderate anemia was recorded for 15.0% (Hb 8.0 to 9.9 g/dL), and severe anemia for 4.0% of patients (Hb < 8.0 g/dL). Although they were anemic at enrollment, 71.7% of these patients did not receive anemia treatment in the prior 30 days. About half (49.5%) of those patients anemic at enrollment were not treated for their anemia during the survey, 13.1% received epoetin, 14.1% received transfusion, and 23.3% received iron only. The mean Hb for

initiation of therapy was 8.8 g/dL for transfusion and 9.0 g/dL for epoetin. For the 858 patients with Hb values for analysis, 79.0% (n=675) were anemic at some time during the survey. Anemia was most frequently reported in patients who received chemotherapy (81.3%), while anemia occurred in 74.6% of patients who did not receive cancer treatment at any time during the survey. *Summary/Conclusions. Results.* from ECAS and MEWACAS show that the prevalence and incidence of anemia are high and correlate with poor performance status. These large-scale prospective surveys provide clinical data indicating that anemia remains common in cancer patients. Anemia may be present early after diagnosis; it may be severe; it is often associated with poor QOL. Importantly, treatment for anemia is not optimized; only a minority of anemic patients is receiving treatment, despite accepted anemia treatment guidelines. Understanding these results may lead to better management of anemia in cancer patients and optimization of patient QOL.

0074

TREATMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA IN INFANTS BELOW 1 YEAR OF AGE WITH RITUXIMAB, A MONOCLONAL ANTI CD20 ANTIBODY

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Autoimmune Hemolytic Anemia (AIHA) with early onset in infancy is a serious life-threatening condition which commonly has a protracted course with a reported mortality of 25%. Few reports in literature focus on treatment options for this population. First-line therapy for presentation with severe anemia is high-dose steroid and, if necessary, exchange transfusions (ET). Prognosis for these infants is very severe if acute hemolysis is not controlled. Second-line treatment including cytotoxic agents (vincristine, cyclophosphamide), immunosuppressive drugs (cyclosporine (CSA), mercaptopurine (6-MP), azathioprine) and splenectomy, are not consistently effective and carry high treatment-associated risk. Since 2001 several reports on the effectiveness of rituximab, a monoclonal anti CD20 antibody rapidly depleting B-cells, in controlling AIHA in children with refractory chronic disease have been published. Only 5 infants treated with rituximab, with refractory AIHA, are mentioned in literature in 3 different reports. We report our single-centre experience on the use of rituximab in four infants below 1 year of age with AIHA who at onset did not show response to steroid therapy. The infants were diagnosed with idiopathic warm-antibody AIHA at the age of 21 days, four, six and nine months respectively. Anemia and hemolysis was severe in all cases (Hb 4.0, 4.1, 2.8 and 2.6 g/dL respectively, with reticulocytosis). Unsuccessful first line treatment was prednisone 6-8 mg/kg/day for respectively 20, 9, and 8 days in three cases and steroids for one day plus ET in another case. All patients, as second line treatment, were given a course of four weekly doses of rituximab (375 mg/m²/dose in 8 hour intravenous infusion). Intravenous immunoglobulins were supplemented every 3 weeks and co-trimoxazole prophylaxis for *Pneumocystis Carinii* Pneumoniae was performed. *Results.* In two infants hemolysis stopped within one and three months and steroid could be tapered off and suspended after 4 and 12 months. They are now well and without immunosuppressive therapy 30 months from diagnosis. In the other two another four weekly doses of rituximab were given 1 and 5 months later, in addition to therapy with CSA, 6-MP and steroid. Of these two, one infant has now tapered off steroids and 6-MP and is only on CSA therapy, with a negative direct antiglobulin test (DAT) at 8 months from diagnosis. The other has, at 33 months from diagnosis, chronic compensated hemolysis, positive DAT, 0-3% CD20+ lymphocytes in peripheral blood and is on therapy with CSA (4 mg/kg/day), steroid (0.1 mg/kg/day) and a maintenance regimen with rituximab infusion every 40 days. *Summary/conclusion* In two infants AIHA looks eradicated after a long-term follow-up; in another, after a shorter follow-up, AIHA is controlled and immunosuppressive therapy reduced. The fourth infant with chronic compensated AIHA remains on rituximab in maintenance regimen. No adverse reac-

tions to rituximab were seen. No severe infectious complications during the phase of B-cell depletion were seen. Our data indicates rituximab as a safe and effective therapy for AIHA early in the course of the disease in infants below 1 year of age.

0075

HEPCIDIN AND HEMOJUVELIN EXPRESSION IN HEMOSIDEROTIC PATIENTS WITH THALASSEMIA MAJOR

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Hepcidin and hemojuvelin play a central role in iron homeostasis. Hepcidin seems to be the common final mediator of both 'erythroid' and 'stores' regulators, and coordinates intestinal iron absorption and iron release from reticuloendothelial macrophages. The 'erythroid' regulator probably dominates over the 'stores' regulator. The functional role of hemojuvelin remains unclear. Homozygotes patients with mutations in the hemojuvelin gene present with juvenile hemochromatosis, which is a severe iron overload disease, and have decreased urinary hepcidin levels. Iron overload in thalassemia major is attributed mainly to blood transfusions and partly to increased iron absorption. Urine hepcidin levels in regularly-transfused thalassemia patients are inappropriately low in regards to their iron stores. Liver hepcidin expression is suppressed in the murine model of human thalassemia (Hbbth3/+). In the present study, the impact of iron stores and erythropoiesis on liver hepcidin and hemojuvelin expression in patients with thalassemia major has been evaluated. Nineteen transfusion-dependent thalassemic patients (14 females) of 20±7.2 years of age underwent liver biopsy. Fourteen patients were seronegative for hepatitis C. Liver iron concentration (LIC) was estimated by atomic absorption spectrometry. Soluble Transferrin Receptor (sTfR) and ferritin levels were assessed with the Advia-Bayer 1650 Clinical Chemistry System. Hepcidin and hemojuvelin mRNA expression levels were estimated by quantitative Real-Time PCR (Lightcycler, Roche) from isolated RNA from liver tissue. Statistical analysis was performed using either the Pearson test or the Spearman test for non-Gaussian distribution of the values. LIC ranged from 3.1 to 18.9 µg Fe/g dry tissue (median 8.3 µg Fe/g dry tissue) and ferritin levels from 990 to 5963 µg/L (median 2174 µg/L). Mean sTfR levels were 3.1±1.7mg/L. Hepcidin expression ranged from 0.08-38.4 (median 1.13). Hemojuvelin expression ranged from 0.01-2.82 (median 0.13). Hepcidin and hemojuvelin expression inversely correlated with sTfR (r=-0.59, p=0.01 and r=-0.5, p=0.03, respectively). The correlation between hepcidin and sTfR was even stronger when patients with infectious hepatitis were excluded from analysis (r=-0.82, p<0.001). However, hepcidin and hemojuvelin expression did not correlate with the degree of hepatic siderosis or ferritin levels. *Conclusions.* Our results provide additional evidence that increased erythropoietic activity (assessed by sTfR measurements) down-regulates hepcidin expression. The lack of correlation between iron stores (assessed by LIC and ferritin levels) and hepcidin expression is in consistency with the hypothesis that increased erythropoietic activity dominates over iron stores in the regulation of hepcidin expression in patients with thalassemia major. Finally, the inverse correlation of sTfR with hemojuvelin expression might indicate that hemojuvelin participates in the regulation of hepcidin expression according to the needs of erythropoiesis, thus being an important component of the *erythroid* regulator pathway.

0076

EFFECT OF BODY WEIGHT ON THE EFFICACY AND SAFETY OF FIXED DOSE EPOETIN ALFA FOR CANCER-ASSOCIATED ANEMIAE. Vercammen¹, C. Ludwig², K.L. Liu¹, L.X. Xiu¹, P. Bowers¹¹Johnson & Johnson, Buckinghamshire, UK; ²Wilhelminenspital, Wien, Austria

Background. Epoetin α for the treatment of cancer-associated anemia is effective given as a weight-based dose (WBD; 150 IU/kg) or fixed dose (FD; 10,000 IU) 3-times weekly (TIW), or a FD (40,000 IU) once weekly (QW). To evaluate effect of body weight on efficacy of epoetin α administered as FD; evaluate if FD regimen produces adequate efficacy across all weight ranges vis-à-vis WBD; assess safety across all weight ranges. **Methods.** Data from FD trial (40,000 IU QW increased to 60,000 IU QW for insufficient response; N = 2964; [Gabrilove, 2001]) and data from 8 WBD studies (150 IU/kg TIW increased to 300 IU/kg for insufficient response; N = 944) were analyzed and compared. Patients were adults with variety of solid and hematologic malignancies. Body weights were defined by quartiles (Q) derived from the European cancer anemia Survey (ECAS) (Ludwig, 2004): Q1, ≤ 60.3 kg; Q2, >60.3 to ≤ 70.0 kg; Q3, >70.0 to 79.5 kg; Q4, >79.5 kg. FD body weights analyzed corresponded closely to the ECAS-based body-weight quartiles Q2 (600 IU/kg dose) and Q4 (450 IU/kg dose).

Table. Hb-change from baseline to last value by body weight.

Change in Hb (g/dL)	Epoetin α	Placebo (n=165)
Quartile 1: ≤ 60.3 kg		
N	30	33
Mean (SD)	3.2 (2.02)	1.2 (1.85)
	3.7	1.3
Quartile 2: > 60.3-70.0 kg		
N	39	37
Mean (SD)	3.4 (2.05)	0.7 (1.27)
Median	3.7	0.5
Quartile 3: > 70-79.5 kg		
N	24	37
Mean (SD)	2.4 (1.90)	0.9 (1.36)
Median	1.8	0.9
Quartile 4: > 79.5 kg		
N	61	50
Mean (SD)	2.3 (1.96)	0.7 (1.39)
Median	2.4	0.7

Efficacy endpoints were erythropoietic response (ER; hemoglobin [Hb] increase ≥ 2 g/dL independent of transfusion) and Hb change from baseline. Safety was evaluated in the patients in the FD trial who received at least 1 dose of epoetin α , with body-weight quartiles similar to the ECAS-based body-weight quartiles. **Results.** ER was somewhat greater in lighter-weight patients (Q2; ≤ 60.3 kg) in the FD group than in heavier-weight patients (Q4; >79.5 kg), 65% versus 61%, respectively. In the WBD group, ER for lighter- and heavier-weight patients was 72% and 63%, respectively. These differences in effect of body weight on ER between FD and WBD dosing regimens were not significant ($p=0.3005$). Adjusted estimates of probability of transfusion or Hb ≤ 8 g/dL by body weight outlier category for FD epoetin α were 11%, 14%, and 18% in the 5th (50.0 kg), 5th to 95th, and 95th (>95.9 kg) weight percentiles, respectively; all remained low. Concerning Hb change from baseline, heavier patients had slightly smaller Hb increases from baseline with FD (2.0 g/dL Q2, 1.8 g/dL Q4) and WBD (2.2 g/dL Q2, 1.9 g/dL Q4); mean Hb change with FD epoetin alfa was >1.5 g/dL, even for patients who weighed ≥ 95.9 kg. Probability of dose increase

with FD was 33% in Q2 and 42% in Q4 ($p=0.034$). Probability of dose increase with WBD was comparable across all body-weight quartiles (range: 28%–35%). Safety of FD epoetin α was similar across all body-weight quartiles, demonstrating that safety was not compromised in lower-weight patients. **Conclusions.** Both fixed (40,000 IU) and weight-based (450 IU/kg and 600 IU/kg) QW dosing of epoetin α effectively increase Hb in lighter-weight and heavier-weight patients. Tolerance of FD epoetin α is excellent regardless of body weight. Overall, FD dosing may provide efficacy and safety comparable to that of WBD, with the added advantage of greater convenience in prescribing.

0077

RELATIONSHIP OF BODY WEIGHT TO EFFICACY OF A FIXED-DOSE REGIMEN OF EPOETIN α VS PLACEBO IN ANEMIC CANCER PATIENTSM. Dicato¹, E. Vercammen², K.L. Liu², L.X. Xiu², P. Bowers²¹Luxembourg Medical Center, LUXEMBOURG, Luxembourg; ²Johnson & Johnson, BUCKINGHAMSHIRE, UK

Background. Fixed-dose (FD) regimens of epoetin alfa at initial dose of 40,000 IU once weekly (QW) (Gabrilove, 2001; Shasha, 2003; Witzig, 2004) have been shown to effectively increase hemoglobin (Hb) levels in anemic cancer patients. **Aims.** To evaluate relationship of body weight to efficacy of a FD dosing schedule of 40,000 IU epoetin alfa QW; determine whether a FD regimen produces adequate efficacy across all weight ranges. **Methods.** Data analyzed were from a prospective, 16-week, randomized, double-blind, placebo-controlled, multicenter study that enrolled patients with advanced cancer undergoing chemotherapy. (Witzig, 2004) Patients received either placebo or a fixed dose of 40,000 IU epoetin alfa subcutaneously QW, with dose escalation to 60,000 IU QW if Hb had not increased by >1 g/dL after 4 weeks. Efficacy analysis was based on body weight quartiles as defined in the European Cancer Anaemia Survey (ECAS): ≤ 60.3 kg, >60.3 kg to ≤ 70.0 kg, >70.0 kg to 79.5 kg, and >79.5 kg. (Ludwig, 2004) Endpoints were erythropoietic response (ER; postbaseline Hb ≥ 12 g/dL or Hb increase of ≥ 2 g/dL from baseline, independent of transfusion), transfusion status after treatment Day 28, and change in Hb from baseline to last value. **Results.** Three hundred thirty-three patients (epoetin alfa, 168; placebo, 165) were evaluated. Demographic and baseline characteristics were generally similar between the treatment groups. Mean baseline Hb was 9.4 g/dL for both groups. Percentages of epoetin alfa-treated patients with ER were 77%, 74%, 58%, and 74% in Quartiles 1 through 4, respectively, compared with 35%, 19%, 30%, and 32%, respectively, for placebo-treated patients. The interaction between treatment and body weight on response was not significant ($P=0.7187$). When analyzed by outlier category, the percentages of responders in the epoetin alfa group were similar for the 5th and 95th weight percentiles, ie, 67% and 74%, respectively; corresponding percentages for the placebo group were 17% and 46%. In Quartiles 1 through 4, 13%, 15%, 29%, and 11% of epoetin alfa-treated patients received transfusions after Day 28, vs 24%, 30%, 27%, and 30% of placebo-treated patients. Interaction between treatment and body weight on response was not significant ($P=0.3209$). Transfusion status by outlier category was similar: in the 5th and 95th weight percentiles, none and 5% of epoetin alfa-treated patients received transfusions after Day 28, vs 17% and 31% of placebo patients. Mean changes in Hb in the epoetin alfa group were substantially higher than those in the placebo group across all body weight quartiles (table). Interaction between treatment and body weight on Hb was not significant ($P=0.3324$). Mean Hb increases in the 5th and 95th weight percentiles were 2.3 g/dL and 2.1 g/dL for the epoetin alfa group vs 0.7 g/dL and 0.6 g/dL for the placebo group. Percentages of epoetin alfa-treated patients with dose increase were 26%, 38%, 46%, and 56% in Quartiles 1 through 4, respectively, vs 62%, 76%, 84%, and 70%, respectively, for placebo-treated patients. **Conclusions.** The results demonstrate epoetin alfa administered at a FD is effective over a broad range of patient weights.

0078**PARALLEL ASSESSMENT OF TRANSFERRIN RECEPTOR-1 AND -2, FERRITIN AND NRAMP2 EXPRESSION DURING HUMAN ERYTHROPOIESIS**M. Samara¹, I. Chiotoglou¹, S. Likousi¹, A. Triantafyllou², S. Bonanou³, N. Vamvakopoulos¹, P. Liakos⁴, P. Kollia¹¹School of Medicine, Univ. of Thessaly, Larissa, Greece; ²Lab. Biochemist. Univ. of Thessaly, Larissa, Greece; ³Lab. Biochemist. University of Thessaly, Larissa, Greece; ⁴Lab. Biochemist., Univ. of Thessaly, Larissa, Greece

Transferrin receptors (TfR)-1 and -2 are critically implicated in cell absorption of iron. TfR2 exists in two alternatively spliced forms (α/β transcripts), with differential expression. In this study, we evaluated mRNA expression of TfR1/TfR2, ferritin H-/L-subunits and DMT1/Nramp2 as well as protein expression of TfR1 in a model system of human erythropoiesis. CD34+ erythroid precursors collected from peripheral blood samples of normal individuals were cultured in free serum StemSpan medium in the presence of SCF and erythropoietin (3/10u/mL). mRNA transcripts for TfR1/TfR2 were analyzed by qualitative and real-time quantitative PCR. Levels of TfR1 mRNA increased significantly during cell proliferation and erythroid maturation (days 6-14: 3-6-fold); in contrast, TfR2a mRNA transcripts were low at the proerythroblast stage, then showed a moderate increase (days 6-14: 1.2-1.3-fold) and eventually remained stable up to terminal differentiation. TfR2b mRNA levels were generally low and did not change throughout erythroid differentiation. Augmented EPO dose (10 u/mL led to further increase in both TfR1 and TfR2a transcript levels (3.0- and 1.5-fold, respectively). TfR1- and TfR2a-mRNA stability was analyzed in cultures treated with actinomycin-D (actD: 5µg/mL) for 24 and 48hrs on day 8 of culture (proerythroblast stage). The levels of both TfR1 and TfR2a mRNA transcripts increased by 1.4-3.0-fold in actD-treated-CD34+ cells. Western blotting experiments were performed with an anti-TfR1-specific antibody on lysates derived from CD34+ cells throughout erythroid differentiation (days 6-14 of culture). These analyses demonstrated only moderate increase of TfR1 protein during erythroid maturation, despite high, progressively increasing TfR1 mRNA levels. Assessment of H- and L-ferritin mRNA content at different stages of erythroid maturation demonstrated slightly higher levels of H-ferritin, albeit without significant changes throughout erythroid maturation. Similar results were obtained for DMT-1/Nramp-2 mRNA. These findings suggest that: (i) TfR1 is highly expressed from early erythroid stages without significant changes throughout erythroid maturation in a context of progressively increasing transcription, alluding to predominantly post-transcriptional mode of regulation; (ii) TfR2a expression is regulated by transcriptional as well as post-transcriptional mechanisms; (iii) Epo mediates the expression of both TfR1- and TfR2a-mRNA transcripts in mature erythroid precursors. These observations suggest that increased iron demands of developing erythroid cells might be met by consistently high expression of TfR-1 and -2 perhaps also mediated by Epo.

0079**SBDS INHIBITS EXPRESSION OF FAS IN RESPONSE TO APOPTOSIS INDUCERS**P. Rujkijyanont, S. Jadko, S. Zhang, Y. Dror
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Background. Shwachman-Diamond syndrome (SDS) is an inherited marrow failure disorder with varying cytopenias and a strong predilection to MDS and AML. Previously, we found that the percentage of CD34+ cells in bone marrows and the in-vitro colony formation from CD34+ cells of SDS patients were markedly reduced. We also showed that patients' marrow cells are characterized by accelerated apoptosis, through the Fas pathway, with increased expression of Fas on marrow cells throughout maturation. **Objectives:** Herein we asked whether SBDS can specifically dysregulate Fas expression, and whether

activation of the Fas pathway is the only mechanism for the accelerated apoptosis in SDS, or the Bax/Bcl-2 apoptosis pathway is also involved. **Methods.** To study whether the SBDS is involved in the dysregulating Fas expression in SDS we generated an SBDS containing adenovirus 5 vector (Ad-pCMV/SBDS) and a control (Ad-pCMV/EGFP), which were used to infect Jurkat-T cells after induction of apoptosis by tumor necrosis factor (TNF)-α and interferon-γ. To study whether an increase in the Bax/Bcl-2 expression ratio also contributes to the apoptosis in SDS, marrow cells from patients and controls were incubated in liquid cultures. Non-adherent cells were harvested after 48h. Cell viability was determined by trypan blue exclusion. RNA was extracted, followed by reverse transcription and multiplex-PCR containing primers for the Bax, Bcl-2, Bcl-XL and GAPDH genes. Multiplex-PCR products underwent agarose gel-electrophoresis, and band intensities were quantified by laser scanning densitometer. For protein analysis, duplicate cultured cells underwent lysis, and band intensity of electroblotted Bax and Bcl-2 proteins on nitrocellulose membranes after SDS-polyacrylamide gel electrophoresis was compared between patients and controls. Last, we asked whether inhibition of Fas or Bax/Bcl-2 apoptosis pathway can rescue SDS cells from apoptosis. For this we studied cell viability after incubating SDS lymphoblasts and control lymphoblasts with either a blocking anti-Fas antibody or the anti-caspase 9 inhibitor Z-IETD-FMK. **Results.** Overexpressing SBDS using Ad-pCMV/SBDS alone did not change the apoptotic rates or Fas expression, however, it diminished Fas expression in response to increasing concentrations of TNF-α and interferon-γ in combination. Next, although cell viability was decreased in the patients, the Bcl-2/Bax gene expression ratio and Bcl-XL/Bax gene expression ratio was increased in the patients. By western blot analysis, BCL-2 protein was higher and Bax protein was lower in the patients compared to healthy controls. Of interest, Fas inhibition in SDS lymphoblastoid cells prominently increased cell viability compared to cultured SDS cells where the Bax/Bcl2 apoptosis pathway was inhibited by caspase 9 inhibitor or compared to lymphoblastoid cells from a healthy control. **Conclusions.** SBDS inhibits Fas expression in response to TNF-α and interferon-γ. In contrast, the Bax/Bcl2 apoptosis pathway manifests pro-survival changes in SDS marrow cells, and does not mediate the accelerated apoptosis. This might be related to a compensatory mechanism for the accelerated apoptosis through the Fas pathway. This is of utmost importance as design of anti-apoptosis agents for the treatment of the cytopenia in SDS should target the Fas pathway and possibly avoid unnecessary alterations in the mitochondrial pathway.

0080**EPIDEMIOLOGICAL OBSERVATIONS ON GENETIC HAEMOCHROMATOSIS IN THE NORTH-EASTERN REGION OF REPUBLIC OF IRELAND**K.M.A. Ramadan¹, S. Ramadan², D. Long²¹Belfast City Hospital, Belfast, UK; ²Our Lady of Lourdes Hospital, Drogheda, Ireland

Background. Genetic haemochromatosis (GH) refers to iron accumulation in the body due to the inheritance of mutations in the HFE gene. This leads to excessive absorption of iron from food. If patients are diagnosed in the pre-cirrhotic, pre-diabetic stage and then life expectancy is normal. It is therefore important to diagnose the condition as early as possible. **Aims.** To study the epidemiological pattern of GH in north-eastern region of Ireland. **Methods.** The clinical notes of patients with GH were studied retrospectively to ascertain the diagnosis, age at diagnosis, sex, family history of GH, presence of possible complications of GH, serum ferritin level at diagnosis and results of genetic testing. **Results.** Clinical notes of 56 patients with GH were reviewed. There were 12 females and 44 males with M:F ratio 3.6:1. The age range at diagnosis was 19-79 years with an average of 50 years. Twenty seven (48.2%) patients have family history of haemochromatosis and 35 (62%) patients had one or more possible complications of HC at the time of diagnosis. High ferritin level was found in 53 (94.6%) patients at diagnosis

sis. The average ferritin level at diagnosis was 1205 µg/mL. Confirmation of diagnosis in patients with hyperferritinemia with genetic testing was done in 47 cases (84% of cases). Out of 47 cases, 39 (83%) patients with haemochromatosis were homozygous for C282Y mutation and 5 (9%) were heterozygous for C282Y. In those 5 cases, H63D mutation was sought in only one case and was detected. 4 (7%) cases were homozygous for H63D mutation. One case (1.7%) was heterozygous for H63D mutation with no C282Y mutation. Nine cases were not tested for genetic abnormalities, of whom 6 patients had liver biopsies and five shown haemosiderotic changes consistent with the diagnosis of haemochromatosis. Thirty five patients (62.5%) with haemochromatosis had one or two complications at the time of diagnosis. Of whom, 11 patients (19.6%) have arthralgia or arthritis, 20 patients (35%) have transaminitis, 2 patients (3.5%) have liver cirrhosis, 11 patients (19.6%) have diabetes mellitus and 2 patients (3.5%) have cardiac arrhythmias. *Summary.* The incidence of GH in the north-eastern region of Republic of Ireland is one of the highest in Europe. Most of patients were males and this might reflect the fact that only half of patients have family history GH and as a result the diagnosis was sought because of presentation with one or more of potential disease complications. It is also evident from this study that in those who were screened for genetic mutations in HFE gene only 84% were homozygous for C282Y mutation which is lower than expected (90% in most of series) and 7% were homozygous for H63D mutation which is higher than many reported series (ie 5%). Haemochromatosis is a treatable condition with normal life expectancy if treated early. In our series 3.5% of patients have cirrhosis and 3.5% have cardiac arrhythmias presumably secondary to GH.

0081

EFFECT OF IRON OVERLOAD (IN VIVO & IN VITRO) ON TELOMERASE ACTIVITY AND PROLIFERATION OF PERIPHERAL BLOOD T-LYMPHOCYTES

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Background. Iron overload enhances oxidative stress in cells of immune system leading to biological molecules malfunctioning; it impairs host defence mechanism by directing influencing the T-lymphocyte function. It cause cellular damage by participating in the generation of the hydroxyl radical, through the Fenton reaction, thought to be the principal effector of oxidative DNA damage. Telomerase is a ribonucleoprotein complex responsible for maintaining telomeres and for repair of DNA strand breaks. Telomerase is involved with cellular proliferation and cellular senescence. Therefore for T-lymphocytes, the ability to undergo extensive cell division and clonal expansion is crucial for effective immune function. *Aims.* The aim of the present study was to investigate telomerase activity in T-lymphocytes from patients with β -thalassemia major (*in vivo* iron overload) and *in vitro* iron overload and to observe its regulation of cellular proliferation and its correlation with immune function. *Methods.* Peripheral blood mononuclear cells (PBMC) were isolated from 20 patients with beta-thalassemia major and 20 healthy donors. Cells were cultured in the presence or absence of phytohemagglutinin (10 µg/mL). To perform *in vitro* iron loading of lymphocytes, the cells were incubated in presence of 500 µM FeSo₄-7H₂O for 24 hours. Telomerase activity was measured by the telomeric repeat amplification protocol-based telomerase polymerase chain reaction enzyme-linked immunosorbent assay at 0 and 72 h of incubation. In addition, DNA synthesis of the cells was assayed using BrdU (5-bromo-2'-deoxyuridine) incorporation. *Results.* We found that telomerase activity in T cells from patients with β -thalassemia major was significantly down-regulated. The DNA proliferation was paralleled by decrease in telomerase activity. There was significant difference between telomerase activity in T-lymphocytes from β -thalassemia major patients and normal donors. The remarkable differences were not observed between *in vitro* iron

overload lymphocytes and lymphocytes of normal donors. *Conclusions.* One possibility is that telomerase is essential for the repair of telomeric DNA following damage by oxygen radicals and clonal expansion of T-lymphocytes. The results account for decreased efficacy of cellular immunity arise from decrease T-cells in patients with β -thalassemia major (*in vivo* iron overload) in reply to antigen.

0082

POLARIZED CTL RESPONSES DETECTED IN PATIENTS WITH AUTOIMMUNE NEUTROPENIA

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Background/Aims. Drugs and intrinsic bone marrow diseases can explain most of the cases of neutropenia and true autoimmune neutropenia (AIN) is a diagnosis of exclusion. Antineutrophil antibodies are not reliable and their absence does not preclude the diagnosis of AIN. Lineage restricted cytopenias including neutropenia are associated with T cell large granular lymphocyte leukemia (T-LGL). But the diagnosis of this condition involves positive TCR rearrangement and flow cytometric identification of a pathologic cytotoxic T cell (CTL) population. These routinely applied methods have a limited sensitivity and rely on the presence of a high frequency of clonal cells in the sample. AIN, similar to T-LGL may be related to a CTL mediated process. We hypothesize that AIN in a portion of patients is a CTL mediated disease in which myeloid progenitors are the targets. Consequently, in those patients, polarized expansions of CTL clones may be detected if efficient and sensitive diagnostic methods will be applied. *Methods.* Previously we developed a diagnostic strategy for the identification and quantification of clonal expansions in T-LGL based on the molecular analysis of TCR repertoire. For detection of CTL expansions in AIN, VB-typing and VB specific RT-PCR were applied followed by PCR cloning and sequencing of a large number of clones, and determination of expanded CDR3 clonotypes. When no expansion was detected by flow cytometry multiplex PCR was used to amplify the whole VB spectrum with subsequent subcloning and sequencing. Clonotype Taqman PCR utilizing a JB-probe and clonotype-specific primer was utilized to track expanded clonotypes. *Results.* We studied a cohort of patients with various degrees of neutropenia (n=22) that was unexplained by clinical grounds and standard laboratory testing. Antineutrophil antibodies were found in 6 of these patients. In 3 patients, serum mediated inhibition (>20%) of colony formation by normal hemopoietic progenitors was found, but there was no correlation between antibodies and serum inhibition. Using our strategy, we found only 2 expanded clones in 24 healthy controls. Those expanded clones accounted for 20% of a given VB family or 0.7% of the CD8+ repertoire. In AIN we detected expansions in 14 of 22 patients (by VB flow cytometry VB expansions were found in 5 patients). Clonal frequency was 40%±13% of a given VB family or 13%±14% of the total CD8+ population. The presence of expanded CTL did not correlate with anti-neutrophil antibodies or serum mediated colony inhibition. Using CDR3-specific Taqman PCR we confirmed patients specific clonotypes and we also were able to detect their expression in some patients and healthy controls, however at significantly lower expression levels than in the original patient sample (< log₃). *Conclusions.* We conclude that using sensitive approaches, CTL expansions can be detected in a significant proportion of patients with AIN. These cases may represent minor variants of an autoimmune process that operates in LGL leukemia. The antigens that trigger these expansions may likely be shared. Clinically, detection of the CTL mediated process in neutropenia may point toward rational immunosuppressive therapy aimed at T cells.

Bleeding disorders (congenital and acquired) I

0083

EPIDEMIOLOGICAL DATA-EXPRESSION OF THE QUALITY OF HEMOPHILIA CARE

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Background. Hemophilia is a hereditary congenital disorder with an evolution potential insignificantly limited through disease under adequate substitution therapy. The absence of substitution has a decisive negative impact on life-expectancy and quality of life for haemophiliacs, as shown by descriptive epidemiological data. **Aims.** The objective of this study is the assessment of the modalities by which the quality of the therapy and implicitly the social-economical status of a country impact on the parameters of descriptive epidemiology of haemophilia. **Methods.** Data was processed from the National Registry and the Regional Registry of haemophilia and correlated with the indices which define the main features of the treatment. **Results.** The National Registry comprises a number of 1266 haemophiliacs, corresponding to a prevalence in the country of 11.4/100,000. The regional indices of prevalence vary between 1.25-26.38/100,000 as a result of major differences in the diagnostic work-up, significantly higher prevalence being found in the university centers ($p < 0.01$). The ratio haemophilia A/B of 8.9 as well as the ratio severe forms / moderate and mild forms of 0.8 are higher than those reported in western European countries (4.4-6.4 and 0.4-0.6 respectively), showing an insufficient diagnostic identification of the forms with more reduced clinical expression. The distribution of the patients by groups of age revealed a proportion of patients with ages over 30 years of only 34.4% in comparison with 50-64.3% as in the countries of the EU. Causes of mortality are dominated by bleeding events with a proportion of 90% whereas these accidents occupy in other countries the 4th-5th place. Survival rates significantly cut-off in our country correlate with the insufficient and delayed character of the substitution, 0.1 IU/capita-consume of factor concentrates in our country being edificatory in this sense. The proportion and profile of patients with blood borne infections also differs from those known in the western European countries: 4.57% with hepatitis B virus (HBV) infection HBsAg+ from which 33.33% with active replicative infection, 59.9% with hepatitis C virus (HCV) infection from which 50% with HCV-RNA present and finally below 3% with HIV infection. These numbers reflect the treatment with wet plasma products (fresh frozen plasma and cryoprecipitate) obtained through small plasma pools from a population with relatively high endemic HBV infection (mean portage proportion of HBsAg of more than 3-4%) but low incidence of HIV in the adult population. **Conclusions.** Our epidemiological data reflect some deficiencies in the diagnostic identification of haemophilia as well as in the quality and quantity of therapeutic substitution which claim for better solutions in the near future.

0084

UNUSUAL COMPLICATION OF SEVERE HAEMOPHILIA A WITH INHIBITOR: TWO CASES WITH ACUTE INTESTINAL OBSTRUCTION DUE TO INTESTINAL INTRAMURAL HAEMORRHAGE

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Background. Haemophilia A is a hereditary bleeding disorder

caused by a deficiency in factor VIII. Severe haemophiliacs have a factor level less than 0.1 IU/mL. Haemophilia A is treated by infusing plasma derived or recombinant factor VIII. This can be complicated by the development of inhibitory antibody to factor VIII. Patients with severe haemophilia A usually presents with joint, gastrointestinal and urinary tract haemorrhage. Bleeding elsewhere is often precipitated by pre-existing pathology or trauma. **Methods.** The clinical notes of two patients with severe haemophilia and inhibitor who presented with acute intestinal obstruction were reviewed to ascertain the radiological findings, the clinical course and management outcome. **Results.** Case 1: A 34-year-old male patient with severe haemophilia A presented with a 48-hour history of increasing abdominal pain and vomiting. He has a high responding inhibitor and receives recombinant factor VIIa (rFVIIa) (Novo-Seven[®]) on demand basis at home. There was a history of gallstones and chronic hepatitis C infection. On examination his abdomen was distended with generalised tenderness. Bowel sounds were absent. CT scan of abdomen showed dilated small intestine with fluid filled loops. There was a long segment in the jejunum with marked transmural thickening with no obvious other abnormalities. These appearances were consistent with intramural haemorrhage in the small intestine as the cause of acute obstruction. He received rFVIIa 90mg/Kg for 3 doses, 2 hours apart on 3 successive days. He was managed conservatively and his abdominal pain settled and passed motions 4 days after his admission to the hospital. Case 2: A 65-year-old male patient with severe haemophilia and inhibitor presented with a 4-day history of acute abdominal pain and vomiting. He has chronic hepatitis C infection. On examination his abdomen was distended with generalised tenderness. CT scan of abdomen showed an intramural haemorrhage in duodenum and upper part of jejunum. He received rFVIIa 90mg/Kg for 3 doses, 2 hours apart on 2 successive days. He was managed conservatively and his abdominal pain settled and passed motions 4 days after his admission to the hospital. **Summary.** It is extremely unusual for haemophiliac patients to bleed into the wall of the small intestine without a history of trauma or pre-existing pathology. The management of acute intestinal obstruction due to intramural bleed in the intestinal wall in haemophiliac patient with inhibitor is rarely described in the literature. CT imaging helped to distinguish between surgical causes of acute abdomen and bleeding. Conservative management with rFVIIa should be attempted before embarking on surgery. It appears that rFVIIa was effective in controlling intramural haemorrhage in the small intestine.

0085

HAEMOPHILIA INHIBITOR ANALYSIS-RESULTS OF MULTICENTRIC INFORMATION SYSTEM HEMIS (HAEMOPHILIA - INFORMATION SYSTEM)

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Background. The project HemIS has three types of outputs: A/ on-line database with patients' data and data from their bleeding episodes, B/ data on clinical aspects of the therapy and its results, and finally C/ expert system that is able to analyse the data and serves as a pool of data for summarized clinical assessment. The system is focused on analyses of main endpoints of the project: therapeutic results reached in different types of bleeding episodes and development of clinically relevant scoring for bleeding episodes, especially in patients with inhibitor. **Aims.** Development of a clinical registry for the standardized

processing of diagnostic, prognostic and longitudinal data from haemophilia patients with inhibitor against factor VIII or IX. Detailed analysis of therapeutic approach stratified according to bleeding severity. **Method:** The register has started since 2003 and full-scaled clinical data from 24 patients with inhibitor (mean age 16 years ranging from 3 to 53 years, median 13 years) were successfully recorded. **Results.** In 92% patients with inhibitors suffered from hemophilia A, and most of these inhibitor patients (72%) had severe type of haemophilia. High responders were diagnosed in 43%. The frequency of bleeding events was in average 12-times per year (with min = 1 and max = 28 events per year). Bleedings were posttraumatic in 35% and spontaneous in 51% of all recorded cases. Severity of bleeding episodes was mild in 59% and moderate in 35%. In 40% of inhibitor patients the bleeding duration ranges from 9 to 24 hours (depending on the severity of haemophilia). Rebleeding (8% of events in total) was resolved within 48 hours in 38% and within 24 hours in 24% of rebleeding events. The reason for rebleeding was delay of therapy initiation (23%) and bleeding severity and intensity (15.4%). Home treatment was applied in 63% of patients (73% of them severe and 27% moderate patients). **Conclusions.** Detailed information on patients with inhibitor is available for clinical use. Further registration continues.

0086

COMPARISON OF A NEW AUTOMATED VWF:ACTIVITY ASSAY WITH AN AGGREGATION VWF:RCO ASSAY IN SCREENING FOR VON WILLEBRAND'S DISEASE

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Background. The HemosILTM von Willebrand Factor (VWF) Activity assay (Instrumentation Laboratory-IL) is an automated latex enhanced, turbidimetric immunoassay for the quantitative determination of VWF Activity, based on monoclonal antibodies against the platelet-binding site of VWF. It was designed as a screening assay for von Willebrand disease (VWD) for use on IL coagulation analysers. **Aim** The HemosILTM VWF Activity assay was evaluated and compared with the VWF:RCo aggregation method. **Methods.** The HemosILTM VWF Activity assay was implemented on a STAC analyser (Roche) using two protocols: one with no sample predilution and one with a sample predilution of $1/2$. In the no-predilution protocol the samples with a value of VWF:Activity > 150% were diluted $\frac{1}{2}$. The VWF:RCo activity with aggregation method was performed on an APACT aggregometer (VWR-Helena) with the Dade Behring von Willebrand reagent and Ristocetine from Biopool. Samples from 20 healthy volunteers and 49 patients were analysed in parallel with the HemosILTM method and the aggregation method. Dilution and imprecision studies were performed respectively with the HemosILTM Calibration Plasma and HemosILTM Test Control material. **Results.** Within-run imprecision (CV%) was 17.2% with a bias of 11.1%. Passing Bablok regression for the 69 samples comparing the HemosILTM VWF Activity Assay (no-predilution protocol) and the aggregation method yielded a slope of 1.33 (95% CI: 1.13-1.49) and an intercept of 0.00 (95% CI: -2.97-0.00). The correlation coefficient was 0.83 (95% CI: 0.74-0.89). 19 patient samples were analysed with both protocols. Correlation between the two protocols was 0.93 (95% CI: 0.83-0.97). Passing Bablok regression showed an intercept of 5.69 (95% CI: -2.98-13.50) and a slope of 0.72 (95% CI: 0.50-0.93). The no-predilution protocol and the predilution protocol have a sensitivity of respectively 95.12% and 100% and a specificity of respectively 92.86% and 50% in comparison with the VWF:RCo aggregation assay. One of the two patients missed with the no-predilution protocol had a borderline VWF:RCo, still within the reference values for blood type O and a normal VWF:Ag. After correction for this sample, the sensitivity was 97.50%. Dilution studies showed a linearity between 12.5-100%. **Conclusions.** The HemosILTM VWF Activity assay, performed on the STAC has an acceptable imprecision and bias. Although the manufacturer recommends to dilute the samples with a value of VWF Activity > 200%, our dilution

studies showed that all samples with a value > 100% should be diluted, because of the limited linearity between 12.5%-100%. When using the sample-predilution- $1/2$ protocol, the sensitivity is 100%, but with a too low specificity of 50%. With the no-sample-predilution protocol, 1 out of 41 patients with a decreased VWF:RCo was missed, which means a sensitivity of 97.5% with a specificity of 93.1%. This makes the the HemosILTM von Willebrand Factor Activity assay with the no-predilution protocol on STAC an acceptable screening assay for Von Willebrand disease. VWF:RCo and VWF:Ag still need to be determined to confirm the diagnosis and for further classification of the VWD.

0087

GENETIC POLYMORPHISM FACTOR XII 46C/T AND OBSTETRIC COMPLICATIONS

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Introduction: decreased levels of coagulation factor xii are associated with fetal loss. Recently, a common genetic polymorphism (46c/t) and an association between the t allele and low plasma levels of factor xii were reported. **Objective:** to evaluate whether factor xii 46c/t genetic polymorphism is associated with obstetric complications. **Methods and patients:** we were analyzed 92 patients with a history of two or more unexplained recurrent miscarriages (first, second and third trimester), intrauterine growth restriction and or pre-eclampsia; and 54 healthy controls (blood donors) the detection of polymorphism factor xii 46c/t by pcr in real time, in liquid phase, in a lightcycler (roche diagnostics) thermal cycler were made. **Statistical methodology,** medians and (p25, p75) were determined for the quantitative variables. Percentages were used for qualitative variables. Ratio of similarity test. **Results.** in the studied patients were detected 9 (10%) cases with homozigote t allele (tt) of the 46c/t polymorphism in factor xii gene, 23 (23.3%) cases are ct and 60 (66.6%) are cc. In the controls there are not homozigote t allele, 14 (25.9%) are ct and 40 (74.1%) are cc. Relationship between patients and controls of the allele t in polymorphism factor 46c/t is significant $p=0.0015$. **Conclusions.** genetic polymorphism factor xii 46c/t and obstetric complications are related.

0088

BORDERLINE FACTOR XI DEFICIENCY: A NOVEL MUTATION IN FACTOR XI GENE

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Background. Congenital factor XI (FXI) deficiency is a rare condition, which is usually manifested clinically as an excessive post-traumatic bleed. The correlation between the plasma level and the severity of haemorrhage is poor. Even patients, who are heterozygotes, with factor levels of 50-70 iu/mL can have severe post-traumatic or surgical bleeding. Factor XI is encoded by a gene located on chromosome 4 and synthesized by the liver. It consists of 15 exons with a number of mutations have been described. There appears to be a relationship between specific defects and the severity of the bleeding tendency. We describe a case of FXI deficiency with a novel mutation in FXI gene. **Methods.** A patient with a borderline FXI level and history of post surgical haemorrhage had genomic DNA extracted from an EDTA-anticoagulated blood sample. The 15 exons of the factor XI gene plus adjacent intronic sequences were amplified by PCR using oligonucleotide primers designed using the

primer 3 programme from: http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi. Amplified DNA was purified and underwent sequencing by the dideoxy chain termination method with forward or reverse PCR primers using a commercially available kit (Big Dye v3; Applied Biosystems, UK). Patient FXI sequences were examined for the presence of mutations by comparison with the human Factor XI gene sequence downloaded from the NCBI database. **Results.** A 40-year-old female patient attended the hemophilia center with a history of spontaneous bruising, menorrhagia and recurrent epistaxis. She had 4 teeth extracted and bled excessively in 3 extractions, which required suturing. There was a history of excessive post-traumatic haemorrhage in her mother but none in her four sisters. Her nephew who has a history of excessive bleeding died in a road traffic accident. Coagulation screen revealed a prolonged activated partial thromboplastin time (APTT) at 41.5 seconds (24-38), normal Prothrombin time, factor VIII levels and von Willbrand factor levels and activity. Factor XI was slightly reduced at 0.56 iu/mL. Analysis of factor XI gene using the above described method revealed a mutation in exon 2 where 168T>A nucleotide substitution led to C38X nonsense mutation. This results in premature termination of translation of factor XI mRNA thus producing truncated factor XI protein. **Summary.** There are more than 45 different mutations described so far in factor XI gene, of which Glu117stop and Phe283Leu are the most common. These two mutations are prevalent in Ashkenazi Jews population where FXI deficiency is more common than other people. In our case this novel mutation is described in a person of northern European descent. It results in truncated functionless protein. The patient is heterozygous for this mutation, which explains the borderline factor XI levels.

0089

ACQUIRED FACTOR VIII INHIBITORS IN NON-HEMOPHILIC PATIENTS-REVIEW OF SEVEN CASES IN CHINESE

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Background. Acquired factor VIII inhibitor in non-haemophilic patients, also known as acquired haemophilia A, is a rare but life-threatening bleeding disorder. Treatment is of two folds-effective and urgent control of bleeding, and ultimate suppression of antibody production. Current management is challenging with the availability of a novel agent, recombinant activated FVII (rFVIIa), which bypasses FVIII. Information of the impact of this agent on the overall outcome of the disease however is still limited. We report seven cases in Chinese. Patients and methods We retrospectively analyzed the clinical features and treatment outcome of seven Chinese patients with acquired haemophilia A between July 2000 and December 2004 in a regional hospital in Hong Kong. **Results.** Seven Chinese patients (M:F = 5:2; median age 77, range 25-88) were analysed. All had no associated conditions like autoimmune disease, malignancy, relevant drug history or pregnancy. All presented with extensive spontaneous bleeding, prolonged aPTT and reduced FVIII level. The FVIII inhibitor titre ranged from 9 to 180 Bethesda Unit(BU). All received high dose steroid and IVIg with or without other immunosuppressants. Five patients received variable doses of rFVIIa. Four patients (57%) with high FVIII inhibitor levels (>30 BU) died of bleeding complications. Of the three (43%) survivors with factor VIII inhibitor levels 10 BU, all received rFVIIa, had uneventful recovery, and no treatment related complication. **Conclusions.** Our experience confirms that significant clinical bleeding is the major presenting symptom of acquired haemophilia A which in our series remained idiopathic, affected mainly the elderly, and was associated with a high mortality due to bleeding. Though rFVIIa is effective for controlling haemorrhage, strict adherence to its recommended dose and frequency of administration is difficult due to its extremely high cost. Its use, if coupled with the use of more

effective immunosuppressive therapy such as anti-CD20 antibody may further improve the outcome of this disorder. Prospective and larger studies are needed to evaluate the most appropriate treatment protocol for this disorder.

Table.

Age/ Sex	Presenting	APTT (sec.)	Inhibitor (BU)	Hem. ther.	Imm. suppr.	Outcome	Imm. cause of death	Survival (Dx to death or last FU)
F/25	Hematuria CNS bleeding	66.1	10	rFVIIa rFVIII	Steroid IVIg Cyclophosphamide	Alive	-	3 years
M/65	Skin ecchymoses	60	9	rFVIIa	Steroid IVIg	Alive	-	3.5 months
F/77	Hematuria ecchymoses Vitreous hemorrhage	59.1	10	rFVIIa rFVIII	Steroid IVIg	Alive	-	3 months
M/74	Hematuria Calf hematoma	68	14	rFVIIa rFVIII	Steroid IVIg, cyclophosphamide	Died	Retro peritoneal	8 days bleeding
M/88	GIB ecchymoses	95.5	44	rFVIIa APCC	Steroid IVIg	Died	Intracerebral hemorrhage	8 days
M/80	Macular hemorrhage	86.2	57	rFVIII	Steroid IVIg	Died	Compartment syndrome	3 days
M/86	Upper limb hematoma	54.2	180	rFVIII	Steroid IVIg	Died	Compartment syndrome	3 days

Hem. ther.: hemostatic therapy; Imm. suppr.: immunosuppressants; imm. cause of death; immediate cause of death.

0090

UNUSUAL HEMORRHAGE IN A PATIENT WITH AL AMYLOIDOSIS

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Bleeding is a well-known complication of AL amyloidosis. It occurs mainly as purpura, bruising and gastrointestinal hemorrhage. Many coagulation abnormalities have been identified, inhibition of coagulation factor X being the most characteristic although not specific. Other causes for bleeding are vessel infiltration, inhibition of thrombin activity, hyperfibrinolytic states, localized amyloidosis and inhibition of other coagulation factors. To date, abnormal platelet function in the absence of renal insufficiency has not been extensively considered among these causes. **Case Report:** We report the case of a 70-year-old male suffering from AL amyloidosis diagnosed through a bone marrow biopsy. The patient complained of intense fatigue and weight loss. Renal function was normal and no signs of neuropathy were identified. Echocardiographic criteria for amyloidotic infiltration were not met. Physical examination was not contributive. The only unusual finding was a normocytic anemia with a slight degree of ferropenia. Coagulation screen showed decreased prothrombin activity, in the range of 45 to 55%, INR ranging from 1,6 to 2,0. Fibrinogen and activated partial thromboplastin times were normal. Of note, the patient was on antiplatelet therapy with 100 mg daily of acetylsalicylic acid due to previous myocardial infarction. With the diagnosis of AL amyloidosis, therapy with pulsed melphalan and prednisone was started, as well as blood transfusions and oral iron supplements to improve anemia. Antiplatelet therapy was maintained since no bleeding signs were found. When a total of three cycles had been administered, the patient started with pain in the thigh, irradiated through the leg, as well as haematuria. The

pain was intense and the patient could not stand up. Magnetic Resonance Imaging showed an intramuscular haematoma in psoas, with 8 cm of maximum diameter and another haematoma next to liver's papillary process. Antiplatelet therapy was stopped but haematuria worsened after cystoscopic examination, with urine cultures being repeatedly negative. In coagulation tests a profound decrease in factor X level was found. Fresh frozen plasma administration did not improve bleeding, despite improved prothrombin activity. Platelet function was assessed using the PFA-100[®] analyser (Dade diagnostics) and platelet aggregation tests, once antiplatelet effect of drugs had disappeared. PFA-100[®] times were substantially prolonged and aggregation with ADP, epinephrine, collagen and arachidonic acid was absent or decreased, while aggregation with ristocetin appeared preserved. Despite the previous history of myocardial infarction, desmopressin was used to improve platelet function and bleeding stopped. The patient now continues on melphalan and prednisone without antiplatelet therapy; both haematomas have been reabsorbed and no new episodes of haemorrhage have appeared. Impairment of platelet function has been identified in amyloidosis as a cause of bleeding which may improve after desmopressin therapy. In this patient, abnormal platelet function appeared along with well-known disorders of haemostasis associated with amyloidosis, and concurred with antiplatelet therapy prompting unusual bleeding at intramuscular sites. The findings in our patient suggest that platelet function should be assessed in patients with amyloidosis in order to establish the risk of severe bleeding and avoid drugs affecting hemostasis.

0091

NEED FOR ORAL ANTICOAGULATION FOR MECHANICAL HEART VALVE IN A PATIENT WITH SEVERE HOMOZYGOUS FACTOR XI DEFICIENCY WITH ACQUIRED FACTOR XI INHIBITOR

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The risk of bleeding in factor XI deficiency is unpredictable even in patients with acquired inhibitor. We describe a patient with severe homozygous factor XI deficiency who developed factor XI inhibitor on treatment with plasma and who showed no increase in bleeding tendency even with invasive surgery. He has been maintained on oral anticoagulation for mechanical heart valve with no undue risk of bleeding. A 56 year old male Ashkenazi Jew with severe homozygous factor XI deficiency (FXI 4 U/dL) with no history of excessive bleeding developed bacterial endocarditis of a bicuspid aortic valve. He received fresh frozen plasma (FFP) during open-heart surgery for the insertion of an St Jude's mechanical aortic valve with no undue intra- or post-operative bleeding. He was maintained on oral anticoagulant therapy with a target INR of 2.5. Nine years later (August 2004), he presented with pain and swelling in the left calf. A Doppler scan showed features of pseudo-aneurysm at the junction of the posterior tibial and peroneal arteries. He underwent an arteriogram that confirmed the findings. His oral anticoagulant therapy was replaced by unfractionated heparin infusion during his hospital stay with short cessations prior to invasive procedures. The invasive arterial imaging was performed using FFP to increase his FXI with good response. His FXI level post 4 units of FFP infusions ranged 23-30 U/dL. Test for FXI inhibitors were negative at the start of his therapy. He received several doses of FFP as attempts were made to occlude the aneurysm using initially local injections of thrombin and later endo-vascular stent. These attempt failed to occlude the pseudo aneurysm. He continued to show good response to the FFP infusions initially. However, seven weeks later his factor XI dropped to less than 1U/dL and he had no further increments in FXI level following FFP infusions. Test for FXI inhibitor was positive with a level of 3.8 Bethesda units. The pseudo aneurysm was eventually excised using open surgery. He was not given any replacement therapy with coagulation factor concentrate and he had excellent haemostasis peri-operatively. He

was re-started on warfarin for his mechanical valve. This case illustrates that clinical history is the most significant parameter in assessing the risk of bleeding in patients with FXI deficiency, even in those with inhibitors. Some of these patients may have equal needs to that of patients with normal haemostasis for oral anticoagulation for mechanical heart valve with no increase the risk of bleeding related to such therapy.

0092

RECOMBINANT FACTOR VIIA (RFVIIA, NOVOSEVEN) AS AN ALTERNATIVE TO BLOOD TRANSFUSION FOR THE TREATMENT OF MASSIVE BLEEDING

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Background. Patients with persistent uncontrolled bleeding have a high mortality despite blood transfusion or surgery. Recombinant factor VIIa (NovoSeven) is an hemostatic agent licensed for the management of bleeding in patients with inhibitors against factors VIII and IX, factor VII deficiency and Glanzmann's thrombasthenia. However, several case series have reported its efficacy in the control of haemorrhages refractory to other conventional treatments. **Aims. and Methods.** We present the experience from one single centre with the use of rFVIIa in patients with massive bleeding. All patients treated at our institution with rFVIIa were identified and data concerning baseline demographics, cause of the hemorrhage, dose and timing of rFVIIa, blood component therapy prior to and in the first 24 hours following administration of rFVIIa and coagulation results immediately prior to and following administration of rFVIIa were obtained. In addition the treatment efficacy (complete, partial or absence of response) and the vital status (dead or alive) at 30 days after the onset of hemorrhage results prior to and after the administration of rFVIIa were compared using a paired Wilcoxon signed rank test. **Results.** Since June 2000, 21 patients between 22 and 87 years old (median age, 59), have been treated with rFVIIa in our institution: 4 patients with bleeding associated with acquired inhibitors, 16 patients with uncontrolled life-threatening bleeding and 2 patients (one of them with previous administration of rFVIIa during a bleeding episode) as pre-operative prophylaxis. Uncontrolled life-threatening hemorrhages occurred in patients undergoing surgery (7 cases), liver disease (5 cases), idiopathic thrombocytopenic purpura (1 case), essential thrombocythemia (1 case), Rendu-Osler disease (1 case) and Non-Hodgkin Lymphoma (1 case). Treatment was effective in all patients with acquired inhibitors, and the inhibitor disappeared after immunosuppressive therapy. 10/16 patients with massive bleeding (62.5%) achieved a complete stop of the haemorrhage after infusion of rFVIIa and 9/16 (56.3%) were alive after 30 days. The use of rFVIIa was associated with a significant reduction in the transfusion requirements and improvement of the accompanying coagulopathy, especially the prothrombin time (Table 1).

Table 1.

Transfusion requirements	Before rFVIIa [median (range)]	24 hours after rFVIIa [median (range)]	p
Red cell (U)	11 (3-27)	3 (0-29)	0.014
Fresh frozen plasma	7 (0-20)	0 (0-11)	0.044
Platelets	1 (0-7)	1 (0-2)	0.073
Coagulation status	Before rFVIIa [median (range)]	After rFVIIa [median (range)]	p
Prothrombin time (%)	49.5 (14.3-87)	66 (35.8-127)	0.004
APTT (s)	48.9 (29.8-180)	45.9 (35-130)	0.33
Fibrinogen (mg/dL)	126 (79-267)	127 (50-235)	0.65

Pre-operative prophylaxis with rFVIIa was associated with

reduced blood loss in both cases (a cystectomy in a patient with FVII deficiency and a pelvic sarcoma tumorectomy with severe bleeding in previous surgery). *Conclusions.* rFVIIa may have an important role in the achievement of an adequate haemostasis in patients with severe life-threatening hemorrhages, in whom other standard treatments have previously failed, emerging as a possible alternative to blood transfusion.

0093**IDIOPATHIC THROMBOCYTOPENIC PURPURA: PROGNOSTIC FACTORS AND SURVIVAL. THE EXPERIENCE OF A SINGLE HEMATOLOGICAL CLINIC**

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Idiopathic Thrombocytopenic purpura (ITP) is a diagnosis that is confirmed by excluding other causes of thrombocytopenia. The peripheral destruction of platelets, that is caused by an autoimmune mechanism verifies the diagnosis. It is characterized by thrombocytopenia and usually by mucosal bleeding. The adult form of ITP and ITP in childhood are two different diseases. Adult's ITP is usually a chronic disease, its invasion is sudden and is twice more common in women than in men. The purpose of this retrospective study is the evaluation of patients (pts) who were hospitalized in our clinic during the period 1997-2004. The study's target was the therapeutic response, the survival and the search of prognostic factors in ITP. *Material and Methods.* Our experience consists of 28 pts, 8 of which were men and 20 were women. The average age was 50 years old (17-87) and the average platelets count at diagnosis was $9000 \times 10^9/L$ (2000-23000). 20 pts were under the age of 60. The average follow-up was 28 months (8-65m). 8 pts were suffering from severe bleeding. All pts were treated at the beginning with corticosteroids. Treatment with immunoglobulin was added to the initial treatment in 3pts. *Results.* 11pts are in complete remission without relapse. 4pts are in partial remission with a platelet count between 30000 and 45000 without bleeding. In 8pts thrombocytopenia was resistant to corticosteroids and they were submitted to splenectomy. Of the above 8 pts 6 had a complete response. The other 2 pts relapsed after the splenectomy (1 and 8 months later) but they responded easily to a second short course of corticosteroids. 5 pts died of massive bleeding. These pts needed high dose corticosteroids in order to preserve their platelet count above 10000 and they presented with major bleeding at the time of diagnosis. *Conclusions.* The limited number of pts and the short time of follow-up do not allow us to come to safe conclusion. Nevertheless, our study indicates that: 1-Corticosteroids are the most effective initial treatment, whereas splenectomy is the second best choice after the failure of corticosteroids. 2-Age over 60 years old does not seem to be an unfavorable prognostic factor. 3-Severe bleeding and resistance to corticosteroids have an unfavorable influence in the survival of patients suffering from ITP

0094**SPINAL HEMATOMA AS A RARE COMPLICATION OF ORAL ANTICOAGULATION THERAPY**

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Background. Oral anticoagulant treatment is related to an increased percentage of hemorrhagic events, specially in the early time of anticoagulation. This complication must be evaluated individually before prescription, and the anticoagulant treatment should be closely controlled. Spontaneous central nervous system hemorrhage in patients receiving anticoagulant therapy is not uncommon, but intraspinal epidural space is a rare and

unfavorable location Case report. A 71 years old man was admitted in hospital because a rapidly progressive interscapular 24 hours back pain followed by bladder sphincter dysfunction and sensorimotor deficit in both lower limbs. Three weeks ago the patient had started receiving oral anticoagulation with acenocoumarol for a high risk of cardiogenic embolization secondary to atrial fibrillation. Hemostatic parameters showed an INR level of 3.5 and a TTPa ratio of 1.53. Physical examination confirmed motor loss of lower limbs, T5 sensitive level and urinary retention. A magnetic resonance imaging was performed, recognizing a posterior epidural hematoma including T1-T2 space, with neural compression. In an emergent actuation we proceed to a rapid correction of anticoagulation with activated factor VII, the hematoma was evacuated by a laminectomy and surgical decompression with extirpation. Epidural venous tissue was biopsied to discard vascular malformations as the origin of hematoma. The recovery of neural dysfunction was progressive and complete after decompression. Posterior successive paroxysmal supraventricular tachycardia episodes were treated with slow pathway ablation. It was not necessary to reinstitute oral anticoagulation after spinal surgery. *Conclusions.* Diagnosis of spontaneous spinal hematoma must be considered in oral anticoagulated patients with progressive neural dysfunction, and the prognosis depends on the length of time between the onset of sensorimotor deficit and surgical decompression. The incidence of that complication might be minimized by close monitoring and tight control of the intensity of anticoagulation. The restitution of anticoagulant therapy after surgery procedure is indicated if necessary, though discontinuation of anticoagulation for several days seems safe in the early postoperative period.

0095**ACQUIRED IMMUNE COAGULATION DISORDERS: DIFFERENT RESPONSES TO IMMUNOSUPPRESSIVE THERAPY**

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Acquired inhibitors of coagulation are auto-antibodies that develop spontaneously in people without pre-existing factor deficiencies or exposure to an external antigen. Most frequently these antibodies neutralize function of clotting factors. They have been associated with a wide array of underlying conditions and can present with bleeding, thrombosis or no symptoms at all. We report on five cases detected between 1997 and 2004 in our hospital. Patients and *Methods.* Range of age: 44-81 years without personal or family history of bleeding dyscrasias. The first patient developed an anti-factor V inhibitor at the 21st day after a liver transplantation, while treated with FK506. The inhibitor disappear spontaneously three days later, without discontinuation of the immunosuppressive therapy. The second patient presented with an acquired haemophilia, eight months after a liver transplantation, despite being treated with cyclosporine. The inhibitor promptly disappeared after steroids therapy. The third patient presented an acquired severe von Willebrand syndrome associated with an IgG κ monoclonal gammopathy of uncertain significance. The syndrome persisted despite treatment with prednisone and cyclophosphamide, though the bleeding was ameliorated. The fourth patient, one year after a matched-related allogeneic bone marrow transplant, and with an extensive chronic graft-versus-host disease (GVHD), presented severe bleeding after minor surgery procedure. No specific inhibitor was evident and only a *de novo* lupus anticoagulant(LA) without hypoprothrombinemia, was observed. The LA disappeared after control of the chronic GVHD. The last case refers to the detection of an acquired haemophilia fourteen months before the presentation of a pancreatic carcinoma. Treatment with steroids was not able to eradicate the inhibitor. In contrast, after surgical excision of the mass, the inhibitor showed a progressive reduction. Three years later it reappeared inhibitor and the tumour (metastasis). *Results.* All cases, except one (anti-factor V inhibitor), had severe cutaneo-

mucous bleeding, that were treated with rFVIIa (median dose: 90µg/kg). The hemorrhagic episodes yielded satisfactorily, with an average of inferior time to six hours after the start of rFVIIa. Only in one case the treatment with rFVIIa had to be discontinued because the appearance of angor pectoris. In transplant-ed patients the immunosuppressor treatment was able to eradicate inhibitor. **Conclusions.** Although acquired inhibitors are rare, their associated morbidity and mortality can make timely diagnosis and treatment extremely important. In patients who undergo transplantation (liver and bone marrow) the development of an inhibitor, despite being treated with immunosuppressive therapy, seems to be related with autoimmune disorders. The inhibitors may be detected even years before the presentation of the underlying disease. rFVIIa is, in most cases, a safe and effective treatment for acute bleeding complications in patients with acquired inhibitors. The use of immunosuppression in acquired inhibitors appears to ameliorate the haemorrhagic manifestations. In our cases, in non-transplanted patients, immunosuppressive therapy was unable to eradicate the inhibitor.

Chronic lymphoblastic leukemia and related disorders - Clinical

0096

A POLYMORPHISM IN INTRON 6 OF THE P53 GENE IS ASSOCIATED WITH ADVERSE PROGNOSTIC FACTORS AND A SHORTER TIME TO TREATMENT FAILURE IN CHRONIC LYMPHOBLASTIC LEUKAEMIA

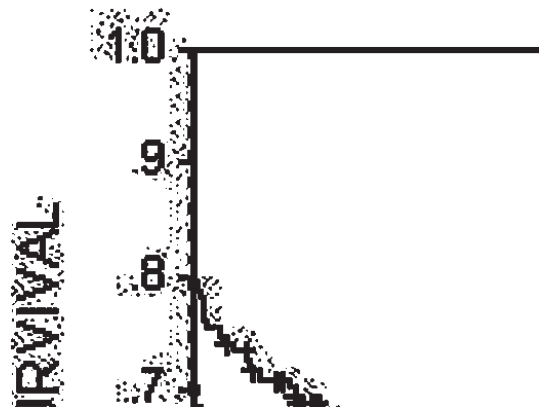
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Background. The tumour suppressor gene p53 is a cell cycle checkpoint control protein that controls cellular homeostasis in different tissues through induction of growth arrest, senescence or apoptosis. Recent studies have shown that a single nucleotide polymorphism at codon 72 in exon 4 leads to changes in apoptotic potential, influencing responses to chemotherapy and is associated with breast cancer (Nature Genetics 33,357-365 (2003)). A polymorphism in intron 6 has also been associated with lung cancer (Lung Cancer 31 (2001) 157-162). **Aims.** To investigate the role of these two p53 polymorphisms in chronic lymphoblastic leukaemia. **Methods.** Genotyping of the two polymorphisms, the exon 4 BstUI and intron 6 MspI, was performed on DNA samples from 274 patients with chronic lymphoblastic leukaemia by polymerase chain reaction (PCR) amplification and restriction enzyme digestion as described previously (Hum Hered 1995; 45:144-149, Anticancer Res 1998; 18:2095-100). The association between these two polymorphisms and patient/disease-related variables namely sex, age (≤ 65 vs > 65 years), lymphocyte doubling time (≤ 12 vs > 12 months), disease stage (A vs B+C) and IgVH mutational status (mutated vs unmutated) were analysed by Fisher exact test. The presence of these polymorphisms was then correlated with overall survival (OS), progression-free survival (PFS) and treatment-free survival (TFS) using the Kaplan-Meier method and log rank test. Subsequently, multivariate analysis according to the Cox proportional hazards regression model was used to explore the independent effect of variables that showed a significant influence on OS, PFS and TFS by univariate analysis. The exon 4 BstUI polymorphism showed no correlation with any clinical or biological variable. We divided patients with the intron 6 MspI p53 polymorphism into two groups: wild type (B2) and heterozygous or mutant (B1 + heterozygous). The B2 genotype was associated with a longer lymphocyte doubling time: 80% of patients with a LDT greater than 12 months have the B2 variant compared to 54% of patients with a LDT < 12 months ($p=0.026$, Fisher's exact test) and stage. 81% of patients diagnosed in stage A have the B2 variant compared to 65% of patients diagnosed in stage B+C ($P=0.039$, Fisher's exact test). In terms

of treatment-free survival, univariate analysis revealed that 4 variables (stage, LDT, IgVH mutational status and intron 6 p53B1 +heterozygotes) had a significant impact on it ($p < 0.0001$ for the first 3, $p = 0.029$ for p53, log-rank test) (Figure 1). Cox multivariate analysis did not show independent significance for this polymorphism. This is the first study to show a correlation with a polymorphism involving intron 6 of the p53 gene and adverse biological behaviour in CLL. This polymorphism is not functionally active and, given that a similar effect has been reported in lung cancer, suggests that it is linkage disequilibrium with an important susceptibility site in or near the p53 gene.



0097

CLINICAL ACTIVITY OF LENALIDOMIDE (L) (REVLIMID™) IN PATIENTS (PTS) WITH RELAPSED (REL) OR REFRACTORY (REF) CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL)

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Background. Of the various cytokines in the CLL microenvironment (ME) TNF- α and VEGF are considered to play a major role in the pathogenesis of the disease. L is an immunomodulatory agent that can down regulate VEGF and TNF- α and thus may be useful in the treatment of CLL. We therefore conducted a pilot study of L in CLL. **Aims.** The aim of this clinical trial is to determine the clinical activity of lenalidomide in pts with rel and or ref CLL. **Method:** Pts with rel or ref CLL were eligible. L was given at 25mg PO QD x 21 days followed by 7 days rest on a 28 day cycle. Absolute lymphocyte counts (ALC) were measured at Day 0, 7 and 30. Response was assessed at day 30 and monthly thereafter using the NCI-WG criteria (1996). Pts with stable disease (SD) or better response are continued on therapy for a maximum of 12 months while those with progressive disease (PD) will receive Rituxan (R) (375mg/m²) added to L. Target enrollment is 29 patients. **Results.** Fifteen pts (13M, 2F; median age 66 years, range 58-74; 10 had Rai III/IV and 5 had I-II) have been enrolled to date. Median baseline ALC was 67k (8-338). Pts had received a median of 3 prior therapies (range 1-10) and all patients enrolled so far have received prior fludarabine. Eight pts have completed 30 days of therapy and are available for toxicity and response evaluation, 7 pts have not completed at least 1 cycle, 1 of whom withdrew consent, 1 died (myocardial infarction with previously documented heart disease) and 5 continue on treatment. The most commonly reported adverse event was fatigue (Grade I/II, n=7; Grade III, n=1) and rash (Grade I/II n= 8). A flare reaction (tender swelling of lymph nodes) and tumor lysis syndrome were noted in 3 and 2 pts, respectively. Grade III/IV thrombocytopenia and neu-

tropenia were noted in 6 and 3 pts respectively. All 8 pts responded at day 30 with either a decrease in ALC or lymph node size and were continued on treatment. Of these, 2 pts achieved CR/CRu, 2 PR and 4 have SD. Of 6 pts with elevated ALC, the median decrease in ALC was 65% (range; 54-94%). None of the pts on treatment have PD and therefore have not yet received R. **Conclusions.** This is the first report of the use of lenalidomide in pts with CLL. Our preliminary results show encouraging clinical activity of lenalidomide in CLL with anti-tumor effects noted as early as the 7th day of treatment. Further follow-up will be needed to establish the durability of these responses. Toxicity profile so far is predictable and manageable.

0098

VEGF PRODUCTION BY CLL CELLS: A PROGNOSTIC MARKER AND A POTENTIAL THERAPEUTIC TARGET

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Background. Angiogenesis appears to play a role in the pathogenesis of B-cell chronic lymphoblastic leukemia (CLL). In particular, vascular endothelial growth factor (VEGF) is present in the serum of CLL patients and has been detected in leukemic cells by immunostaining of bone marrow biopsies and both events have been correlated with a poor prognostic likelihood. Numerous prognostic factors have been proposed in CLL patients; in particular, the expression of CD38 and ZAP-70, and the unmutated IgVH status are indicative of progressive CLL. **Aims.** To study the angiogenic potential of purified CD19+ CLL cells in relationship with other prognostic parameters and the possibility of overcoming this CLL-associated property by specific inhibitors. **Methods.** The diagnosis of CLL was based on morphologic and immunophenotypic criteria (CD5/CD20+, CD22±, CD23+, CD79b-, FMC7-, low expression surface Ig). The CD38 antigen was evaluated in quadruple staining together with CD3, CD20 and CD5. ZAP-70 protein (Upstate, Lake Placid, NY) expression was evaluated by immunocytochemistry on leukemic cells. The IgVH status was analyzed by automated sequencing. An Angiokit test (TCS Cell Works, Buckingham, UK) was utilized to evaluate the capacity of negatively purified CD19+ CLL cells to stimulate the growth and tubule formation of human endothelial cells. Leukemic cells were seeded on the monolayer of endothelial cells, at direct contact or in transwell, for 10 days. The angiogenic effect induced by leukemic cells was measured also after addition to the culture medium of the anti-VEGF specific antibody, an anti-IL8 antibody and a proteasome inhibitor (Bortezomid). The quantification of endothelial tubule formation was carried out using a specific software. Six untreated patients were evaluated: in 3 the leukemic cells were CD38+, ZAP-70+ and showed an unmutated IgVH status; in the remaining 3 patients the leukemic cells were CD38-, ZAP-70- and with a mutated IgVH status. **Results.** In CLL patients with poor prognostic factors, CD38+, ZAP-70+ and unmutated IgVH status, purified leukemic cells showed a major capacity of stimulating the growth and the tubule formation of human endothelial cells compared to leukemic cells obtained from CLL patients with good prognostic factors, i.e. CD38-, ZAP-70- and a mutated IgVH status. In fact, 1506, 1507 and 4204, tubules were detected in the 3 patients with poor prognosis, while 702, 745 and 375 tubules were found in the cultures set up with leukemic cells from patients with favourable prognostic factors. The growth control in the culture was represented by VEGF (3062 tubules). The growth of endothelial cells was inhibited (>80%) in all cases by the anti-VEGF antibody. On the contrary, anti-IL8 and Bortezomid were ineffective. **Conclusions.** VEGF levels released by purified leukemic cells are associated with poor prognostic factors in CLL patients. The specific inhibition of VEGF represents a potential therapeutic option for the management of patients with CLL.

0099

THE ZAP-70/SYK MRNA RATIO CORRELATES STRONGLY WITH IMMUNOGLOBULIN VH GENE MUTATIONAL STATUS AND RISK FOR DISEASE PROGRESSION IN PATIENTS WITH CHRONIC LYMPHOBLASTIC LEUKEMIA

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Background. Chronic lymphoblastic leukemia (CLL) patients with progressive disease usually lack somatic mutations in their immunoglobulin heavy-chain variable region genes (VH) and express ZAP-70, a T-cell specific protein tyrosine kinase (PTK). Recent studies have shown that the presence of ZAP-70-positive cells above a certain threshold correlates strongly with the need for treatment and shorter survival. However, the percentage of ZAP-70 positive cells in many cases is relatively near the threshold, which may impose difficulties in standardizing the flow-cytometric procedure in laboratories that lack sufficient operator experience with the employed gating strategies. **Aims.** To develop and evaluate an alternative approach for quantification of ZAP-70 expression that will be applicable for routine analysis in the clinical setting. **Methods.** The coding sequence of ZAP-70 is highly homologous to the coding sequence of the related PTK Syk, which is expressed in all CLL B-cells and functions as a key mediator of proximal B-cell receptor signaling. We took advantage of this homology to develop an RT/PCR procedure which amplifies both mRNA transcripts with equal efficiency. The relative amounts of ZAP-70 and Syk mRNA were determined in purified B-cells from 90 CLL patients and correlated with the VH gene mutational status and the clinical course of the patients. **Results.** We observed that ZAP-70 is expressed in the majority of cases with unmutated VH genes (87%), but also at lower levels in a substantial fraction of cases with mutated VH genes (41%). To identify the level of ZAP-70 expression that could best discriminate the mutated from the unmutated cases, we used a receiver operating characteristic plot. A ZAP-70/Syk mRNA ratio of 0.25 was the best cutoff that correctly assigned the VH gene mutational status in 87% of cases. Using this cutoff, 81% of the unmutated and only 10% of the mutated samples were classified as cases with high ZAP-70 expression. Median treatment free survival (TFS) in the group of patients with high ZAP-70 mRNA levels was 18 months while median was not reached yet for the group with low or undetectable ZAP-70 ($p < 0.001$). **Conclusions.** Measurement of the ZAP-70/Syk mRNA ratio can accurately predict the VH gene mutational status and risk for disease progression in CLL and represents a valuable alternative to flow-cytometric analysis of ZAP-70 expression.

0100

OUTCOME PREDICTION IN CLL PATIENTS BASED ON A DISEASE-SPECIFIC OLIGONUCLEOTIDE MICROARRAY

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The outcome prediction in CLL may improve thanks to the inclusion of new additional biological variables. This could allow a more reliable risk stratification of patients. With this aim, we have analysed with oligonucleotide chips two different series of CLL patients in a two-step process. RNA extracted from tumoral samples in an initial, exploratory, series of 26 CLL patients, was hybridised using commercial 22K oligonucleotide chips (Agilent Technologies), and 135 genes whose expression was associated with changes in survival probability

were identified. An additional review of published data yielded a total number of 497 genes whose expression has been related with outcome in CLL. A CLL specific chip was then built including, besides this, control genes until 1023 genes. This new CLL-chip was then hybridised with RNA extracted from peripheral-blood samples in a new series of consecutively diagnosed 60 patients. Cox's univariate and multivariate analysis were performed to look for molecular variables associated with time to treatment as endpoint. FDR significance levels were also used when appropriate, for adjusting the associated p-value. The results, analysed with different approaches, allow to identify a set of 87 genes whose expression was associated with changes in outcome. A multivariate analysis disclosed a three-gene model that distinguishes two groups of patients with survival probability at 5 yrs of 87% and 26% respectively.

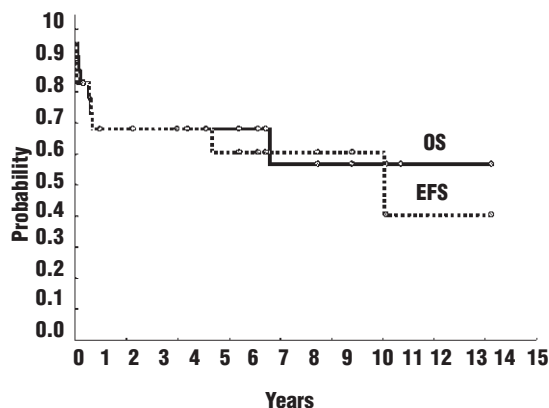
0101

PROLONGED RESPONSE AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION (ALLO-SCT) FOR HIGH-RISK CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL): LONG TERM OUTCOME OF A SERIES FROM A SINGLE INSTITUTION

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Background. Prognosis of patients with CLL with high-risk features or failing to treatment with purine analogues is poor, with a significantly shortened life expectancy. In this regard, alloSCT is reported to result in a high proportion of responses, in part due to induction of graft-versus-CLL effect, although data on long-term outcome are scarce. **Aim of the study:** To analyse the outcome of 23 consecutive patients with poor-prognosis CLL who underwent alloSCT in a single institution. **Patients and Methods.** Twenty-three patients (61% male; age: 46, 29–58) who received an alloSCT for high-risk CLL during the period 1991–2004 have been included in the study. Median number of lines of therapy was 2 (1–5) and median interval from diagnosis was 42 months (6–104). At time of SCT, 13 patients were considered to have refractory disease. Conditioning regimen consisted of cyclophosphamide (120 mg/kg) and TBI (13 Gy) in 17 patients and a reduced-intensity conditioning in the remaining 6 (fludarabine/melphalan, 5; fludarabine/TBI, 1). Donor was an HLA-identical sibling in 19 patients and unrelated donor in 4 cases. Source of stem cells was peripheral blood in 17 and bone marrow in 6, and 9 patients received a T-cell depleted allograft,



An unmutated IgVH configuration was found in two-thirds of the patients with available biological samples at diagnosis, and a poor-prognosis cytogenetics was identified in 7 patients. Response to alloSCT was evaluable in 21 of the 23 patients: 14 achieved CR (62%), and 7 died due to a transplant-related cause

(1-year TRM: 32% \pm 10). After a median follow-up of 72 months (6–163), two patients relapsed at 53 and 121 months after alloSCT, this resulting in a 5-year relapse risk of 11% (\pm 10). Overall survival (OS) and event-free survival (EFS) at 5-year was 68% (\pm 10) and 60% (\pm 11), respectively (figure). Within the subset of refractory patients, 5-yr OS and EFS were 58% (\pm 15) and 43% (\pm 16), respectively. **Conclusions.** Despite its remarkable toxicity, alloSCT results in long-lasting responses in a significant fraction of patients with poor-prognosis CLL and is the best therapeutic option for this other otherwise incurable subset of patients; late relapses, however, are of concern.

0102

CHRONIC LYMPHOBLASTIC LEUKEMIA WITH MUTATED VH GENES PRESENTING WITH BINET STAGE B OR C DISTINGUISHES A SUBGROUP WITH INFERIOR OUTCOME

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Background. The immunoglobulin VH gene mutation status is a strong prognostic indicator in B-cell chronic lymphocytic leukemia (CLL), since unmutated VH genes correlate with short survival. However, the traditional cut-off level dividing mutated and unmutated cases, e.g. more or less than 2% mutation, has been questioned and other cutoffs have been suggested. **2. Aims.** We here wanted to investigate whether an alternative cutoff should be applied as well as the relation of the mutation status to another prognostic marker, eg. Binet staging. **3. Methods.** The VH gene mutation status was assessed in 332 CLL cases by PCR amplification and nucleotide sequencing and was further correlated with overall survival and Binet stage. By calculating the Youden index for different mutation levels (1–7%) we investigated which mutation cutoff best predicted outcome in our CLL material. **4. Results.** After testing of different mutation borders, the 2% cutoff remained the best discriminative level for determining prognosis. Interestingly, an improved division was revealed by combining the VH gene mutation status with Binet staging: unmutated cases (all stages, n=151, median survival 82 months), mutated cases presenting with stage A (n=77, 179 months) and mutated cases with stage B or C (n=37, 74 months). **5. Summary/conclusions** We could confirm the 2% cutoff as the best discriminator of outcome in CLL. Furthermore, CLL cases displaying mutated VH genes with Binet stage B or C had significantly shorter survival similar to unmutated cases, when compared to mutated stage A CLLs. Our result reveals clinical heterogeneity within the VH mutated CLL group by inclusion of Binet stage data; a finding which is of importance when considering surrogate marker (s) for the VH mutation status.

0103

INCIDENCE OF ZAP70 POSITIVITY IN FRESH SAMPLES FROM UNSELECTED PATIENTS WITH CLL AND OTHER B-LPD

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Background. Recently there has been much interest in ZAP70 as a surrogate marker for immunoglobulin VH status in chronic lymphocytic leukemia (CLL). Individuals with unmutated IgVH genes have a poorer overall survival and strong correlations have been shown with the expression of ZAP70. Previous

studies have predominantly used frozen material to enable retrospective analysis and published analyses have largely focussed on the CLL group. **Aims.** We wanted to avoid any potential biases of using stored material by utilising only fresh peripheral blood specimens. Additionally, we expanded our study beyond CLL to include all B-cell lymphoproliferative disorders (B-LPD) at our centre. **Methods.** ZAP70 was analysed on fresh whole blood using flow cytometry in 267 consecutive cases of B-LPD. A combination of ZAP70 (Upstate Biotechnology), CD3 PE and CD56 PE (Coulter Immunotech) antibodies (Ab) were used to exclude T and NK cells. CD3 and CD56 staining was followed by fixation and permeabilisation (Dako Intracellular Antigen Kit) and addition of anti-ZAP70 Ab. Indirect labelling of ZAP70 was performed using a secondary conjugate IgG (H+L)-FITC (Southern Biotechnology). Positivity was established at the 20% threshold. **Results.** 211 cases of CLL were examined for ZAP70 expression, 61 (29%) were positive. In 2 cases, the ZAP70/CD3/CD56 combination expressed values of less than 20% (6% and 13%), however, quenching was deemed to have occurred in these cases (based on the results of the staining of ZAP70 alone). The ZAP70 negative cases of CLL had a median value of 1% (range 0-14%). 56 non CLL cases were examined for ZAP70 expression-mantle cell lymphoma (MCL, 20 cases), hairy cell leukaemia (HCL, 6), diffuse large B cell lymphoma (2) and 28 cases of B-cell LPD that could not be categorised. Only 5 non CLL cases were positive for ZAP70 expression and all these were cases of MCL (24-39% positivity). The ZAP70 negative cases of MCL had a median value of 0% (range 0-2%). No other non CLL B-LPD showed levels of expression >10%. **Summary and Conclusions.** Our data shows that 29% of CLL cases and 25% of MCL cases are positive for ZAP70. In utilising fresh material, we have avoided any potential bias of the freeze/thawing process and have obtained results comparable with published data for CLL. Furthermore, we show that no other B-LPD group, aside from MCL, is positive for ZAP70. ZAP70 may therefore be useful as a diagnostic tool to help distinguish difficult CLL cases from non CLL (other than MCL).

0104

HUMAN LEUKOCYTE ANTIGENS HLA DRB1*01 AND DRB1*02 INFLUENCE CLINICAL OUTCOME OF CHRONIC LYMPHOBLASTIC LEUKEMIA

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Background. The etiology of chronic lymphocytic leukemia (CLL) remains unknown, though a genetic susceptibility within MHC class II region has been suggested. **Aims.** and **Methods.** We investigated here whether human leukocyte antigens class II (HLA DRB1) might influence incidence and clinical characteristics of CLL by analyzing 90 patients and 94 ethnically-matched healthy controls using PCR-SSO (sequence specific oligonucleotides) DNA assay. **Results.** The HLA DRB1 allele frequency was similar in patients and control groups and their distribution was consistent with Hardy-Weinberg equilibrium, therefore it is unlikely that they confer susceptibility to CLL occurrence. There were no associations between HLA DRB1 alleles and clinical characteristics of CLL patients at diagnosis or response to first-line treatment. In patients with HLA DRB1*02 null allele, a trend towards a higher progression rate was observed ($p=0.59$). The mortality rate was significantly higher in patients carrying HLA DRB1*01 [$p=0.014$ OR 7.81 (95% CI 1.36-44.7)], HLA DRB1*02 null allele ($p=0.01$), or both genetic markers [$p=0.009$ OR 12 (95% CI 1.4-104)]. With a median follow-up of the surviving patients of 40 months (range 0.07-53 months), the subgroups of patients with HLA DRB1*02 null and HLA DRB1*01 or both genetic markers had significantly shorter overall survival (OS), ($p=0.009$, $p=0.0017$ and $p=0.001$, respectively). To further characterize HLA DRB1 linkages with survival in CLL patients, we analyzed both DRB1*02 splits (DRB1*15 and DRB1*16) and found that only DRB1*16 null

allele remained significantly associated with longer OS ($p=0.02$). However, neither of these genetic markers was found to be associated with freedom from progression in CLL patients. After incorporating HLA DRB1*01, HLA DRB1*02 null alleles and prognostic parameters reported to influence CLL outcome, in a multivariate Cox regression model, the HLA DRB1*01 remained the one independent factor predicting for shorter OS survival ($p=0.003$). **Conclusions.** Our results suggest that genetic factors within or close to human MHC class II region may influence CLL clinical outcome.

0105

IMMUNO CHEMOTHERAPY (ANTI CD20 AND FLUDARABIN/ CYCLOPHOSPHAMIDE) AND HIGH DOSE CHEMOTHERAPY WITH ASCT INCREASES MOLECULAR REMISSION RATE BUT CANNOT PREVENT RELAPSES

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Background. CLL is considered to be incurable disease by standard therapeutic approaches including chemotherapy and autologous stem cell transplantation (ASCT) as well as combination of immunotherapy (monoclonal antibody) and chemotherapy. **Aims.** We have decided to explore the combination of all methods-antibody (anti CD20), chemotherapy (fludarabine and cyclophosphamide) and high dose therapy (HDT) with ASCT in CLL. **Methods.** Twenty five pts with B-CLL (median age 57 years (32-62)) have been recruited. Pts were eligible for study if they failed previous therapy, progressed without previous tx or if they exhibit at least two adverse prognostic factors at dg. The mutational status was examined since 2002. The treatment protocol consists of 4 cycles of R-Flu/Cy (rituximab 375 mg/m², fludarabine 3x25 mg/m², cyclophosphamide 3x300 mg/m²), followed by mobilization regimen ESHAP with harvest of progenitor cells and HDT (BEAM) with ASCT. Pts were reassessed after R-Flu/Cy regimen and after HDT therapy. Molecular monitoring was based on CDRIII rearrangement assessment by PCR. Twenty one patients were examined before therapy or were informative and they are followed. Fourteen out of 25 pts were treated because of disease progression or previous tx failure. **Results.** Seven CR, 14 nPR and 4 PR were achieved after R-Flu/Cy therapy. Molecular remission was confirmed in 6 out of 21 evaluable pts (29%). Twenty four pts proceed to mobilization and the harvest was successful in 20 (83%). HDT with ASCT was performed in 18 pts (2 refusals) with an excellent CR rate (17 CR and 1 PR). Thirteen out of 15 informative patients (87%) have reached PCR neg. after HDT. The clinical response rate based on intent to treat (ITT) analysis of all pts (25 treated with R-Flu/Cy, 24 mobilized, 18 transplanted, 15 reassessed after HDT) was as follows: 18 CR, 4 nPR and 3 PR. Based on ITT analysis 14 out of 21 informative pts (21 treated with R-Flu/Cy, 20 mobilized, 15 transplanted, 15 reassessed after HDT) have reached PCR negativity (67%). 10 relapses/progressions and one death were observed with median of follow up 32 months from start of tx and 43 from diagnosis. 8 out of 10 relapses were found as unmutated CLL, four occurred in patient without HDT and ASCT (including the only one mutated case). Out of 7 progressions in PCR informative cases, 4 occurred in PCR neg group (57%) and 3 in PCR pos. pts (43%). The median time to progression among patients who progressed was 24 month. The 2y PFS probability from time of therapy is 75% and 4 y PFS probability from dg is 70%. **Conclusions.** The combined therapeutic approach is very well tolerated and has a high response rate including molecular biology responses in CLL and in unmutated cases as well. Relapses however have been observed regardless PCR status and they occur mainly in unmutated cases. The addition of anti CD20 to HDT approach in these subgroup of patients does not seem to be curative, but it can prolong remission.

Chronic myeloid leukemia I

0106

A PROGNOSTIC SCORE INCLUDING THE TIME-DEPENDENT VARIABLE CYTOGENETIC REMISSION FOR RISK-STRATIFIED COMPARISON OF SURVIVAL BETWEEN IMATINIB-TREATED PATIENTS AND HISTORICAL CONTROL DATA ON IFN-TREATMENT

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Background. In a large clinical trial, most patients randomised to a treatment based on interferon- α (IFN) crossed over to treatment with imatinib. It is to be expected that a comparison in survival between IFN- and imatinib-treated patients within the same randomised trial will hardly be realisable. Only second best to a randomised trial but still a possible help out would be the comparison between imatinib-treated patients and a historical control sample of IFN-treated individuals. In such a case, it is particularly important to ensure that survival probabilities of both patient samples are subject to the same risk structure or are compared by a risk-stratified test statistic. At diagnosis, the New CML score (Hasford *et al.*, *JNCI* 1998;90:850-8) allocates IFN-treated patients to three risk groups with statistically significantly different survival probabilities. However, non-high-risk patients achieving an MCR (0-35% Philadelphia chromosome-positive (Ph+) metaphases) by IFN treatment are the only group likely to be able to compete with survival probabilities expected for imatinib. Hence, under consideration of cytogenetic remission, an alternative CML score was developed. **Methods.** In a sample of 743 IFN-treated patients, the correlation between survival times and ten explanatory variables was analysed by multiple Cox regression. Apart from cytogenetic remission, all variables were measured at diagnosis. In developing the prognostic score, specific attention was paid to the time-dependence of the remission variable, correctness of model assumptions, and goodness of fit but also to clinical usefulness and ease of application. **Results.** Cytogenetic remission, age, spleen enlargement, haemoglobin, eosinophils and basophils (both peripheral blood) were statistically significant in the multiple model. A prognostic system with four risk groups based on five dichotomised baseline variables and two dummy variables, for partial remission (PCR, 1-35% Ph+ metaphases) and complete remission (CCR, 0% Ph+ metaphases), was identified. At diagnosis, the *highest-risk* group comprises patients with all five baseline values in the more unfavourable categories or with high risk according to the New CML score, since these patients are not likely to benefit from MCR. Patients with three or four values in the worse categories form the *higher-risk* group, patients with less unfavourable baseline values the *lower-risk* group.

Table 1. Prognostic CML score including cytogenetic remission. Landmark 18 months.

Patients observed for ≥ 18 months after start of therapy	No. of patients (no. died after 18 months)	Median survival	10-year survival probability (95% confidence interval)
<i>Lowest-risk patients</i>	106 (15 died)	not observed	0.76 (0.65; 0.86)
<i>Lower-risk patients</i>	278 (103 died)	81 months	0.27 (0.14; 0.41)
<i>Higher-risk patients</i>	197 (116 died)	63 months	0.13 (0.04; 0.22)
<i>Highest-risk patients</i>	89 (69 died)	43 months	not observed

Independent of a later MCR, patients with highest risk remain in this group. As soon as PCR is observed, patients of the two more favourable groups move up to the next better risk group. Thus, formerly lower-risk patients establish a *lowest-risk* group which is completed by originally lower- and higher-risk patients after observation of a CCR. Survival probabilities can be described by Kaplan-Meier starting at chosen Landmarks (e.g. 18 months after start of therapy, Table 1).

0107

IDENTIFICATION OF GENES INVOLVED IN IMATINIB RESISTANCE IN CML-A GENE EXPRESSION PROFILING APPROACH

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The use of the tyrosine kinase inhibitor imatinib, which blocks the enzymatic action of the BCR-ABL fusion protein, has represented a critical advance in chronic myeloid leukemia (CML) treatment. However, this treatment fails in a subset of patients. Use of c-DNA microarray expression profiling allows the identification of genes whose expression is associated with imatinib resistance. Thirty-two CML bone-marrow samples, collected prior to imatinib treatment, were hybridized with a c-DNA microarray containing 6,500 cancer genes, and analyzed using bootstrap statistics. Patients refractory to IFN- α treatment were evaluated for cytogenetic and molecular responses for a minimum of 12 months. Imatinib induces complete hematological response after a 12-month follow-up in 100% of patients and a greater cytogenetic response in 65% of patients (20 vs. 11 patients). In terms of molecular response 30% of patients show a poor response (BCR-ABL/ABL >0.1), 40% show an intermediate response, and 30% show a good response (BCR-ABL/ABL <0.01). In terms of classical predictable variables, the presence of additional chromosomal abnormalities, the lack of complete hematologic response and the level of Ph chromosome were significantly associated with the lack of a major cytogenetic response at 6 months ($p=0.005$, $p=0.03$, and $p=0.031$, respectively). There was no such association for cytogenetic response at 12 months and for the molecular response. The expression profile study gives a set of 53 genes differentially expressed in imatinib responders and nonresponders. This set includes genes involved in cell adhesion (TNC and SCAM-1), drug metabolism (COX1), protein tyrosine kinases, and phosphatases (BTK and PTPN22) and NF κ B activation (IKKBK, XIAP, CFLAR). COX 1 expression was highly correlated with the failure of molecular response ($p<0.05$). In order to obtain a prediction model with a low number of genes, factorial analysis was carried out. A 6-gene prediction model was constructed, capable of distinguishing responder's vs non-responders cases with an accuracy of 80%. The model was confirmed in an independent group of patients treated with imatinib as a first-line therapy. This study identifies a set of genes that may be involved in primary resistance to imatinib, suggesting BCR-ABL-independent mechanisms.

0108

GROWTH-INHIBITORY EFFECTS OF THE HO-1-TARGETING COMPOUND PEG-ZNPP ON IMATINIB-RESISTANT CML CELLS

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Background. Chronic myeloid leukemia (CML) is a myelo-

proliferative disease characterized by the BCR/ABL oncogene and an increased survival of leukemic cells. The BCR-ABL tyrosine kinase inhibitor imatinib has successfully been introduced in the treatment of CML. However, despite high expectations and encouraging initial results, the long term outcome is not known, and recent data suggest that resistance against Imatinib can occur. Therefore, current studies focus on novel drug-targets in CML cells. **Aims.** We have recently identified heme oxygenase-1 (HO-1) as a novel BCR/ABL-dependent survival-molecule in primary CML cells. In this study, we analyzed signal transduction pathways underlying BCR/ABL-induced expression of HO-1 and evaluated the role of HO-1 as a potential new target of drug therapy. **Methods.** Growth inhibitory effects of HO-1-targeting compounds were determined by 3H-thymidine incorporation assay. Signal transduction pathways were characterized using pharmacologic inhibitors and constitutively activated or dominant negative signaling molecules. **Results.** We found that the PI3-kinase inhibitor LY294002 and MEK inhibitor PD98059 counteract expression of HO-1 in CML cells. In addition, constitutively activated Ras- and Akt -mutants were found to promote expression of HO-1 in Ba/F3 cells suggesting involvement of the PI3-kinase/Akt as well as the MAPK pathway. To establish a role for HO-1 in survival of CML cells, expression of HO-1 was silenced by siRNA which resulted in apoptosis of K562 cells. In a next step, HO-1 was targeted in CML cells by pegylated zinc protoporphyrin (PEG-ZnPP). Exposure to PEG-ZnPP resulted in growth inhibition and induction of apoptosis in primary CML cells as well as in the CML-derived cell lines K562 and KU812 with IC50 values ranging between 1-10 µM. The growth-inhibitory effects of PEG-ZnPP were not only observed in CML cells responsive to imatinib, but also in imatinib-resistant K562 cells and Ba/F3 cells expressing various imatinib-resistant mutants of BCR/ABL (T315I, E255K, M351T, Y253F, Q252H, H396P). Moreover, imatinib and PEG-ZnPP were found to exert synergistic growth inhibitory effects on Imatinib-resistant leukemic cells. **Conclusions.** Together, these data suggest that HO-1 represents a novel interesting target in CML.

0109

FOLLOW-UP OF 52 PATIENTS WITH CLONAL CHROMOSOMAL ABNORMALITIES IN PHILADELPHIA NEGATIVE (PH-) CELLS DURING GLEEVEC® TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML)

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Background. Imatinib mesylate, also called Gleevec® (Novartis, Hanover, NJ) a specific inhibitor of the BCR-ABL tyrosine kinase was introduced into the therapy of CML. Few cases of emergence of clonal chromosomal abnormalities after therapy with Gleevec® has been reported but the incidence of such abnormalities differs and their explanation and clinical significance have to be clarified. We report here a large series of 52 CML patients treated with Gleevec® who developed of new Ph- clones. **Methods.** Between September 2000 and December 2004; 52 CML patients (29M/23F) of 57.7 years mean age and treated with Imatinib developed Ph négatif clone. When Imatinib treatment was started: 43 patients were in chronic phase, 9 in accelerated phase. Nine patients were treated with Imatinib in up-front. The others patients were previously treated: 3 with hydroxyurea alone (HU), 40 with interferon α (INF) alone or associated with cytarabine (Ara-C), 2 received autologous stem-cell transplant. The mean duration of disease was 36

months (range 0-108 months) before imatinib. **Results.** All patients responded to Imatinib and 37 patients achieved major or complete cytogenetic response (CCyR). Clonal abnormalities in Ph- cells appeared 12.5 months (range 3-36 months) after Imatinib initiation. The most common cytogenetic abnormalities were a change in the number of chromosome: trisomy 8 (18), monosomy 7 (7), Y loss (8) gain of chromosomal marker (2) and trisomy 15 (1). Structural chromosomal abnormalities included four reciprocal balanced translocations and 7 deletions: 3 del (20) (q11) 2 del (7) (q21q36), del (11q), del (7p). With a mean follow-up of 33 months (range 10-40 months), 32 patients are still in CCyR, 6 patients manifested hematological or clinical signs of progression of CML and 9 relapsed. Patients with Y loss clone are in CCyR and 3 achieved low BCR/ABL transcript level. Among patients with trisomy 8: 4 manifested an accelerated or leukemic phase, 3 relapsed and 2 are Imatinib non-responders, 5 achieved CCyR associated in 2 cases with good molecular response. Among patients with -7/7q defect only 2 about 9 are still in CCyR. In contrast, among patients with other anomalies: only one developed an accelerated phase; the evolution of patients with +8 or -7/7q- is significantly different from the evolution of patients with the others abnormalities. **Discussion /Conclusions.** Gleevec®, a specific ABL kinase inhibitor, produces sustained complete hematologic and major cytogenetic responses in CML patients but long-term outcomes for these patients are not yet known. The emergence of the clonal chromosomal abnormalities in Ph- cells develop in a significant higher proportion of patients treated with imatinib (4%) than observed in patients with other treatments. The clinical significance of these abnormalities is not yet completely clarified. These clonal abnormalities seems to be benign and transient in half of the patients when a long follow-up is obtained. But patients who developed Ph- trisomy 8 or -7/7q- clones can progress to a more advanced phase or relapsed and should be regularly monitored.

0110

LOW-LEVEL EXPRESSION OF PRO-APOPTOTIC BIM IN CML CELLS: ROLE OF BCR/ABL, CHARACTERIZATION OF UNDERLYING SIGNALING PATHWAYS, AND RE-EXPRESSION BY PHARMACOLOGICAL AGENTS

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Background. Chronic myeloid leukemia (CML) is a myeloproliferative disease in which BCR/ABL enhances survival of leukemic cells through modulation of pro- and anti-apoptotic molecules. Recent data suggest that pro-apoptotic Bim plays a role as a tumor-suppressor in myeloid cells, and that leukemic cells express only low amounts of this cell death activator. **Aims.** In the current study, we have investigated expression of Bim in primary CML cells and BCR/ABL-transformed cell lines, and examined mechanisms and signal transduction pathways underlying Bim expression in leukemic cells. **Methods.** Expression of Bim was analyzed in primary CML cells, the CML-derived cell lines K562 and KU812, and Ba/F3 cells inducibly expressing BCR/ABL on exposure to doxycycline (TonB.210-X cells). In addition, Ba/F3 cells expressing imatinib-resistant mutants of BCR/ABL were examined. Bim mRNA expression was determined by Northern blotting, and expression of the Bim protein by Western blot analysis. Signaling pathways contributing to Bim expression in leukemic cells were examined by pharmacologic inhibitors of MEK (PD98059), PI3-kinase (LY294002), and mTOR (rapamycin). Proliferation of CML cells was investigated by measuring 3H-thymidine uptake. **Results.** Primary CML cells were found to express significantly lower amounts of bim mRNA and lower levels of the Bim protein compared to normal bone marrow cells. The BCR/ABL-inhibitors imatinib (Novartis Pharma AG) and AMN107 (Novartis Pharma AG) were

found to promote Bim expression in CML cells at pharmacologically relevant concentrations. In addition, BCR/ABL was found to down-regulate expression of Bim in TonB.210-X cells. The BCR/ABL-induced decrease in expression of Bim in leukemic cells was found to be a post-transcriptional event that depended on signaling through the MAP kinase pathway, and was abrogated by the proteasome-inhibitor MG132. Interestingly, MG132 up-regulated Bim-expression and suppressed the growth of Ba/F3 cells containing either wild-type BCR/ABL or various imatinib-resistant mutants of BCR/ABL including the T315I mutation that is resistant to all kinase inhibitors currently used in clinical trials. *Conclusions.* Our data identify BCR/ABL as a Bim-suppressor in CML cells and suggest, that re-expression of Bim by proteasome inhibition or by targeting of signaling pathways downstream of BCR/ABL may be an attractive therapeutic approach in imatinib-resistant CML.

0111

DEVELOPMENT OF ABL KINASE MUTATION AND LONG-TERM CYTOGENETIC RESPONSE IS DETERMINED BY THE LEVELS OF IMATINIB DRUG TRANSPORTERS *hOCT1* AND *MDR1* IN IMATINIB TREATED CHRONIC MYELOID LEUKEMIA (CML)

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Although imatinib is effective in treating CML, some patients become resistant, which is often due to the emergence of leukaemic clones bearing ABL kinase domain mutations. We and others have shown that imatinib is a substrate for the efflux pump P-glycoprotein, the gene product of the *MDR1* gene. We have recently shown that cellular imatinib uptake is also an active process, mediated by human organic cation transporter 1 (*hOCT1*). The balance of influx (*hOCT1*) and efflux (*MDR1*) transporter expression may therefore be an important determinant of intracellular imatinib concentration. In this study, we have correlated the mRNA expression of *hOCT1* and *MDR1* with outcome in 67 imatinib treated CML patients (50 in chronic phase and 17 in accelerating phase). Samples were collected before and at 4 weeks, 3 months and 6 months after imatinib treatment. Gene expression was measured by real-time quantitative RT-PCR on a LightCycler, using GAPDH as an internal control. *hOCT1* and *MDR1* expression was normalised to their expression in VBL100 cells, defined arbitrarily as 1.0. After 6 months of therapy, 40 patients had achieved a major cytogenetic response (MCR; of which 36 were complete (CCR)) and 27 had no cytogenetic response (NCR). Samples were available for 5 of the 7 MCR patients that subsequently lost their response, and all 5 were found to have a mutation in the BCR-ABL kinase domain at progression (though not at earlier time points). None of the other MCR and no NCR patients had ABL kinase mutations at their latest follow up. No significant difference in baseline WBC was seen at between MCR and NCR patients. During the first 4 weeks and 3 months of imatinib treatment, both *hOCT1* and *MDR1* expression increased ($p < 0.01$). Prior to commencement of imatinib, *hOCT1* expression levels were greater in patients who subsequently achieved MCR than in NCR patients ($p = 0.025$). Moreover, 22 of 40 patients destined to achieve MCR expressed *hOCT1* at a level of greater than 800 arbitrary units, whilst only 7 of 27 NCR expressed *hOCT1* at this level ($p = 0.02$, odds ratio = 3.49). The baseline and early (4 week/3 month) *MDR1* expression did not influence the achievement of MCR. However, 5 of 7 MCR patients who at their latest follow-up had lost their cytogenetic response had high (greater than 10% arbitrary units) *MDR1* expression, while only 4 of 33 MCR patients in sustained response had *MDR1* expression at this level ($p = 0.002$, odds ratio = 20.63). We conclude that: 1) high baseline expression of *hOCT1* predicts the achievement of MCR by 6 months, and 2) in patients who subsequently achieve MCR, low baseline/early (4 weeks/3 months) expression of *MDR1* predicts the loss of cytogenetic response and the

development of an ABL kinase mutation. The data support the view that high intracellular imatinib levels correlate with sustained MCR; low levels with NCR, and intermediate levels with initial MCR but the subsequent development of an ABL kinase mutation. The data also imply that suboptimal imatinib dosing may increase the risk of developing an ABL kinase mutation.

0112

ABL MUTATIONS IN CP-CML PATIENTS RESISTANT TO IMATINIB ARE ASSOCIATED WITH SIGNIFICANTLY SHORTER TIME TO PROGRESSION AND SURVIVAL, WITH P-LOOP MUTATIONS CONFERRING A PARTICULARLY POOR PROGNOSIS

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Background. ABL kinase domain (KD) mutations have been associated with resistance to imatinib (IM) in chronic myeloid leukemia (CML) patients (pts.), but their clinical and prognostic relevance is still controversial. *Aims.* To shed further light on the clinical and prognostic significance of ABL KD mutations, we retrospectively analyzed an homogeneous cohort of chronic phase (CP) CML pts enrolled in the CML/002/STI571 multicenter clinical trial of the GIMEMA Working Party on CML, who showed either primary or secondary cytogenetic resistance to IM. *Methods.* Using denaturing-high performance liquid chromatography and sequencing, we screened for ABL KD mutations 65 CP CML pts treated with IM, who did not reach a CCgR at 12 months (n=41) or who lost CCgR at any time while on IM therapy (n=24). All pts had failed previous \square -IFN therapy (12 pts being intolerant of \square -IFN, 18 pts showing hematologic resistance, 35 pts showing cytogenetic resistance). IM was administered at the dose of 400 mg/d until progression or grade 4 non-hematological toxicity. The median age at the time of IM start was 54 years (range, 29-73) and the median time from diagnosis to IM start was 3.6 years (range, 1-13.2). Median follow-up is 37.5 months (range, 10-51). *Results.* KD mutations were found in 27/65 (41.5%) pts and mapped to 10 codons (Y253F/H, 5 pts; E255K/V, 4 pts; G250E, 4 pts; M351T, 4 pts; F359V, 2 pts; M244V, 2 pts; E355G, 2 pts; F317L, 2 pts; F311L, 1 pt; H396R, 1 pt). There were no significant differences between pts with and pts without mutations as far as sex (M/F: 15/12 vs. 17/21, respectively), median age at the time of IM start (53 vs. 55 years, respectively) and disease history (intolerance/hematologic resistance/cytogenetic resistance to \square -IFN: 5/7/15 vs. 7/11/20 pts, respectively) were concerned. Median time from diagnosis to the start of IM therapy was significantly longer for pts with mutations with respect to pts without mutations (4.8 vs. 2.8 years, Mann-Whitney U Test $p = 0.02$). At 3 and 6 months, the CHR rate was 81% (22/27) and 89% (24/27), respectively, for pts with mutations, as compared to 82% (31/38) and 89% (34/38), respectively, for pts without mutations. However, presence of a KD mutation was significantly associated with a greater likelihood of subsequent progression to accelerated phase/blast crisis (Log-Rank $p = 0.0005$) and shorter survival (Log-Rank $p = 0.006$). Pts carrying P-loop mutations (codons 250, 253, 255; n=13 pts.) showed a particularly poor outcome both in terms of time to progression (Log-Rank $p = 0.02$) and in terms of survival (Log-Rank $p = 0.01$). *Conclusions.* These results in a homogeneous and relatively large cohort of IM-resistant CP CML pts support the concept of a gradual accumulation of a pool of BCR-ABL mutants over time, which expand under the selective pressure of IM therapy if favored by a lower affinity for the inhibitor. Moreover, they provide strong evidence that, irrespective of the hematologic response, regular monitoring for emerging mutations may help in identifying those CP pts with worse prognosis, for whom a revision of the therapeutic strategy should be considered.

0113**A NOVEL 4-ANILINO-3-QUINOLINECARBONITRILE DUAL SRC AND ABL KINASE INHIBITOR (SKI-606) HAS IN VITRO PRO-APOPTOTIC ACTIVITY ON CML PH+BLAST CELLS RESISTANT TO IMATINIB**T. Grafone¹, M. Mancini¹, E. Ottaviani¹, M. Renzulli¹, F. Boschelli², L. Ferraretti¹, M.A. Santucci¹, M. Amabile¹, S. Soverini¹, C. Terragna¹, A. Poerio¹, S. Colarossi¹, G. Rosti¹, M. Baccarani¹, G. Martinelli¹¹University of Bologna, Bologna, Italy; ²Department of Oncology, Wyeth Research, Pearl River, New York, USA

A new class of compounds, 4-anilino-3-quinolinecarbonitrile Src kinase inhibitors, has been synthesized. One member of this class, SKI606, is a dual-specificity inhibitor of both Src family and Abl kinases. To investigate the effect *in vitro* of SKI-606, we analyzed human cell lines from CML patients in blast crisis (K562, MK2, LAMA) and CD34+ from patients in CML blast crisis using a wide range of concentrations (0.01mM-1mM) of this novel agent. In K562, MK2 and LAMA84 we observed a decrease in cell viability after treatment with SKI. The effects of this compound on cell cycle progression show an accumulation of G1/S phase in our spermental model. First we observed a reduced Lyn and Hck phosphorylation in whole cell extracts from K562 cells after treatment with SKI 0.1uM. We also demonstrated an hypophosphorylation of AKT and a consequently dephosphorylation of BAD on ser 136. Finally we observed a decrease of Bcl-xl expression and a subsequently activation of Caspase-9. We evaluated the effects of SKI-606 in CML primary progenitors sensitive or resistant to STI-571. In the patients resistant to STI571 with point mutation F359V, Y253H, E255V and E255K was evident a modification of cell cycle progression with an accumulation in G1phase and increment of subdiploid peak. The effect of STI571 not was relevant. Cytofluorimetric analysis of non mutated patients showed an accumulation sensitive CML patients by treating SKI606 (0.1-1 mM) alone or in combination STI571 of cells in phase G1 of cell cycle and a few increment of apoptosis when treated with 1uM of SKI. Data more significative were obtained treating the cells. In one patient with a point mutation F359V we observed a general decrease of bcr-abl protein expression and a reduced protein phosphorylation in tyr 245. In the same patient an hypophosphorylation of Hck and Lyn was observed. We obtained the same results we for two patients in which we didn't find any mutation. Our study thus showed a potential therapeutic usefulness of the drug in treatment of CML, particularly in blast crisis phase. Ongoing gene expression profiles will contribute to further understanding of the drug mechanism.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2004, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna.

0114**COMPARISON BETWEEN PATIENTS WITH PH+ CHRONIC PHASE CML WHO OBTAINED COMPLETE CYTOGENETIC RESPONSE WITHIN 1 YEAR OF IMATINIB THERAPY AND THOSE WHO ACHIEVED SUCH A RESPONSE AFTER 12 MONTHS OF TREATMENT**I. Iacobucci¹, G. Martinelli¹, M. Amabile¹, A. Poerio¹, F. Castagnetti¹, N. Testoni¹, D. Cilloni², B. Izzo², S. Soverini¹, S. Colarossi¹, C. Terragna¹, F. Pane³, G. Saglio², G. Rosti¹, M. Baccarani¹¹University of Bologna, Bologna, Italy; ²University of Turin, Orbassano, Turin, Italy; ³University of Naples, Naples, Italy

Imatinib mesylate is a potent, specific inhibitor of BCR-ABL, the constitutively active tyrosine kinase protein critical for the pathogenesis of chronic myeloid leukemia. Real time Q-RT PCR is increasingly used to monitor responses in leukemia. It has been demonstrated that the BCR-ABL transcript level is an accurate surrogate of the contemporaneous bone marrow cyto-

netic response. We reviewed 284 patients with late chronic phase Philadelphia-positive chronic myeloid leukemia treated with imatinib 400 mg daily after interferon- α failure. In a retrospective study we evaluated the pattern and rapidity of the response to imatinib, comparing the cytogenetic and molecular responses in patients who obtained a complete cytogenetic response very quickly, i.e. within one year of treatment (early responders, n=114), and in patients where a complete cytogenetic response was detected only later, after 12 months of treatment (late responders, n=37). After 3 or 4 years of treatment the molecular response of the late cytogenetic responders was similar to that of the early cytogenetic responders. At 36 months of treatment the amount of residual disease measured by standardized quantitative reverse-transcriptase polymerase chain reaction was 0.00047 in late responders versus 0.00022 in early responders (median values, *p* value n.s.), and at 48 months it was 0.00019 versus 0.00026 (median values, *p* value = n.s.). We also analyzed whether there was any difference between early and late responders in terms of progression-free survival and overall survival and found no difference. Though late responders achieved complete cytogenetic remission after 1 year of imatinib therapy, they showed a progression-free survival rate and an estimated 4-year overall survival similar to or even better than those of early responders (88% versus 100% and 92% versus 100%, respectively). These results suggest that the sensitivity and the response to imatinib may require 1 year or more and that late responders are by no means rare. In conclusion, the long-term follow-up results of patients with chronic-phase CML treated with imatinib mesylate after IFN- α failure continue to improve in terms of the rates and durability of the complete cytogenetic response and major or complete molecular response, and in terms of improved survival compared with previous accepted treatments for CML.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2004, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L.

0115**ACQUIRED MUTATIONS IN THE BCR-ABL KINASE DOMAIN MAY NOT EXPLAIN IMATINIB RESISTANCE SEEN IN CML PATIENTS**J.S. Khorashad, M. Anand, D. Marin, S. Saunders, B. Swale, S. Marley, J.V. Melo, J. Apperley, J.M. Goldman, J.S. Kaeda
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Background. It is generally accepted BCR-ABL kinase mutations confer imatinib mesylate (IM) resistance. **Aims.** To study if the observed mutated alleles are indeed the cause of the IM resistance by quantifying the mutant BCR-ABL in serial samples. **Methods and Results.** The latest cDNA from a heterogenous group of 217 CML patients treated with IM were tested for P-Loop mutations by Pyrosequencing. A Single stranded DNA templates were prepared by alkali denaturation of the biotinylated PCR products, to which a sequencing primer was annealed. Pyrophosphate released on incorporation of a complementary nucleotide to the sequencing primer is converted to ATP by sulfurylase. The ATP generated is utilised as a co-enzyme for luciferase that catalysis the conversion of luciferin to oxyluciferin and in doing so emits light. The intensity of the light is proportional to the level of the single nucleotide polymorphism (SNP) in a given sample. Of the 217 patients 18 (1 E255K; 5 G250E; 7 M244V; 2 Y253F; 3 Y253H) had P-Loop mutations, i.e. between residues 242 and 260. All the mutations found have been reported to be associated with resistance. No other BCR-ABL kinase domain mutations were found in these patients by direct sequencing. For 9 of the 18 patients 5 or more sequential cDNA samples were available. We subjected all the available samples for these individuals to quantitative single nucleotide polymorphism (Q-SNP). Three different patterns of kinetics were observed. (1) Non-mutated allele, i.e. the sensitive clone, decreased rapidly as the overall BCR-ABL quantified by QR-PCR decreased, whilst the mutated allele (the resistant

clone) increased relatively and became the dominant clone. (2) A reduction in the sensitive clone and a relative increase in the resistant clone. But, thereafter the increase in total BCR-ABL was reflected by an expansion in the mutant allele levels, whilst the non-mutated allele continued to diminish. These data imply an increase in tumour load was directly due to increasing levels of mutated mRNA. (3) Third pattern showed the wild type clone to be the major component of the total mRNA. In contrast mutated allele was the minor proportion of the total tumour load. Whilst, these data confirm the mutated clone is resistant to IM, does not contribute significantly to the total BCR-ABL, implying another mechanism for resistance associated with the non-mutated allele, i.e. the dominant clone. *Conclusions.* The *in vivo* data show that reported mutations are associated with heterogeneous phenotype. The data show for the first time that the mutated clone is not always the dominant clone and therefore unlikely to contribute directly to disease progression. Furthermore, the kinetics varied among patients with the same mutations and between those with different mutations.

0116

MOLECULAR RESPONSES AND MUTATION ANALYSIS IN IMATINIB-RESISTANT PATIENTS WITH PHILADELPHIA POSITIVE (PH+) LEUKEMIA TREATED WITH THE DUAL SRC/ABL KINASE INHIBITOR BMS-354825

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Background. In patients with CML, imatinib-resistance is frequently associated with BCR-ABL mutations that interfere with the ability of imatinib to inhibit BCR-ABL kinase activity. Real-time quantitative PCR (RQ-PCR) of BCR-ABL transcripts is a precise and sensitive method to monitor imatinib response and can be used as a screen to identify patients harbouring mutations. BMS-354825 is a novel kinase inhibitor that targets ABL and SRC. It is 300 to 1300-fold more potent than imatinib at killing leukemic cells and has preclinical efficacy against all of the imatinib resistant mutations tested so far except for the T315I mutation. *Aim* We report the molecular analysis of imatinib-resistant/intolerant patients treated at UCLA in a Phase I dose escalation trial of BMS-354825. We sought to gain preliminary insight into a number of issues including whether patients achieve significant reductions in the BCR-ABL level; the effect of BMS-354825 on mutant BCR-ABL clones *in vivo*; whether a rise in BCR-ABL level signifies the evolution of a mutation; and if the selection of the T315I mutation will be a factor for resistance. *Methods.* The BCR-ABL levels were measured approximately monthly by RQ-PCR. A 1-log reduction from the standardized baseline corresponds to a major cytogenetic response. Mutations were detected by direct sequencing. *Results.* A) Eight patients with advanced phase CML or Ph+ALL received between 1 to 8 months of BMS-354825. Three patients had detectable mutations prior to commencing BMS-354825 and one had the T315I mutation. This patient came off study after one month due to lack of a response. All other patients achieved a 1 to 3-log reduction of BCR-ABL within 3 months. In 6 of these 7 patients the T315I mutation became detectable at the last 2 analysis timepoints (at 2 to 7 months of BMS-354825). Three of these patients had a rise in BCR-ABL of greater than 2-fold and hematological relapse. The BCR-ABL levels remained low in the remaining 3 patients with the T315I mutation. B) Fifteen patients with chronic phase CML had a median of 8 months follow up since commencing BMS-354825 (range 3 to 15 months). Thirteen of the 15 patients had detectable mutations prior to commencing BMS-354825 and one patient had the T315I mutation. This patient came off study after 3 months. At 3 months, 5 patients achieved at least a 1-log reduction of BCR-

ABL. This number increased to 7 patients with longer follow up. At the last analysis, 2 patients had achieved a 3-log reduction and one of these patients had a mutation (S417Y) prior to commencing BMS-354825. The mutations have remained the predominant sequence in 12 of the 13 patients with mutations that were detectable prior to commencing BMS-354825. The T315I mutation has not become detectable in any chronic phase patient. *Conclusions.* Substantial reductions of BCR-ABL were evident in the majority of imatinib-resistant patients treated with BMS-354825. In almost all patients with mutations prior to commencing BMS-354825, the mutation remained the predominant sequence. The T315I mutation has only evolved in advanced phase patients.

0117

BCR-ABL RNA LEVELS AT THE TIME OF A COMPLETE CYTOGENETIC RESPONSE (CCR) PREDICT THE DURATION OF CCR IN IMATINIB-TREATED CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. Most patients with chronic phase CML who receive imatinib achieve complete cytogenetic remission (CCR) and low levels of BCR-ABL transcripts. CCR is durable in the majority of patients, but relapse occurs in a subset. To determine the potential of quantitative RT-PCR (qPCR) of BCR-ABL to predict cytogenetic relapse, we serially monitored residual disease in 97 CML patients with an imatinib-induced CCR. *Methods.* and patients: mRNA was prepared from total nucleated cells from blood or bone marrow, and cDNA was synthesized using random hexamer primers. Relative BCR-ABL expression was measured by real-time quantitative PCR normalized for β 2-Microglobulin (β 2M). The lowest level of detectability of the method is 0,00001. Patients with late chronic phase CML after IFN- α failure were treated with imatinib 400 mg daily. During the imatinib follow-up time of 30 months (median), disease monitoring occurred by cytogenetics and qPCR. The CCR was achieved after 6 months (median) therapy of imatinib therapy. *Results.* At the time of first achieving CCR, BCR-ABL RNA levels had decreased by a median of 2 logs below the median baseline level. During further follow-up, 19 patients (20%) experienced cytogenetic relapse (defined as any Ph-positive metaphase cell) at a median 18 months after CCR and a median 24 months after starting imatinib. There was no difference in the imatinib treatment time, the time to achieve CCR, or the post-CCR follow-up period between the patients with and without subsequent cytogenetic progression. The reduction of BCR-ABL transcript level at the time of first achieving CCR was significantly less in those patients with a subsequent cytogenetic relapse (median 1 log) compared to those with a sustained CCR (median 2 logs) ($p=0,0051$). In the 78 patients with a sustained CCR, the molecular response progressively improved over time to reach a median reduction of 3 logs at 30 months (median) after starting imatinib. Of the 19 patients achieving at least a 2-log reduction of BCR-ABL RNA at the time of first reaching CCR, only 3 (16%) had a subsequent cytogenetic relapse. In comparison, 12 of 37 patients (32%) with less than 2 log reduction at the time of achieving CCR had a subsequent cytogenetic relapse. *Conclusions.* We conclude that, in the majority of imatinib-treated CML patients reaching CCR, the level of BCR-ABL RNA at the time that the CCR is first achieved is a sensitive predictor of the durability of the CCR.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2004, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L.

0118**LONG TERM SIGNIFICANCE OF ACHIEVING A MAJOR MOLECULAR RESPONSE FOR FIRST AND SECOND LINE IMATINIB TREATED CHRONIC PHASE PATIENTS WITH CML ENTERED IN THE IRIS STUDY**T. Hughes¹, J. Radich², B. Kiese³, C. So³, S. Branford¹, A. Hochhaus⁴, J. Kaeda⁵, J. Goldman⁵¹IMVS, Adelaide, Australia; ²Fred Hutchinson Cancer Center, Seattle, USA; ³Novartis Pharmaceuticals, Basel, Switzerland; ⁴Klin Med der Uni Heidelberg, Mannheim, Germany; ⁵Hammersmith Hospital, London, UK

Background. The IRIS study established that chronic phase CML patients treated with imatinib as first line therapy had a low probability of disease progression in the short term if they achieved a major molecular response (MMR) by 12 months. The study did not establish whether patients who crossed over to imatinib after IFN/Ara-C failure or intolerance also had a low probability of disease progression if a MMR was achieved. **Aims.** The longer term value of molecular response as a predictor of progression free survival and duration of complete cytogenetic response (CCR) in imatinib-treated first line as well as second line CML patients was determined. **Methods. and Results.** 1106 patients with chronic phase CML were randomized to receive either imatinib (400 mg/day) or IFN/Ara-C. 64% crossed over from IFN/Ara-C to imatinib after varying periods of their initial treatment (second line imatinib group). Patients who achieved a CCR (equivalent to a 2 log reduction in BCR-ABL) were monitored serially by real-time quantitative PCR for BCR-ABL transcripts. A standardised protocol coordinated between 3 laboratories used BCR transcripts as an internal control; results for individual patients were compared to a median value for untreated patients and expressed as a ratio of BCR-ABL/BCR transcripts on a log scale. A >3 log reduction in BCR-ABL levels from the standardised baseline was defined as MMR. By 12 and 24 months of imatinib therapy, 40% and 55% of first line patients achieved a MMR respectively. Patients were classified by the degree of transcript reduction after 12 months of imatinib. The estimated progression free survival after 42 months of imatinib therapy for those who had achieved (1) no CCR, (2) CCR but <3 log reduction of BCR-ABL, and (3) >3 log reduction of BCR-ABL was 75%, 90% and 98% respectively (p<0.001). The probability of remaining free of accelerated phase or blast crisis at 42 months was 91%, 95% and 100% respectively in these three groups (p=0.001). The probability of remaining in CCR at 42 months was 96% for patients with >3 log reduction of BCR-ABL at 12 months versus 88% for those in CCR but <3 log reduction. For patients receiving imatinib as second line therapy, 31% achieved >3 log reduction after 12 months of imatinib therapy. The estimated progression free survival at 39 months from the start of imatinib for those who had achieved (1) no CCR, (2) CCR but <3 log reduction of BCR-ABL, and (3) >3 log reduction of BCR-ABL was 86%, 90% and 100% respectively (p=0.24). Probability of remaining in CCR at 30 months in second line patients was 100% for patients with >3 log reduction at 12 months and 84% for those in CCR but <3 log reduction (p=0.09). **Summary.** With more than 3 years of follow up, first and second line imatinib treated patients who achieved MMR by 12 months have maintained a very high progression free survival and have rarely lost CCR. Achieving a MMR should be the initial therapeutic target for first and second line imatinib recipients.

0119**SENSITIVE DETECTION OF CLONES HARBORING BCR-ABL MUTATIONS PRIOR TO HEMATOLOGIC RELAPSE IN CML PATIENTS ON IMATINIB THERAPY BY D-HPLC**

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The advent of the selective BCR-ABL tyrosine kinase inhibitor imatinib mesylate (Gleevec®) has substantially changed

treatment of chronic myelogenous leukemia (CML). However, mutations of the BCR-ABL tyrosine kinase domain constitute the leading cause of resistance in CML patients on imatinib monotherapy. The impact of individual mutations on the inhibition of BCR-ABL activity and cell proliferation by imatinib is highly variable. We sought to investigate the dynamics of the mutated clones in 52 CML patients (23 chronic phase, CP, 18 accelerated phase, AP, ten myeloid and one lymphoid blast crisis, BC) with hematologic relapse associated with BCR-ABL mutations applying sequential sensitive denaturing-high performance liquid chromatography (D-HPLC; WAVE technology, Transgenomic, Omaha, NE, USA). This method is based on the formation of heteroduplexes from normal and mutated BCR-ABL sequences which exhibit a change of the elution profiles compared to homoduplexes. Twenty-three different mutations affecting 20 amino acids of the BCR-ABL kinase domain were revealed by direct sequencing at the time of hematologic relapse. In five patients, two different mutations were observed in parallel. D-HPLC was optimized to detect 0.1-0.5% BaF3BCR-ABL cells harboring various mutations in a background of normal BaF3BCR-ABL cells. In comparison, the detection limit for a point mutation by conventional sequencing was 10%. The mutated clone was tracked back towards start of imatinib therapy by D-HPLC in a total of 382 peripheral blood samples collected every three months. Hematologic relapse occurred at a median of 11.9 months (range 0.9-43.7) after start of imatinib therapy. However, BCR-ABL mutations became first detectable at a median of 5.8 months (range 0-25.0) after commencing imatinib. Eleven patients (21%) showed evidence for BCR-ABL mutations even before imatinib was administered (T315I, n=4; M244V; M351T, n=2 each; K247R; Y253H; L324Q, n=1 each). These patients were in early CP, n=1; CP after prior therapy with IFN, n=5; AP, n=3; my. BC and ly. BC, n=1 each; prior to imatinib therapy. Mutations were first detectable by D-HPLC at a median of 6.6 months (range 0-41.4) before hematologic relapse occurred. In order to determine the predictive value of minor clones harboring mutations for consecutive relapses, samples of 18 CML patients in CP in continuous complete cytogenetic response after imatinib monotherapy for more than two years were screened for BCR-ABL mutations by D-HPLC before and six months after commencing imatinib therapy. No mutations were detected in any of these samples. We conclude that (i) D-HPLC is a reliable and sensitive method to screen for BCR-ABL mutations before and during therapy with tyrosine kinase inhibitors; (ii) the observation of BCR-ABL kinase domain mutations during imatinib therapy is predictive for hematologic relapse; (iii) mutations may be detectable several months before hematologic relapse, and (iv) early detection of mutations could provide clinical benefit by allowing early intervention such as dose escalation, combination therapy, or administration of second-generation tyrosine kinase inhibitors.

0120**SIMULTANEOUS DETECTION OF MULTIPLE BCR-ABL MUTATIONS USING RFMP (RESTRICTION FRAGMENT MASS POLYMORPHISM)**E.H. Lee¹, H.R. Moon¹, S.P. Hong², S.O. Kim², W.D. You², S.Y. Park³¹Green Cross Reference Laboratory, Yongin Gyunggi-Do, South-Korea; ²GeneMatrix Inc., Seoul, South-Korea; ³Seoul National University Hospital, Seoul, South-Korea

Background. Imatinib (Gleevec) is a very effective therapy for BCR-ABL positive chronic myeloid leukemia (CML) patients. However, resistance has been reported, particularly in patients with advanced-stage CML or Ph-positive acute lymphoblastic leukemia (ALL). Point mutations have been detected in ATP-binding domain of ABL gene which disturbs the binding of Gleevec to this target leading to resistance. **Aims.** We established a very sensitive and effective RFMP method to detect eleven point mutations (Y253H, Y253F, E255K, E255V, T315I, M351T, F317L, E355G, F359V, H359P, H356R) simultaneously. **Methods.** We evaluated 15 patients with hematologic and cytogenetic resistance on imatinib therapy at 400 mg orally daily for the

detection of BCR-ABL kinase domain mutations by RFMP of 12 CML and 3 ALL patients. We used a reverse transcription-polymerase chain reaction (RT-PCR) strategy to amplify the ABL kinase domain of BCR-ABL, and then fragmented those PCR products by restriction enzymes, Fok I, Bst F5I. After fragmentation, we identified eleven kinds of point mutation using MALDI-TOF mass spectrophotometry by each fragment DNA molecular weight. **Results.** Six of 15 patients with imatinib resistance showed point mutations, and one of these 6 patients was primary resistance (E255V) and the remaining 5 patients were secondary resistance (Y253H, E255K, T315I, M351T present in 2, 1, 1, and 1). **Conclusions.** Using RFMP, we were able to detect 11 point mutations at one time. Since high-dose imatinib is suggested another therapy to overcome disease-poor response to conventional doses and the response to the high-dose imatinib is different according to point mutation type, simultaneous multiple mutation detection assay using RFMP will be of great utilization in clinical management of imatinib resistance. Furthermore, this RFMP can measure the relative amount of wild and mutant type, so that the early detection of point mutation is possible before a resistance mutant type becomes dominant.

Conditioning for allogeneic transplantation

0121

SUCCESSFUL ENGRAFTMENT FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION IN VERY HIGH-RISK PATIENTS WITH BUSULFAN AS A SINGLE AGENT

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Background. Busulfan is the most commonly used myeloablative alkylating agent, but it is usually considered as a poor anti-lymphocyte agent. In addition to adequate immunosuppression, engraftment of allogeneic stem cells depends also on successful hematopoietic competition. Moreover, residual lymphocytes of host origin may play a beneficial role in vetoing graft-versus-host disease (GVHD). **Aims.** Considering those facts we have decided to apply the use of de-escalated doses of oral busulfan as a single agent for conditioning of stem cell transplantation (SCT) in recipients of matched related, matched unrelated donor or partially mismatched family members. **Methods.** Fifteen patients (age 25-66, median 42 years) with hematological malignancies were conditioned with busulfan alone, 4 mg/kg/day for 2, 3, or 4 consecutive days. No additional pre- or post-transplant immunosuppressive agents were used. **Results.** Conditioning was well tolerated and 3-lineage engraftment was documented in all patients and none exhibited immune-mediated rejection. Time to recovery of absolute neutrophil count (ANC) $>0.5 \times 10^9/L$ and $1.0 \times 10^9/L$ was 12-38 (median 15) days and 12-41 (median 15) days, respectively. The time interval to platelet recovery >20 and $>50 \times 10^9/L$ ranged from 0 to 26 (median 11) days, and from 0 to 83 (median 14) days, respectively. Surprisingly, moderate or severe hepatic veno-occlusive disease (VOD) did not occur in any of the patients. **Summary:** Our data suggest that using busulfan alone for preparation of patients for stem cell transplantation (SCT) may be sufficient for engraftment, thus providing an alternative low-cost conditioning for successful SCT for high risk patients.

0122

MORBIDITY AND MORTALITY WITH NONMYELOABLATIVE COMPARED TO MYELOABLATIVE CONDITIONING BEFORE RELATED STEM CELL TRANSPLANTATION

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Background. Nonmyeloablative regimens for allogeneic hematopoietic cell transplantation (HCT) have been developed for patients ineligible for myeloablative conditioning to reduce the regimen-related toxicities (RRT) and non-relapse mortality (NRM). **Aims and Methods.** In this retrospective analysis we describe our results after the first HCT among patients with acute myeloid leukemia and chronic myeloid leukemia after reduced intensity conditioning (RIC), we compare RRT and NRM with balanced group of patients after myeloablative conditioning (MC). The RIC and MC groups were balanced on diagnosis and disease stage: AML in complete remission, n=12 vs 14 and CML in the first chronic or accelerated phase n= 16 vs 18. The RIC patients significantly differed from MC patients in median age (53.5 years vs 37.5 years). The reduced intensity regimen consisted of fludarabine (30 mg/m²/d; 5 days), busulfan (total dose 8-12 mg/kg), and ATG Fresenius (10 mg/kg/d; 4 days), post-transplant immunosuppression was mostly cyclosporine alone.

	Reduced intensity conditioning	Myeloablative conditioning	p Binomic test
AML/CML	48% (n=12) 52% (n=16)	44% (n=14) 56% (n=18)	NS
Median follow-up (days)	841	589	□
Pts. without any RRT	32%	6%	0.0116
Pts. with RRT gr.1	50%	22%	0.0271
Pts. with RRT gr.2-4	18%	72%	0.0001
Mortality/non-relapse mortality	14%/3.5%	37.5%/19%	0.0442/NS
Early/Late TRM	3.5%/3.5%	12%/6%	NS/NS
Complete chimerism achieved	86%	72%	NS
CML pts. with molecular remission	94%	84%	NS

The conventional regimen was busulfan (total dose 14-16 mg/kg) cyclophosphamide (120 mg/kg) followed by posttransplant cyclosporine and methotrexate. Both groups have mostly peripheral blood mononuclear cells as stem cell source from related donor. RRT were graded using Seattle toxicity criteria. **Results.** The RIC vs MC group had median follow up 841 vs 589 days. Nine pts (32%) vs two pts (6%) didn't have any RRT, severe RRT (gr 2-4) was in 5 pts (18%) vs 23 pts (72%), the highest grade of toxicity in RIC group was 3 in two pts (7%) vs MC group which had two pts (6%) with RRT gr 4. Four pts (14%) didn't achieve complete chimerism (CC) (3 AML with progression, one CML day+ 133 after SCT) vs 9 pts (28%) (6 AML, 3CML; 8 of them because progression or they died, one of them: AML day+219 after SCT who was in time of follow up in complete remission). Median time to achievement CC was 91 vs 95 days. Median time to achievement molecular remission (MR) for pts with CML was 216 days and one pts didn't reach MR (he was in time of follow up 133 days after SCT) in RIC group vs 42 days in MC group, where 3 pts died before assessment of MR. Six patients with AML (50%) relapsed in median 383 days and 3 of them died in RIC group vs 6 pts with AML (42%) relapsed of their disease in median 209.5 days and all of them died in MC group. In the first group 4 pts. with CML

(25%) relapsed in median 1030.5 days, each of them reached new MR, but one of them died for infection short time after MR. In the second group nobody with CML relapsed. Mortality from any cause was 14% vs 37.5%, NRM was 3.5% vs 19%, early TRM was 3.5% for progression vs 12.5%, late TRM was 3.5% vs 6%. *Conclusions:* Reduced-intensity conditioning containing fludarabine, busulfan, ATG is well tolerated, with lower RRT, NRM, early and late TRM than conventional myeloablative regimens. Relapses of AML were similar in both group, relapses of CML were higher in RIC group.

0123

A MODIFIED MYELOABLATIVE REGIMEN COMBINING FLUDARABINE AND ABLATIVE DOSES OF INTRAVENOUS BUSULFAN IN PATIENTS WITH AML/MDS NOT ELIGIBLE FOR STANDARD CONDITIONING: MYELOABLATION WITH REDUCED TOXICITY

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Allogeneic stem cell transplantation (SCT) is a potentially curative approach in patients (pts) with AML and MDS, however it is associated with relatively high risks of morbidity and mortality, especially in elderly and medically infirm pts. Reduced-intensity conditioning (RIC) has allowed extension of allogeneic SCT to these pts, but has a limited role in active and refractory leukemia. Intravenous busulfan (Busulfex, ivBu) was shown to reduce treatment-related mortality (TRM) and improve outcome after standard ablative conditioning (ivBuCy). In this study we evaluated the feasibility and outcome of SCT using a modified myeloablative regimen consisting of fludarabine (160 mg/m²) and myeloablative doses of ivBu (12.8 mg/kg) in pts not eligible for standard myeloablative conditioning (n=20) and compared SCT outcomes to standard RIC consisting of fludarabine (150 mg/m²) and reduced doses of ivBu (6.4 mg/kg; n=36). The median age of recipients of modified myeloablation was 52 years (range, 18-64), 8 pts were over 55 years and 4 over 60 years at SCT. The donor was a matched sibling (n=8) or matched unrelated (MUD, n=12). Pts were considered not to be good candidates for standard myeloablative conditioning because of advanced age (>55 for sibling SCT and > 50 for MUD; n=15), poor performance (n=2), extensive prior therapy, organ dysfunction or active fungal infection (n=1, each). Disease status at SCT was high-risk CR1 (n=4), induction failure (n=7), CR2 (n=1), refractory relapse (n=3) or previously untreated (n=5). Overall, 14 pts had active disease with more than 10% marrow blasts at SCT. With a median follow-up of 1 year, 11 pts are alive and 9 pts have died; 7 of disease relapse and 2 of acute GVHD. There was no regimen-related mortality. The estimated 1-year overall (OS) and disease-free survival (DFS) were 55% (95%CI, 32-79) and 45% (95%CI, 14-72), respectively. The 1-year cumulative incidence of TRM and relapse was 11% (3-41) and 36% (19-69), respectively. The pts in the RIC group had similar criteria for exclusion of myeloablative conditioning. OS, DFS, TRM and relapse rates were 55% (27-83), 51% (33-69), 8% (3-25), 42 (28-63), all not significantly different from the modified ablative regimen. However, when we analyzed the subgroup of pts with active disease (>10% marrow blasts) at SCT, the OS was 49% (20-79) after myeloablative ivBu compared to 14% (0-20) after reduced ivBu (p=0.07). The difference was related to increased relapse rate with the later regimen (71% compared with 43%) while TRM rates were similar. Among the subgroup of pts in remission at SCT, OS was higher in the RIC regimen (79% Vs 60%), not reaching statistical significance. In conclusion, pts considered ineligible for standard myeloablative conditioning tolerated the modified ablative regimen consisting of fludarabine and high-dose intravenous busulfan, relatively well, with low TRM, similar to that of pts conditioned with RIC regimens. RIC had a limited role in pts with refractory or active leukemia at the time of SCT, yet a significant fraction of these pts could be salvaged with the modified myeloablative regimen. These observations need further confirmation in a larger comparative trial.

0124

EQUALLY HIGH CURE RATES IN OLD AND YOUNG PATIENTS (18-74 YEARS) WITH AML OR MDS BY ALLOGENEIC TRANSPLANTATION FROM UNRELATED OR RELATED DONORS AND ALKYLATOR-BASED AGE ADAPTED CONDITIONING REGIMEN

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Background. The upper age limit for allogeneic haematopoietic cell transplantation (HCT) used to be in the range of 50 to 55 years, well below the age with the highest incidence of myeloid malignancies. *Aims.* Here we present data on 177 adult patients (pts) with AML or MDS (median age 44; range 18-74) transplanted from a matched related (n=68) or unrelated (n=109) donor. *Methods.* All pts were either conditioned with standard busulfan 16 mg/kg / cyclophosphamid 120 mg/kg (up to age 50) or with the uniform, dose-adapted, myeloablative conditioning FBM regimen consisting of fludarabine 150mg/m², carmustine 300 mg/m² and melphalan 110mg/m² for older pts. GvHD prophylaxis consisted of cyclosporine A and Mtx or mycophenolate mofetil with additional rabbit anti-T lymphocyte globulin (ATG) in the unrelated setting. Graft source was marrow in 47 pts and filgrastim mobilized peripheral blood in 130 pts. Remission at transplant was CR1 in 53 pts, and advanced disease in 124 pts (CR2: 14; primary refractory: 33, untreated: 33; relapsing disease: 44). *Results.* After a median follow up of 520 days (range 3-3245 days), the estimated probability of 2 year overall survival (OS) was 60% and 53% for pts in CR1 and advanced disease, respectively. In the advanced disease group no difference in OS was seen when comparing related with unrelated donor transplantation or comparing pts younger with those older than 50 years (<50 years (18-50): 2 and 5 year OS: 53% and 43%; >50 years (51-74): 53% and 43%). *Conclusions.* We conclude that HCT using the dose-adapted FBM protocol is a reasonable treatment option for elderly pts with active advanced myeloid malignancies resulting in outcome as good as in young adults conditioned with Bu/Cy. HCT from unrelated donors results in outcomes comparable to HCT from related donors. Allogeneic HCT in patients 50 to 70 years is indicated in a similar way as in younger patients.

0125

RESULTS OF ALEMTUZUMAB-BASED REDUCED-INTENSITY ALLOGENEIC TRANSPLANTATION FOR ADVANCED CHRONIC LYMPHOBLASTIC LEUKEMIA

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Background. Although the course of chronic lymphocytic leukaemia (CLL) is extremely variable, patients with advanced disease have a median survival of 24-72 months with conventional chemotherapy. Prolonged remissions have been achieved with allogeneic haematopoietic cell transplantation (HCT), but this procedure is only suitable in a small proportion of patients. In an effort to reduce transplant-related mortality, several reduced-intensity conditioning (RIC) regimens have been developed. The UK Collaborative Group's protocol incorporates alemtuzumab in order to allow engraftment without excessive regimen toxicity or graft-vs-host disease (GVHD). In addition, alemtuzumab has proven activity in progressive CLL, even if refractory to fludarabine, and therefore has added value in this setting. *Aims.* To assess the feasibility and efficacy of an alemtuzumab-based RIC regimen in patients with advanced CLL. *Methods.* We describe 14 consecutive CLL patients conditioned with fludarabine 150 mg/m², melphalan 140 mg/m² and alemtuzumab 100 mg. Age at transplant ranged from 37 and 58 years (median 52 years) and median time from diagnosis was 54 months (range 13-109 months). Pre-transplant treatment was heterogeneous with a median of 2 (range 1-5) prior chemotherapy regimens. Six patients (43%) were refractory to fludarabine and in 2 others (14%) it had to be stopped due to autoimmune haemolytic anaemia. Also, 5 patients (36%) had relapsed fol-

lowing a previous autologous HCT. At the time of transplant, 3 patients (21%) had chemorefractory disease and 11 patients (79%) had some response to their last chemotherapy course, but only 2 of them were in complete remission (CR). Donors were 10 HLA-matched siblings and 4 unrelated volunteers (2 of them mismatched). Median follow-up was 15 months (range 2.5-62.5). **Results.** All patients had initial haematopoietic recovery except 1 who had refractory immune thrombocytopenia pre-transplant. Median intervals to neutrophil ($> 0.5 \times 10^9/L$) and platelet ($> 20 \times 10^9/L$) recovery were 16 (range 12-27) and 12 (range 9-89) days, respectively. Five patients (36%) relapsed post-transplant and received escalated donor lymphocyte infusions, but only 1 of them had a sustained response. Three more patients (21%) had delayed graft rejection that responded to a second stem cell infusion. Grade I-III acute GVHD and limited chronic GVHD were observed in only 5 (36%) and 3 (21%) patients, respectively. Four patients had CMV reactivation out of 8 patients at risk (50%). Five patients (36%) have died, 1 of progressive disease and 4 in CR. Causes of death in these 4 patients were post-transplant lymphoproliferative disorder, grade III GVHD + fungal pneumonia, RSV pneumonia and ARDS. Therefore, 4-year overall survival and non-relapse mortality were 59% (95% IC 45%-73%) and 31% (18%-44%), respectively. **Conclusions.** Our alemtuzumab-based RIC regimen is feasible and effective in patients with poor-risk CLL with a relatively low rate of GVHD. However, non-relapse mortality remains relatively high as a result of a variety of viral and fungal infections. Ongoing studies are trying to address the efficacy of reduced doses of alemtuzumab in this group of very immunosuppressed patients.

0126

INTRAVENOUS VERSUS ORAL BUSULFAN AS PART OF BUSULFAN/THIOTEPA/CYCLOPHOSPHAMIDE PREPARATIVE REGIMEN FOR ALLOGENEIC STEM CELL TRANSPLANTATION: DECREASED INCIDENCE OF NON-HEPATIC REGIMEN RELATED TOXICITY

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Some studies have suggested that the better pharmacokinetics profile of intravenous busulfan (IV-BU) rather than oral BU results in a lower regimen-related toxicity (RRT) and transplant-related mortality (TRM) after conditioning with BU and cyclophosphamide (CY). The purpose of this retrospective comparison was to evaluate veno-occlusive disease of the liver (VOD), non-hepatic regimen related toxicity (RRT), and 100-day treatment related mortality (TRM100) in patients (pts) undergoing allogeneic stem cell transplantation (allo-SCT) after conditioning with Bu plus cyclophosphamide (Cy) and thiotepa (TT) in which either oral or IV Bu was administered. Thirty-one patients (23 men, 8 women; median age 32 years) who underwent alloSCT between November 2002 and June 2004 were included. Conditioning regimen consisted of TT 5 mg/kg/d on days -9 and -8; IV-BU 0.8 mg/kg every 6 hs on days -7 to -5 and CY 60 mg/kg/d on days -3 and -2. RRT was graded according to the scale of Bearman et al. Veno-occlusive disease (VOD) of the liver was diagnosed according to the Jones criteria, and its severity was graded according to the McDonald criteria. **Results.** were compared with a group of historical controls matched (1:2) for age, type of transplant (related vs. unrelated donor), and disease status at transplant. Controls received the same infectious prophylaxis, supportive care, and conditioning regimen except for the use of oral BU (total dose: 12 mg/kg). A total of 93 patients (31 cases and 62 controls) were included in the study. Significant differences between the two groups were found for the median year of transplant (2003 vs. 2000; $p < 0.001$) and for the use of MTX for GVHD prophylaxis (36% vs. 13%; $p = 0.01$). The rate of VOD was 4/31 (13%) and 5/62 (8%) after IV and oral BU respectively ($p = NS$). Severe VOD was observed in 1 (3%) and 3 (5%) patients in the IV and oral group respectively. Patients receiving oral BU experienced a higher rate of grade

2 or higher mucositis (61% vs. 41%; $p = 0.07$) and non-hepatic RRT (77% vs. 48%; $P = 0.005$), as well as a higher incidence of acute GVHD grade 2 or higher (55% vs. 37%; $p = 0.08$). Overall TRM100 was 16% and 27% after IV and oral BU respectively ($p = 0.2$). **Conclusions.** These results show that the use of IV-BU reduces non-hepatic RRT of a conditioning regimen consisting of BU, CY, and TT and suggest that IV-BU should replace oral BU for patients receiving that conditioning regimen prior to alloSCT.

0127

HIGH TRANSPLANT RELATED MORTALITY IN HEAVILY PRETREATED ACUTE LYMPHOBLASTIC LEUKAEMIA PATIENTS

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The role of allogeneic hematopoietic stem cell transplantation (HSCT) in acute lymphoblastic leukaemia (ALL) is still controversial because of its doubtful risk/benefit ratio, and the main policy is to reserve it for high-risk or advanced patients (pts.). To evaluate the efficacy and the mortality of HSCT, we analysed the results of 79 consecutive pts. affected by ALL referred to our BMT Unit for transplant from March 1989 to October 2004. 49 were male, 30 female; median age was 29 years (range 16-54). 53 pts. had B-lineage ALL, 26 had T-lineage ALL. The Philadelphia chromosome (Ph) was present in 20 pts. (25%). According to the German ALL risk classification, 28 pts. were classified as standard risk, 31 high risk and 20 very high risk. 43 pts. (54%) received transplantation in $> CR1$; 35 pts. (44%) received more than 3 cycles of chemotherapy before the transplant procedure. The donor was in 52 patients an HLA-related identical sibling (IS) and in 27 pts. an unrelated matched donor (UD). Stem cell source was in the majority bone marrow (82%). The conditioning regimen was iTBI 1320 cGy and CY120 (addition of VP-16 50 mg/mq in 7 advanced IS pts.). GvHD prophylaxis was CYA/MTX; in UD transplant group ATG was added. As regard the disease status, pts. in $> CR1$ were 27 (51%) in the IS group and 16 (59%) in the UD group; 19 (37%) and 16 pts. (59%) had received more than 3 cycles respectively in the IS and in the UD group. With a median follow-up of 12 months (range 1-191), the event free survival at 3 years (EFS) was 37% (median 7 months). The EFS for IS was 43% (median 12 months) and for UD 26% (median 6 months) ($p = 0.04$). The overall TRM was 23%; 12% in IS transplant vs 44% in UD ($p = 0.0001$); according to disease status, TRM was 11% for pts. transplanted in first CR vs 33% for pts. in more advanced phase of disease ($p = .04$). A significant difference in term of TRM was also documented in pts. that received more than 3 courses of chemotherapy compared to the others (43% vs 7%) ($p = 0.0007$). In multivariate analysis, the factors influencing TRM were the number of chemotherapy courses ($p = .007$; H.R.: 12.6; CI 95%: 2.07-79.29) and the type of transplant ($p = .02$; H.R. 5.3; CI 95%: 1.25-23.08) while the disease status at transplant was not significant ($p = 0.9$; H.R. 0.92; CI 95%: 0.15-5.61). In our experience, in order to reduce TRM myeloablative allogeneic transplantation, particularly in the unrelated setting, should be performed, when possible, in patients not heavily pretreated.

0128

REDUCED-INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FROM MATCHED RELATED AND UNRELATED DONORS IN RELAPSED AND REFRACTORY HODGKIN'S DISEASE

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Background. Allogeneic stem cell transplantation (allo-SCT) with myeloablative conditioning is associated with a prohibitive transplant-related mortality in relapsed/refractory

Hodgkin's disease (HD). *Aims.* To assess feasibility of employing reduced-intensity conditioning (RIC) allo-SCT in relapsed/refractory HD. *Methods.* Forty patients with relapsed/refractory HD underwent allo-SCT following a fludarabine-based conditioning regimen from an HLA-identical sibling (n=20) or a matched unrelated donor (n=20). The median age was 31 years (range 18-58). Disease status at allo-SCT was refractory relapse (n=14) or sensitive relapse (n=26). The median number of chemotherapy regimens received prior to allo-SCT was five (range 2-9). Thirty (75%) and thirty (75%) patients had received prior radiotherapy or a prior autologous SCT, respectively. The conditioning regimens employed were fludarabine (25 mg/m² sq IV x 5 days)-cyclophosphamide (1 g/m² sq IV x 3 days)±antithymocyte globulin (30 mg/kg IV x 3 days) (FC±ATG) (n=14), a less intensive regimen, and fludarabine (25 mg/m² sq IV x 5 days)–melfalan (70 mg/m² sq IV x 2 days) (FM) (n=26), a more intensive one. The two groups had similar demographics and prognostic factors. *Results.* The median time to neutrophil recovery (i.e. absolute neutrophil count ≥ 500/mL) was 12 days (range 10-24). The median time to platelet recovery (i.e. platelet count ≥ 20,000/mL) was 17 days (range 7-132). Chimerism studies indicated 100% donor-derived engraftment in 26/26 (100%) FM patients and in 9/13 (69%) evaluable FC±ATG patients. Day 100 and cumulative (18-month) transplant-related mortality (TRM) were 5% and 22%, respectively. The incidence of acute (grade II-IV) GVHD was 38%. The incidence of chronic GVHD at 18 months was 69%. There was a trend for a lower relapse rate after the occurrence of GVHD (hazard ratio 0.8; *p*=0.6). Twenty-four patients (60%) are alive (fourteen in complete remission) with a median follow-up of 13 months (4-78). Sixteen patients expired (TRM n=8, disease progression n=8). FM patients had significantly better overall survival (73% vs. 39% at 18 months; *p*=0.03), and a trend towards better progression-free survival (37% vs. 21% at 18 months; *p*=0.2). *Summary/conclusions:* RIC allo-SCT from matched related and unrelated donors is feasible in relapsed/refractory HD patients with a low TRM. The intensity of the preparative regimen affects survival.

0129

SINGLE CENTRE COMPARISON OF INTRAVENOUS VS ORAL BUSULPHAN AS PART OF FLUDARABINE-BUSULPHAN-CAMPATH (ALEMTUZUMAB) REDUCED INTENSITY CONDITIONING (RIC) HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Background. Busulphan has been extensively used as a conditioning agent in allogeneic HSCT. Busulphan has a narrow therapeutic window, with increased levels associated with a significant risk of hepatic veno-occlusive disease (HVOD) and early mortality. Lower doses have been associated with graft rejection and disease relapse. Intravenous (IV) busulphan has reported improved bioavailability with lower variability of plasma levels compared with oral busulphan. *Aims.* To evaluate the impact of the use of oral vs IV busulphan as part of RIC HSCT on toxicity as well as engraftment. *Methods.* Single center retrospective review was performed of 44 patients receiving fludarabine (30 mg/m² x 5 days), alemtuzumab (20 mg x 5 days) and either oral busulphan (4 mg/kg x 2 days) or IV busulphan (3.2 mg/kg x 2 days). 22 patients received IV busulphan from Feb-Dec 2004. A comparative group of 22 patients receiving oral busulphan between Jul 2003-Aug 2004 was selected. All patients were being treated for myeloid malignancies: AML-23, MDS-11, CML-4, CMML-4, MF-2. No patients had a previous history of hepatic impairment. Median age was 53.6 years (22-72). There were 9 vs 10 sib matched HSCT, 10 vs 9 matched VUD HSCT and 3 vs 3 1-major antigen mismatch VUD HSCT in oral vs IV busulphan group. Both groups were closely matched for diagnosis, age and all patients were in complete remission/good partial remission

at HSCT. Median follow-up was 266 days vs 146 days in the oral vs IV groups respectively. In the oral group 9/22 (40.9%) patients were classified as heavily pre-treated (3 or more prior intensive chemotherapy/prior HSCT) vs 11/22 (50%) in the IV group. 3 patients (oral) and 1 patient (IV) received previous Myelotarg. *Results.* 21/22 (95%) of the oral busulphan group received PBSC with a median CD34 dose of 4.12 (0.7-14.6) x 10⁶/kg compared with 18/22 (81%) of the IV busulphan group with a CD34 dose of 5.69 (1.0-13.9) x 10⁶/kg. Median time to neutrophil (>0.5x10⁹/kg) and platelet (>20x10⁹/kg) regeneration was 13.0 vs 13.8 days, 13.3 vs 15.4 days in the oral and IV groups respectively. 22/22 patients in the oral group and 21/22 patients in the IV group had donor engraftment. 1 patient receiving IV busulphan failed to engraft. 2 (9%) patients receiving oral and 1 (4.5%) patient receiving IV busulphan developed HVOD. Number of patients achieving full donor chimerism at days 28 and 100 was 14/22 (63.6%) vs 8/22 (36.3%) and 11/19 (57.9%) and 5/14 (35.7%) for the oral and IV groups respectively. At day 100, there were 2 deaths (HVOD/sepsis) and 1 disease relapse in the oral busulphan arm, and 1 death (sepsis) and 4 relapses in the IV busulphan group (all 3 patients with prior HSCT in the IV busulphan group did not relapse). The actuarial 100 day OS and DFS was 90.9% vs 90.9%, and 90.9% vs 70.1% (*p*=0.08) for oral vs IV busulphan. *Summary.* RIC HSCT with both oral and IV busulphan appears to be safe and well tolerated regimen with a low incidence of HVOD. Longer term follow-up is required to assess if there is a difference in outcome using oral vs IV busulphan.

0130

A PREVIOUSLY FAILED AUTOGRAFT SIGNIFICANTLY INFLUENCES NONRELAPSE MORTALITY AND OVERALL SURVIVAL IN PATIENTS OVER 55 YEARS RECEIVING REDUCED-INTENSITY CONDITIONING AND ALLOGENEIC TRANSPLANTATION

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Background. Allogeneic stem cell transplantation (SCT) represents a potentially curative treatment for several hematologic malignancies, but is associated with a relevant nonrelapse mortality (NRM); in particular, older age and a previously failed autograft are considered risk factors associated with a NRM ranging between 50% and 80%. *Aims.* We report the results of a prospective multicenter study investigating the impact of age and previously failed autograft on the NRM of 150 patients affected by hematological neoplasms. *Methods.* Patients received the same reduced intensity conditioning (RIC) (Thiotepa 10mg/Kg, Fludarabine 60 mg/m² and Cyclophosphamide 60 mg/kg and) and GVHD prophylaxis (cyclosporin 2 mg/kg and short course methotrexate). They were divided in two cohorts according to the age: 90 patients were younger and 60 older than 55 years. Pre-transplant characteristics were fairly balanced between the two cohorts. *Results.* At a median follow-up of 927 days, the estimated 5-year OS was 66% for younger and 61% for older patients (*p*=0.25); the estimated 5-year NRM was 13% for younger and 19% for the older cohort (*p*=0.1). On univariate analysis, a statistically significant association was found between a previously failed autograft and higher NRM in the elderly cohort: in fact estimated 5-year NRM was 11% for patients not having failed a previous autograft versus 37% (*p*=0.01). Also older patients with refractory disease had a significantly higher risk of NRM as compared to chemosensitive patients (31% vs 8%, *p*=0.03). In multivariate analysis, a statistically significant interaction was found between classes of age and both disease status (*p*=0.03) and previous autografting

($p=0.02$), indicating that older patients with refractory disease or a failed autograft had a NRM rate higher than younger ones. Moreover the association of elderly age and previously failed autograft had a significantly negative impact on OS ($p=0.002$) if compared with younger patients. A significantly higher NRM was observed in patients developing grade III-IV aGVHD in both cohorts. When the effect of chronic GVHD on NRM was evaluated, we found a difference between limited and extensive cGVHD: it was statistically significant for younger patients (NRM rate of 0% versus 28%, $p=0.03$) while there was a trend in the elderly (NRM rate of 0% versus 31%, $p=0.05$). On univariate analysis the onset of grade III-IV aGVHD represented a significantly negative predictor for OS: 5-year OS was 42% and 21% for young and older patients with grade III-IV aGVHD instead of 60% ($p=0.05$) and 85% ($p<0.001$) for patients experiencing grade I-II aGVHD, respectively. Also chronic GVHD had an impact on survival: in fact in the young group OS was 82% for patients with limited cGVHD compared to 45% for pts. with extensive cGVHD ($p=0.2$); in the elderly OS was 100% vs 33% ($p=0.007$) respectively for patients with limited and extensive cGVHD. Summary. Our results indicate that RIC transplants show a rather low NRM, age above 55 years per se cannot be considered a risk factor anymore. Timing of transplant and novel strategies for GVHD prevention could further improve the patients' outcome.

0131

CLINICAL PREDICTORS OF TRANSPLANT RELATED MORTALITY AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Reduced intensity conditioning regimens and improvements in supportive care have allowed for the expansion of allogeneic stem cell transplantation to those previously ineligible. However, such patients remain at significant risk for regimen related toxicity. **Aims.** We examined whether clinical predictors that influence toxicity after standard chemotherapy, such as age, comorbidity and functional status similarly predict increased transplant related mortality (TRM) and decreased survival after reduced-intensity allogeneic stem cell transplantation (RIST) with *in vivo* T-cell depletion. **Methods.** Patients with high-risk and refractory hematologic malignancies underwent RIST from 2002 to 2004. The conditioning regimen consisted of fludarabine 30 mg/m²/d (Days -7 to -3), alemtuzumab 20 mg/d (Days -7 to -3), and melphalan 140 mg/m² (Day -2) with tacrolimus given for post-transplant immunosuppression. Comorbidity was scored from a retrospective chart review using the Charlson Comorbidity Index (CC) and the Kaplan-Feinstein scale (KF). Eastern Cooperative Oncology Group performance status (PS) and age at transplant were tabulated. TRM was defined as any death occurring without disease progression. **Results.** We analyzed 81 consecutive patients whose median age was 51 years (range 17-68). Fifty-five percent had HLA-identical sibling donors, 5% had mismatched related donors, 35% had matched unrelated donors and 5% had mismatched unrelated donors. KF served as a more sensitive indicator of comorbidity than CC. Fifty-three percent of patients scored at least 1 point for a comorbid condition by KF, as opposed to 25% by CC ($P < 0.001$ by χ^2), and 28% scored at least 2 points by KF versus 8% by CC ($p < 0.001$ by χ^2). PS was 0, 1, and >1 for 61%, 28%, and 11% of patients, respectively. The cumulative incidence of 100 and 180 day TRM was 21% and 30%, respectively, with an overall survival (OS) of 15.4 months. Comorbidity, PS, and age > 50 predicted increased TRM and decreased OS. In a multivariable model incorporating these predictors (using either CC or KF), age and PS were significant for TRM, while comorbidity and PS were significant for OS. **Summary/conclusions:** Simple measures of comorbidity, functional status and age predict adverse outcomes after transplantation for hematologic malignancies,

even with a reduced intensity regimen. Prospective studies could provide a more accurate estimation of regimen tolerability, particularly in older or sicker patients.

Table.

	Transplant-related mortality				Overall survival		
	Incidence*	UHR	UV-P	MV-P	UHR	UV-P	MV-P
Performance Status							
PS=0	0.86	4.2	0.002	0.007	0.78	0.003	0.016
PS>0	0.53						
Co-morbidity							
CC, none	0.76	2.3	0.042	0.089	0.25	0.014	0.03
CC, =1	0.52						
KF, none	0.85	2.7	0.027	0.183	0.74	0.004	0.03
KF, =1	0.56						
Age							
Age < 50	0.80	3.5	0.13	0.47	0.73	0.98	0.34
Age > 50	0.63						

0132

FLUDARABINE, MELPHALAN AND ALEMTUZUMAB CONDITIONING IN ADULTS WITH ADVANCED AML AND MDS: EXCELLENT OUTCOMES IN PATIENTS WITH STANDARD RISK DISEASE

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Background. Allogeneic transplantation is a curative procedure for patients with acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS), but its efficacy is partially offset by a high incidence of treatment-related mortality resulting from the toxicity of pre-transplant conditioning and post-transplant methotrexate, as well as a high incidence of severe or fatal acute and chronic graft-versus-host disease (GVHD). **Aims.** In order to limit such complications, we conducted a prospective study of fludarabine melphalan conditioning and *in-vivo* T-cell depletion with alemtuzumab, followed by post-transplant tacrolimus. **Methods.** Patients with AML or MDS received pre-transplant conditioning consisting of fludarabine (25 mg/m² on Days -6 to -3), alemtuzumab (20 mg on Days -6 to -3), and melphalan (70 mg/m² on Days -3 and -2), followed by allogeneic transplantation with peripheral blood stem cells. Tacrolimus was administered on Day -2 through Day 100, and high-dose valacyclovir was given for cytomegalovirus prophylaxis. **Results.** Here we report the results in 52 consecutive adults with AML and MDS. Median age was 52 (range, 17-71) and the majority of patients had high risk disease, comorbidities, and/or modest reduction in performance status. Fifty-six percent had unrelated or mismatched related donors. After a median follow-up of 18 months (range, 2-34), one year survival was 48% (95% CI, 34-61), progression free survival was 38% (95% CI, 25-52), the relapse rate was 27% (95% CI, 15-40), and treatment related mortality was 33% (95% CI, 20-46). The cumulative probability of extensive chronic GVHD was 18% (95% CI, 8-40); extensive chronic GVHD was only observed in recipients of unrelated donor transplantation. Performance score and disease status were the major predictors of outcome. High risk disease (i.e. active AML or MDS with >5% blasts) or even modest decreases in performance status were associated with poor outcomes. By contrast, patients with standard risk leukemia (in either first or second complete remission) or MDS (with <5% blasts) had excellent outcomes despite unfavorable disease characteristics. **Summary/conclusions:** We conclude that the fludara-

bine melphalan regimen when combined with *in vivo* alemtuzumab is a promising transplant regimen for patients with AML or MDS and low tumor burden. For patients with active disease, this regimen provides, at best, modest palliation. Even with a low incidence of GVHD, transplantation is still associated with considerable non-relapse mortality in patients with decreased performance status.

0133**TOXICITY-REDUCED CONDITIONING WITH DOSE-ESCALATED TROUSOLFAN AND FLUDARABINE PRIOR TO ALLOGENEIC BLOOD STEM CELL TRANSPLANTATION FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)**

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Allogeneic stem cell transplantation is a potentially curative treatment approach for patients with AML. The median age of patients presenting with AML is 60 years, in large transplantation registries the median age of patients transplanted for AML is more than 20 years lower. We have, therefore, developed a toxicity-reduced but full intensity conditioning regimen using treosulfan and fludarabine. We have retrospectively analyzed 50 patients, not eligible for conventional conditioning, with a median age of 55 years (range, 22-71) suffering from primary (n = 32) or secondary AML (n = 18) who were treated with fludarabine 50-30 mg/m² d -6 to d -2 and treosulfan 3 x 10 (n = 15), 3 x 12 (n = 18) or 3 x 14 g/m² (n = 17) d -6 to d -4 prior to allogeneic transplantation. 32 patients (64%) were in 1., 2. or 3. CR, 10 patients (20%) were in PR (1. or 2.), 2 patients suffered from progressive disease and 6 patients (12%) were in relapse (n = 3) or untreated (n = 3). Peripheral blood stem cells (n = 40) or bone marrow (n = 10) were donated by matched related (MRD, n = 19) or unrelated donors (MUD, n = 31). In the case of MUD transplantation additional immunosuppression was applied using ATG, ALG or campath. All patients except one (2%) had a primary engraftment. With a median follow-up of 16 months (range; 1-69) an estimated overall survival (OS) and event-free survival (EFS) of 59% respectively 54% after one year and 50% respectively 49% after three years was observed. After MRD transplantation, a one year OS and EFS of 83% respectively 70% and a three year survival of 70% (OS, EFS) was estimated. MUD transplantation resulted in a one year OS/EFS of 42% respectively 43% and a three year OS/EFS of 35% respectively 36%. Patients transplanted in 1. CR (n = 21) had an OS of 65% and an EFS of 60% after one and three years. The relapse rate of all patients was 27% after one and three years, the non-relapse mortality was 17% at day +100, 24% after one and 30% after three years. Reasons for non-relapse mortality were infections (n = 10, MUD patients only), GvHD (n = 1, MUD) and a myocardial infarction in a MRD patient suffering from a pre-existing severe coronary heart disease. These results compare favorably with published data of younger patients and conventional conditioning. Taking into account that all patients included into this retrospective evaluation were considered unfit for standard transplantation therapy and were significantly older, the conditioning with treosulfan and fludarabine is considered suitable for high risk patients and for patients with normal risk factors. A multicenter phase II study is underway to test this hypothesis.

0134**FLUDARABINE AND BUSULFAN (FLUBU REGIMEN) AS MYELOABLATIVE CONDITIONING REGIMEN FOR ALLOGENEIC PBSCT IN 71 LEUKEMIC PATIENTS (A UNICENTRIC STUDY)**

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Background. Following 3 other centers in USA, Canada and Germany, we are evaluating fludarabine (40 mg/m² on days -6 to -2) and busulfan (4 mg/kg/day on days -5 to -2) as a new conditioning regimen for allogeneic peripheral blood stem cell transplantation in leukemic patients with matched related donors. **Methods.** Seventy one patients were enrolled, 18 with high and 53 with standard risk (18 ALL, 35 AML, 16 CML and 2 MDS; F=29 M=42). The median patient age was 23.7 years (range, 2.4- 46.7). Cyclosporine was used as a prophylactic agent for GVHD (3 mg/kg IV till +4, 10 mg/kg oral from day +5). Despite of other studies used ATG and MTX for GVHD prophylaxis and conditioning regimen, we didn't use these two drugs in order not to ameliorate anti-leukemic effect of SCT. **Results.** The median follow-up was 269 days (range, 50-459 days). 91.5% and 15.5% developed mucositis and hepatic toxicity respectively which resolved with conservative therapy. There was no cardiac toxicity (except one patient with mild pericardial effusion and another with tachycardia). The median of highest serum creatinine level during hospitalization were 1.6 mg/dL (range, 0.8-3.7; 24.3% with Cr>2) and serum cyclosporine level, at the same time, was 246 ng/mL (range, 9-814). 7% experienced hemorrhagic cystitis (infection was ruled out) and 36.6% experienced moderate to severe headache. 38% and 14.1% of the patients showed grade 1, 2 and grade 3 acute GVHD respectively. Grade 4 acute GVHD was found in one patient. 50% and 6% showed limited and extensive chronic GVHD. 27% of patients became CMV+ (min +17, max +69). The median time for neutrophil and platelet recovery were 10 (min 0, max +26) and 12 (min 0, max +30) days. In day +38, 86.7% of the patients had 90% or more, mononuclear chimerism (with STR-PCR technique; median, 97%; range, 25-100). 5 ALL and 8 AML patients relapsed (18.3% of all patients) and 6 (8.45%) died after relapse. Nonrelapse mortality was 13% (9 patients; acute GVHD grade IV=1, CMV infection and GVHD=2, CMV infection=2, pneumonia=2, infection=2). With a median follow up of 9 months (range, 1.6-15.3 months), the probability of overall survival and disease free survival were 79.68% and 81.26% respectively. **Conclusions.** It could be beneficial to use fludarabine versus cyclophosphamide in standard conditioning regimen for leukemic patients because of reduced toxicity, low incidence of acute GVHD and facilitated donor engraftment.

0135**SALVAGE THERAPY USING ANTI-THYMOCYTE GLOBULIN +/- CYCLOPHOSPHAMIDE CONDITIONED SECOND ALLOGRAFT FOR ENGRAFTMENT FAILURE FOLLOWING ALLOGENEIC PROGENITOR CELL TRANSPLANT FOR HEMATOLOGIC MALIGNANCY**

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Background. Graft failure or rejection following allogeneic transplantation (AlloSCT) is uncommon and may be expected to occur in around 9% of patients receiving sibling allograft but is associated with high mortality. There is no consensus on how this difficult problem should be tackled and within transplant centres in the UK no single protocol has emerged. **2. Aims.** To report engraftment and survival outcome following second stem cell infusion using an immunosuppressive regimen (anti-thymocyte globulin ATG +/- cyclophosphamide) in 12 patients

experiencing graft failure/rejection in two UK transplant centres. 3. *Methods.* 12 patients, median age 44 years (range 17-62), were identified in two centres from 1997 with either primary (n=4) or secondary (n=8) graft failure following conventional Cy/TBI MUD alloSCT (n=4) or reduced-intensity FLU/Mel/Campath allograft (n=8, MUD n=4, sibling n=4). 8 patients had CR $\frac{1}{2}$ acute leukaemia (AML n=5, lymphoblastic lymphoma n=2, CLL lymphoid blast crisis n=1) and 2 CLL, 1 myeloma. Two had major ABO mismatch. 6 were seropositive for CMV. 9/12 patients had 100% donor chimerism at graft failure, the remainder 38-98% chimerism. Time to secondary graft failure (n=8) was 54-574 days. A protocol for top-up transplantation was defined using antithymocyte globulin median dose 7.5 mg/kg (range 5-30 mg/kg), +/- cyclophosphamide 60 mg/kg x2 doses (n=3). Whilst 7 were MUD allografts, all donors contacted during the time of this study consented to second stem cell harvest, one patient had an alternative matched donor. Second CD34+ cell dose was a median 2.25×10^6 /kg (range 0.51-9.56 $\times 10^6$ /kg). Time from transplant/ secondary graft failure to top-up stem cell infusion was median 63 days (range 365-16 days). 4. *Results.* Successful engraftment following second SCT occurred in 10/11 evaluable patients (91%) with time to neutrophil engraftment 15 days, range 7-20 days, to platelet engraftment 35 days (range 9-247 days). This was sustained beyond 100 days in 8 patients and VNTRs were 100% in 9 patients. Whilst there was encouraging sustained engraftment rates and full donor chimerism established following this procedure, with 92% survival beyond 100 days, at 2.5 years follow-up from top-up transplant, longer-term outcome in this group was poor with 66% mortality at a median of 121 days (range 15-328 days) following top-up. Deaths were due to viral infections in 4 patients (CMV/adenovirus/EBV), 2 patients disease relapse, 1 died from GVHD and 1 from cerebral oedema at 15 days. Overall CMV reactivation occurred in 5/5 (100%) evaluable patients at risk. 5. *Conclusions.* There is no consensus on approach to graft failure. The use of this ATG protocol allows successful engraftment in 91%. This approach has high long-term mortality, largely due to infectious complications including CMV and adenovirus and EBV-related post-transplant lymphoproliferative disorder. In acute leukaemia, there is a high relapse-related mortality, probably reflecting graft failure as a precursor to disease relapse. This has led to a review of immunosuppression used in this group as the benefits from reduction in GVHD/rejection may be considerably outweighed by the delayed immune reconstitution.

0136

IMPROVING PRETRANSPLANT CONDITIONING THERAPY THROUGH BUSULFAN PHARMACOKINETICS

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Background. We postulated that the highly reproducible pharmacokinetics of intravenous (IV) Busulfan (Bu) could be used for very precisely targeted systemic exposure (SE) if a test dose with plasma pharmacokinetics was incorporated into the dosing regimen. Further, this would make dose intensification safer, assisting to assure engraftment and disease control while minimizing inter-individual variability in systemic drug exposure. Our previously reported fixed-dose Bu program (130 mg/m²) yielded a mean daily systemic exposure (AUC) of 4,925 μ Mol-min (range 2,900-8,300). We selected a daily target AUC for Bu of 6,500 μ Mol-min ($\pm 12\%$), yielding a median Bu-SE intensification of 30% compared with the fixed-dose approach. *Methods.* Based on PK parameters from a 32 mg/m² test dose (given 48 hr prior to starting the high-dose therapy). We then administered IV Fludarabine (Flu) (40 mg/m²) over 60 min followed by IV Bu over 3 hr to achieve a target AUC of 6,500 μ Mol-min daily x 4. AML/MDS patients were eligible. To date 24 patients have been treated. Twelve had active disease, 12 were in CR (CR1: 6, CR2: 5, CR3: 1). Their median age was 46 years (range, 13-65). Twenty subjects had family donors; 18 were HLA-matched and 2 had

a one antigen-mismatched donor. Four patients had matched unrelated donors. Treatment was well tolerated. All patients engrafted neutrophils in a median of 15 days (12-24). Treatment-related mortality occurred in 3 patients; fungal pneumonia with multi-organ failure (6 weeks, n=1), hepato-renal syndrome progressive to multi-organ failure (10.4 weeks, n=1), and TTP-HUS (26 weeks, n=1). Twenty patients are alive in continuous CR (CCR), and one CR2 pat who relapsed at 5 mos is on salvage treatment. Of 12 patients transplanted with active disease 9 are alive in CCR; of 12 patients treated in CR1-3 11 are in CCR. The median follow-up is 6 months (range, 6 weeks-13 months). Comparison of the individual Bu plasma clearance estimates from the test dose and the therapeutic doses demonstrates close agreement. The mean Bu clearance estimate from the therapeutic doses was within 6% of those predicted by the test dose with a slight bias (75% were higher than the test dose estimate) toward over prediction of clearance by the test dose. The magnitude of error in the prediction of Bu plasma clearance, on an individual basis, was small being < 15% in all patients. Prospective estimation of Bu clearance was used to determine starting therapeutic doses to achieve an AUC of 6,500 μ Mol-min; in 23/24 patients the mean achieved daily exposure was 6435 ± 386 μ Mol-min, close to the prospective targeted daily AUC of 6,500 μ Mol-min over the 4-day treatment course. We conclude, that the use of a test dose strategy for administration of the IV Bu treatment dose allows highly accurate and safe dose intensification. The value of intensified pretransplant conditioning therapy to improve overall and disease-free survival in high-risk patients with myeloid malignancies can now be investigated.

Cytokine signaling and transcriptional I

0137

ISOZYME-SPECIFIC MEMBRANE INTEGRATION OF PROTEIN KINASE C IN ERYTHROCYTES

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Background. Protein kinase C (PKC) is a family of serine/threonine kinases that are pivotal in cellular regulation. It is generally thought that it is the peripheral membrane pool of PKC that is activated by virtue of its translocation from the cytosol to the plasma membrane upon stimulation. Although an *in vitro* 'membrane-inserted' form of PKC was reported some 15 years ago, no such tight-membrane bound form of PKC in cells/tissues has ever been reported. *Aims.* To study whether an integral membrane form of PKC exists in mammalian cells and/or tissues. If they do, what functions it performs. *Methods.* We used Triton X-114 phase partitioning and extraction by alkali, urea and high salt to determine the presence of an *in vivo* pool of integral plasma membrane pool of PKC. *Results.* Both native PKC from quiescent cells and catalytically inactive PKC mutants are integral membrane proteins, indicating the membrane integration is a constitutive process. In erythrocytes, PKC-delta and PKC-zeta but not PKC- η integrate with the plasma membrane. The integral membrane PKCs are catalytically active and are subject to acute regulation by typical PKC activators. At least for PKC- η , the C2-V3 region at the regulatory domain of the kinase is responsible for membrane integration. In cells, only the wild-type PKC- η but not membrane integration-deficient PKC- η mutants is able to mediate phorbol ester-stimulated translocation of myristoylated alanine-rich protein kinase C substrate, to activate mitogen-activated protein kinase and to augment melatonin-stimulated neurite outgrowth. *Conclusions.* 1—For the first time in the 50 year's protein phosphorylation research, we reveal that mammalian ser/thr kinases can exist as both soluble and integral plasma membrane proteins. 2—For the first time in 25 years PKC research, we discover a novel integral plasma membrane pool of PKC that play important roles in signaling events elicited by classic PKC activators. 3—Our data suggest that, contrary to the prevailing dogma, at least some of the key

cellular functions of PKC are mediated by the integral membrane pool, but perhaps not the peripheral membrane pool or cytosolic pool of PKC *in vivo*. The isozyme-specific membrane integration of PKC is controlled by biochemistry and physiology of the cells/tissues in question. *E-mail: bchduanw@nus.edu.sg*

0138

SOLUBLE VEGF/sFLT1 RATIO IS INDEPENDENT PREDICTOR OF AML PATIENT OUTCOME

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Angiogenesis, the formation of new blood vessels from existing one, and is controlled by a balance between positive and negative angiogenic regulatory factors. Soluble vascular endothelial growth factor receptors 1,2 (Flt-1,KDR) are negative counterpart of vascular endothelial growth factor (VEGF) signaling pathway, which has been characterized as one of the most important endothelial regulator in human angiogenesis. In the present work, we tested the differential prognostic relevance of soluble vascular endothelial growth factor (VEGF), their receptors 1 (Flt-1), 2 (KDR), and the ratio between sVEGF/sFlt-1 in 43 AML patients. sVEGF and its soluble receptors were assessed using an ELISA. Soluble VEGF, sFLT-1, sKDR concentration levels was significantly higher in AML patients at diagnosis as compared to that in normal control. sVEGF, sFlt1 and sVEGF/sFlt1 ratio were significantly higher in non responders as compared to responders (P<0.001 for all). However, there is no significant difference regarding sKDR levels (P>0.05). sVEGF, and sVEGF/sFlt1 ratio and not sFlt1, sKDR levels were significantly elevated in non survivors as compared to survivors. sVEGF, sFlt1 levels were significantly correlated to WBCs counts (R=0.93, p=0.000, R=0.56, p=0.000 respectively); bone marrow blast cell counts (R=0.92, p=0.000; R=56, p=0.000 respectively); peripheral blood blast cell counts (R=0.91, p=0.000; R=0.52, p=0.000, R=0.37, p=0.014 respectively); but sKDR was only correlated to peripheral blood blast cell counts (R=0.37, p=0.014). Cox regression analysis results with sVEGF, sFlt1, sKDR, sVEGF/sFlt1 ratio suggest that the most important predictor for AML outcome is sVEGF/sFlt1 ratio. In conclusion: sVEGF/sVEGF ratio is independent predictor of AML patient out come, and is preferred to be assess before the decision to use antiangiogenic therapy.

0139

SPECIFIC ATTENUATION OF TRANSFORMING GROWTH FACTOR-BETA SIGNALING PATHWAYS ALTERS ERYTHRO-MEGAKARYOCYTIC DIFFERENTIATION OUTCOME OF CORD BLOOD PRIMITIVE HEMOPOIETIC STEM CELLS

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Aims. investigation of the requirement of transforming growth factor-β (TGF-β) signaling pathways proteins [the Smad and the mitogen-activated protein kinases (MAPKs)] for erythroid and megakaryocytic differentiation. *Methods and Results.* Employing K562 cell line that represents multipotent leukemic hemopoietic stem cells (HSC), TGF-β1 treatment phosphorylates Smad2/3 and MAPK proteins including ERK, p38 and JNK, leading to erythroid differentiation. Stable overexpression of Smad7 prevented Smad2/3 activation and differentiation induced by TGF-β1 and enhanced the nuclear ploidy of K562-derived megakaryocytes. RNA interference of endogenous Smad7 enhanced erythroid differentiation of K562 cells in response to treatment with physiological doses of TGF-β1.

Inhibitor of activation of TGF-β type I receptors (SB505124) or p38 (SB203580) but not inhibitor of MEK (PD98059 and U1026)) or JNK (SP600125) MAPK abrogated intracellular hemoglobin synthesis by TGF-β1. Interestingly, MEK inhibitors induced Smad2/3 phosphorylation and cell hemoglobinization that was prevented by prior treatment with SB505124, revealing cross talk between Smad and ERK-MAPK in erythroid differentiation. Using cultures of CD34+ Lineage negative (Lin-) cord blood (CB), neutralization of autocrine TGF-β1 using anti-TGF-β, in stem cell factor (SCF)-stimulated serum-free cultures, leads to the development of tryptase -ve glycophorin A+ve erythroid progenitors while, cells stimulated with SCF alone developed to tryptase +ve mast cells. Of interest, among inhibitors tested, treatment of thrombopoietin- (50 ng/mL) plus interleukin-3- (20 ng/mL) stimulated CB-CD34+Lin- cells with SP600125 improved proplatelets formation, cell ploidy and expression of megakaryocytic markers (CD41a, CD61 and CD42b). *Conclusions.* we conclude that specific attenuation of TGF-β signaling using pharmacological inhibitors and molecular agents would alter differentiation outcome of HSC.

0140

SYNTHESIS OF RIBOSOMAL RNA AND REVERSIBLE UNRAVELLING OF THE RIBOSOMAL MULTICOPY GENE IN LYMPHOBLASTS TREATED WITH ACTINOMYCIN D

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Background. The blastic transformation of quiescent lymphocytes is accompanied by a mobilization of polymerase I transcription machinery and powerful rRNA synthesis supported by an extensive unwinding of the multicopy rDNA gene into the structure of dispersed transcription units (TU). Despite the easy demonstration of this remarkable transformation, its molecular mechanism remains to be elucidated. *Aims.* With the goal to contribute to the understanding of molecular dynamics and the useful effect structural changes has on the ribosomal gene, we have investigated the effect of aggregation of TUs, induced by inhibition of ribosomal transcription in lymphoblasts treated with actinomycin D. *Method:* Lymphocytes were stimulated 48 hrs with 8 ug/mL of phytohemagglutinin (PHA). 10 ng/mL of actinomycin D (AMD) was added in time intervals of up to 45 hrs after PHA addition. Cells were examined for viability by BrdU incorporation ELISA and for rRNA synthesis by Run-On assays. The process of dispersion or association of TUs, manifested by varying number and size of UBF positive spots, was examined by anti-UBF immunofluorescence microscopy.

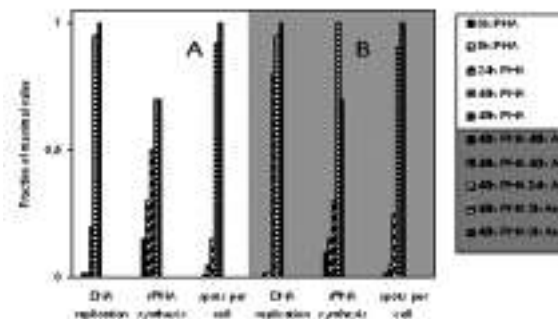


Fig 1. A) The rate of BrdU incorporation, the rate of synthesis of ribosomal RNA, and unwinding of rDNA transcription units evaluated as a number of UBF positive spots in lymphocytes stimulated with PHA for 0, 24, 48, and 48 hrs and B) in lymphocytes stimulated with PHA for 48 hrs in

Transcription of mRNAs coding for proteins taking part in ribosomal biogenesis was examined using RT-PCR. *Results.* PHA stimulated entry of quiescent lymphocytes into the G1 phase was accompanied by a mobilization of polymerase I machinery represented by Pol I, TIF-1A, UBF, TBP, and TAFs and by increas-

ing synthesis of ribosomal rRNA. As shown in Figure 1, incorporation of BrdU into the chromosomal DNA in the presence of AMD suggested that after 20 hrs of stimulation without AMD, cells were committed to replication and the subsequent addition of AMD did not inhibit G1/S transition. However, transcription of rRNA was inhibited by AMD (except for the short-term presence of AMD which temporarily activated rRNA synthesis), and for the majority of cells the unravelling of the ribosomal gene was reversed and an association of transcription units predominated. Summary/conclusions: In lymphocytes stimulated to the blastic transformation the synthesis of ribosomal RNA is likely promoted by opening the structure of the ribosomal gene. In quiescent lymphocytes hundreds of transcription units of rDNA are clustered in one small domain with a low active fibrillar center. Once PHA has been added, the synthesis of rRNA increases and the structure unwinds into separated TUs. The addition of actinomycin D inhibits the ribosomal synthesis and the majority of G1 lymphoblasts restores the coiled structure of the fibrillar center characteristic of quiescent lymphocytes. The transformation takes place without attenuation of cell cycle progress. Changes in activity of RNA polymerase I likely play an active role in the reversible transformation of the structure of fibrillar centers and the steric effect of increasing the number of rRNA transcripts in nucleolus probably contribute to stabilizing the expanded structure. We speculate that short-term activation of ribosomal synthesis by 10 nM AMD can originate in common affinity of UBF and AMD for GC rich domains of rDNA.

Grants of MHCR: IGA NR7998-3 and CEZMZ 0023736001.

0141

MARROW FAILURE IN CHRONIC IDIOPATHIC NEUTROPENIA IS ASSOCIATED WITH DEFECTIVE LYMPHOCYTE PRODUCTION INDICATED BY LOW IL-7 LEVELS AND DECREASED T-CELL NUMBERS WITH T-CELL RECEPTOR EXCISION CIRCLES

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Background. We have previously shown that a significant proportion of patients with hypoplastic chronic idiopathic neutropenia (CIN) display decreased number and increased activation status of peripheral blood T-lymphocytes compared to age and sex-matched healthy controls. The cause and underlying mechanism (s) of lymphopenia remain largely unknown. **Aims.** To probe the mechanism of lymphopenia in CIN patients by evaluating the production of naive T-cells in the thymus and the apoptosis-induced T-cell death in the peripheral blood. **Methods.** We studied 22 patients with CIN, aged 27-68 years and 20 age- and sex-matched healthy individuals. The lymphocyte production in the thymus was indirectly evaluated by determining the T-cell receptor excision circles (TRECs) in immunomagnetically sorted peripheral blood CD4+ and CD8+ cells from patients and controls by means of Real Time PCR. The levels of interleukin-7 (IL-7), a cytokine that induces T-cell production in the thymus, were determined in the supernatants of long-term bone marrow cultures (LTBMC) by means of ELISA. The proportion of naive CD45RO+/CD27+ and memory CD45RO+/CD45RA- cells in the CD4+ and CD8+ cell fractions was estimated by flow-cytometry. The proliferation status of peripheral blood CD4+ and CD8+ cells was evaluated using flow-cytometry by quantifying the proportion of cells expressing the Ki-67 nuclear antigen. The percentage of apoptotic cells within the CD4+ and CD8+ cell compartments was estimated by flow-cytometry and the use of 7-amino-actinomycin-D stain under steady state conditions and following 5-days of culture. **Results.** The proportion of CD45RA+/CD27+ cells was significantly lower in patients compared to controls within the CD4+ cell fraction (5.75%±3.78% versus 10.95%±6.66%, $p=0.0196$) while a trend towards lower CD45RA+/CD27+ cell numbers were also observed in patients' CD8+ cell population (5.63%±2.94% versus 7.49% ±2.95% P

= 0.1576). No significant difference was found between patients and controls in the proportion of CD45RO+/CD45RA- cells. The TREC content of CD4+ and CD8+ cells was significantly lower in CIN patients (6.44x10⁻³ copies/cell±5.14 and 3.31x10⁻³ copies/cell±1.35 respectively) compared to controls (25.4x10⁻³ copies/cell±2.15 and 15.2x10⁻³ copies/cell±11.04, respectively; $P < 0.0001$ and $P = 0.0024$, respectively), suggesting lower T-cell production in patients thymus. In keeping with this finding are the statistically significant lower IL-7 levels in patient LTBMC supernatants compared to healthy controls (0.3pg/mL±0.35pg/mL versus 0.64pg/mL±0.58pg/mL, $P = 0.0277$). The nuclear Ki-67 expression was significantly increased in patients compared to controls in both the CD4+ and CD8+ cells ($P < 0.05$ and $P < 0.05$, respectively) suggesting increased number of proliferating T-cells in patients' peripheral blood. However, the proportion of apoptotic cells detected in the CD4+ and CD8+ cell compartments did not differ significantly from the respective of the healthy controls under steady-state conditions or following 5-day incubation. **Conclusions.** Lymphopenia in CIN is due, at least in part, to defective IL-7 production in the BM. We speculate that the increased T-cell activation, previously reported in CIN, that probably results in a degree of T-cell extravasation cannot be counterbalanced by increased IL-7-induced CD45+/CD27+ T-cell thymic production, finally resulting in lymphopenia in the patients.

0142

A NOVEL SMALL MOLECULE (NIP-004) WITH THROMBOPOIETIN MIMETIC ACTIVITIES

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Background. Thrombopoietin (TPO) is a critical humoral regulator of megakaryopoiesis and thrombopoiesis. TPO induces proliferation and maturation of hematopoietic stem cells and megakaryocyte progenitors by stimulating its cognate receptor, c-Mpl. **Aims.** We newly developed nonpeptidyl small molecules with TPO mimetic activities by screening our chemical compound libraries. In this study, we evaluated the biological activities of a novel TPO mimetic molecule, NIP-004, *in vitro* and *in vivo*. As NIP-004 is specific for human c-Mpl, we created a new experimental animal model of human megakaryopoiesis using immunodeficient NOG (NOD/SCID/ β chain null) mice. **Methods.** [*in vitro* assay]: The proliferation assay was performed by using TPO-dependent human megakaryocytic cell lines, UT-7-EPO/Mpl. After the immunoprecipitation with an antibody against phosphotyrosine, Western blotting was performed by antibody against human c-Mpl or Stat5. The CFU-Meg assay was performed by using human bone marrow-derived CD34-positive cells in collagen based semi-solid culture. [*in vivo* assay]: After 2.4 Gy-irradiation, 1x10⁶ of human umbilical cord blood-derived CD34-positive cells were intravenously injected into NOG mice. The chimerism of human platelets in NOG mice was measured by flowcytometry using species-specific antibodies against CD41. **Results.** [*in vitro* assay]: NIP-004 could stimulate the proliferation of UT-7-EPO/Mpl, EC50=10 nM, and induce tyrosine phosphorylation of c-Mpl and Stat5 in the cells. We also confirmed 1 μ M of NIP-004 by itself induced maturation of megakaryocytes from human bone marrow-derived CD34-positive cells in the serum free cultures. [*in vivo* assay]: The chimerism of human platelets in NOG mice was kept between 0.3% and 2.0% for 6 months. We found that NIP-004 (30 mg/kg s.c., for 14 days) induced statistically significant increase of human platelets (10.1+/-2.0x10⁹/L [NIP-004], 3.6+/-1.2x10⁹/L [vehicle], n=3, $p < .01$, Student t-test). After cessation of the drug, the platelet counts returned to the counts before the treatment. **Conclusions.** Our novel compound NIP-004 has TPO mimetic activities *in vitro* and *in vivo* and has strong potential for future clinical development.

0143**ALTERATIONS OF HEMATOPOIESIS IN MICE AFTER LONG-TERM TREATMENT WITH G-CSF**

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G-CSF is widely used for leukemia priming before treatment, for shortening neutropenia period after aggressive chemotherapy and, of course, for mobilization of peripheral blood stem cells for transplantation purposes. Treatment of mice with low G-CSF doses (25mcg/kg) known to be insufficient for mobilization of peripheral blood stem cells for 4 days leads to 2-fold decrease of bone marrow primitive hematopoietic stem cells (P-HSC) at the end of the treatment, while the CFU-S number in the bone marrow doesn't change. At the same time the number of peripheral blood CFU-S doubles whereas leukocyte count and the proportion of granulocytes remain unchanged. The aim of this study was to reveal whether the decrease of P-HSC after G-CSF treatment is hematologically relevant. Female mice CBF1 (CBA/Lac x C57Bl/6) F1 and DBF1 (DBA/2 x Balb/c) F1 were injected with G-CSF (25 mcg/kg) for 4 days once a month with blood cell count and cytology measured before and after the course. A month after the end of 7th course (7 months since the beginning of the experiment) the bone marrow was obtained from anesthetized mice and then tested for the CFU-C and the LTC-IC frequency. There were no changes in blood cell count for the whole period of observation. CFU-C (7 days) concentration in the bone marrow didn't differ among control and experimental mice either, while the concentration of CFU-C counted on the 14th and 21st day after the plating, representing more immature hematopoietic precursors, increased more than 2-fold in the bone marrow of G-CSF treated mice while LTC-IC frequency decreased 2-fold in the latter group. Obviously hematopoietic precursors on various stages of maturation respond to the G-CSF treatment with pharmacological doses in different ways. As the dose of G-CSF used was not sufficient for mobilization, decrease in LTC-IC frequency in the bone marrow cannot be explained by the recruitment of these precursors into the peripheral blood. Increase in frequency of more mature hematopoietic precursors suggests stimulation of differentiation of LTC-IC as a probable cause of the effect. Hematopoiesis occurred does not seem to be effective as neither frequency of the mature bone marrow myeloid precursors nor leukocyte count in peripheral blood increased. During 7 months of experiment overall survival of G-CSF treated mice was 50% versus 100% in untreated group. Two out of ten CBF1 mice developed different myeloproliferative disorders (defined on classification proposed in Blood, 2002; 100:238-245). MPD-like myeloid leukemia with histiocytic sarcoma occurred in one of these mice after 4th course of G-CSF treatment. Bone marrow and liver cells of the mouse are retransplantable, recipients become moribund within 21-32 days since cells injection. Another mouse developed myeloid leukemia without maturation after 7,5 month since the beginning of the experiment. Stable decrease of early hematopoietic precursors' frequency and the development of inducible leukemias suggest the occurrence of serious alterations in the hematopoietic system under the G-CSF treatment. The mechanism of their formation is being scrutinized.

0144**CHROMATIN REMODELLING ENZYMES IN CHLORAMBUCIL-TREATED LYMPHOID CELL LINES**

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Chronic lymphoid malignancies (eg CLL) have for many years been treated with alkylating agents either alone (particularly chlorambucil (CLB)) or in combination with corticosteroids. The basis of drug resistance observed in many patients

treated with CLB is complex and may be related to defective chromatin remodelling mechanisms that may include abnormal methylation and expression of apoptotic genes (eg TRAIL receptors, caspase-8, Bcl-2) and derangement of histone acetylation/deacetylation. These processes are regarded as possible therapeutic targets. *Aims.* To explore the possible association between the apoptotic response of lymphoid cells to CLB and the expression of chromatin remodelling enzymes, DNA methyltransferase 1 (DNMT1) and histone acetylase 1 (HAT1). *Methods.* Lymphoid cell lines LP1 and NCI-H929 cultured in RPMI1640/10% FBS, were treated with 20 microM CLB, 10 microM dexamethasone (Dex) or vehicle alone (0.1% ethanol) for up to 72 hours. Induction of apoptosis was assessed by flow cytometry using Annexin V-propidium iodide method. Expression of DNMT1 and HAT1 relative to phosphoglycerate kinase-1 (PGK1) was measured using real time PCR. *Results.* CLB induced apoptosis in both cell lines. Necrotic (propidium iodide positive) cells comprised 73.7% (+/- 6.47 SEM, n=5) of CLB-treated LP-1 cells at 72 hrs (vehicle alone 22.0% +/- 5.62, n=5, p=0.0007) and 80.8% +/-9.92, n=5 in NCI-H929 cells (vehicle alone 23.4+/-4.75, n=5, p=0.0008). DNMT1 expression was elevated 1.94 fold (+/-0.246, p=0.0245) at 48 hrs in LP-1 cells (relative to control) but not at 72 hrs (p=0.3975). DNMT1 expression in NCI-H929 cells did not change significantly at any time. HAT1 mRNA was elevated in LP-1 cells 1.525 fold (+/- 0.157, p=0.0165) at 48 hrs but not at 72 hrs (p=0.206). Similar changes in HAT1 expression profile were observed in NCI-H929 cells (1.636+/- 0.136, p=0.0087, n=5 at 48 hours and non-significant afterwards). Dex induced significant apoptosis in NCI-H929 cells only. No changes in DNMT1 and HAT1 expression could be observed following Dex treatment. *Conclusions.* Changes in expression of chromatin remodelling enzymes, DNMT1 and HAT1, may be associated with induction of apoptosis by CLB but are unlikely to be significantly involved in Dex-induced cell death at least in these two lymphoid derived cell lines under the test conditions. Similar experiments using fresh B-lymphocytes from CLL are in progress.

0145**DISTINCT ONCOGENIC PROPERTIES OF NUP214-ABL1 IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA**

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Background. BCR-ABL1 is frequently associated with chronic myeloid leukemia and B-cell acute lymphoblastic leukemia, but is rare in T-cell acute lymphoblastic leukemia (T-ALL). We recently identified two novel ABL1 fusions in T-ALL: NUP214-ABL1, associated with episomal amplification of ABL1 (6% of T-ALL cases), and EML1-ABL1, associated with the cryptic translocation t(9;14)(q34;q32) (1 case). *2.Aims.* We investigated whether NUP214-ABL1 and EML1-ABL1 display similar oncogenic properties, imatinib sensitivity and downstream signalling as BCR-ABL1. *3.Methods.* These ABL1-fusions were expressed in Ba/F3 cells and NUP214-ABL1 was studied in the ALL-SIL cell line. *4.Results.* All 3 fusions transformed Ba/F3 cells to IL3 independence, but NUP214-ABL1 transduced cells needed a significantly longer period to obtain the same proliferation level as EML1-ABL1 and BCR-ABL1 transformed cells. This observation was due to the fact that only the Ba/F3 cells with the highest expression levels of NUP214-ABL1 could proliferate. At lower expression levels, NUP214-ABL1 cells were unable to phosphorylate endogenous ABL1 and SRC kinases. In addition, NUP214-ABL1 expressing Ba/F3 cells were significantly more sensitive to imatinib (IC50~3 nM) than EML1-ABL1 or BCR-ABL1 transformed Ba/F3 cells (IC50~200 nM), suggesting a lower kinase activity of NUP214-ABL1. This observation is in agreement with our inability to detect phosphorylation of tyrosine 412 of ABL1 in the NUP214-ABL1 fusion, while EML1-ABL1 and BCR-ABL1 show phosphorylation at this tyrosine in the activation loop of the kinase domain. These data are compatible with the finding that NUP214-ABL1 is always amplified in

NUP214-ABL1 positive T-ALL patient samples and cell lines, and suggest that amplification of *NUP214-ABL1* is strictly required for transformation. This may also be related to our observation that fusion of the coiled-coils of *NUP214* to *ABL1* did not cause activation of the kinase domain, suggesting a different activation mode than *BCR-ABL1*, where the coiled-coils of *BCR* are sufficient to activate *ABL1* kinase activity. In *Ba/F3* cells (B-cells), *ABL1* fusion kinases activate the SRC kinase *Lyn*, which was recently shown to be required for B-cell transformation by *BCR-ABL1*. To test if this was also true for T-cell transformation, we studied the SRC kinases in the *NUP214-ABL1* expressing T-ALL cell line ALL-SIL. In addition to its imatinib sensitivity, ALL-SIL was sensitive to the SRC family kinase inhibitor PP2, suggesting a role for SRC family kinases in *NUP214-ABL1* signalling. ALL-SIL cells only expressed the LCK and FYN SRC family kinases, and phosphorylation of LCK decreased upon PP2 treatment. We are currently working on siRNA experiments and primary T-cell models to further unravel the role of LCK and FYN in the observed PP2 sensitivity.

5. Conclusions. We describe significant differences between *NUP214-ABL1* and *BCR-ABL1* with respect to activity and imatinib sensitivity, which may explain why *NUP214-ABL1* is associated with high level amplification in T-ALL patients and cell lines. However, despite its amplification, *NUP214-ABL1* positive T-ALL cells remain sensitive to imatinib, since *NUP214-ABL1* is more sensitive to imatinib than *BCR-ABL1*. Finally, we show that *NUP214-ABL1* positive T-ALL cell lines are sensitive to SRC kinase inhibitors, suggesting new therapeutic strategies for treatment of *NUP214-ABL1* positive T-ALL.

0146

ERYTHROPOIETIN SIGNALING IN TUMOR CELLS: DIFFERENTIAL ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASES AND THEIR EFFECT UPON CELL SURVIVAL

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Erythropoietin (EPO) regulates the proliferation and differentiation of erythroid progenitors via its receptor, EPO-R and through various Mitogen activated protein kinase (MAPK) pathways. The role of EPO on other cell types is still unknown. In the present study we have elected to examine this aspect in a transformed pancreatic cell line, AR42J cells. We investigated the activation of two MAPKs, namely extracellular regulated kinase (ERK) and c-jun NH2-terminal kinase (JNK) after exposure of AR42J cells to 1 U/mL EPO using the Western blot analysis. We also examined cell viability during EPO exposure by high sensitivity fluorometric method using CCK8. We found a rapid activation of ERK^{-1/2} in AR42J cells reaching the maximum of 3.3 fold in 5 min, while it took 30 min for JNK^{-1/2} to reach the maximum. In the absence of EPO, addition of specific JNK inhibitor, SP600125, reduced cell viability to 50% at a dose of 20 μ M while with ERK inhibitor, UO126, cell viability was not reduced even up to 60 μ M of the drug. To examine the effect of induction of MAPK by EPO on AR42J cell survival, cells were treated with inhibitors to ERK or JNK 1h prior to EPO addition and the cumulative cell survival were calculated from day 1 through 4. EPO addition to AR42J cells resulted in significantly higher cumulative cell survival of 1.00 \pm 0.04 unit absorption compared to the value of 0.35 \pm 0.02 unit absorption seen in controls without EPO. When cells were treated with EPO and ERK inhibitor a significantly higher cumulative cell survival of 1.50 \pm 0.04 unit absorption was observed ($p < 0.01$) indicating ERK to be less effective in their survival. On the other hand, samples treated with EPO and JNK inhibitor had significantly lower cell survival (0.55 \pm 0.06 unit absorption, $p < 0.01$) than the EPO control indicating an essential role of JNK in their survival. These results indicate that in EPO mediated survival of AR42J cells, activation of JNK appears to be more important than ERK.

0147

THE TYROSINE KINASE-TARGETING DRUGS PKC412 AND AMN107 INHIBIT GROWTH OF NEOPLASTIC MAST CELLS EXPRESSING THE D816V MUTATED VARIANT OF KIT

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Background. In the majority of patients with systemic mastocytosis (SM) including aggressive SM and mast cell leukemia (MCL), neoplastic cells express the oncogenic C-KIT mutation D816V. This mutation activates the tyrosine kinase (TK) of the KIT receptor which thus represents an attractive target of therapy. However, most of the available TK inhibitors including STI571 (imatinib; Novartis Pharma AG), fail to block TK activity of KIT D816V at pharmacologically relevant concentrations.

Aims and Methods. In an attempt to identify new promising drugs for the treatment of SM, we examined the effects of various TK inhibitors on 3H-thymidine uptake in KIT D816V-transformed cells.

Results. We provide evidence that the TK inhibitors PKC412 and AMN107 (Novartis Pharma AG) counteract the TK activity of D816V-mutated KIT, and inhibit the growth of *Ba/F3* cells with doxycycline-induced expression of KIT D816V, and neoplastic human mast cells (mast cell leukemia cell line HMC-1) expressing this C-KIT mutation. PKC412 was found to be highly active, with IC50 values of 50-100 nM, and without differences seen between HMC-1 cells exhibiting or lacking KIT D816V. AMN107 was also highly active against HMC-1 cells lacking KIT D816V (IC50 5-10 nM) and maintained some activity against KIT D816V-expressing HMC-1 cells: IC50 1-5 μ M). In contrast, imatinib exhibited potent growth-inhibitory effects only on HMC-1 cells lacking the D816V-variant of KIT (IC50: 10 nM), but showed no effect on growth of HMC-1 cells exhibiting KIT D816V (IC50 >10 μ M). Corresponding results were obtained with *Ba/F3* cells exhibiting wild-type KIT or D816V-mutated KIT. The growth-inhibitory effects of PKC412 and AMN107 on HMC-1 cells were associated with TK inhibition of KIT and induction of apoptosis, as assessed by conventional morphology and electron microscopy.

Conclusions: These data show that PKC412 and AMN107 counteract the growth of neoplastic mast cells expressing the SM-related D816V-mutated variant of KIT. Both drugs may therefore be considered as novel promising agents for targeted therapy in patients with aggressive SM and MCL.

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0148

SEMI-SYNTHETIC HOMOHARRINGTONINE TRIGGERED RAPID MCL-1 DOWNREGULATION AND INDUCED APOPTOSIS VIA THE MITOCHONDRIA IN MYELOID LEUKEMIA CELLS

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Introduction: Homoharringtonine (HHT) is a cephalotaxine-ester, initially extracted from the bark of *Cephalotaxus* (natural HHT, nHHT), active in chronic and acute myeloid leukemias. J.-P. Robin was the first to semi-synthesize HHT (ssHHT) from cephalotaxine extracted from dry leaves of *Cephalotaxus*. The ssHHT has higher purity (99.7%) than the nHHT. The action of the ssHHT (Stragen, Lyon, France) is now being evaluated in CML and AML patients. We have investigated the mechanism of apoptosis induced by ssHHT in myeloid leukemia cells.

Materials and Methods. The studies were carried out with the HL60 myeloid leukemia cell line, and its derivative, the HL60/MRP daunorubicin-resistant sub-clone, overexpressing MRP1 gene and lacking Bax expression, as well as the fresh leukaemia cells

from AML patients. Annexin V staining was used for the analysis of apoptosis, Western blotting for protein expression studies, DiOC6 (3) incorporation for evaluation of the change of mitochondrial membrane potential. **Results.** We have shown that ssHHT induces apoptosis in HL60 and HL60/MRP cell lines in a time and dose dependant manner and independently of bax expression. The apoptosis induced by ssHHT is mediated through the mitochondria, because we could observe the decreased mitochondrial membrane potential by DiOC6 (3) incorporation and the release of cytochrome C by Western Blotting after exposure of HL60 and HL60/MRP cell lines to ssHHT. Furthermore, we demonstrated the increase of caspase 3 and caspase 9 activities, but not of caspase 8 activity in cells treated with ssHHT. Therefore, we suggested that the apoptosis induced by ssHHT was caspase 8 independent and caspase 9 dependent. We also investigated the expression of the Bcl-2 family proteins in the attempt to unveil the relation between ssHHT and the change induced to the mitochondria. We have shown that ssHHT decreased Mcl-1 expression and induced Bcl-2 cleavage in both HL60 and HL60/MRP cells. Bcl-2 cleavage could be inhibited by the Z-VAD.fmk caspase inhibitor. However, Mcl-1 turnover was very rapid and occurred before caspase activation. The Mcl-1 turnover could be restored by proteasome inhibitors, so we propose that ssHHT triggered the Mcl-1 turnover through the proteasome. Finally, we confirmed that ssHHT induced apoptosis also in 15 AML patient cells. The median apoptosis rate was about 80%, ranging from 46% to 90% at the concentration of 15ng/mL of ssHHT, which is within the range of plasma concentration of ssHHT in patients receiving the drug. We have also confirmed the release of cytochrome C and rapid turnover of Mcl-1 in these patient cells, taking place only in apoptotic cells induced by ssHHT, but not in cells undergoing spontaneous apoptosis. **Conclusions.** Semi-Synthetic Homoharringtonine triggered rapid Mcl-1 turnover via the proteasome and induced apoptosis via the mitochondria in myeloid leukemia cells. Our results encourage ssHHT clinical trials in AML patients.

0149

GENOTYPE AND ALLELE FREQUENCIES OF C3435T POLYMORPHISM OF THE MDR1 GENE IN VARIOUS JEWISH POPULATIONS OF ISRAEL: IMPLICATIONS ON THE PROGNOSIS AND TREATMENT IN HEMATOLOGICAL MALIGNANCIES

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Intrinsic and acquired resistance to multiple chemotherapeutic drugs is a major clinical obstacle in the treatment of hematological malignancies. Over-expression of the multidrug resistance (MDR1) gene product, P-glycoprotein (Pgp), has been associated with poor prognosis in leukemia and other hematological malignancies. Pgp is a membrane-bound efflux-transporter conferring resistance to various cytotoxic drugs. The MDR1 gene is polymorphic with over 20 known single nucleotide polymorphisms. The single nucleotide, C3435T polymorphism at exon 26 was previously associated with different expression levels of the MDR1 gene and substrate uptake in mononuclear cells of the peripheral blood. Individuals with the CC genotype had an approximately two-fold higher Pgp expression in comparison to subjects with the TT genotype, while heterozygous subjects had an intermediate Pgp expression level. Differences in allele frequencies of the C3435T polymorphism have been previously demonstrated between racial groups. **Aims.** In this study, 500 individuals from five Jewish populations of Israel (Ashkenazi, Yemenite, North-African, Mediterranean, Near-Eastern) were examined for C3435T polymorphism. **Methods.** C3435T polymorphism has been identified using PCR-RFLP based technique to calculate genotype- and allele-frequencies. **Results.** Frequencies of the C allele were quite similar among the Ashkenazi (0.65), Yemenite (0.645) and North-African (0.615) Jewish populations. However, the frequency of this allele was slightly lower among

Mediterranean Jews (0.58) and significantly lower among Near-Eastern Jews (0.445). The frequency of the C allele among Near-Eastern Jews is, therefore, significantly different from all the other tested Jewish populations. In comparison to previously studied non-Jewish populations, the frequency of this allele among Near-Eastern Jews is different from West Africans (0.91), but is similar to Whites (0.497). However, the C allele frequencies among the other four Jewish populations are significantly lower than that found among non-Jewish West Africans and significantly higher than among non-Jewish Whites. **Conclusions.** These findings may have implications on Pgp-related prognosis and on differential selection of treatment protocols against hematological malignancies in Jewish populations.

0150

7-COLOR MULTIDIMENSIONAL FLOW CYTOMETRY (MDF) TO IDENTIFY NORMAL AND ABNORMAL MYELOID AND MONOCYTOID POPULATIONS IN MYELOPROLIFERATIVE DISORDERS (MPDs) AND MYELODYSPLASTIC SYNDROMES (MDSS)

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The diagnosis of MPDs, MDSs and acute myeloid leukemia (AMLs), has relied on combining characteristic clinical information with morphological features of the peripheral blood and bone marrow and with the typical patterns of surface antigen expression (CD antigens). MDF represents a highly reproducible and objective way of assessing the antigen expression in myeloid or monocytoid maturation as well as in benign reactive expression on a single cell to identify normal or abnormal patterns proliferation or maligne proliferation such as marrow regeneration. The aim of this study is to correlate the normal antigen expression during hematopoietic development as determined by MDF with the dysregulation of hematopoiesis observed in MDSs, MPDs and AMLs. Furthermore we want to compare MDF immunophenotyping with conventional clinical parameters, and to determine the prognostic incidence of MDF. **Methods.** bone marrow aspirates and peripheral blood from 10 patients without hematological disease, and from 50 patients with a diagnosis of *de novo* MPDs, MDSs, AMLs were analysed by flow cytometry. All the specimens were processed using lyse-no-wash methodology. Flow cytometric immunophenotyping was performed on flow cytometer Cyan TM (Dakocytometry, Ft. Collins, CO, USA) by collecting from 50000 to 100000 viable ungated list mode events. We have elaborated a 6-7-color immunofluorescence protocol to generate 8-9 parameters per cellular event. These 8-9 parameters defined the coordinates for that event in 8-9 dimensional dot plot. The following panel of antibodies was used in all of the cases: (CD66b-fitc/CD235a-Pe/CD45Pe-TXred/CD34Pe-Cy5/CD11b-Cy7/CD71APC); (CD13-Fitc/CD15-Pe/CD45-Pe-TXred/CD38-Pe-Cy5/CD33-Pe-Cy7/CD117-APC/CD16-APC-Cy7); (TdT-Fitc/CD56Pe/CD45Pe-TXred/CD10-Pe-Cy7/Mpo-APC/CD34-Pe-Cy5). **Results.** The patterns of antigen expression on the pathological population cells, maturing monocytes were compared with the patterns typically seen in normal myeloid and monocytoid populations. The coordinate variation in the expression of CD33, CD13, CD11b, CD15, CD16, CD66b that occur as the myeloid and monocytoid precursor mature to both neutrophils and monocytes make the combination of anti-CD33, anti-CD13, anti-CD11b, anti-CD66b, anti-CD15 and anti-CD16 particularly useful for identifying maturational anomalies or asynchronous antigen expression. Maturational asynchrony is determined by the contemporary expression of antigens that are specific of immature myeloid cells and of antigens exclusively present on mature neutrophils or monocytes. In conclusion we have assumed that is very important not to report only the percentage of expression of single antigen but to use a subjective determination about the presence or absence of an antigen, qualified by the intensity of expression and the pattern of antigen expression. The variability observed in the analysis of patterns of antigen expression may bear some relationship to

mechanisms of disease dissemination and progression. Furthermore, we have demonstrated that MDF is capable of distinguishing normal from abnormal myelopoiesis and in experienced laboratories, 7-color flow cytometry can play a central role in the diagnostic workup of MPDs, MDSs and AMLs.

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PHILADELPHIA (PH)-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) CELLS EXHIBIT HIGH LEVELS OF HEAT-SHOCK-PROTEIN 90 (HSP90) AND ARE HIGHLY SENSITIVE TO 17-ALLYLAMINO-17-DEMETHOXY GELDANAMYCIN (17-AAG)

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The 90-Kda heat shock protein (HSP90) is constitutively expressed in most mammalian cells, even in the absence of stress, and is involved in the conformational maturation and degradation of a large variety of tyrosine kinases. In cancer cells, high levels of HSP90 are often observed, the protein being moreover in activated (phosphorylated) form. The ansamycin antibiotic geldanamycin and its analogue 17-AAG selectively block the activity of HSP90. In this study we tested the expression of HSP90 and the effects of 17-AAG in two B-lymphoblastic leukemia lines, Reh (common-ALL) and SUPB15 (Ph-positive ALL), and in cells collected at diagnosis from pediatric and adult patients with B-cell ALL. Twenty-eight patient samples were explored, including 14 with common (CD10-positive) ALL (c-ALL) without Ph-chromosome, 10 with Ph-ALL and four with lymphoblastic transformation of chronic myeloid leukemia (CML). Protein expression was tested by flow cytometry and (for HSP90) western blot. Short term (24 and 48 hours) cultures were performed in the presence or absence of 17-AAG, adriamycin and/or imatinib mesilate. Apoptosis was assessed by activated caspase3 expression and annexinV binding. The levels of HSP90 were significantly higher in Ph-ALL and transformed CML (72% positive cells by flow cytometry) than in c-ALL (42%), and in SUPB15 (92%) than in Reh (40%). In short-term liquid culture, 17-AAG decreased cell survival in a dose-dependant fashion. Furthermore the percentages of surviving cells were significantly lower for Ph-ALL and transformed CML than for c-ALL: respectively 46% vs 43% at 24 H and 0% vs 24% at 48H in the presence of 5 µM 17-AAG, 0% vs 27% at 24H in the presence of 10µM 17-AAG. Similar results were obtained for ALL lines. Exposure to 17-AAG induced apoptosis in ALL cells, which was higher in Ph-ALL and SUPB15 line. 17-AAG also downregulated anti-apoptotic bcl-2 and bcl-xL proteins, and upregulated proapoptotic protein bax. This effect was again more pronounced in Ph-ALL. Finally, 17-AAG increased the effects of adriamycin on c-ALL cells, and of imatinib mesilate on Ph-ALL cells. In conclusion we show that 17-AAG exhibits a high antileukemic in-vitro activity in ALL, particularly in Ph-ALL and lymphoblastic transformation of CML.

0152

BORTEZOMIB INDUCES SELECTIVE APOPTOTIC CELL DEATH IN MONOCLONAL B LYMPHOCYTES

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B-cell lymphocytic leukaemia (B-CLL) is the most frequent leukaemia in the Western World. It is an incurable disease with an indolent course, characterized by a low growth fraction and

progressive expansion of a subpopulation of monoclonal B lymphocytes (LLC-B cells), which are functionally inactive and resistant to apoptotic cell death. Thus, failure to undergo apoptosis is important in LLC-B development, which may have direct implications for LLC-B therapy. The advent of new technologies and the knowledge of biochemical pathways involved in cancer development have contributed to the discovery of new therapeutic targets. Recently, it was verified that deregulation of the ubiquitin-proteasome pathway activity is involved in the pathogenesis of several diseases, including certain cancers. Proteasome inhibitors may be a promising therapeutic approach for treating human cancers, namely haematological neoplasias. Recently, a promising new drug, bortezomib, a dipeptidyl boronic acid proteasome inhibitor, was approved by the US FDA and EMEA for the treatment of patients with progressive multiple myeloma. Extensive preclinical data are being developed to study the potential therapeutic benefit of this new drug in other cancers. The aim of this study is to evaluate the potential of the proteasome inhibitor, bortezomib, in the treatment of B-CLL. For this purpose, mononuclear cells isolated from 12 patients with LLC-B (6 without and 6 with conventional therapy) were cultured in the absence and presence of bortezomib (ranging concentration from 1–30 nM), as a single agent, or plus 150 µM fludarabine, over 24 hours. Directly conjugated monoclonal antibodies to CD5 and CD19 were used to identify LLC-B cells. Cell death was evaluated by annexin V incorporation and detected by flow cytometry. Our preliminary results show that bortezomib induces a marked decrease in the viability of LLC-B cells by increasing the percentage of apoptotic cells (> 70%). Modest sensitivity to bortezomib was observed in normal mononuclear cells (< 30% apoptosis). The apoptotic effect of bortezomib seems to be independent of previous therapy. We observed higher levels of apoptosis in LLC-B cells treated with bortezomib compared with levels observed in untreated cells (average difference of 72.9±5% increase in apoptosis) or in response to treatment with fludarabine (average difference of 70.8±5.8% increase in apoptosis). However, unlike the findings of prior studies, we observed identical levels of apoptosis in LLC-B cells in the presence of bortezomib plus fludarabine. On the other hand, compared with the results obtained with fludarabine alone, an increase in the level of apoptosis was detected (average difference of 69.5±28% increase in apoptosis). These preliminary results support the idea that bortezomib induces apoptosis with some selectivity for the transformed (LLC-B) cells, suggesting that this proteasome inhibitor may be useful as a single agent for treatment of B-CLL patients. This work is supported by Jansen-Cilag and Millennium Pharmaceuticals, Inc.

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GLOBAL ANALYSIS OF HYDROXYUREA THERAPY ON HUMAN ENDOTHELIAL CELLS TRANSCRIPTOME

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Sickle cell disease (SCD) is characterised by recurring painful vasoocclusive episodes and multiple organ damages. Although the polymerisation of haemoglobin S under deoxygenated conditions is the base of the pathophysiology, the vascular endothelium seems to play an essential role in vascular occlusion. In fact, endothelial cells are able to modulate the vascular flow by their adhesion properties and the secretion of vasomodulators and inflammatory mediators. Hydroxyurea (HU) is the only drug which has measurable clinical benefits for SCD patients by reducing the frequency of vaso-occlusive crisis. Initially, HU was administrated to induce hemoglobin F (HbF) expression but there is no correlation between the clinical benefit in these patients and enhanced HbF expression. As endothelial cells (EC) play a crucial role in SCD pathogenesis, our laboratory investigates the effect of HU therapy on EC. We have shown previ-

ously that HU affects the expression of specific endothelial genes. These genes seem to be implicated in the pathobiology since they encode for adhesion molecules (*VCAM-1*), vaso-modulators (endothelin-1) and cytokines (IL-6, IL-8). Here we present a more systematic screening of HU's endothelial target genes. We opted for a global analysis of the human transcriptome based on the use of the Applied Biosystems Expression Array System. This novel technology allows to evaluate the expression level of the whole human genome since 31700 genes are detectable and then to establish a data collection of genes expressed by EC. We analysed the effect of HU on a human endothelial cell line derived from bone marrow microcirculation (TrHBMEC) during a 24 h treatment period. Five microarrays were hybridised for each condition. Pearson correlations of the replicate experiments averaged around 0.980. Genes were considered as detected if their signal/noise ratio was superior to 3. The criterium applied for calling a gene significantly modulated in its expression was that the combined variance of both signals had to be inferior to the signal distance. This measure of robustness was applied to all genes with a fold change superior or equal to 2. The modulation range of genes is comprised between 2 et 150 fold. As a testimony to the reliability of the novel technology applied here we confirmed HU target genes which had previously been characterised by the laboratory. This observation led us to validate the array system results. Around 1000 new potential targets genes were identified. Experiments of RQ-PCR on a representative sample are in due process to confirm the new HU target genes. In a first time, the expression modulation of thrombospondin-1 and von Willebrand factor gene is being evaluated. These two proteins seem to be implicated in the pathobiology since patients have higher plasma levels. Our preliminary results already allow us to rough out a HU pathway of signaling, and are being completed by new experiments (kinetics, inflammatory conditions hypoxia,...). A better understanding of HU's molecular effects and downstream pathways will lead to the emergence of improved alternative therapies.

0154

MULTIPARAMETERS FLOW CYTOMETRY IMMUNOPHENOTYPING OF MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHIES OF UNCERTAIN SIGNIFICANCE

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Background. Monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) are the most frequent forms of monoclonal gammopathies. It remains controversial whether all cases of myeloma evolve from a MGUS condition or not. Aim of this study is if flow cytometric parameters may help us to understand plasma cells biology and pathology. Immunophenotyping is an attractive technique for evaluating changes in the plasma cell (PC) compartment in patients with MM and MGUS because it allows discrimination between myelomatous PCs and normal PCs. In bone marrow plasma cells can be identified as CD38/CD138 positive cells, assays of concomitant expression of CD19 and CD56 allow to distinguish between normal and atypical cells and can detect malignant plasma cells even in MGUS or in pre-myeloma states. Normal PCs from healthy donors express a typical phenotype: CD19⁺ and CD56 negative whereas malignant plasma cells do not express CD19 and generally express CD56. **Methods.** we have applied flow cytometric analysis to evaluate the bone marrow plasma cells in 38 patients with MGUS and 55 patients with multiple myeloma, of which 35 pts have been evaluated at diagnosis and after therapy, 10 after peripheral stem cell transplantation and 10 in progression disease. A flow cytometric technique based on 7-color multidimensional flow cytometry using □□/CD38/CD19/CD56/CD138/CD45/CD2 and two-step acquisition procedure was performed on cytometer Cyan TM (Dako-

cytometry, Ft Collins, CO, USA). In the first step 50.000 events corresponding to the total of BM MNC were acquired. In the second step only those events included in the 'live-gate' drawn on SSC/CD38 bright fraction were acquired. **Results.** in MGUS we found that the CD38 bright cells percentage was <5.5% of bone marrow nucleated cells and three distinct PC populations could be identified: CD138⁺CD20⁻CD56⁻CD19⁻=40%±32%, CD138⁺CD20⁻CD56⁻CD19⁺=25%±15% and CD138⁺CD20⁻CD56⁻CD19⁺=17%±14%. In 22 patients with MM at diagnosis we found a percentage of CD38 bright of 10%-60% of bone marrow nucleated cells with CD56⁻/CD19⁻ cells=67%±30%, normal PCs with CD56⁻/CD19⁺ phenotype were <2%±0.4% and CD56⁺/CD19⁺<1%. After therapy CD38 bright percentage was much lower (5%-10%) and the relative populations CD56⁻/CD19⁻, CD56⁻/CD19⁺ and CD56⁺/CD19⁺ were respectively 48%±32%, 18%±10% and 8%±3%. In the 8 MM patients in progression disease CD38^{bright} increased to 20-50%; CD56⁻/CD19⁻, CD56⁻/CD19⁺ and CD56⁺/CD19⁺ were respectively 75%±20%, 5%±2% and 3%±2%. In 5 patients undergone PBPC autologous transplantation CD38^{bright} were about 5% and CD56⁻/CD19⁻, CD56⁻/CD19⁺ and CD56⁺/CD19⁺ were respectively 40%±35%, 33%±15% and 8%±4%. So we observed an evident normal CD56⁻CD19⁻CD20⁺ phenotype population growth in disease control phases; we showed that the malignant phenotype CD56⁻CD19⁻CD20⁺ remains the most representative. **Conclusions.** our results, even if preliminary, show that 7-color multidimensional flow cytometric analysis can be useful in qualitative and quantitative bone marrow plasma cells assay and it allows their monitoring in various disease's phases.

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CLINICAL AND FACS CHARACTERIZATION OF PEDIATRIC LEUKEMOID REACTION

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The leukemoid reaction (LR), defined as a white blood cell (WBC) count of > 50□10⁹/L is more common in the pediatric population. LR is a reason for anxiety and concern for families and medical staff, often resulting in extensive investigations. **Aims.** to correlate clinical features and etiology with the antigenic characterization by flow cytometry (FC) of WBC in LR. **Patients:** Children Age: 3 mo.- 18 yrs, with no known underlying disorder, diagnosed with LR. **Methods.** Laboratory investigation included a complete blood count and morphology, Viral and M. Pneumoniae serology, blood cultures and a Chest Xray (CXR). Antigenic determinants of WBC were tested by using the 4-colour FC. **Results.** Seventeen children (10 M, 7 F, median 30 months, range 3-90) were enrolled after obtaining informed consent. The presenting symptoms at diagnosis of LR were: fever in 65% (11/17), respiratory symptoms in 41% (7/17), gastrointestinal symptoms in 18% (3/17), skin infection in 12% (2/17), musculoskeletal symptoms and tonsillitis each in 6% (1/17). Lymphoid LR (L-LR) was observed in 59% (10/17) while myeloid LR (M-LR) was observed in 35% (6/17). One patient had eosinophilic LR. The clinical diagnosis in all children was infectious. Pneumonia was diagnosed in 6/6 children of the M-LR group (2M. Pneumoniae). The child with eosinophilic M-LR had scabies (*sarcoptes scabii* mite). In the L-LR group, 2 children had viral gastroenteritis, 2 had EBV infection, 2 had M. Pneumoniae (one had co-infection of EBV & M. Pneumoniae), 1 each had Adeno virus, CMV, E. Coli (UTI), Giardia Lamblia, and Tinea Capitis. The average WBC counts at diagnosis was 60,855/mL (range 50,100-101,400). CXR confirmed lobar consolidations in all 6 cases in the M-LR group, with no other abnormalities in the rest. FC analysis was preformed in 12 children (9 L-LR and 3 in the M-LR groups). Five children's WBC dropped to normal range within a few hours and FC was not performed. FC analysis confirmed the left shift of immature myeloid cells in all children with M-LR. FC results of the 9 patients with the L-LR revealed elevation of immature B cells (CD10⁺/CD20⁺) in 5 children, simul-

taneous co-elevation of CD19⁺ B & CD3⁺ T cells in 2 patients, elevation of immature B cells (CD10⁺/CD20⁻) and high levels of double positive T cells (CD4⁺/CD8), elevation of CD4⁺ T cells in one child with Tinea Capitis infection. **Conclusions.** LR is a benign phenomenon representing an acute response to an underlying disorder (in our study all had an infectious etiology). The most prevalent infection was M. Pneumoniae, a finding not reported previously. FC demonstrated the presence of circulating abnormal immature B cells and double positive (CD4⁺/CD8⁺) immature T cells. Further research using FC is needed to define the role of immature lymphoid cells in LR, and to correlate those findings to clinical etiologies.

0156

INSERTIONAL MUTAGENESIS BY SV40 LARGE T-ENCODING RETROVIRAL VECTORS REVEALS ONCOGENIC COMPLEMENTATION GROUPS

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The identification of oncogene complementation groups is an important task to reveal targets for molecular diagnostics of and therapeutic intervention against malignancies. Replication-defective retroviral vectors (RDRV) are widely used to study the impact of oncogenes on tumor evolution, and to define the minimal number of lesions required for tumorigenesis in different cell types and organisms. Recently, we and others have reported that insertional proto-oncogene activation by RDRV may contribute to tumor manifestation. In this study we wanted to test whether insertion sites of RDRV encoding proto-oncogenes contribute to clonal tumor evolution. **Methods.** and **Results.** We retrovirally introduced the large tumor antigen (TAg) of simian virus 40 into murine hematopoietic stem/progenitor cells (HSC). TAg inactivates the tumor suppressor proteins p53 and Rb by virtue of a chaperone-like activity. However, these mechanisms are not sufficient for tumorigenesis. When introduced into primary HSC, retroviral TAg primarily induced histiocytic sarcoma (average survival of 21 weeks). Retroviral transfer of TAg into pretransformed 32D cells generated a monocytic leukemia, with faster kinetics (~8 weeks). As all tumors were clonal, we sequenced retroviral insertion sites. In histiocytic sarcomas, we found hits in all known cooperation pathways, acting mitogenic and/or apoptosis-modulating (BclX, Crk, Eras/Pim2, Csf1/Pdgfrb, Osm/Lif, Axl, Fli, Sema4b, Sla, Sox4) or encoding a co-chaperone (Dnajb9). 32D-derived monocytic leukemias showed hits in Eras/Pim2 and Max proto-oncogenes, or even intriguingly, the chaperone Hspa4, plus additional signaling genes. **Summary.** We demonstrated that vector-mediated insertional mutagenesis revealed a broad spectrum of potential TAg complementation genes, in a comparatively small sequence sample (n=29). These findings have important implications for the use of RDRV in cancer research, and the expression of signaling genes in somatic gene therapy.

Supported by the Deutsche Krebshilfe grant no.:10-2090 Li 4 and DFG KFO 110.

0157

COMPARISON OF MRNA ABUNDANCE QUANTIFIED BY GENE EXPRESSION PROFILING AND PERCENTAGE OF POSITIVE CELLS USING IMMUNOPHENOTYPING FOR DIAGNOSTIC ANTIGENS IN ACUTE AND CHRONIC LEUKEMIAS

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Microarray analysis is considered a future diagnostic tool in leukemias. While data accumulate on specific gene expression

patterns in biologically defined leukemia entities data on the correlation between flow cytometrically determined protein expression, which are essential in the diagnostic setting today, and microarray results are limited. **Methods.** We compared results obtained by microarray analysis using the Affymetrix GeneChip HG-U133 system in parallel with flow cytometric findings of 36 relevant targets in 815 patients with newly diagnosed acute and chronic leukemias as well as in normal bone marrow samples. In a total of 21,581 individual comparisons between signal intensities obtained by microarray analysis and percentages of positive cell as determined by flow cytometry coefficients of correlation in the range of 0.171 to 0.807 were obtained. In particular, the degree of correlation was high in the following genes critical in the diagnostic setting: CD4, CD8, CD13 (ANPEP), CD33, CD23 (FCER2), CD64 (FCGR1A), CD117 (KIT), CD34, MPO, CD20 (MS4A1), CD7; range of r, 0.589 to 0.807. **Conclusions.** The present data prove the high degree of correlation between findings obtained by microarray analysis and flow cytometry. They are in favor of a future application of the microarray technology as a robust diagnostic tool in leukemias.

0158

DETECTION OF MOLECULAR TARGETS ON THE SURFACE OF CD34⁺/CD38⁻/CD123⁻ STEM CELLS IN VARIOUS MYELOID MALIGNANCIES

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Recent data suggest that myeloid neoplasms are organized hierarchically in terms of self renewal and maturation of early progenitor cells, similar to normal myelopoiesis. This new concept which is based on repopulation studies in NOD/SCID mice using FACS-purified progenitor cell populations of neoplastic cells, has important consequences for the development of antibody-based therapies. In fact, for the design of curative therapies, it seems essential to eliminate all neoplastic stem cells in a given malignant clone. In acute myeloid leukemia (AML), the NOD/SCID mouse-repopulating leukemic stem cells usually co-express CD123 and several other antigens with CD34, but lack CD38. Some of the expressed antigens, like CD33 (Siglec 3 = acceptor for gemtuzumab-ozogamicin, Mylotarg) may serve as targets for antibody-based therapeutic approaches. So far, however, little is known about expression of other markers and targets on neoplastic stem cells in AML or other myeloid neoplasms. **Aims.** In the present study, we examined expression of CD117 (c-KIT), CD13 (CMVR), CD33 (Siglec-3), CD133 (AC133), CD203c (E-NPP3), CD243 (MDR), CD123 (IL-3R), CD44 (Pgp-1), PAR-2, CD164, CD71 (TfR), HLA-DR, CD90 (Thy1), and CD116 (GM-CSFR) on CD34⁺/CD38⁻ (stem) cells in patients with AML (n=22), myelodysplastic syndromes (MDS, n=9), chronic myeloid leukemia (CML, n=9), and systemic mastocytosis (SM, n=7). **Methods.** Bone marrow aspirates were analyzed by multi-color flow cytometry using directly conjugated monoclonal antibodies. Non-fractionated bone marrow aspirates were incubated with various antibody-combinations. Erythrocytes were lysed using FACS Lysing Solution. **Results.** Independent of the type of disease, the vast majority of the CD34⁺/CD38⁻ stem cells co-expressed CD13 and CD44 in all patients examined. The IL-3R=CD123, was found to be expressed on CD34⁺/CD38⁻ cells in a majority of all patients with AML, MDS, and SM, but was only detectable in a subset of patients (roughly 50%) with CML. In all groups of patients, CD34⁺/CD38⁻ progenitor cells expressed variable amounts of CD33, GM-CSFR=CD116, KIT=CD117, and AC133=CD133. **Summary.** In conclusion, neoplastic stem cells in various myeloid neoplasms appear to display a similar phenotype including important target antigens such as CD33. Since most of these targets are not expressed on all stem cells in all patients, the elimination of the entire clone may require combinations of targeted antibodies.

Hemoglobinopathies

0159

COMPREHENSIVE α - AND β -THALASSEMIA GENOTYPING BY MEANS OF REVERSE-HYBRIDIZATION TESTSTRIPS

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α - and β -thalassemia (thal) are among the most common inherited diseases throughout Southeast Asia, India, the Middle East, parts of Africa and the Mediterranean area. Mutations in the α -globin gene, or in one or both of the two β -globin genes, are leading to structural abnormalities (e.g. sickle cell anemia) or to haemoglobin imbalance due to the reduced synthesis or complete absence of the respective globin chains. Unlike the prevalence of point mutations in beta-thal, the majority of α -thal alleles are derived from single or double gene deletions. We have developed reverse-hybridization assays (StripAssays) for the rapid and comprehensive genotyping of α - and beta-thalassemia. The tests are based on multiplex DNA amplification (including gap-PCR) and hybridization to teststrips presenting a parallel array of allele-specific oligonucleotide probes for each variant. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation. The tests are simple and convenient, and require very small amounts of samples, which is of particular importance for prenatal diagnosis. Although the spectrum of α - and beta-thal mutations is known to be highly population-specific, the broad range of variants covered by the StripAssays should make them globally useful diagnostic tools.

0160

HAPLOTYPES LINKED TO THE HB D PUNJAB [α 121 (GH4) GLU: GLN] IN WESTERN IRAN

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The polymorphism of the α -globin gene haplotypes are useful in the determination of the unicentric and multicentric origin of a mutational event and the elucidation of populations affinities. To study the α -globin gene haplotypes associated with α D-Punjab and α A chromosomes in Western Iran. We have studied 25 individuals from nine unrelated families in Western Iran. All subjects were Kurdish people from Western Iran including the provinces of Kermanshah (15 cases), Ilam (7 cases) and Kurdistan (3 cases). They were 15 individuals carrying one Hb D-Punjab chromosome, one subject homozygous for Hb D-Punjab, one patient was a compound heterozygous for Hb D-Punjab and α -thalassemia and one patient had α -thalassemia trait. The seven remaining individuals were normal. The Hb D-Punjab status of all cases was confirmed by PCR followed by digestion with EcoRI. Haplotype of α -globin gene cluster was determined by PCR-RFLP procedure. Also, the haplotype background of α A chromosomes was determined in 18 normal sub-

jects from the same area. α -globin gene haplotype analysis demonstrated that all α D-Punjab genes (18 genes) were linked to the haplotype I [+—+—+], while one α thal chromosome was associated with haplotype I and one other α thal chromosome was linked to the haplotype [-++++-]. Among the 36 α A chromosomes, 15 chromosomes (41.6%) were associated with haplotype I. The frequency of the remaining haplotypes was: haplotype II, 5 chromosomes (13.8%), haplotype V, 4 chromosomes (11.1%), haplotypes III, VII, each 5.6%, and haplotype IX, 1 chromosome (2.8%). Also, the haplotype [-++++-] was found in 3 α A chromosomes (8.3%), haplotype [-+++++] was detected in 2 α A chromosomes (5.6%) and haplotypes [-+—+—] and [-+—+—] were found in two α A chromosomes (each 2.8%). 5. Summary/conclusion: The α D-Punjab gene in Western Iran seems to be associated with a single mutational event(unicentric origin) and has arisen on the same chromosomal background common in the local population.

0161

THE NOVO 4 BP DELETION IN THE CODONS 20/21 AT THE FIRST EXON OF THE (α)-GLOBIN GENE CAUSING A (BETA)⁰-THALASSEMIA IN A SPANISH MALE

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(α)-thalassemias are a heterogeneous group of autosomal recessive disorders characterized by deficient(α +) or absent(α ⁰) (α)-globin chain synthesis. The large majority of the (α)-thalassemia defects are single nucleotide mutations that affect one of the different molecular processes involved in (α)-globin gene expression (transcription, pre-mRNA processing and translation). Only a minority of (α)-thalassemia are produced by deletions of the structural gene. More than 150 different(α)-gene mutations have been described so far. The largest number of (α)-thalassemia results derived from so-called *nonsense* and *frameshift* mutations which interrupt the normal translation of mRNA. AIMS We described a novel mutation in the coding region of the (α)-gene. This mutation is a frameshift deletion of 4 bp (-TGGA) in the codons 20-21 of the first exon of this gene. *Methods*. An asymptomatic 29-year-old man was studied following a routine blood smear analysis which had revealed mildly microcytic and hypochromic red cells. Diagnosis of (beta)-thalassemia was performed measuring red cell indices on an automated blood cell counter. The HbA2 and Hb F levels were obtained by high performance liquid chromatography (HPLC-VARIANTT). DNA was extracted using the salting-out-extraction procedure. Screening of the most prevalent(α)-thalassemia mutations in the Mediterranean populations was carried out by Real Time PCR (RT-PCR Light Cycler) and PCR-ARMS and the characterization of the mutation by sequencing in an ABI-PRISM DNA Automated Sequencer employing the Big Dye Terminator Cycle Sequencing Kit. The propositus exhibited an elevated red blood cell count(6.39 \times 10¹²/L); a lower MCV (59.7fL); a reduced MCH (19.6pg); an increased HbA2 concentration (5.1%) and a normal Hb F level (1.3%). The subject did not show ferropernia. Initial screening, performed by RT-PCR and PCR-ARMS, testing the most frequent(α)-thalassemia mutations in the Mediterranean area were negative. Sequencing in both directions established that the propositus is heterozygous for a 4 bp deletion at codons 20/21 (-TGGA). We report here a novel (α)-thalassemia mutation [deletion of 4 bp (-TGGA) at codons 20-21] that creates a termination codon at position 24, resulting in a truncated (α) chain. This heretofore unreported frameshift mutation could give raise to a (α)⁰ phenotype. Given the negative family history, it is possible that this represents a *de novo* mutation. Detection of novel mutation, such as the one reported here, allows us to describe a growing range of molecular defects causing (α)-thalassemia, thus providing molecular and genetic information for prenatal diagnosis.

0162**DETECTION OF NOVEL DELETIONS CAUSING α - AND β -THALASSEMIA BY HIGH RESOLUTION THREE-COLOR MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION**

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Thalassaemias are inherited hemoglobin disorders characterized by a quantitative reduction of the α - or β -globin chains. The molecular tests commonly used to identify deletions causing α - and β -thalassemia are gap-PCR, Southern blot analysis and fluorescent *in situ* hybridization (FISH) analysis. The applicability of these techniques is limited to known deletions, dependent upon the hybridization probes available and may involve time consuming and laborious cell culture to generate metaphase chromosome spreads. The development of a rapid and simple technique based on Multiplex Ligation-Dependent Probe Amplification for diagnostic and research purposes to detect rearrangements in the α - and β -globin gene clusters in DNA of patients suspected for deletion types of thalassemia or hereditary persistence of fetal hemoglobin (HPFH). *Methods.* Two sets of 35 and 51 probes were designed, covering a region of 700 kb of the α - and 500 kb of the β -globin gene cluster respectively, amplified by primers labeled with up to three different fluorophores. Out of the 38 patient samples tested for rearrangements involving the α -globin gene cluster, 11 different deletions were found in 19 samples. Six were not previously described, including two *de novo* deletions. Similarly, 31 out of 51 patient samples were found to carry 10 different deletions involving the beta-globin gene cluster, of which three were not previously described. Because of its robustness and simplicity, this technique is highly suitable for the detection of (large) rearrangements causing hemoglobinopathies. It can readily be implemented in laboratories capable of performing automated DNA fragment analysis in the size range of 80-125 bp. In addition, this approach as described here is a rapid and sensitive method for high resolution analysis of any region of the genome.

0163**EFFECTS OF LOCAL NITRIC OXIDE APPLICATION ON MICROCIRCULATORY BLOOD FLOW AND OXYGEN TENSION IN THE SKIN OF PATIENTS WITH SICKLE CELL DISEASE**P. Telfer¹, T.C. Ekwebelem², M. Heath¹, M. Radfar², A. Tucker³¹Royal London Hospital, London, UK; ²St. Barts & The London Medical, London, UK; ³St. Bartholomew's Hospital, London UK

Sickle cell patients commonly develop chronic and recurrent lower limb ulceration. This is thought to be due to poor microcirculatory flow and low tissue oxygen levels in the distal parts of the lower limbs. Conventional treatment with dressings and transfusion is often unsatisfactory. Oxygenated haemoglobin scavenges NO out of the circulation by converting NO to nitrate and deoxygenated haemoglobin binds to NO to form heme-nitrosyl-haemoglobin. It has been shown that plasma cell-free haemoglobin released from lysed erythrocytes in Sickle cell patients inactivates NO, so abolishing local NO-mediated vascular control. A novel system of generation and delivery of nitric oxide (NO) and related higher oxides of nitrogen (NO_x) has been developed, composed of 2.5g each of sodium nitrite and ascorbic acid suspended in aqueous gel and contained within a semi-permeable membrane patch. The system has previously been demonstrated to have potent vasodilator and anti-microbial activity. *Aims.* To determine the safety and tolerability and the effects of the NO/NO_x system on skin microcirculatory physiology in the lower limbs of patients with Sickle cell disease. *Methods.* Ten adult patients with homozygous sickle cell disease, in steady state, and with no lower limb ulceration were

selected. The NO/NO_x generation system was applied to the skin of the medial aspect of the calf for 60 minutes and measurements taken prior to and during application. Pulse oximetry was measured at the great toe of the treated leg. Heart rate and blood pressure were continuously measured during the session. Microcirculatory responses were assessed non-invasively by simultaneous measurements using Laser Doppler fluxmetry (LDF) and a transcutaneous gas probe (PO₂/PCO₂) in the region of the patch application. *Results.* The transmembrane NO/NO_x system was effective in enhancing microcirculatory blood flow and local tissue oxygen availability. LDF showed significant increases ($p=0.01$). Enhancement of microcirculatory velocity was associated with elevation of tissue oxygen levels. The increases in Tc-PO₂ measurements were significant ($p=0.01$) and clinically relevant (mean baseline 70mmHg to mean maximum response 91mmHg). In contrast, transcutaneous Tc-PCO₂, pulse oxymetry and blood pressure measurements remained unchanged ($p>0.2$). No reported adverse reactions were noted during the study and the system was well tolerated in all subjects. *Summary.* This study further elucidates potential applications of the NO/NO_x-generation and delivery system. This novel and evolving system may potentially be beneficial in the management of lower limb ulceration associated with Sickle cell disease. These findings, linked with the well-defined antimicrobial activity of NO/NO_x indicate that this system may be advantageous for surgical prophylaxis and wound care management. As such a cheap, effective and safe wound management system would be particularly suited to the global Sickle cell population.

0164**EFFECT OF ZOLEDRONIC ACID ON THE OSTEOPOROSIS OF THALASSEMIA**E.G. Gurkan¹, M. Evran², A. Tuli², F. Baslamisli³, Y. Kilinc¹¹Cukurova University Medical School, Adana, Turkey; ²Adana, Turkey; ³Fikri, Adana, Turkey

Osteoporosis remain as a prominent cause of morbidity, even in well-transfused and iron-chelated patients with thalassemia major. Impaired osteoblastic function and increased osteoclastic activities are among the possible contributory mechanisms of osteoporosis seen in thalassemia major. The aim of our randomized, placebo-controlled study was to investigate the effect of zoledronic acid on the osteoporosis of thalassemia. *Methods.* The effects of treatment on biochemical markers of bone remodeling and bone mineral density (BMD) were studied in 38 patients with beta-thalassemia and osteoporosis. Thalassaemic patients (mean age 23.1±8.2 years) were randomly divided to receive either zoledronic acid i.v. at a dose of 4 mg every 6 months over 12 months or calcitriol 0.25 mcg/day. All patients received 1000 mg of elemental calcium. Twenty-five healthy subjects were also included as controls. *Results.* Insulin-like growth factor 1 (IGF-1) levels which were significantly lower in zoledronic acid and vitamin D groups compared to controls ($p<0.001$) at baseline, increased significantly at the end of treatment in both groups ($p=0.04$ and $p=0.006$, respectively). At the end of the study, the lumbar spine and femoral neck BMD had increased significantly in the zoledronic acid group ($p=0.02$ and $p=0.01$) whereas in the placebo group neither of the measurements changed significantly ($p=0.3$ and $p=0.1$, respectively). No relevant side effects were recorded during our study. *Conclusions.* These data suggest that in patients with thalassemia-induced osteoporosis, administration of zoledronic acid is an effective treatment.

0165

GENETIC MODIFIER IN SICKLE CELL ANEMIA: NO INTERACTION BETWEEN α -THALASSEMIA AND UGT1A1 POLYMORPHISM IN THE OCCURENCE OF CHOLELITHIASIS

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High levels of erythrocyte destruction in sickle cell anaemia (SCA) result in chronic hyperbilirubinemia, with cholelithiasis occurring in a subset of patients. We have recently reported that the promoter polymorphism of UDP-Glucuronosyltransferase 1A1 (UGT1A1) gene, encoding a key enzyme in bilirubin catabolism, is a major genetic risk factor modifying the cholelithiasis prevalence in SCA patients. The promoter region of UGT1A1 gene contains a run of thymine-adenine repeats, (TA)_n (n = 5 to 8). We have shown that SCA patients can be classified into 3 risk groups according to UGT1A1 genotype-group 1 [homozygous (TA)⁶/ (TA)⁶ and heterozygous with at least one (TA)⁵ allele], group 2 [heterozygous (TA)⁶/ (TA)⁷ and (TA)⁶/ (TA)⁸] and group 3 [homozygous (TA)⁷/ (TA)⁷ and heterozygous (TA)⁷/ (TA)⁸]-presenting respectively low, intermediate and high unconjugated bilirubin levels and risk of cholelithiasis. We have pursued this study by analysing the influence of α -thalassaemia, another known genetic factor modulating the haemolytic rate in SCA. The 3,7 kb α -thalassaemia deletion and UGT1A1 promoter genotype have been determined in 169 SCA children and 145 SCA adults, followed by the sickle cell centre of Guadeloupe (French West Indies). Haematological and clinical data have been compared between patients stratified according to their α -globin status. In order to analyse the combined effect of UGT1A1 polymorphism and α -thalassaemia, we have compared hematologic and clinical data between patients stratified according to their α -globin gene status within each previously described UGT1A1 group. Compared to patients with 4 α -globin genes, patients with α -thalassaemia (64 children and 51 adults) show higher haemoglobin levels (7.8±1 vs. 8.2±1 g/dL, p=0.003; 8.2±1.4 vs. 9±1.5 g/dL, p=0.0007) and lower unconjugated bilirubin concentrations (50±29 vs. 41±26 μ M, p=0.007; 55±29 vs. 43±29 μ M, p=0.02). Nevertheless, the frequency of cholelithiasis is not statistically different between patients with and without α -thalassaemia, both in children and in adults (respectively 30% vs. 39%; 71% vs. 57%). The effect of α -thalassaemia on unconjugated bilirubin level can be detected only in the UGT1A1 group 1 (24.8±9.7 vs. 32.9±13.5 μ M, p=0.04; 25.5±13 vs. 35.4±14.2 μ M, p=0.016) suggesting an overload of the conjugation pathway in the two other UGT1A1 groups. No association is observed between α -thalassaemia and cholelithiasis frequency in none of the 3 risk groups established according to UGT1A1 genotypes in children and adult patients. Our study shows, as expected, that α -thalassaemia is associated with lower haemolysis rate and unconjugated bilirubin level in homozygous SS patients. There is no association between occurrence of cholelithiasis and α -thalassaemia. This suggests that the effect of α -thalassaemia on haemolysis is not sufficient to protect SCA patients from cholelithiasis. UGT1A1 gene remains the determinant genetic modifier of this complication.

0166

HYDROXYUREA TREATMENT IN α -THALASSEMIA INTERMEDIA AND MAJOR

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The clinical presentation of α -thalassaemia intermedia (TI) range from an almost asymptomatic disease through a severe disease similar to α -thalassaemia major (ThM). Most severe cases needed regular blood transfusions. Hydroxyurea (HU) increases the Hb F production in sickle cell anemia and protects these patients from recurrent crises. Several trials suggested a beneficial effect of HU alone or together with erythropoietin in TI patients. HU was also reported as a treatment for reducing extramedullary erythropoiesis. We report the results of HU treatment in 15 patients with TI and 3 with ThM treated in three pediatric haematology units. Sixteen patients required between three to 36 units of blood per year, median 7.5, in the two years period before HU treatment started. Two patients were not transfused before HU treatment, in one HU treatment was started because severe bone deformities and multiple RBC antibodies that prevent the use of blood transfusions and in another one treatment was initiated in an attempt to improve the Hgb level and induce healing of a chronic ulcer cruris. The mean age was 22.6±7 years. The patients were treated for a mean period of 46±25 months, median 50 months. Two patients failed to response. The HU dose was 0.5g/day 4 to 7 days a week, mean daily dose 10/9±3 mg/kg/day, a larger dose was not tolerated because neutropenia. Since the initiation of HU treatment no further blood transfusions were needed in most of the patients (12.7 units/year/patient before treatment vs 0.5 units/year/patient). The Hgb F level increased from a mean of 32±28% before HU to 49±31% after one year of treatment (p=0.1).

	Hgb (g/dl)
Before	7.4±0.7
3 months	7.68±0.6
6 months	7.71±1.1

When the baseline Hgb F was compared to the Hgb F level after one month or one year of treatment in each group of patients, α^0 and α^+ , the differences were not significant. In the Hgb level and in red blood cell indexes we found significant differences between patients with α^0 and α^+ thalassaemia. HU in α -TI and several patients with ThM induced a significant and long-standing increase in Hgb F which is comparable to the baseline Hgb F. No significant increase in Hgb or other RBC parameters was achieved, but in selected patients this treatment can replace BT in order to maintain an acceptable Hgb level with good normal daily activities. Larger trials are needed to prove the real efficacy of HU in the treatment protocol of α -thalassaemia patients.

0167**FAMILIAL POLYCYTHEMIA OWING TO Hb LA CORUÑA (β)38 (C4)THR-ILE. NEW HEMOGLOBIN STRUCTURAL VARIANT**P. Ropero¹, C. Fernández Lago², F. González¹, C. Benavente¹, M.P. Marta Polo¹, O. Briceño¹, M. Salvador¹, A. Villegas¹¹Hospital Clínico San Carlos, Madrid, Spain;²Hospital Juan Canalejo, La Coruña, Spain

The accurate behaviour of hemoglobin (Hb) is regulated by modifications in the quaternary structure. These changes determine both states of Hb T (deoxygenated) and R (oxygenated). Any anomaly that disturbs the regulation could lead changes in the function of Hb. In this way the (β)¹(β)² contact plays a very important role in promoting the cooperative oxygen binding. This contact is closely connected with the heme group therefore any change in this contact area or group heme contact could easily influence the environment of the heme and alter the oxygen affinity of the hemoglobin. A main group of hematologic disorder associated with abnormalities of hemoglobin function is familial polycythemia. AIMS We report new hemoglobin variant which was identified during investigating the case of moderate erythrocytosis. This Hb variant has an elevated oxygen affinity. A new mutation (β)38 (C4)Thr-Ile has been found which has been named Hb La Coruña. Blood samples were collected with EDTA and red cell indices were determined by routine automated cell counting. Hemoglobin was analyzed by cation exchange high performance liquid chromatography (HPLC), isoelectrofocusing (IEF) and reversed phase HPLC (RP-HPLC). Functional studies were carried out with a Hemox-Analyzer. Molecular characterization: genomic DNA was extracted using the salting-out-extraction procedure. The (β)-gene was sequenced by direct polymerase chain reaction automatic sequencing. The patient was a Spanish male whose family living in La Coruña, in the northwest of Spain. His mother was a carrier too. The propositus and his mother exhibited an elevated Hb (19.5 and 15.7 g/dL) and PVC (0.6 and 0.5 L/L); normal HbA2 and F levels and reduced P50 (16.5 and 17.5 mmHg). Electrophoretic techniques, IEF and cation exchange HPLC, did not detect any abnormal Hb. RP-HPLC revealed the presence of an abnormal (β)-globin chain (≥40%). Iso-propanol test was negative. Sequencing of the (β)-gene showed a substitution of ACC-ATC at codon 38. This produces the Thr-Ile change at position 38 of the (β)-globin chain. Hb La Coruña is the third Hb variant resulting from a mutation at(β)38 (C4) Thr-Ile, the other two Hb variant are Hb Hazebronk (-Pro) and Hb Hinwill (-Asn). Electrophoretically is a silent hemoglobin because substitution of Thr by Ile does not involve changes in the electric charge of the molecule, although is easily to recognize by RP-HPLC. This mutation causes high oxygen affinity. (β)38 (C4) is a heme contact residue, moreover the neighboring amino acids enter for (β)1 (β)2 contact, which is very important in promoting the cooperative oxygen binding. So, the replacement of Thr by Ile could bring structure modifications of shape in the quaternary structure of Hb. These changes could activate the mechanism that induces the formation of oxihemoglobin. In the case of Hb Hinwill the small change in polarity by substitution of Thr-Asn displaces the heme ring slightly towards R-form and its oxygen affinity is elevated. However, in the Hb Hazebronk the substitution of Thr by Pro produces hemolytic anemia and low affinity oxygen. Pro in position 38 fixes the switch in the T structure.

0168**YRUVATE KINASE DEFICIENCY: CLINICAL, HAEMATOLOGICAL AND MOLECULAR FEATURES IN 61 CASES**

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PK deficiency is the most common glycolytic enzyme defect associated with chronic non-spherocytic hemolytic anemia, with a prevalence of 51 cases per million white population as assessed by gene frequency studies. Aims. We report the clinical, haematological and molecular characteristics of all PK defi-

cient patients referred to our Centre in the last 30 years. Patients and Methods. 61 PK deficient patients (28M, 33F) from 54 families (44 of Italian origin, 4 from Northern Europe, 1 Gypsy, 5 from Australia, Pakistan, Tunisia, Oman, Guinea respectively). Median age at diagnosis was 16 years (range 1 day to 65 years). Consanguinity was reported in 4 cases. Haematological parameters and red cell enzyme activity were determined according to standard methods. The entire coding region and promoter of the PK-LR gene were amplified by PCR and automatically sequenced. Clinical characteristics of the patients are reported in Table1. The early onset of symptoms was usually associated with a severe clinical course: 16/25 exchange-transfused newborns subsequently required multiple transfusions and/or splenectomy. One case died during exchange transfusion soon after birth and in another a family history of intrauterine death was present. Median haemoglobin concentration was 9.8 g/dL in not splenectomised patients and 7.3 g/dL in candidates to splenectomy. Splenectomy usually resulted in stabilisation of the haemoglobin to a slightly higher levels (median Hb increase 1.8 g/dL, range 0.4-3.4) and in a conspicuous rise of reticulocytes (5-fold). Iron status parameters were increased in 33/49 patients (15 of whom never transfused): 18 had increased serum ferritin (SF) alone, 14 increased SF and transferrin saturation (TS), 1 TS alone. Autohemolysis and red cell osmotic fragility were normal in 75% of patients. Red cell PK activity was decreased (median 35% of normal) in all patients but four. Molecular characterisation of PK-LR gene was performed in 53 patients. 43 different mutations were detected among the 97 mutated alleles identified: 28 missense, 7 splice site, 1 nonsense, one 4 nucleotide duplication, 4 small deletion; two large deletions have been identified, the Gypsy deletion of 1149bp which results in the loss of exon 11 and a deletion of 5006bp which results at the cDNA level in the loss of exons from 4 to 11; 14 patients were found to be homozygous. In three patients no PK-LR gene mutations were detected and in three only one mutated allele was identified. The most frequent mutations in unrelated Italian patients were 1456T (19 alleles), 994A (7 alleles) and 1529A (6 alleles). Conclusions. PK deficiency is a very heterogeneous disease from both molecular and clinical point of view. A severe syndrome was commonly associated with some missense mutations (in particular 994A and 1529A) at the homozygous state, or with disruptive mutations such as stop codon in the first part of the protein (for example 721T), frameshift, splicing mutations or large deletions, or with missense mutations involving the last part of the protein.

Table 1.

Anemia	55/61
Jaundice	43/61
Neonatal jaundice	33/56
Exchange transfusion	25/56
Splenomegaly	47/58
Splenectomy	18/61
Transfusions	38/59
Desferal treatment	16/58

0169**MOLECULAR AND FUNCTIONAL CHARACTERISATION OF A LETHAL VARIANT OF PYRUVATE KINASE DEFICIENCY**E. Fermo,¹ P. Bianchi,¹ L.R. Chiarelli,² R. Rodwell,³ G. Valentini,² A. Zanella¹¹Fondazione IRCCS Ospedale Maggiore, Milan, Italy; ²University of Pavia, Pavia Italy; ³Mater Misericordiae Hospital, Queensland, Australia

PK deficiency is an autosomal recessive disorder associated with haemolytic nonspherocytic anaemia. To date, detection of mutations at the gene level advances rapidly and the avail-

ability of a system to easily obtain recombinant mutants of PK helps to better define the molecular basis of the defect and to correlate genotype to clinical phenotype. *Aims.* We describe a case of severe haemolytic anaemia due to PK deficiency caused by a missense mutation and a large deletion in the PK-LR gene. To investigate the contribution of the missense mutation in the severity of clinical pattern we have functionally characterised this variant by mutagenesis and *in vitro* expression of the protein. The patient was an Australian baby affected by PK deficiency. At birth he presented hepatomegaly, haemoglobin 8.9 g/dL, bilirubin 275 µmol/L, and ferritin levels > 4000 ng/mL. The baby died during a double exchange transfusion. Mutation analysis of PK-LR gene was performed by direct sequencing on baby's DNA and father cDNA extracted from reticulocytes. Expression and purification of the mutant protein were performed according to the method previously reported (Valentini *et al.*, 2002, *JBC*, 277). The kinetic, allosteric and thermostability parameters were evaluated and related to the clinical pattern. The direct sequencing of PK-LR gene performed on the propositus led to the detection of a missense mutation G409A (Ala137Thr) apparently at the homozygous level. The mutation was present at the heterozygous level in the mother but not in the father. The study of father reticulocytes mRNA revealed a large cDNA deletion encompassing exon 4 and exon 11 included. DNA analysis of father and baby showed the presence of a large deletion of 5006bp extending from intron 3 to the last nucleotides of exon 10. Ala137Thr mutant enzyme purified to homogeneity exhibited a specific activity of 320U/mg. The oligomeric state of the mutant protein was identical to that of the wild-type enzyme. Similarly, the kinetic parameters and the thermostability properties appear to be substantially unaffected by the Ala137Thr replacement. Furthermore the enzyme is less sensitive to ATP inhibition (IC₅₀ 2.3 mM vs 0.53 mM for the wild-type enzyme). *Conclusions.* the large deletion of 5006bp could be considered one of the most severe abnormality in PK deficiency so far reported. However, the biochemical data obtained for the recombinant mutant enzyme Ala137Thr build up by identical subunits would not explain the very severe clinical pattern found in the patient hemizygous for this mutation. We can suppose that in the PK-deficient erythrocyte the aberrant subunits generated by the deleted allele could interfere in the tetramer assembly of Ala137Thr enzyme, that would be rapidly degraded; alternatively, additional defects other than PK deficiency could exacerbate the clinical pattern.

0170

AHSP (α HEMOGLOBIN STABILIZING PROTEIN) GENE EXPRESSION DURING NORMAL AND α-THALASSEMIC ERYTHROID DIFFERENTIATION

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α-thalassemia severity is strictly related to the amount of free α-chains depending at least in part from different α-thalassaemic genotype. Recently a new erythroid-specific protein, named AHSP (α Hemoglobin Stabilizing Protein), able to form a stable complex with free α-hemoglobin, has been identified. This protein probably acts as a molecular chaperone to prevent the harmful aggregation and precipitation of α-globin that occurs during normal erythropoiesis and to a greater extent in different α-thalassaemias. The aim of this study was to evaluate if the AHSP expression in erythroid cells depends on free α-chains amount. In order to verify this hypothesis, we studied the relative expression of AHSP and globin genes during differentiation of erythroid progenitors derived from peripheral blood of both normal subjects and beta-thalassaemic patients with different genotypes. Erythroid cultures were set up accordingly to the two phases liquid culture method described by Fibach. Total cytoplasmatic mRNA was extracted by the method of Chomczynsky-Sacchi and quantitative real-time PCR was performed using SYBRGreen to evaluate AHSP, α, β and γ globin genes expression.

Table.

Genotypes	Day 13 □/non □ ratio	Day 13 AHSP gene expression	Day 13 vs. day 9 AHSP gene expression
Normal	0.78*	292*	-65%
□*□*	2.4*	699	-15%
□°□*	2.8*	1435	+150%
□°□°	3.6*	549	+57%
	10	8447	-17%
	3.3	48.5	-93%

*means of three different experiments.

To quantify gene expression a mathematical model using calibration data was used and quantification of the house-keeping gene S14 was the internal control. In normal subjects AHSP expression was first detected on day 3 of culture, peaked on day 9 and declined to low levels by day 13 (a decrease of 65% vs day 9 expression). Globin genes expression reached a steady state (□/non-□ ratio very near to a clinical equilibrium) on day 13. In this situation few free □-globin are detectable explaining the lower need of AHSP expression. In beta-thalassaemic subjects we observed a persistence of globin gene unbalance with an excess of free □-chains also in the advanced differentiation steps: interestingly the worst was the genotype, the highest was the globin ratio and lower was AHSP expression. In □*/□* thalassaemic patients AHSP expression declined on day 13 (a decrease of 15% vs day 9 expression) and globin gene ratio was 2.4, expression of a persistence of free □-chains excess. In □°/□* patients globin gene ratio on day 13 was 2.8 and AHSP expression increased of 2.5 times. Finally in □°/□° patients globin gene ratio was very high with values up to 9-10; AHSP expression profiles instead were different between different subjects and related not only to globin unbalance but also to ineffective erythropoiesis rate and □-globin chains persistence. All these findings underline an evident relation between AHSP and globin genes expression, suggesting a probable function of this protein during erythropoiesis.

0171

NATURAL HISTORY OF HB H DISEASE

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Three out of 4 α-globin genes are inactive in HbH disease because of deletions with or without concomitant nondeletional mutations and only one residual α-globin gene is functional. Hb H (b4) is relatively unstable and precipitates, causing mainly hemolysis and some ineffective erythropoiesis. The affected individuals usually have moderate anemia and marked microcytosis and hypochromia. However, there is a marked phenotypic variability, also in patients with identical α-globin genotypes. To define the natural history of Hb H disease in Mediterranean population. We have studied 248 Sardinian patients (133 females and 115 males) aged 2 to 82 years. Two-hundreds and twelve patients had a deletional HbH disease (-/-□) and 34 a nondeletional form (12 -/-□Hph□ and 22 -/-□Nco□). In 7%, diagnosis was made in the neonatal period, in 10% between 1 month and 1 year, in 23% between 1 and 6 years, in 16% between 6 and 18 years and in 44% in adult age. Fifty-four paediatric patients (0-16 years) have been followed longitudinally. Mean Hb was significantly lower and reticulocytes significantly higher in nondeletional than in deletional Hb H disease [8.6±0.7 vs 9.1±0.8 g/dL, 4.5±2 vs 2.9±1.3% respectively (*p*<0.0001)]. Unconjugated bilirubin was slightly higher in patients with the nondeletional type [1.1±0.5 vs 0.8±0.6 mg/dL (*p*=0.09)]. Four children (7.4%) had neonatal anemia and 3 (6.4%) neonatal jaundice. In 18.4% growth retardation (length z score <1.8) was found. Hepatomegaly was present in 18% and splenomegaly in 66% of patients. Twenty-six% had a mild heart dilatation, probably due to chronic anaemia. Eleven chil-

dren (20%) received sporadic transfusions because of hemolytic crisis during infections (5), aplastic crisis by *Parvovirus B19* (1), neonatal anaemia (4), surgery (1). Three children with HbH disease experienced gallstones. Mean serum ferritin in paediatric patients was 94 ± 89 ng/mL (range 13-666). In the adults with HbH disease the following complications have been detected: heart disease (33%), colic lithiasis (16%). The risk of developing gallstones was slightly higher in patients with UDPG-glucuronyl-transferase genotype associated with Gilbert syndrome. Forty-five patients became pregnant and 18% received transfusions during pregnancy. Other reasons for transfusion were haemolytic crisis in 7 cases, fall of hemoglobin in 7 cases, aplastic crisis by *Parvovirus* in two cases, surgery in 2 cases and infection in one case. With the exception of pregnancies, the prevalence of patients transfused in adult age was 9.7% (19/195). Considering both adults and children with HbH disease, the percentage of transfused patients was higher among patients with non-deletional of HbH disease (29% vs 13%). Mean serum ferritin in adults was 376 ± 281 ng/mL (range 13-1750 ng/mL). *Conclusions.* HbH disease should be suspected in case of hemolytic anaemia with hypochromia and microcytosis. The molecular analysis is important in order of a prognostic evaluation. Because of the possible complications, affected patients need to be accurately followed, especially in paediatric age.

0172

BRAIN PERFUSION ABNORMALITIES IN NEUROLOGICALLY ASYMPTOMATIC ADULT PATIENTS WITH SICKLE-CELL DISEASE. A VOXEL-BASED ANALYSIS OF BRAIN SPECT IMAGING

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Neurological dysfunction is a well known complication in patients with sickle cell disease (SCD), usually manifested as an acute cerebrovascular accident, headache, seizure or cognitive decline. Asymptomatic patients may be affected by silent infarcts. Since microvascular damage is prevalent in patients with SCD and that structural lesions are preceded by insidious perfusion deficits, we analyzed the hypothesis of an insidious brain perfusion compromise in SCD patients. *Methods.* Forty two SCD patients distributed as 33 HbSS (14 males and 19 females), 6 HbSC (1 male and 5 females) and 3 HbS, (3 females), mean age $33.4 (\pm 10.55)$, range 18-60 years) with mean steady state hemoglobin level of $8.5 (\pm 1.73)$; $5.2-13.5$) and a mean hematocrit of $25.1 (\pm 4.85)$; $15.6-38.5$) were submitted to brain perfusion SPECT using the rCBF tracer ethyl-cisteinate-dimer labeled with technetium-99m ($^{99m}\text{Tc-ECD}$). The group of patients with SCD was compared to a group of healthy control subjects (29 females and 20 males, mean age 31 ± 8 ; range 25-49 years) recruited within the local community, who were submitted to the same imaging protocol used in the SCD. Reconstructed transaxial images datasets were converted into ANALYZE format and corrected for the orthogonal plane of acquisition using MRICro software. Voxel-based analysis was performed using SPM2 (Wellcome Department of Cognitive Neurology). Images were spatially normalized to standard anatomical template defined in the atlas of Talairach and Tournoux. The normalized smoothed images underwent group comparison of regional tracer uptake using paired t-test. Contrasts were defined in order to estimate the probability of each voxel to have an increased or decreased tracer uptake in images from patients with SCD compared to images from the normal control group. Reduced tracer uptake was observed in many areas of the brain when compared to normal controls. These areas corresponded predominantly to watershed regions and other brain areas supplied by micro vasculature. We observed a reduced tracer uptake in the

central basal forebrain, which includes the basal ganglia and thalami, the anterior frontal region and the watershed region of the temporo-parietal-occipital transition. These regions were significantly hypoperfused when compared to controls areas (false discovery rate correction of $p < 0.05$). The findings of our study demonstrate that neurologically asymptomatic adult SCD patients exhibit a pattern of reduced $^{99m}\text{Tc-ECD}$ tracer uptake demonstrated by SPECT. Early diagnosis of such cerebral vasculopathy has prognostic implications and can be determinant in considering therapeutic alternatives to avoid increasing in brain lesion load and progressive disability.

0173

COMBINED CHELATION IN THALASSAEMIA MAJOR: REVERSING CARDIAC FAILURE AND PREVENTING FURTHER COMPLICATIONS

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The ultimate goal of iron chelation therapy in thalassemia patients (TmP) is to prevent iron-induced toxicity resulting from iron overload. Heart is the target organ of both iron toxicity and mortality. Desferrioxamine (Desferal[®]) monotherapy, SC or IV, is inadequate to prevent heart-associated premature death in TmP. Deferiprone monotherapy (Ferriprox[®]) appears to have a cardioprotective effect when the recommended dosage of at least 75 mg/kg is administered. On the other hand, combined therapy with Desferal[®] and Ferriprox, results to an additive or synergistic chelating effect that cannot be achieved with either drug alone, placing all patients in net negative iron balance. *Aims.* to manage cardiac function with combined chelation, preventing and reversing cardiac complications. Patients: 50 transfusion-dependent TmP received combined chelation of oral Ferriprox[®] (25-30 mg/kg t.i.d) and Desferal[®] (20-50 mg/kg, 8-12h SC or IV 2-6days/week), in a regimen adjusted on individual needs. L-carnitine and antioxidants were administered to all patients as supplements. For the study purpose TmP were divided in 2 groups: a) 23 TmP (46%), 8 females mean age 32,3y, & 15 males mean age 30,6y, with pre-existing cardiac disease (symptomatology, treatment, LV dysfunction) b) 27 (54%), 17 females & 10 males, without cardiac disease *Methods.* The following combination of tests was used: - The trend of monthly determinations of serum Ferritin - Cardiac Echo 2-4 times/year for group a) and annually for group b) - Signa-MRI at 1.5 Tesla, multi-echo T2 sequences to quantify Heart iron overload *Results.* 1) None of the 50 TmP died since combined chelation was implemented, while with Desferal[®] monotherapy mortality fluctuated between 13,3-14,3% in the last decade. 2) Group a) in all patients a reversal of symptoms occurred, LV function normalized and TmP stopped all heart medication. The improvement of all laboratory parameters was statistically significant ($p < 0,0001$). The recovery was faster (after 1 year of combined chelation) in 63% of females, that were less susceptible to cardiac disease (potential role of oestrogen protection). 3) Group b) no new onset or overall worsening was recorded and all parameters normalized with a statistically significant difference ($p < 0,0001$). *Conclusions.* The use of combination of tests to manage cardiac function and combined chelation is imperative because heart disease is asymptomatic until cardiomyopathy becomes advanced, hence resulting in poor outlook. Although Ferritin is not a reliable index, we consider as a safety threshold a cut-off level of 300 ng/mL. Any decrease $>10\%$ of LVEF or LVEF $<45\%$ is indicative of cardiac dysfunction. Heart MRI provides insight into who is at greatest risk of developing heart disease prior to the onset of clinical symptoms. Combined chelation with Ferriprox[®] & Desferal[®] seems to be the choice treatment for cardiac complications in TmP because of increased efficacy in a minimally intrusive way. Compliance to treatment remains of key significance to the efficacy outcome. It may be promoted by the obvious improvement of cardiac function and the normal life style that TmP can achieve while receiving it.

0174

RENAL DYSFUNCTION AND OSTEODYSTROPHY IN PATIENTS WITH SICKLE CELL THALASSEMIA UNDER LONG-TERM TREATMENT WITH HYDROXYUREAE. Voskaridou¹, E. Terpos², A. Margeli³, E. Hantzi³, J. Meletis⁴, I. Papassotiriou⁵, D. Loukopoulos⁶¹Laikon General Hospital, Athens, Greece; ²5¹ General Airforce Hospital, Athens, Greece; ³Aghia Sophia' Children Hosp., Athens, Greece; ⁴Univ. of Athens Medical School, Athens, Greece; ⁵Aghia Sophia' Children's Hospital, Athens, Greece; ⁶Academy of Athens, Athens, Greece

Hydroxyurea (HU) is presently considered as the main treatment for the reduction of sickle-cell crises; however, information regarding the potential of HU to inhibit progressive organ failure is scarce. The gradually failing renal function and renal osteodystrophy are well known complications of sickle cell disease (SCD). The pending question is whether administration of hydroxyurea over very long period of time may delay or prevent the appearance of these abnormalities. To this effect we evaluated the renal function and bone metabolism in 85 patients with HbS/α-thal (α⁰ and α⁺). Forty-six patients (22M/24F; median age 39 years, range: 23-67 years) received hydroxyurea (1 g/daily) continuously for 13 to 166 months up-to-date (mean±SD: 86.5±42.4 months), while 39 patients (19M/20F; median age 41 years, range: 24-70 years) had never received HU and were followed-up in our centre during the same period of time. In addition to conventional renal biochemistry we measured the levels of serum and urinary beta2-microglobulin (b2M), serum cystatin C (specific and sensitive index of glomerular filtration rate), and urine N-acetyl-β-D-glucosaminidase (NAG; reflecting the distal tubular cells function). The extent of renal osteodystrophy was evaluated by DEXA scans assessing bone mineral density (BMD), and by assaying various markers of (a) osteoclast function [soluble receptor activator of nuclear factor-κB ligand (sRANKL), osteoprotegerin (OPG), and tartrate resistant acid phosphatase isoform 5b (TRACP-5b)], (b) bone resorption [C-telopeptide of collagen type I (CTX)], and (c) bone formation [bone-alkaline phosphatase (bALP) and osteocalcin (OC)]. The above parameters were also evaluated in 16 age- and gender-matched controls. We found no differences in terms of number of patients who had increased serum creatinine levels, >300 mg/day protein excretion in the urine, microalbuminuria, and elevated cystatin C, NAG, serum and urinary b2M between the studied groups (Table).

Table. Results of renal function.

	Patients on HU n=46	Patients without HU n=39	p value
Parameters	n (%)	n (%)	
In the urine			
Protein > 300 mg/24h	12 (26.0)	10 (25.6)	> 0.97
Creatinine clearance <80 mL/1.73 m ²	14 (30.4)	16 (41.1)	> 0.48
B ₂ M > 0 mg/L	1 (2.1)	2 (5.1)	> 0.48
NAG/creat. < 5U/g Cr	19 (41.34)	22 (56.4)	> 0.41
In the serum			
Creatinine > 1.3 mg/dL	2 (4.3)		
Cystatin C > 0.96 mg/dL	15 (32.6)		
B ₂ M > 1.8 mg/L	28 (60.8)		

In addition, 14/46 patients who received HU (30%) had osteoporosis/osteopenia in DEXA scans, comparable to 11/39 HbS/α-thal patients (28.2%) who did not receive HU. Furthermore, all patients displayed significantly elevated levels of OPG ($p=0.001$), sRANKL ($p<0.01$), sRANKL/OPG ratio ($p=0.022$), and CTX ($p=0.02$), while significant correlations were found between serum cystatin C vs. both serum OPG and b2M levels as well as between cystatin C and the sRANKL/OPG ratio. Not

only do these results suggest that HU does not prevent renal dysfunction in this cohort of patients but also highlight the role of RANKL/OPG pathway in the renal-induced bone disease of sickle cell syndromes. Furthermore, NAG, cystatin C and OPG may be useful as early biochemical markers for the assessment of renal impairment in SCD patients.

0175

A TOOL FOR PREDICTING HAEMOGLOBIN VARIANTS-AN AID TO DNA DIAGNOSTICSB.E. Clark¹, J. Cunningham², B.J. Wild¹, S.L. Thein²¹Kings College Hospital, London, UK;²GKT School of Medicine, London, UK

King's College Hospital (KCH) is a referral centre for Hb variants and uses automated HPLC (BioRad Variant II) as the first line screening test followed by electrophoretic separations (isoelectric focusing (IEF), cellulose acetate membrane (CAM) and acid gel electrophoresis (AGE)) if a variant is present. Definitive diagnosis is then achieved by either mass spectrometry or DNA analysis. The former method is quick and uses small quantities of blood but the equipment is expensive and requires a high degree of expertise, making it unavailable to many institutions. PCR based DNA diagnostics is routine in many laboratories but is hampered by the cost of consumables and the time it takes. *Aims.* To minimise costs KCH has developed a database that statistically evaluates the phenotypic separations and predicts which Hb variant is present based on past data. A single entry from any one of the phenotypic separations is enough to generate a prediction, and for some variants this may be enough for a presumptive identification. The prediction can be used to direct definitive PCR-based diagnosis to a single nucleotide, obviating the need for DNA sequencing and thereby reducing costs and turnaround times. *Methods/Results.* The database contains the phenotypic separation data (collected over 6 years) of >850 variant samples, comprising >125 different variants, all definitively diagnosed. The electrophoretic separations are entered as distances (mm) the variant has travelled with respect to Hb A and the HPLC data is entered as the elution time of the variant. Each sample is only identifiable by a laboratory number and the date of diagnosis is automatically updated when the definitive diagnosis is entered. KCH will continue to enter variant data to increase the data set and improve the tool's accuracy. The tool continually records its prediction accuracy as definitive diagnosis data is entered. This allows the tool's accuracy to be statistically evaluated and highlights areas for continuing improvement. Summary At present the database is in routine use and has proven to be a useful tool in directing the DNA diagnosis and cataloguing all the definitively diagnosed variants. In time it is hoped that the phenotypic data will be enough to make a presumptive identification for all Hb variants, as is done for the common variants. KCH would now like to invite international groups to discuss collaborating to share the tool and data. If there is sufficient interest we aim to make the tool available on the Internet. This would expand the repertoire of variants and increase the dataset, improving the tool's power and accuracy. Laboratories would then be able to retrieve predictions based on their own past data or the entire database. If enough data is entered the tool could become a useful means of monitoring the frequency of the different globin mutations around the world.

0176

PNEUMOCOCCAL CARRIAGE AND RESISTANCE IN CHILDREN WITH SICKLE CELL DISEASE (SCD)Alexander¹, H. Rashid², R. Booy², P. Telfer²¹St. Thomas's Hospital, London, LONDON, UK; ²Queen Mary University, LONDON, UK

Background. In the UK, the 7-valent pneumococcal conjugate vaccine (PCV-7) was recommended for all children with SCD in 2002. 23-valent pneumococcal polysaccharide vaccine (PPV-23)

has been offered at the age of 2 for at least ten years. Serotype surveillance of *Streptococcus pneumoniae* (pneumococcus) carriage is critical to follow any changes in the prevalence and resistance of vaccine and non-vaccine serotypes associated with vaccination. *Aims.* To determine the carriage rates of vaccine- and non-vaccine serotypes of pneumococcus, and of penicillin resistant isolates, in children aged under 5 years with SCD prior to, and following the introduction of PCV-7. *Methods.* Nasopharyngeal (NP) swabs were taken from children attending routine haematology clinics, between April 2001 and April 2002 and again between June and December 2004. Pneumococcal isolates, antibiotic resistance patterns, and serotyping were done using conventional methods. *Results.* Study 1: 82 swabs were collected from 72 patients prior to use of PCV-7. None of the patients had received prevar. 17 samples (21%) grew pneumococcus with 1 sample containing 2 different isolates. All 18 isolates were potentially covered by PCV-7 (6B x4, 6A x2, 9V x1, 14x5, 19F x3, 19A x1, 23F x1), and 4 (22%) were intermediate penicillin resistant. Study 2: 50 NP swabs were collected from 44 patients after the introduction of PCV-7. 24 (48%) had received at least one dose of PCV-7. Seven samples (14%) grew pneumococcus. Only three could be serotyped (23F, 15B and 19A), the rest being untypable. Serotype 23F is covered by both PCV-7 and PPV-23 while serotypes 15B and 19A are covered by PPV-23 alone. Four isolates (57%) were of intermediate penicillin resistance. *Conclusions.* Nasopharyngeal carriage rate decreased after introduction of PCV-7, however, there was an increase in intermediate penicillin-resistant isolates and in non-serotypable isolates in those with nasopharyngeal carriage of pneumococcus. These findings may indicate that modern pneumococcal prophylaxis is encouraging emergence of more resistant strains.

Hodgkin's lymphoma

0177

CASPASE-3 EXPRESSION IN HODGKIN'S LYMPHOMA

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Background. Caspase-3 is a member of caspases family which mediates several complex proteolytic cascades responsible for execution of various stimuli of apoptotic cell death. *Aim of Work:* The aim of this study was to evaluate caspase-3 expression in Hodgkin's lymphoma patients, by immunohistochemical technique, with correlation to the available clinicopathological data aiming at estimating its prognostic value. *Material and Methods.* The study was carried out on 51 cases of HL that comprises 46 cases of classical Hodgkin's lymphoma (CHL) and 5 cases of nodular lymphocyte predominance Hodgkin's lymphoma (NLP-HL). CHL cases was formed of 23 cases nodular sclerosis (NS), 11 cases mixed cellularity (MC), 6 cases lymphocyte rich (LR) and 6 cases lymphocyte depletion (LD). Additional 10 cases of reactive follicular hyperplasia for control purpose were also included. *Results.* There were no significant statistical differences between different CHL subtypes as regard clinical comparison ($P > 0.05$). However, LR subtype was the most favorable histologic subtype, it showed the least mean of Hodgkin/Reed-Sternberg (H/RS) cells, mitotic count and represented with partial nodal effacement. While, LD was associated with largest mean of H/RS cells and of mitotic count. Different subtypes of CHL showed significant statistical differences regarding type of H/RS cells and its variants and the polymorphic cellular infiltrates. CHL cases were common (90.2%) than NLP-HL cases (9.8%). Classic RS cells were higher in CHL than in NLP-HL ($P < 0.05$). L&H (popcom) cells expressed significantly in NLP-HL cases. CHL cases showed tissue eosinophilia while NLP-HL cases did not show any eosinophilia ($P < 0.05$). CHL subtypes were significantly differentiated as regard caspase-3 immunostaining in the background cellular infiltrate ($P < 0.05$). In LD-CHL 66.7% showed >50% positivity in the

background cellular infiltrate while, in LR-CHL, 66.7% showed <25%. In CHL cases, 93.5% of the cases were caspase-3 positive in H/RS cells while all NLP-HL cases were negative. Positive caspase-3 (>10%) expression in H/RS was statistically correlated with increased apoptosis and favorable prognosis in CHL ($P < 0.05$). While negative caspase-3 in background cellular infiltrate was correlated with good outcome ($P < 0.05$). On multivariate analysis, stage at presentation of CHL followed by caspase-3 expression in H/RS cells were the most independent indicators of survival in CHL. *Conclusions.* NLP-HL has a distinct morphological and immunohistochemical characters differentiate it from other CHL subtypes. Stage at presentation was the most independent indicators of survival in CHL followed by caspase-3 expression. Caspase-3 expression could be used as a good prognostic marker in CHL. *Key words:* Caspase-3, Hodgkin's lymphoma, survival, immunohistochemistry.

0178

EXPRESSION OF MCM6 IN CLASSIC HODGKIN LYMPHOMA (CHL): COMPARISON WITH KI67 AND CORRELATION WITH CLINICAL STAGE AND SUBTYPES; AN IMMUNOHISTOCHEMISTRY STUDY IN 28 CASES

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Background. KiMCM6 has recently been introduced as a unique marker of complete cell cycle since it is expressed in early G1 phase, a cell cycle fraction that is not covered by antibodies specific to Ki67. Tumoral cells in HL display an increased growth fraction and diminished apoptosis. We investigated Proliferation Index (PI) immunohistochemically by MCM6 and compared it with PI by Ki67 expression and assessed its correlation with clinical stage and subtypes in 29 paraffin embedded lymphnode biopsies of patients with CHL referred to our Pathology Department, as a referral center for Mediastinal and Lymph node specimens. *Methodology.* Our samples were 29 paraffin embedded lymph node biopsies from 28 cases (10 male and 18 female males) of CHL with age ranged 13-68 with median age of 22 in stages of II=11, III=10 and 7 in stage IV, Nodular sclerosis subtype (NS)=19 and Mixed cellularity subtype (MC)=9. They were investigated immunohistochemically with monoclonal antibodies against KiMCM6 (with permission from Prof. R. Parwaresch, Department of Haematopathology and Lymph Node Registry, Kiel, Germany), and Ki67 (Ki55, DAKO). Antigen Retrieval was achieved by boiling the sections in Tris buffer PH=9 in autoclave. The Hodgkin's and Reedsternberg cells with clear nuclear staining were counted as positive and the percentage were calculated, blindly. *Results.* MCM6 expression was 27.2% more than Ki67 and the difference was statistically significant (78.5 ± 18 vs. 51.4 ± 34.3 , $p < 0.002$). Neither MCM6 nor Ki67 expression were associated with clinical stages (II, III, IV). MCM6 expression was more than Ki67 by 27% (97.45 ± 19 vs. 56 ± 33.2 , $p < 0.006$) in stage II and by 42% (80 ± 18 vs. 38 ± 38 , $p = 0.004$) in stage III. In stage IV, MCM6 (75.6%) was expressed more than Ki67 (64%) with no statistical significance. In MC subtype MCM6 was expressed more than Ki67 by 20.6% which was not significant statistically (75 ± 23 vs. 54.4 ± 30 , $p = 0.067$) and by 30% in NS subtype (80 ± 16 vs. 50 ± 37 , $p = 0.000$). In patients with bulky mass (X) MCM6 was expressed more than Ki67 by 22.2% (82 ± 20 vs. 59.5 ± 35 , $p = 0.005$) and by 33% in patients without bulky mass (75 ± 17 vs. 42.4 ± 34 , $p = 0.003$). *Conclusions.* The MCM6 values in this study reflects the growth fraction of tumoral cells in HL about 27% more than Ki67. As is seen in all analyses performed the Ki67 counts showed more dispersion than MCM6 counts. Although, there was no statistically meaningful difference between MCM6 expression and clinical stages and subtypes, in comparison with Ki67, there was significant higher expression of MCM6 in stages II and III. and NS HL. All non significant results were analysed for power of the test and all were low so may be by increasing sample size the results be changed may be not. The immunohistochemical staining of MCM6 is an easy, more reliable and accurate tool to evaluate PI of tumoral cells in HL.

0179**THE ASSOCIATIONS OF HLA-SYSTEM WITH HODGKIN'S DISEASE AND THEIR PROGNOSTIC VALUE**

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Hodgkin's disease (HD) is the first disease, for which the associations with HLA system was established. But for present day the result of investigations is ambiguous, also there is a problem of the search of supplementary factors for the discrimination of patients with unfavorable prognosis. The aims of our work was the determining of immunogenetic markers of predisposition and resistance to the HD development, and the connections of those markers with HD prognosis. *Methods.* 112 patients with HD were investigated. Controls were 332 donors of blood components. HLA-A, -B and -Cw specificities were determined by standard microlymphocytotoxic test. HLA-DRB1 and -DQB1 specificities were done by PCR-SSP methods. Frequency of HLA-specificities and RR (related risk of disease development) were analyzed, significance of association was determined by exact Fisher's test, Kaplan-Meier estimates were used to describe survival. *Results.* There were positive associations between HD development and the presence of Cw7 (RR=3.0) and DRB1*11 (RR=6.0). Negative associations took place between HD and DRB1*07 (RR=0.14). DRB1*01 (RR=0,31) was negatively associated with HD in variant of nodular sclerosis. The presence of this specificity in genotype diminished the risk of HD development in young people. DRB1*04 was associated with complete remission. The frequency of DRB1*04 in patients with remission was significantly higher than in patients insensible to treatment (20.0% vs. 8%). Event-free survival significantly distinguished in DRB1*04-positive (78%) and DRB1*04-negative (42%) patients. DRB1* -14 Æ -08 were associated in HD patients with resistance to treatment, their frequencies were 16.7% and 12.5% accordingly vs. 6.8% and 2.3% in patients with remission during 5 and more years. *Conclusions.* There are significant associations between HLA-system and predisposition/resistance to HD development, its histological variants, the age of the HD beginning and the answer to treatment.

0180**HIGH TOPOISOMERASE IIA (TOPOIIA) EXPRESSION IS AN INDEPENDENT PROGNOSTIC FACTOR HODGKIN'S LYMPHOMA (HL) TREATED WITH ABVD AND EQUIVALENT REGIMENS WITH OR WITHOUT RADIOTHERAPY (RT)**T.P. Vassilakopoulos¹, I.A. Doussis-Anagnostopoulou¹, D. Karandrea¹, P. Korkolopoulou², M.K. Angelopoulou¹, M.P. Siakantaris¹, S.I. Kokoris¹, E.M. Dimitriadou¹, M.N. Dimopoulou¹, C. Kalpadaki¹, M.-C. Kyrtsonis¹, E. Patsouris¹, C. Kittas¹, G.A. Pangalis¹¹Laikon General Hospital, GOUDI, ATHENS, Greece; ²University of Athens, GOUDI, ATHENS, Greece

Introduction: Topoisomerases constitute a family of highly conserved nuclear enzymes, which control, maintain and modify the topologic states –structure and function- of DNA. They are classified into topoI and topoII, based on their ability to cleave one or two DNA strands respectively. TopoII is found in two isoforms; whereas topoII, is relatively constant over cell and growth cycles, topoII α increases in rapidly proliferating cells, being expressed in the S and G2/M phases. Besides its role as a proliferation marker, topoII α is a target for several cytotoxic agents used in the treatment of HL, such as doxorubicine, epirubicine, etoposide, and mitoxantrone. High topoII α expression has been associated with adverse prognosis in some neoplasms. In contrast, in the single study conducted in 42 patients with HL, high topoII α expression was a favorable feature. *Aims.* To evaluate the expression of topoII α in patients with HL and investigate its potential association with clinical features and prognosis. *Patients and Methods.* We evaluated the immunohistochem-

ical expression of topoII α in lymph node biopsy sections of 235 patients with HL, who had been treated with ABVD or equivalent regimens with or without RT. Immunohistochemistry was performed with the streptavidin-biotin-peroxidase method, using the monoclonal antibody KiS1 (DAKO, Denmark, dilution 1:50) and diaminobenzidine as substrate. The proliferation marker Ki-67 was also evaluated in 74 patients, by the monoclonal antibody MIB1 (YLEM, dilution 1:100). In brief the median age of the patients was 30 years (15-82), 49% were males, 23%, 49%, 17% and 12% had stage I,II,III and IV (47% advanced stages defined as IB/IIB/III/IV), 36% had B-symptoms, 15% had ≥ 5 involved sites, 38% had anemia, 16% leukocytes $\geq 15 \times 10^9/L$, 7% severe lymphocytopenia, 37% serum albumin < 4 g/dL, and 32% elevated LDH levels. *Results.* topoII α and Ki-67 expression was demonstrated in all cases. The percentage of topoII α + Hodgkin-Reed-Sternberg (HRS) cells followed a normal distribution with a mean \pm SD value of $63 \pm 19\%$ (5%-98%). The corresponding figures for Ki-67+ HRS cells were $80 \pm 17\%$ (17%-99%). TopoII α and Ki-67 were loosely correlated (Spearman's rho 0.265, p=0.02). topoII α expression was not correlated with any clinical or laboratory parameter except of gender (higher in males, p=0.04). The percentage of topoII α + HRS cells was $< 30\%$ in 15 patients (6%), 30-74% in 149 (63%) and $\geq 75\%$ in 71 (30%) patients respectively. The 10-year failure free survival (FFS) for these 3 groups was 100%, $85 \pm 3\%$ and $67 \pm 7\%$ (p=0.002). In multivariate analysis topoII α was an independent adverse prognostic factor, when evaluated as continuous covariate. At a cutoff value of 75%, high topoII α expression was the most important independent predictor of FFS (p=0.002), followed by advanced stage disease and involvement of ≥ 5 anatomic sites. *Conclusions.* topoII α is variably expressed in all patients with HL. It appears to be a "primary" prognostic variable, because it is not correlated with the established conventional prognostic factors. Under standard treatment with ABVD or equivalent regimens, high topoII α expression provided strong and independent prognostic information, which may reflect the role of this molecule in cell proliferation.

0181**VERY LATE RELAPSES IN PATIENTS WITH HODGKINS LYMPHOMA**

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Background. It is generally accepted that most patients with Hodgkin's lymphoma (HL), who are destined to relapse, do so within the initial 5 years. However occasional patients relapse even later. The incidence of these very late relapses is not precisely known. Although relapses occurring > 1 year after primary treatment have a relatively favorable outcome, the prognosis of patients with very late relapses has not been established. *Aims.* To evaluate the actuarial incidence and risk factors for relapse in patients with HL, who had been in complete remission (CR) for 5 years after the diagnosis and initiation of treatment with chemotherapy (CT) or combined modality therapy (CMT), and investigate the outcome of these very late relapses after salvage therapy. *Methods/Results.* Among 485 patients with HL, who received CT/CMT and achieved a CR lasting for 5 years after treatment initiation, 443 remain in CR, 15 died from second malignancies and 27 experienced a subsequent relapse. The 10, 15, and 20-year relapse rate (RR), provided that a CR had been sustained for 5 years, was $4.4 \pm 1.0\%$, $9.0 \pm 1.9\%$ and $11.1 \pm 2.8\%$, respectively. The 15-year RR for patients with early stage (IA/IIA) or advanced stage (IB/IIB/III/IV) disease at diagnosis was $7.6 \pm 2.5\%$ vs $10.5 \pm 3.0\%$ (p=0.24). Among the baseline demographic, clinical, conventional laboratory, and first-line treatment characteristics, a higher risk of very late relapse was predicted in univariate analysis

by the higher number of involved sites (NIS ≥ 5 , $p=0.007$), non-nodular sclerosing histology (non-NS, $p=0.05$), stage IV ($p=0.02$), administration of MOPP vs anthracycline-based CT ($p=0.09$) and CT alone vs CMT ($p=0.03$). In multivariate analysis only a NIS ≥ 5 ($p=0.008$) and non-NS histology ($p=0.03$) were independently associated with the incidence of very late relapses. All 27 patients with very late relapses were treated with conventional salvage therapy (17 with non-cross resistant CT, 7 with the same CT regimen and 3 with RT alone). Two patients died of toxicity of salvage therapy, 5 did not achieve a second CR (CR2) and 20 achieved CR2. Among them 9 relapsed again and 11 remain in continuous CR2 for a median of 22 months (2-143). The 5-year freedom from second progression rate (FF2P) was $29\pm 11\%$ and the 5- and 10-year survival after relapse (SAR) was $64\pm 10\%$ and $41\pm 11\%$. B-symptoms, extranodal involvement and anemia at relapse were associated with inferior outcome after salvage therapy in univariate analysis. **Conclusions.** Among patients with HL, who were in continuous CR for 5 years after the initiation of first-line CT or CMT, approximately 9% experience very late relapses during the subsequent 10 years. This was more frequent in patients with higher disease burden and non-NS histology. The outcome of very late relapses was not satisfactory after conventional salvage therapy, with less than 30% of patients achieving a durable CR2. Thus, high-dose therapy should not probably be spared in this subgroup based simply on the length of CR1.

0182

THE PROGNOSTIC SIGNIFICANCE OF SERUM LACTATE DEHYDROGENASE (LDH) LEVELS IN PATIENTS WITH HODGKIN'S LYMPHOMA (HL)

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Background. LDH is considered a strong prognostic factor for aggressive non-Hodgkin's lymphomas. Some research groups have attributed an independent prognostic role for serum LDH levels in patients with HL, but these observations were not widely reproduced. **Aims.** To examine the impact of serum LDH levels on the outcome of 459 patients with HL, who were treated with ABVD or equivalent regimens with or without radiotherapy (RT). **Methods.** LDH was measured in pretreatment serum samples, by standard assays. LDH levels were considered elevated, when they exceeded the upper normal limit ($>1.0 \times N$), and significantly elevated, when they exceeded the upper normal limit by >1.5 times ($>1.5 \times N$). In multivariate analysis LDH levels were examined along with the number of involved sites (NIS), B-symptoms and the 7 parameters included in the International Prognostic Score (IPS). **Results.** Elevated LDH levels were recorded in 111/459 patients (24%), being moderately elevated (1.0-1.5xN) in 78 (17%) and significantly elevated ($>1.5 \times N$) in 33 (7%). Elevated LDH levels positively correlated with clinical and laboratory features that reflect increased tumor burden, such as clinical stage ($p<0.001$, but not stage IV, since they were elevated in 10%, 24%, 44%, and 27% of patients with stages I, II, III, and IV), B-symptoms ($p<0.001$), NIS ($p<0.001$), bulky disease ($p<0.001$), anemia ($p<0.001$), leukocytosis ($p=0.005$), severe lymphopenia ($p=0.001$), serum albumin $<4g/dL$ ($p=0.008$), $ESR \geq 50$ ($p<0.001$), and $IPS \geq 3$ ($p=0.01$). They were also higher in patients with nodular sclerosing histology ($p=0.009$). The 8-year failure free survival (FFS) rate was $81\pm 3\%$ vs $69\pm 5\%$ for patients with normal and elevated serum LDH levels, respectively ($p=0.006$), being $74\pm 6\%$ vs $59\pm 10\%$ for those with moderately and significantly elevated levels. Among 266 patients with early stage HL (IA/IIA), serum LDH levels were not predictive of FFS (8-year rates $87\pm 3\%$ vs $83\pm 7\%$, $p=0.39$). Similarly they were not predictive of FFS among 193

patients with advanced stage HL (IB/IIIB,III,IV) (8-year rates $67\pm 5\%$ vs $61\pm 7\%$, $p=0.26$). The 8-year overall survival (OS) was $88\pm 3\%$ vs $74\pm 7\%$ for patients with normal and elevated serum LDH levels, respectively ($p=0.005$). In multivariate analysis elevated LDH levels did not add independent prognostic information for FFS either in the whole patient population or in subgroups defined by stage. Furthermore they were not of independent significance for OS in the whole patient population or those with advanced disease. Although they appeared to independently predict OS in early stages, this should be interpreted with caution, due to the low number of deaths and the lack of association with FFS in this subgroup. **Conclusions.** Our data suggest that serum LDH levels correlate strongly with markers of tumor burden and aggressive biological behavior. These associations explain the disappearance of their adverse effect on the outcome, when multivariate analysis was performed.

0183

HODGKIN'S LYMPHOMA (HL) IN THE ELDERLY: OUTCOME AFTER TREATMENT WITH ANTHRACYCLINE-BASED CHEMOTHERAPY WITH OR WITHOUT RADIOTHERAPY (RT) AND COMPARISON WITH THE SUBGROUP OF YOUNGER PATIENTS

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Background. Published data on the effect of advanced age on the outcome of patients with HL are contradictory. Reported differences in prognosis between elderly and younger patients may have been due to differences in treatment intensity, inadequate staging, inferior efficacy of salvage therapy and presence of comorbid conditions. **Aims.** To compare the demographic, clinical and laboratory profile as well as the outcome of patients with HL > 60 years old in comparison with younger patients, provided that they had been treated with ABVD or equivalent regimens with or without RT. **Results.** Among 732 patients who were treated with ABVD or equivalent chemotherapy with or without RT in our Unit, 77 (12%) were 60 years or older. In detail, 33 of them (43%) were 60-66 years old, 31 (40%) were 67-74, and 13 (17%) were 75 years old or older. When compared with younger patients, elderly patients (≥ 60 years) had a borderline higher incidence of advanced disease (55% vs 46%, $p=0.16$). However several potentially adverse prognostic features were significantly more prevalent in elderly patients, including B-symptoms ($p=0.03$), bone marrow ($p<0.001$) and liver ($p=0.002$) involvement, iliac/inguinal involvement ($p=0.03$), and elevated beta2-microglobulin levels (78% vs 32%, $p<0.001$). On the contrary elderly patients had a lower prevalence of bulky mediastinal masses (8% vs 24%, $p<0.001$) which was probably the result of the lower frequency of nodular sclerosis (47% vs 70%, $p<0.001$). Laboratory features were similar in the two groups. IPS was clearly higher in elderly patients (≥ 3 in 53% vs 21%, $p<0.001$). There was no difference in the use of 7-8 drug regimens with respect to age but elderly patients received RT less frequently (50% vs 74%, $p<0.001$). Despite the relatively unfavorable prognostic profile of elderly patients, their 10-year failure free survival (FFS) was not significantly different from that of younger ones ($78\pm 2\%$ vs $75\pm 6\%$, $p=0.33$). However elderly patients had a higher incidence of toxic deaths (5.2% vs 0.3%, $p<0.001$), and inferior 10-year overall survival ($86\pm 2\%$ vs $44\pm 8\%$, $p<0.001$), HL-specific survival ($90\pm 1\%$ vs $71\pm 7\%$, $p<0.001$), and survival after failure ($41\pm 6\%$ vs 0%, $p<0.001$). Further subgrouping of elderly patients according to age did not demonstrate differences in FFS. **Conclusions.** When treated with anthracycline-based chemotherapy, elderly patients have similar failure rates with younger ones. However the higher incidence of toxic deaths and the poor results of salvage therapy result to inferior overall and HL-specific survival rates.

0184**PROGNOSTIC VALUE OF INTERLEUKIN 10 (IL10) SERUM LEVELS IN PATIENTS WITH ADVANCED HODGKIN'S LYMPHOMA**

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The aim of the study was to assess the predictive value of serum soluble Interleukin 10 (sIL10) at diagnosis in patients with Hodgkin's lymphoma and its correlation with clinical, laboratory features, and event free survival (EFS). Patients and *Methods*. Between February 1996 and December 1999, 60 consecutive patients with Hodgkin's lymphoma (32 man / 28 woman) were evaluated. All patients were clinically staged according to the Ann-Arbor system. The following parameters were evaluated for prognosis: age, sex, histology, clinical symptoms, extra-nodal disease, stage, treatment, blood counts (haemoglobin, white blood cells, lymphocyte and platelets counts), blood chemistry (including: β 2microglobulin, LDH and thymidine-kinase levels) and serum soluble IL10 and IL6 using an ELISA method. Statistics: Continuous variables were compared by using the U Mann-Withney test and dichotomised with optimal cut-off values using the CART method. EFS was estimated by the Kaplan and Meier method. Cox multiple regression analysis was performed to determinate the independent contribution of the variables. *Results*. The median age was of 36,5 years (range 15-82), 20 (33%) patients presented B symptoms. 23 out of 60 patients (38%) were considered as having advanced disease (II-B, III-B, and IV stages according with the local definition). Bulky disease (>10 cm) was present in 35%, and bone marrow was involved in 7%. The predominant histology was nodular-sclerosis (65%). COPP as upfront chemotherapy was used in 18 patients (30%), hybrid schedule in 21 (35%), AVBD in 15 (25%) and radiotherapy in 6 (10%). sIL10 levels were higher in patients with anaemia (Hb < 10,5 g/dL, $p=0.002$), low serum albumin (< 3,5g/L, $p=.021$), advanced disease ($p < 0.001$), B symptoms ($p < .001$), serum β 2microglobulin > 3mg/L ($p=.008$), ESR > 30 mm ($p < .001$), and thymidine-kinase > 10 U/L ($p < 0.001$). Estimated EFS was 68% at 7 years with the median follow-up of 60 months. In the univariate analysis the presence of a IV stage, extra-nodal disease, bone marrow involvement, hypoalbuminaemia, increased β 2microglobulin, and sIL10 > 44 pg/mL (all with $p < 0,05$) showed prognostic value for EFS. Only sIL10 > 44 pg/mL had independent predictive value for EFS in the multivariate analysis. Three groups could be established combining stage and sIL10: a) patients with early disease (84% EFS at 5 yr), b) patients with advanced disease and low sIL10 (69% EFS at 5 yr), and c) patients with advanced disease and sIL10 elevated (14% EFS at 4 yr) ($p=0.001$). *Conclusions*. Serum levels of IL10 can help to identify a subgroup of patients with advanced disease and poorer EFS who may benefit from more aggressive upfront therapy.

0185**SAFETY AND EFFICACY OF VINORELBINE/GEMCITABINE COMBINATION (NAGEM) FOR REFRACTORY LYMPHOMAS**

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The prognosis of Hodgkin Disease (HD) or Non-Hodgkin lymphoma (NHL) refractory to salvage therapy or autologous stem cell transplantation (ASCT) remains poor. We retrospectively evaluated the efficacy and safety of the Vinorelbine/Gemcitabine (NaGem) combination in a series of 36 patients with primary refractory or relapsing disease (HD =26, NHL=10). They had received a median of 4 lines of treatment (range, 1-7) and 20 had already underwent ASCT. A total of 110 cycles (median, 2; range, 1-6) of Gemcitabine at 1250 mg/m² and Vinorelbine at 30 mg/m² (days 1 & 8, q28 days) were administered. Response was observed in 12/26 HD patients vs. only

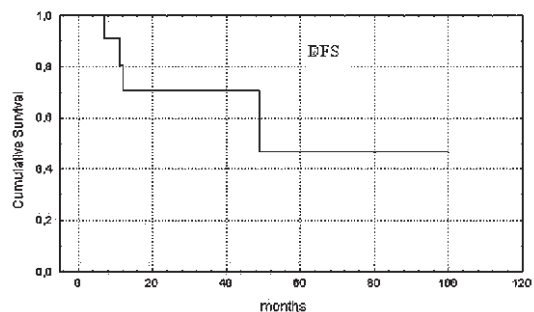
1/10 NHL patients. The 2-year Overall Survival (OS) and Event Free Survival (EFS) for all patients were 15% and 12% respectively. Among responding patients, 5/13 attained complete remission (CR; all HD patients) while the remainder attained a partial remission. The 2-year EFS and OS for responding patients were 29% and 38% respectively. Especially, in the HD cohort, 2-year EFS and OS rates were 18% and 29% respectively. On multivariate analysis [tested variables: IPI/IPS, disease type and stage, prior treatment, performance status (WHO), age], EFS was adversely affected by poor performance status and NHL diagnosis; IPI/IPS was the only adverse prognostic variable for OS. Nine patients without response to or progression after intermediate intensity regimens for stem cell mobilization received NaGem. Interestingly, 3/9 responded (CR: 1, PR: 2) and finally underwent ASCT. Myelotoxicity > gr.3 was observed in 18 patients; deep vein thrombosis and incomplete ileus attributed to vinorelbine developed in, respectively, one and seven patients; treatment was interrupted in three out of seven patients with incomplete ileus. In conclusion, the NaGem combination proved an effective salvage regimen for patients with refractory HD with good performance status, and was associated with an acceptable toxicity profile in this group of heavily pretreated patients. In this retrospective study the above combination seems ineffective for refractory NHL. Further studies are needed to establish its efficacy and define its role in the management of lymphoma patients.

0186**LATE RELAPSE IN HODGKIN'S DISEASE: TREATMENT RESULTS**

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With current treatment modalities, most patients (pts) with early stage Hodgkin's disease (HD) can be cured. Relapsing pts, usually do so within 3 years after treatment completion. Pts who have a brief duration of first remission have a poor outcome. Recurrences of HD after 5 years after initial therapy are rare and pathological, biological and clinical features in this subset of pts are to be assessed. Aim of this study is to describe the characteristics and the outcome of pts who had late relapses, defined as relapses occurred 5 or more years after first complete remission. *Methods*. Of 498 consecutive pts with classical HD treated at our Institute from 1985 to 1999, 452 pts (91%) had complete remission, but 150 had a relapse (135-29.8%- early and 15 -3.3%- late). The histologies of the 15 pts in late relapse were mixed cellularity in 7 (46.7%) and nodular sclerosis in 8 (53.3%). The median age was 40 yrs (16-70). 8 pts (53.3%) had stage I-II. International prognostic score were 0-1 in 9 pts (60%) and 2-3 in 6 (40%). 12 pts (80%) presented B symptoms at diagnosis, and 7 (46.7%) had bulky disease.



Results. Late relapse occurred after a median disease free interval of 7 yrs (range 5-18) and it involved sites of previous disease in 10 pts (66.6%). Salvage treatment induced a complete response in 14 pts (93.3%) and a prolonged complete remission in 11 (73.3%). At a median of 4 years after therapy for late relapse, 9 pts (60%) are still alive and free of disease and 6 (40%)

died (1 of HD and 5 of toxicity). The probability of disease free survival at 4 years was 70%. Late relapse is associated with a better survival than relapse occurring within the first 4 yrs from the time of diagnosis. Late recurrences of HD usually regarded as relapses of initial disease at least part of these may represent *de-novo* HD due to an increased constitutional risk.

0187

POLYMORPHISMS IN THE XENOBIOTIC METABOLIZING ENZYME CYP1A1 AND RISK FOR HODGKIN'S LYMPHOMA

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Background. Polymorphisms in xenobiotic genes can modify an individual's susceptibility to the carcinogenic effect of environmental and pharmacological agents. The role of these enzyme systems in the pathogenesis of lymphomas is not well known. Generally, these enzyme systems are divided in a phase I class, to which the activating cytochrome P-450 (CYP) enzymes belong, and a phase II family, including conjugating enzymes such as the glutathione-S transferases (GSTM1 and GSTT1). We have previously shown an increased frequency of deletions of GSTT1 gene in patients with Hodgkin's lymphoma (Clin Cancer Res 2003; 9:3435). **Aims.** We assessed the role of polymorphisms in the CYP1A1 gene to modify the risk for Hodgkin's lymphoma. **Methods.** CYP1A1 mutations T6235 (m1), A4889G (m2) and C4887A (m4) were characterized by a PCR-RFLP approach. These mutations define the different alleles: CYP1A*2A, CYP1A*2B and CYP1A*4. DNA was extracted from peripheral blood from 130 patients with Hodgkin's lymphoma (median age 33 years, range 13 - 71 years; 52 females and 78 males) and compared the genotypes frequencies to a control group of 260 normal blood donors matched for age and sex (median age 39 years, range 18-72; 103 females and 157 males). **Results.** In the control group, the CYP1A*4 variant was present in 12% (31/260) of normal donors, while in patients with Hodgkin's lymphoma the prevalence was 52% (129) ($p < 0.001$, Fisher's exact test). Using the logistic regression model, this translates into an odds ratio of 4.8 (95% C.I., 2.9-7.8) for individuals with the CYP1A*4 variant to develop Hodgkin's lymphoma ($p < 0.01$). The risk for Hodgkin's lymphoma was not increased in individuals with the CYP1A*2A and CYP1A*2B variant. Genotype frequencies were not different in the patient and control group: 15% vs 17% and 9% vs 9%, respectively. We also examined for associations between CYP1A1 alleles and patient characteristics, including histotype, stage of disease, presence of B-symptoms, bulky disease and abnormal laboratory parameters. Using the Fisher's exact test, no such associations became evident. **Conclusions.** These data suggest a role for CYP1A*4 as risk factor in the pathogenesis of Hodgkin's lymphoma. stefan.hohaus@rm.unicatt.it

0188

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR RELAPSED OR REFRACTORY HODGKIN'S DISEASE IN CHILDREN AND ADOLESCENTS: A SINGLE CENTER EXPERIENCE

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High-dose chemotherapy with autologous hematopoietic stem cell transplantation (HSCT) is becoming the standard approach for the management of the relapsed and refractory Hodgkin's disease (HD) in adults. Aim. Since the experience in children and adolescents is limited, this retrospective study was undertaken to assess the outcome of children with HD who have undergone a HSCT in a single Center over a 20-year period. **Methods.** Twenty-seven HD patients (19 males and 8 females) aged 7 to 17 years (median 13.2) at diagnosis, with relapsed or refractory HD underwent a HSCT between March 1984 and April 2004 at the Division of Hematology, 'La Sapien-

za' University, Rome. Twenty-three patients were treated with salvage chemotherapy before HSCT. Thus, at the time of transplantation, 11 (41%) patients were in 2nd complete remission (CR), 3 in 3rd CR, 9 (33%) in partial remission (PR) (6 in 1st and 3 in 2nd) and 4 patients (16%) presented active disease (AD). All patients received chemotherapy-based conditioning regimens: the carmustine, etoposide, citarabina and melphalan (BEAM) regimen was employed in 14 (52%) patients and the cyclophosphamide, carmustine and etoposide (CBV) protocol in 13 (48%). Peripheral blood (PB) was the source of progenitor cells in 16 patients and bone marrow (BM) in 11. **Results.** All but 1 patient engrafted. The median time to reach neutrophils $> 500 \times 10^9/L$ and platelets $> 20 \times 10^9/L$ was 11 days after PB vs 18 and 21 days after BM, respectively. At a median follow-up of 4.1 years, (range: 0.1-17.4), of 26 evaluable patients, 19 (73%) are alive (17 in CR), while 7 patients have died (4 of transplant-related complications). The projected 5-years overall survival (OS) and progression free-survival (PFS) rates are 64% and 54%, respectively. With regard to the disease status at time of transplantation, no statistical difference was found between patients transplanted in CR and those in PR or with AD. On the other hand, the stem cell source significantly affected survival: the 5-year projected OS rates is 94% for patients who received PB cells and 45% for those who reinfused BM cells ($p = 0.0424$). **Conclusions.** The results of our study indicate that HSCT is an effective treatment modality that can result in long-term response and cure for children and adolescents with relapsed or refractory HD, also for those with AD. Moreover, the PB cell source of hematopoietic stem cells has a significant influence on the disease outcome, reducing the transplant-related complications.

0189

IMMUNOHISTOCHEMICAL EXPRESSION OF VEGF, CD34 AND CD44 IN BONE MARROW INFILTRATION BY HODGKIN'S LYMPHOMA

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Bone marrow (BM) infiltration is present in 5-15% of patients with Hodgkin's lymphoma. It is usually associated with prominent fibrosis and proliferation of blood vessels. Limited information exists about the significance of neoangiogenesis and expression of *homing receptor* CD44 in Hodgkin's lymphoma. **Aims.** In this study we analyzed immunohistochemical expression of VEGF, CD34 and CD44 in BM infiltration by Hodgkin's lymphoma. **Methods.** We identified 38 patients (28 male and 10 female, median age 45 years) with BM infiltration by Hodgkin's lymphoma. The most frequent histological type was nodular sclerosis (26/38). Slides from paraffin embedded BM biopsy were stained with hematoxylin&eosin and reticulin fibers (Gordon-Sweet). BM microvessels were visualized by immunohistochemical staining for CD34. We counted the number of vessels per 400x high power field (HPF) in the area of most dense vascularization. All samples were further analyzed for the expression of VEGF and CD44 in neoplastic Hodgkin-Reed-Sternberg (HRS) cells. The intensity of staining was graded as weak, moderate and strong. **Results.** In majority of patients (30/38) we found marked increase of microvessels density, which correlated with the number of VEGF-positive HRS cells. We observed that mean vessels count per HPF was 10.5. The intensity of VEGF expression in HRS cells was usually moderate. Immunohistochemical expression of CD44 was high in HRS cells of majority of patients (25/38). **Conclusions.** Our findings suggest that neoangiogenesis in BM infiltration by Hodgkin's lymphoma was extensively increased. Therefore, the density of microvessels and expression of VEGF may provide useful prognostic information and basis for the therapeutic investigation of anti-angiogenic agents. The results of CD44 expression might suggest causal involvement of this marker in the progression of Hodgkin's lymphoma, especially nodular sclerosis type.

Infectious complications I

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CLINICAL AND LABORATORY MANIFESTATIONS OF PATIENTS WITH PRIMARY IMMUNODEFICIENCY DISORDERS ASSOCIATED NEUTROPENIA; THE REPORT FROM IRANIAN PRIMARY IMMUNODEFICIENCY REGISTRY

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Primary immunodeficiency disorders are relatively rare disorders, characterized by an unusual susceptibility to infections. Some of these conditions may also feature neutropenia as a consequence of either an intercurrent infection or an autoimmune disease. Neutropenia is characterized by decrease in the absolute number of circulating neutrophils and increased susceptibility to infections. *Aims.* The current study was performed in order to explain the clinical and laboratory findings of primary immunodeficient patients with associated neutropenia. *Methods.* The patients' records of 56 neutropenic cases out of 474 patients with primary immunodeficiencies, who had been enrolled to Iranian primary immunodeficiency registry during a 24-year period (1980-2004), were reviewed. *Results.* Fifty-six cases (32 males and 24 females) with the mean age of 10.7±5.7 years were associated with neutropenia (11.8%). The median age for the onset of disorder was 6.5 months (1-134) and the median age at the time of PiD diagnosis was 3 years (2 months-13 years), with a median diagnosis delay of 23 months (<1 month - 12 years). The most common disorders with neutropenia were shwachman-diamond syndrome (7 of 7), cyclic neutropenia (7 of 7), Kostmann disease (6 of 6), chediak-higashi syndrome (5 of 10), hyper IgM syndromes (3 of 8), severe combined immunodeficiency (3 of 19), hyper IgE syndrome (2 of 15), and common variable immunodeficiency (13 of 109). The other less common primary immunodeficiency disorders with neutropenia were: chronic mucocutaneous candidiasis (1 of 12), x-linked aglobulinemia (3 of 45), leukocyte adhesion defects (1 of 17), ataxia-telangiectasia (3 of 75), and chronic granulomatous disease (2 of 81). The mean of absolute neutrophil count was 666.0±423.1 cells/mm³ (range: 25-1376/mm³), at the time of diagnosis.

Classification	Number of patients	Cd concentration (100-300 mg/l)	C1q concentration (80-120 mg/l)	C1 INH concentration (124-240 mg/l)	C1 INH function
Atypical Anaphylactoid					
	8	Normal	Normal	Normal	Normal
Nonallergic Angioedema					
Hereditary Angioedema Type I (HAE-I)	4	Low	Normal	Low	Normal
Hereditary Angioedema Type II (HAE-II)	2	Low	Normal	Normal	Low
Idiosyncratic nonhistaminergic angioedema	12	Normal	Normal	Normal	Normal
Acquired Angioedema (AAE)	2	Low	Low	Normal/Low	Normal/Low

The median count of white blood cells and monocytes were: 3635/mm³ (range: 1620-9600/mm³) and 204/mm³ (range: 24-1480/mm³), respectively. According to the classification of neutropenia, 26 patients suffered from severe, 16 moderate, and 14 mild neutropenia, respectively. Also, 32 out of these patients showed leukopenia (57.1%), 24 had anemia (42.9%), 11 thrombocytopenia, and only 3 patients had monocytosis. The most commonly occurred manifestations in patients with neutropenia were: pneumonia (30 cases), otitis media (28 cases), acute diarrhea (28 cases), abscess (24 cases), oral candidiasis (23 cases), oral ulcers (17 cases), cutaneous infections (16 cases), and

sinusitis (12 cases). Other less frequent infections were: periodontitis, conjunctivitis, cystitis, meningitis, and osteomyelitis. Seven neutropenic patients have died because of recurrent infections. Neutropenia may occur in any of the primary immunodeficiency disorders. Persistent or severe infections always raise a suspicion, which deserves further evaluation for detecting an underlying immune deficiency syndrome and neutropenia, as a delay in diagnosis may result in a serious organ damage or even death of the patient.

0191

SEVERE HEMATOLOGICAL ABNORMALITIES IN PATIENTS WITH RICKETTSIOSIS

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Rickettsiosis due to *Rickettsia conorii* (Mediterranean spotted fever) is endemic in the island of Corfu, with more than five new cases annually. Although the clinical manifestations and the course of the disease is characteristic, differential diagnosis is sometimes difficult, especially when typical clinical manifestations are absent. 26 cases of rickettsia infection were diagnosed and treated in our hospital the last eight years. All patients had fever and headache and all but one had a rash (maculopapular or petechial). One patient presented with coma. The characteristic eschar at the site of the tick bite with or without regional lymph node enlargement was found in four patients. Splenomegaly and liver enlargement was detected in 7 patients. All patients had anemia, mild elevation of CPK, mild to moderate elevation of erythrocyte sedimentation rate (48-120 mm), C-reactive protein (4,5-25 mgr/dL), LDH and abnormalities of the liver (bilirubin and Alanine aminotransferase) and renal function. One patient had thrombocytosis (PLT = 750.000/mL). Cerebrospinal fluid was examined and found normal in one patient. In 6 cases bone marrow examination was done, and reveal normal to hypercellular marrow with hyperplasia of macrophages which presented active phagocytosis. In six patients the initial presentation of the disease was with severe hematological complications. Thrombotic thrombocytopenic purpura was the initial diagnosis in patient one. Disseminated intravascular coagulation (DIC) with hypofibrinogenemia in patient two. Severe thrombocytopenia with platelet number less than 12.000/m³ plus agranulocytosis and mild anemia in patient three, four and five. Direct antiglobulin test positive severe hemolysis in patient six. Elisa test (IgM or IgG) became positive in all after the second week of the disease and the diagnosis was confirmed by the detection of antibodies to *R. conorii* by the microimmunofluorescence test. Treatment with doxycycline or chloramphenicol plus supportive care (when necessary), was effective and prompt in all patients. Recovery was complete with no permanent sequelae in all our patients. *Conclusions.* In patients with severe hematological abnormalities when an association with acute infection is suspected, *R. conorii* should be considered in the differential diagnosis especially in areas where *R. conorii* is endemic.

0192

INVASIVE FUNGAL INFECTIONS AND ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN: A SINGLE-CENTER RETROSPECTIVE STUDY.

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Invasive fungal infections (IFI) are an increasing cause of morbidity and mortality after Hematopoietic Stem Cell Transplantation (HSCT). We retrospectively analysed the incidence and outcome of IFI in our population. *Materials and Methods.* From October 1991 to June 2004, 90 allogeneic HSCTs were performed in 86 paediatric patients (pts) in our unit. In 61 pts (67.7%), HSCT was done as part of the treatment for haema-

tological malignant diseases, 12 pts were treated with HSCT for SAA/Fanconi anaemia (13,3%) and 17 pts had HSCT for other non-malignant diseases (18,8%). 53 pts were boys and 36 were girls with a mean age of 6,65 years (range 3 months -16 years). The donor was matched unrelated (MUD) in 44 cases (48,8%); 36 patients received stem cells from an HLA- identical sibling (40%); 4 were transplanted with matched related donors other than sibling and 6 donors were haploidentical. All patients were nursed in single rooms with HEPA filtration and received oral Amphotericin B up to 3 months after transplant. The diagnosis of IFI was based on results from blood cultures, biopsy or autopsy material and bronchoalveolar lavage together with findings on high resolution computer tomography. IFI was defined according to the criteria from the EORTC/ IFICG consensus as *proven, probable and possible*. Nine patients, 5 boys and 4 girls with a median age of 8.5 years, developed a proven (6 patients) or probable (3 patients) IFI. 5 patients received grafts from an MUD and 4 from an identical sibling, 3 out of these 9 pts received more than one allogeneic HSCT. 1 patient developed a systemic infection due to *Candida albicans* during the neutropenic phase (< 50days post transplant). 5 pts had an invasive aspergillosis (4 lung aspergillosis and 1 cerebral aspergillosis) as a late complication (4,5,11,12 and 44 months post transplant) while receiving immunosuppressive treatment for chronic GvHD in 2 pts, or during reinduction for relapsed disease in 3. Three other pts had an aspergillus infection (2 lung localization and 1 sinus localization) prior to HSCT. 2 out of these pts died due to transplant related toxicity. **Conclusions.** The incidence of IFI after allogeneic HSCT was 10% in a single-centre pediatric population. 4 out of 9 pts died from invasive fungal infections despite specific treatment. Transplant related mortality in these patients was 77% vs. 25% in the group without IFI (Chi square =7.779 $p=0.005$). The most frequent IFI was *Aspergillus* and was predominantly seen as a late complication in patients under steroids and/or immunosuppressive treatment.

0193

ANTIFUNGAL PROPHYLAXIS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES-A SINGLE CENTRE EXPERIENCE

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Background. Fungal infections are common in patients with haematological malignancies and have a high case-fatality rate. Studies on the effectiveness of antifungal prophylaxis in haematological malignancies show varied results. **Aims.** The purpose of the study was to evaluate the effectiveness of antifungal prophylaxis at our centre. **Methods.** 104 patients admitted between January and July 2004 were stratified into low- risk and high-risk groups. The low risk group included autologous transplants, standard leukemia induction, treatment with corticosteroids and purine analogues. The high risk group included autologous transplants with total body irradiation, allogeneic transplants, treatment with gemtuzumab or campath, prolonged neutropenia (<0.5 for > 2 weeks) and graft- versus- host disease for the duration of immunosuppression. Agents used for primary antifungal prophylaxis since diagnosis and the development of fungal infections were reviewed. **Results.** Among 49 patients in the low risk group, 22 had received fluconazole, 3 itraconazole, 1 ketoconazole, 1 voriconazole and 12, a combination of agents. Among 55 patients in the high-risk group, 13 received fluconazole, 5 itraconazole, 1 ketoconazole, 4 voriconazole and 23, a combination of agents. 3 patients in the high-risk group received amphotericin also. 12 patients in the low risk group and 4 patients in the high-risk group did not receive any prophylaxis. Voriconazole was predominantly used as secondary prophylaxis for proven, probable or possible aspergillosis. There was a trend towards a higher incidence of aspergillosis (proven or probable) as well as candidiasis (invasive or non invasive) in the high-risk group ($p > 0.05$). The incidence of fungal infections in both groups was similar irrespective of the prophylaxis they had received. Itraconazole drug levels were done only in 31.5%

of patients and there was a higher incidence of fungal infections in whom itraconazole levels were not done ($p > 0.05$). **Conclusions.** Fungal infections are more common in high-risk patients with haematological malignancy. However the agent used for antifungal prophylaxis does not seem to influence the incidence of fungal infections in either of the groups. The increased incidence of fungal infections in those patients in whom itraconazole drug levels were not done could have been due to inadequate drug levels.

0194

ANTIFUNGAL THERAPY IN NEUTROPENIC PATIENTS: A SINGLE CENTER RETROSPECTIVE ANALYSIS

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Infections are the most frequent complication during chemotherapy-induced neutropenia and fungal infections are a major cause of morbidity and mortality. We have retrospectively analysed our patients who received an antifungal treatment for fungal infection. Between April 1998 and December 2004 we analysed 693 consecutive patients admitted at our Institution. The diagnoses were: acute leukemia (AL) 236, lymphoma 146, multiple myeloma (MM) 200, chronic leukemia (CL) 19, severe aplastic anemia (SAA) 12, solid tumors (breast, renal, testis cancer) 44, multiple sclerosis 5, others 31. Among them 440 (63.5%) were at high risk for infections. The rationale for the antifungal therapy was for a first short period an empirical treatment (when fever persisted more than 4 days despite antibiotic therapy during neutropenia); after, only when another sign (clinical or radiological or microbiological) of fungal infection was present they received an antifungal treatment. We treated also a small cohort of patients with a secondary prophylactic regimen (they were patients who developed a fungal infection during a previous treatment). Seventy-one patients received an antifungal treatment (10% of all patients and 16% of high-risk patients). The infection was possible (empiric treatment) in 4 cases, probable (presumptive therapy) in 35 cases, proven in 15 cases; 17 cases of secondary prophylaxis. The first drug was amphotericin B deoxycholate (AMB) in 31/71 cases (44%), abelcet (ABCT) in 6/71 (8%), liposomal amphotericin B (LAMB) in 18/71 cases (25%), voriconazole (VCZ) in 3/71 cases (4%) and caspofungin (Caspof) in 13/71 cases (19%). The schedule of treatment was: AMB 0.7 mg/Kg in 6 hours, ABCT 5 mg/Kg in 3 hours, LAMB 3 mg/Kg in 1 hour, VCZ 6 mg/Kg bid iv in 2 hours for 3 days then 4 mg/Kg bid orally, Caspo 70 mg iv the first day and then 50 mg/Kg in 1 hour. For the empiric treatment the first drug was AMB 3 and Caspo 1; for presumptive therapy AMB 18, ABCT 4, LAMB 4, VCZ 1 and Caspo 8. For proven infections AMB 8, ABCT 1, LAMB 5, VCZ 1; for secondary prophylaxis AMB 2, ABCT 1, LAMB 9, VCZ 1 and Caspo 1. The isolated fungi were *Candida albicans* 4, *Aspergillus* spp 3, *Scedosporium* 2, *Fusarium solani* 1, others (only histological isolation) 5. The days of treatment were 7.64 for AMB, 6.88 for ABCT, 14.22 for LAMB, 14.1 for Caspo and 30 for VCZ. Adverse events with AMB and ABCT were renal insufficiency, fever, ipokalemia, chills; with VCZ visual disturbances and mild hepatic failure; no adverse events with Caspo and LAMB were noted. AMB was discontinued 9/31 times (29%), ABCT 1/6 (17%) for adverse events. Our conclusions are that AMB and ABCT are problematic drugs for their poor tolerability, the necessity of an important premedication and of a hyperhydration regimen and a long-time administration; moreover for a great cohort of patients we have had to discontinue the drug. The other drugs seems to be better tolerated; no organ failures were seen and the treatment duration was longer for Caspo and LAMB.

0195**INVESTIGATION OF RESPIRATORY VIRAL INFECTIONS IN PATIENTS WITH HAEMOBLASTOSIS AND MIELODEPRESSIONS BY PCR**

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Community respiratory viruses are recognized to be an important cause of infectious complications in immunocompromised leukemia patients (pts). The aim of the work was to study of frequency and clinical peculiarities of respiratory viral infections by means of PCR. Fifty one pts with haemoblastosis and mielodepressions were studied. The clinical specimens (nasal swabs and peripheral blood) were collected in these patients during the episodes of respiratory illnesses. The sensitive polymerase chain reaction was used for detection nucleic acids of Adenoviruses, Respiratory Syncytial virus (RSV), Influenza A and B viruses and Coronaviruses (CoV). Viral infection was diagnosed in 27 (52,9%) pts. Adenoviruses in nasal swabs were detected in 23,5% pts, CoV in 13,7%, Influenza A and B in 9,8 and 5,9% comparatively, RSV in 3,9% pts. Viruses were determined in 4 (10,2%) blood specimens: 3 (7,7%) adenoviruses and 1 Influenza B (2,6%). The clinical features of infection ranged from common cold and fever to severe exacerbations of chronic bronchitis. The infectious episodes were diagnosed mostly in the period of high dose antileukemical treatment and profound neutropenia. In several cases activation of herpes infection was diagnosed during the period of viral respiratory illnesses. So the community respiratory viruses as well as herpes group viruses must be carefully controlled in immunocompromised leukemia pts. PCR is adequate method for monitoring viral infections in this group of patients.

0196**DETECTION OF CATHETER-RELATED BLOODSTREAM INFECTIONS BY THE GRAM STAIN-ACRIDINE ORANGE LEUKOCYTE CYTOSPIN TEST IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENT**A. Abdelkefi¹, W. Achour², L. Torjman², A. Lakhal², S. Ladeb², T. Ben Othman², A. Ben Hassen², A. Ben Abdeladhim²¹Centre National de Greffe de Moelle, Tunis, Tunisia; ²Centre de Greffe de Moelle, Tunis, Tunisia

In patients with central venous catheters (CVCs), catheter-related bloodstream infections (CRBI) are a prominent cause of morbidity, excess hospital costs, and in some cases mortality. *Aims.* The aim of the present study was to determine the rate of CRBI in stem cell transplant patients and whether the Gram stain-Acridine Orange Leukocyte Cytospin (AOLC) test could offer accuracy comparable to that of difference in time to positivity (DTP) for the diagnosis of CRBI in this patient group. *Methods.* This prospective study was conducted between May 2002 and December 2004 at the 'National Center for Bone Marrow Transplantation', Tunisia. Patients were eligible for the study if they were between 4 and 60 years of age and had a non-tunneled CVC. Exclusion criteria were the presence of a CVC at admission, a catheterization for less than 7 days, a contraindication to the use of subclavian catheterization due to major blood coagulation disorders (ie, platelet count < 50 × 10⁹/L, disseminated intravascular coagulation). CVCs were externalized, non-tunneled, polyurethane double lumen catheters (Arrows, Readings, USA). All CVCs were placed in the subclavian vein by infraclavicular approach, in the operating room. Catheters were inserted percutaneously, using the Seldinger technique. Study catheters were not exchanged over guidewires. Catheter-related bloodstream infection was defined according to Infectious Disease Society of America guidelines: 1 bacteremia or fungemia in a patient who has an intravascular device and 1 positive result of culture of blood samples obtained from the peripheral vein, clinical manifestations of infection

(e.g., fever, chills, and/or hypotension), no apparent source for bloodstream infection (with the exception of the catheter), and a DTP of 120 minutes or more. *Results.* A total of 245 consecutive patients [125 males and 120 females; median age: 30 years (4-59 years)] were included during the 32-month study period. Twenty-six of the 245 patients (10.6%) had CRBI as determined by the DTP method. The median number of days between the insertion of the CVC and diagnosis was 26 days (10-35 days). In 2 of 26 patients (7.6%) with a positive DTP result, the Gram stain-AOLC was positive. Microorganisms involved in CRBI were: coagulase-negative Staphylococci (16 cases), *Candida albicans* (2 cases), *Pseudomonas aeruginosa* (2 cases), and *Staphylococcus aureus*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Escherichia coli*, *Stenotrophomonas maltophilia*, *Corynebacterium* spp (1 case respectively). Only one death (*Stenotrophomonas maltophilia*) was attributed to CRBI. The CVC was removed in all patients with CRBI, and an appropriate systemic antimicrobial therapy was administered. *Conclusions.* Our results suggest that Gram stain-Acridine Orange Leukocyte Cytospin test is not useful for the diagnosis of catheter-related bloodstream infection in hematopoietic stem cell transplant recipients.

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0197**DESCRIPTIVE ANALYSIS OF 268 CONSECUTIVE EPISODES OF FEBRILE NEUTROPENIA IN OUR CENTER**E.M^a. Donato¹, R. García Boyero¹, M.C. Mas¹, A. Escolá¹, M. Guinot¹, J. Marco¹, A. López², E. Herrera¹, I. García¹, T. Gozalbo¹, J. Amela¹, G. Cañigral¹¹General Hospital of Castellon, Castellon, Spain;²Arnau de Vilanova Hospital, VALENCIA, Spain

Neutropenic patients with haematological diseases may develop episodes of fever and infection during chemotherapy-induced aplasia. Febrile neutropenia is the most important problem to face during the treatment of these patients. *AIMS* The aim of this study was to report our experience about febrile neutropenia in our center. *Methods.* The study presents a retrospective analysis of 268 episodes of febrile neutropenia along a 7-year period (from January 1997 to December 2003). The median age of the patients was 61 (range 16-90), with 176 episodes in males (66%). Most episodes occurred in patients with acute leukemia (n=178). Intensive chemotherapy was applied in 205 episodes, 29 of them corresponding to autologous transplantation. The neutropenic episodes were classified as high, moderate or low risk depending on their duration (>14 days, 8-14 days or <7 days, respectively). In 162 cases the patient received antibiotic prophylaxis with quinolones and azoles. The analysis included 38, 65 and 165 episodes of low, moderate and high risk febrile neutropenia respectively. Most of them (82%) were severe neutropenias (<100 neutrophils/mm³). The episodes were classified as bacteremias (30%), clinical infections (35%), fever of unknown origin (29%) and infections with microbiological documentation without bacteremia (7%). The most frequent initial clinical focus were in lung, vascular catheter access sites, skin and soft tissues, digestive and oropharynx with 33, 26, 24, 21 and 19 episodes, respectively. Gram-negative bacilli was the most frequently isolated pathogen (n=53), followed by Gram-positive bacteria (n=49), fungus (n=15), virus (n=2) and toxoplasma (n=1). The most common Gram-negative pathogens were *E.Coli* (n=31), *Kleb pneumoniae* (n=7), *Pseud aeruginosa* (n=6) and *A. Baumannii* (n=6). Respect to Gram-positive pathogens *Staph epidermidis* (n=27), *Staph haemolyticus* (n=10), *Staph aureus* (n=6) and *Strept viridans* (n=4) were the most frequently isolated. The infections due to *Candida* species were the most common of the fungal infections (n=14). Empirical antibiotic therapy was effective in 220 episodes and it was necessary to add an antifungal drug to the regimen in 36% of the episodes. There were 25 septic shocks

and 48 deaths (26 of them (10%), directly related to the infection). **CONCLUSION** The etiology of bacteremia in febrile neutropenic patients in the past few decades has shifted from gram-negative to gram-positive organisms. Potential reasons include the use of indwelling catheters, local environmental conditions, and the administration of specific antibiotic agents, especially as prophylaxis. The present study has allowed us to obtain information about clinic-microbiologic profile of febrile neutropenia in our hospital. In comparison to that previously observed, we have detected an increasing proportion of gram-negative bacillus and emerging fungal infections with a reduction in the incidence of gram-positive infections.

0198

RISK FACTORS OF BACTEREMIA IN 178 EPISODES OF FEBRILE NEUTROPENIA IN PATIENTS WITH ACUTE LEUKEMIA

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Neutropenic patients with haematological malignancies are at high risk of contracting life-threatening infections. Bacterial infections are the major cause of morbidity and mortality among neutropenic patients. Bacteremia is one of the most important problems to face during the treatment of these patients. **AIMS** In this study, 178 febrile neutropenic episodes were overviewed retrospectively. Our aim was to know which are the factors that contribute to the development of bacteremia in neutropenic patients with acute leukemia in our hospital. **Methods.** Over a 7-year period, from January 1997 to December 2003, 178 consecutive episodes of febrile neutropenia were presented in patients with acute leukemia. The median age was 59 (range 16-82), with 113 males (64%). There was intensive chemotherapy in 147 episodes (83%) with refractory disease in 76 cases (43%). The febrile neutropenia was intrahospitalary in 128 episodes (72%) with a median of 10 days (range 0-59) since hospital admission to the start of empiric antibiotic treatment. In most cases the duration of neutropenia was >14 days (76%) and neutrophil count <100 cells/mm³ (87%). Antibiotic prophylactic treatment started concomitantly with chemotherapy in 119 episodes. A 44% of the patients had Hickman's catheter. The statistical analysis was performed with Kaplan-Meier test, log rank test and Cox regression using the statistical program SPSS v10.0. A total of 60 episodes of bacteremia were found, in which 37 gram-negative bacilli (*E. Coli* was the most common pathogen, n=26), 32 gram-positive bacteria (*Staphylococcus epidermidis*, n=16) and 7 fungal infections (*Candida* species, n=7) were identified. Septic shocks appeared in 19 cases, any of them by gram-positive bacteria. In univariate analysis the variables with statistical significance ($p < 0.05$) associated with bacteremia were: intrahospitalary acquisition ($p = 0.0006$), intensive chemotherapy ($p = 0.0001$), antibiotic prophylaxis ($p = 0.026$), administration of a granulocyte colony-stimulating factor (G-CSF) ($p = 0.015$), >10 days in hospital before febrile neutropenia ($p = 0.0002$), parenteral nutrition ($p = 0.009$), and Hickman's catheter ($p = 0.003$). Multivariate analysis showed that the variables with statistical significance ($p < 0.05$) were intensive chemotherapy ($p = 0.022$) and intrahospitalary acquisition ($p = 0.035$). In gram-negative bacteremia the factors with statistical significance were intrahospitalary acquisition ($p = 0.017$) and Hickman's catheter ($p = 0.027$). In gram-positive bacteremia and in fungal infections only intrahospitalary acquisition ($p = 0.013$) and parenteral nutrition ($p = 0.024$), respectively were found statistically significant. **CONCLUSION** Intrahospitalary acquisition, intensive chemotherapy, Hickman catheter and parenteral nutrition were the main risk factors of bacteremia in our center.

0199

THE USE OF SUBJECTIVE GLOBAL ASSESSMENT IN DETERMINING THE NUTRITIONAL STATUS OF HEMATOLOGIC MALIGNANCIES

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The degree of malnutrition is one of the most important criteria in patients with hematological malignancies that affects both survival and the quality of life. Whether during the process of diagnosis or after initiation of treatment, hospitalized patients become deprived of good nutrition for a various number of reasons. As a result their nutritional status fail to keep up with the already increased catabolic rate, causing severe results such as progressive fatigue, electrolyte imbalances, poor wound-healing and immune suppression. Determining which patients are under nutritional risk could gain importance especially in hematological malignancies in which diagnosis and treatment should be planned dynamically. For this purpose the use of Subjective Global Assessment (SGA) could be convenient, a method that refers to the overall evaluation of a patient by an experienced dietician that correlates the subjective and objective aspects of the patient's medical history and physical examination. **Methods.** We used the SGA method to determine the nutritional status and malnutrition risk of 59 patients randomly selected among all hematological malignancies admitted to Ibn-i Sina Hospital Hematology Service during the period April 2003-May 2004.

Diagnosis	No. of cases	Female/male	No. of SGA-A	No. of SGA-B+C
AML	28	14/14	14	14
ALL	13	4/9	5	8
MM	6	2/4	4	2
NHL-HH	5-2	1-0/4-2	2-2	3-0
CML	4	4/2	2	2
CLL	1	1/0	1	0

Patient demographics are shown in Table. Review of the patients' history by the Nutrition Committee included an assessment of weight and weight change, dietary intake, gastrointestinal symptoms, disease state, physical activity and the presence of fever and stress. The patients were classified as well nourished (A), mild-to-moderately malnourished (B), or severely malnourished (C). The C-reactive protein (CRP), prealbumine and transferrine levels at the time of the evaluation were also recorded. After evaluation 30 (50.8%) patients were distinguished as A, 22 (37.3%) as B and 7 (11.9%) as C. When status A patients were compared to status B+C according to their diagnosis, stage of disease and whether they were in relapse or remission, no significant difference was found. The prealbumine levels were found to be significantly diverse between the two groups, while CRP and transferrine levels remained inconsequential. **Conclusions.** The relationship between the type and stage of the hematological malignancy and the patient's SGA score thus his malnutrition risk could be better understood by increasing the number of cases and prolonging the follow-up of the study. This study showed us how we could be informed of our patients' nutritional status by following the laboratory parameters closely.

0200**PROSPECTIVE EVALUATION OF HEPATITIS B, C AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION AS A SEQUEL OF ANTINEOPLASTIC TREATMENT IN 860 SARCOMA PATIENTS**

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Cancer patients very often require supportive therapy with transfusions of blood products. These may be contaminated with viruses, such as hepatitis viruses or the human immunodeficiency virus (HIV). Routine donor blood screening for hepatitis viruses and HIV has been implemented in many countries in order to prevent the transfusion of infectious blood products to patients. *Aims.* The aim of the current study was to prospectively analyse the cumulative incidence of infections with hepatitis B and C (HBV, HCV) viruses and HIV acquired under antineoplastic therapy in a well-defined cohort of 860 sarcoma patients. *Methods.* The Late Effects Surveillance System (LESS) of the German Society for Paediatric Oncology and Haematology (GPOH) prospectively registers - since 1998 - late effects in soft tissue-, osteo- and Ewing's sarcoma patients of all ages treated within the therapy optimisation trials EICESS-92/EURO-E.W.I.N.G.-99, CWS-96/CWS-2002P, COSS-96/EURAMOS-1 in Austria, Germany and Switzerland. The follow-up is conducted at local clinics or general practitioners in accordance with the LESS guidelines. Data is gathered and analysed by the LESS centre. The LESS follow-up protocol prescribes serological examinations for HBV, HCV and HIV at the end of treatment and at 6 months thereafter. There were 860 patients eligible for this analysis, out of a total of 1780 sarcoma patients in LESS. There were 487 male and 373 female patients. There were 319 osteosarcoma, 339 soft tissue sarcoma and 202 Ewing's sarcoma patients.

Table 1 Characteristics of the three patients with relevant viral infections. All patients had acquired the infection before antineoplastic therapy.

Patient	Gender	Tumour	Age at diagnosis in years	Follow up in months	Virus infection
1	male	osteosarcoma	18	55	Hepatitis B Hepatitis C
2	male	osteosarcoma	17	64	Hepatitis B
3	female	Ewing's sarcoma	10	56	Hepatitis C Hepatitis G

The median age at diagnosis was 13,3 years (range: 0-62 years) and the median follow-up was 25,3 months (range: 6-74 months). *Results.* None of the patients acquired an infection with HBV, HCV or HIV in the line of their antineoplastic treatment. There were three patients that had been diagnosed as having a relevant infection before commencing treatment for their malignancy at the initial staging examinations. One patient had a hepatitis C and G virus infection, one patient had a hepatitis B and C virus infection and one patient had a hepatitis B virus infection. All these patients were immigrants and had acquired the respective infection in their land of origin. *Conclusions.* Blood donor screening and cautionary measures employed in Austria, Germany and Switzerland to prevent infections of cancer patients with HBV, HCV and HIV are very effective and prevented fully new infections in a large cohort of paediatric and adult sarcoma patients.

0201**FATAL VISCERAL VARICELLA-ZOSTER INFECTION FOLLOWING RITUXIMAB IN A PATIENT WITH REFRACTORY AUTOIMMUNE HAEMOLYTIC ANEMIA**

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Rituximab is an anti-CD20 murine/human chimeric antibody that has been used in the last years in the treatment of autoimmune diseases. Rituximab has shown to be effective in childhood and adult autoimmune haemolytic anaemia (AIHA). Most adverse events are mild and related to infusion. Viral infections during treatment have been seldom reported. We report the first case, to our knowledge, of fatal visceral varicella-zoster virus (VZV) infection in a patient with refractory autoimmune haemolytic anaemia after administration of rituximab and corticosteroids. A 30-year-old man had been diagnosed as having Evan's Syndrome (autoimmune haemolytic anaemia and immune thrombocytopenic purpura) in March 1999. Association with autoimmune diseases was excluded. The initial episode and several recurrences were successfully treated with corticosteroids. In November 2003 splenectomy was performed for recurrence of thrombocytopenia. Post-splenectomy, four recurrences of autoimmune haemolytic anaemia were successfully treated with corticosteroids. For persistence of recurrences, rituximab was started (375 mg/m², weekly) in February 2005. 4 days after second dose was infused, the patient was admitted to our hospital with back pain. Physical examination only revealed intensive lumbar pain. Blood count showed 17 x 10⁹/L leukocytes (80% N), 15.7 g/dL of hemoglobin and 520 x 10⁹/L platelets. Lumbar CT scan and MRI were normal. At 3rd hospital day, he developed a generalised papulo-vesicular rash, dyspnea and oliguria. Despite intensive treatment, the patient died twenty hours later. Autopsy revealed cytopathic changes suggestive of herpes virus infection in skin, lungs, liver and bowel. PCR was positive for VZV. High incidence of herpes infections have been described in patients with rituximab treatment. No patient receiving acyclovir prophylaxis developed herpes infection in an open-label study of rituximab in combination with fludarabine chemotherapy in low-grade or follicular lymphoma. However fatal visceral varicella-zoster virus infection have rarely been observed, acyclovir prophylaxis should be prescribed in all patients after rituximab treatment.

0202**VERY LATE INVASIVE PULMONARY ASPERGILLOSIS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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Invasive aspergillosis (IA) is a well-known complication in patients with hematologic malignancies. The incidence is rising because of the increasing use of high-dose chemotherapeutic agents and hematopoietic stem cell transplantation (HSCT). Prolonged neutropenia is the main risk factor for the development of IA. Other important risk factors include graft-versus-host disease (GVHD), steroids, cytomegalovirus disease and immunosuppressive agents. In patients undergoing allogeneic HSCT, IA generally occurs during early post-transplant period and if GVHD occurs. We report the case of a 53-year old woman diagnosed in 1999 of chronic myeloid leukemia. She was initially treated with α -interferon subsequently adding subcutaneous cytarabine. After 1 year of therapy without achieving response, treatment was withdrawn. On July 2001 she underwent HLA-identical sibling allogeneic stem cell transplantation, with T lymphoid depletion. She received cyclophosphamide and total body

irradiation conditioning regimen, and achieved a complete molecular response. In early post-transplant period, infections of *Serratia marcescens*, coagulase-negative *Staphylococcus* and cytomegalovirus were detected and successfully treated. Furthermore, grade I graft-versus-host disease was treated with steroids with complete response. On May 2004 antimicrobial cotrimoxazole prophylaxis was stopped. On July 2004 she began with progressive discomfort, asthenia, anorexia, weight loss, urinary discomfort and fever. A urinary infection was diagnosed and treated with amoxicillin/clavulanate. Five days after, her condition worsened and she had lower right side and throat pain. On physical examination the blood pressure was 90/60 mm Hg, the temperature 38.4°C and the oxygen saturation was 94%. Except for throat candidiasis the rest of physical examination was normal. A chest-x ray showed an increased lung opacity and bilateral interstitial affection. A thoracic computed tomographic (CT) scan revealed a ground glass attenuation pattern mainly in the right upper and medium lobes and in the left upper lobe. Blood counts, biochemical parameters and coagulation screen were normal. The blood culture was negative. Leukemic relapse was ruled out, as well as CMV infection, *Streptococcus pneumoniae*, *Legionella pneumophila* and other bacterial infections. *Aspergillus* galactomannan antigen test was positive in two determinations. A bronchoscopic examination with bronchoalveolar lavage (BAL) was performed. In BAL specimen culture growth of *Aspergillus sp.* was detected. The patient began oral voriconazole therapy, achieving complete resolution of the invasive aspergillosis by 10 days of treatment. **Conclusions.** In this patient, development of IA was largely unexpected since risk factors such as prolonged steroid treatment or extensive chronic GVHD were absent and the infection appeared three years post-transplant. Also we want to emphasize the importance of an early diagnosis using the new diagnostic methods like galactomannan antigen test and CT scans

0203

ROLE OF SURVEILLANCE BLOOD CULTURES IN MANAGEMENT OF INFECTIOUS COMPLICATIONS IN HAEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Central venous catheters (CVC) are broadly used in patients with haematological malignancies, raising the risk of infectious complications during cytotoxic therapy inasmuch in both AML-induction and haematopoietic stem cell transplant (HSCT) settings. A common practice is the use of surveillance blood cultures for early detection of infection. The care and surveillance by the physicians and nurses of infectious signs of CVC and the early beginning of antibiotic therapy may be a more useful measure. The aim of our study was to analyse the feasibility of surveillance blood cultures versus observation by physicians and nurses in the detection of CVC infection in patients following HSCT. We have retrospectively studied those patients following HSCT since January 2003 to December 2003 in our centre. All of them were Hickman carriers and a surveillance culture was taken at time of hospitalization prior to conditioning regimen for transplant. As principal variable we did analyse the infectious signs of CVC and fever through transplant procedure by studying medical and nursery records, microbiology reports and radiology records. **Results.** We have included 39 patients, 59% males and 41% females, median age 44 (range, 19-65), with the diagnosis as follows: 27 patients with lymphoma (21 NHL and 6 HD), 5 patients with multiple myeloma, 5 with acute leukaemia (4 AML and 1 ALL), 1 with CLL and 1 patient with primary myelofibrosis. All cases underwent peripheral blood stem cell transplantation (79% autologous and 21% allogeneic). The median time from catheter insertion was 36 days (range, 4-243). The twenty-four percent of surveillance blood cultures obtained by CVC prior to conditioning were positive whereas fever was documented in 97.4% of patients. In the blood cultures obtained through CVC the more frequent microorgan-

isms isolated were *Staphylococcus coagulase negative* and *Staphylococcus epidermidis*. When surveillance cultures were positive therapy with vancomycin or teicoplanin was initiated despite afebrile status. All febrile patients did respond well to broad-spectrum antibiotic empiric therapy, and only in a case was necessary to remove the catheter to solve the fever. No patient died by infectious complications during the transplantation. The surveillance blood cultures prior to transplant and the prophylactic measures initiated did not avoid the infectious complications during the transplant. The nursery cares and the surveillance of infectious signs of CVC are equally effective to treatment of CVC-associated infections, which is a more cost-effective measure. According to these results, in our centre we have suppressed the surveillance blood cultures in absence of fever or infectious signs.

0204

EPIDEMIOLOGY OF FUNGAL INFECTIONS IN HEMATOLOGICAL STEM CELLS TRANSPLANTED PATIENTS: SEIFEM 2004 STUDY (SORVEGLIANZA EPIDEMIOLOGICA INFEZIONI FUNGINE NELLE EMOPATIE MALIGNE)

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To evaluate the epidemiology and the outcome of fungal complications in patients who underwent autologous or allogeneic hemopoietic stem cells transplantation (HSCT) and admitted in multiple Italian centres. A retrospective study, conducted over 1999-2003, in bone marrow transplant recipients, admitted in 18 hematology divisions in tertiary cares or university hospitals, who developed fungal infections. **Results.** we evaluated 4,139 patients who underwent HSCT in 18 different Italian hematology divisions during the period 1999-2003: 1,505 (36.4%) allogeneic and 2,634 (63.6%) autologous transplant recipients (TR). A fungal infection occurred in 78 patients, with an incidence of 1.9%; in particular we registered 59 episodes sustained by moulds (incidence 1.4%) and 19 by yeasts (incidence 0.5%). The incidence rate depends upon the type of transplant (3.8% in allogeneic HSCT, 0.8% in autologous HSCT). Among moulds, the detected specific etiologic agents were *Aspergillus spp.* in 58 episodes (incidence 1.4%). Among yeasts, we registered 19 episodes caused by *Candida spp.* (incidence 1.6%). As for aspergillosis, the identification of the specific subtype was possible only in the 31% of cases; *A.fumigatus* was identified in the 6 cases (10.4%), *A. flavus* in 2 (3.4%), *A. terreus* in 7 (12%), *A. niger* in 3 (5.2%). The mortality registered in our population was about 64%, with differences between allogeneic TR (56%) and autologous TR (7.7%). The etiologic agent also influenced the patients outcome: the mean mortality rate due to *Aspergillus spp.* was about 69% (78% in allogeneic TR and 12.5% in autologous TR), while that one due to *Candida spp.* was about 47.4% (66% in allogeneic TR and 38.5% in autologous TR). **Summary/conclusions:** among HSCT recipients, fungal infections represent a common complication, in particular in patients undergoing allogeneic transplantation. *Aspergillus spp.* is the most frequent agent detected in our population. The mortality rate due to fungal infection was higher in allogeneic TR than in the autologous one. Patients affected by aspergillosis have a poor outcome more frequently than those affected by candidemia.

0205**LOW THRESHOLD FOR ANTIFUNGAL TREATMENT IS EFFECTIVE IN PREVENTING SEVERE FUNGAL INFECTIONS IN ALLOGENEIC BONE MARROW TRANSPLANTED PATIENTS**

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Bone marrow transplantation (BMT) is often complicated by invasive fungal infections (IFIs). Prevalence of IFIs in BMT patients reaches 15%. Known risk factors for the development of IFIs before day 40 after BMT are previous fungi infection, age (>45 years), prolonged neutropenia, advanced disease, severe mucositis, absence of HEPA filters, intensive immunosuppression for acute GVHD; chronic GVHD and his treatment, mismatched unrelated donors and age are associated with IFIs after day +40. We performed a retrospective analysis in order to evaluate the prevalence of IFIs (before day 40 and after day 40) in our series of allogeneic matched related transplanted patients. Patients and methods Forty-four patients, with a mean age of 47 years, were transplanted at our institution for hematological malignancies between 1999 and 2004. Three patients died before day 40 and were excluded from the analysis. The known risk factors for IFIs, the prevalence of major bacterial and viral infections, the antifungal prophylaxis and the treatment were evaluated. Diagnosis of IFIs was done according to the guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). *Results.* All the patients underwent BMT in a sterile room with positive air pressure and HEPA filters; 21 were more than 45 years old, 12 with advanced disease, one patient had a probable visceral candidiasis in the induction phase, 32 experienced a neutropenia lasting more than 10 days, 19 severe mucositis (grade III or IV) and 5 were treated for a refractory acute GVHD. Forty patients received prophylaxis with aerosolized deoxycolate Amphotericin B (dAmB), 15 mg bd for a mean period of 15 days, coupled with fluconazole (31/41, 400mg for 20 days) or itraconazole (5/41, 300 mg for 23 days). Prophylaxis with liposomal AmB due to previous IFIs, or to epatotoxicity was done in 4 patients. Fungi were isolated only in surveillance swabs in 19 patients. Fever lasting for 3 days developed in 20 patients, severe infections in 5 (2 pneumonias, SIRS, sepsis, meningitis). 16 patients required empirical antifungal treatment for a mean period of 23 days (until neutrophil recovery and resolution of acute GVHD). A probable aspergillosis was diagnosed after day 40 only in one patient allergic to AmB and transplanted twice for a refractory leukemia. None died for IFIs. Comment The low threshold for antifungal empirical treatment in a cohort of allogeneic BMT patients results in an effective reduction in IFIs. Despite the low number of patients we recommend an adequate antifungal prophylaxis according to the individual risk factors and possibly integrated by aerosolized dAmB. Currently a cost analysis is ongoing in order to evaluate the economic impact of this strategy.

0206**ABERRANT ALTERNATIVE SPLICING OF INTERFERON REGULATORY FACTOR-1 (IRF-1) IN MYELODYSPLASTIC HEMATOPOIETIC PROGENITOR CELLS**

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Interferon regulatory factor-1 (IRF-1), a tumor suppressor gene, originally identified as an interferon γ (IFN γ) promoter binding factor, is involved in the control of cellular proliferation and malignant transformation. Deletions and somatic mutations of IRF-1 gene have been implicated in the multiphase process of human myelodysplasia and leukaemic evolution. In addition, alternative mRNA splicing that results to functional inactivation of IRF-1 has been reported to participate in the development of hematopoietic and non-hematopoietic malignancies,

including myelodysplastic syndrome (MDS). *Aims.* In order to address the possible involvement of IRF-1 alternative splicing in the MDS development, we examined the IRF-1 mRNA expression profile (qualitatively and quantitatively) in bone marrow mononuclear cells (BMMCs), CD34⁺ progenitor and CD71⁺ erythroid cells. In addition, the protein expression pattern of IRF-1 was studied using Western blot analysis and immunohistochemistry. The study group consisted of 45 well documented MDS patients and 20 iron deficiency controls. *Methods/Results.* We demonstrate that bone marrow mononuclear cells, as well as CD34⁺ and CD71⁺ subpopulations, present an identical IRF-1 mRNA expression pattern in MDS patients. This pattern is characterized by the absence or the reduction of the full length IRF-1 mRNA that is accompanied by the expression of multiple alternative splicing variants. These variants lack exon 2 and frequently exon 3 that encode the DNA binding domain. Exons 6, 7, 8 and 9 were absent in a limited number of splicing variants, while exons 4 and 5 that encode the nuclear localization sequences (NLS), were present in all the examined variants. Furthermore, in these truncated variants were detected several base replacements that were located solely in exon 1, an untranslated region of the IRF-1 mRNA. These were mainly non-sense transversions from A to C (i.e. -23A \rightarrow C), or transitions from T to C (i.e. -40T \rightarrow C). Quantitative analysis by real-time PCR, which was designed to amplify all the alternative transcripts of IRF-1, did not reveal any statistically significant differences in IRF-1 mRNA expression between MDS patients and controls. Immunoblotting analysis of the IRF-1 protein molecules revealed the expression of two truncated protein forms, 47 and 45 kDa, respectively by bone marrow mononuclear cells of MDS patients. By means of immunohistochemistry, we detected the presence of IRF-1 protein in paraffin-embedded bone marrow biopsy tissues. *Summary/conclusions:* The role of IRF-1 mRNA splicing variants, as well as of the truncated IRF-1 protein forms, remains elusive. Possibly, they act as negative regulators of the full length IRF-1 protein. Given the role of IRF-1 gene in tumor suppression inactivation and myeloid differentiation, the existence of IRF-1 alternate transcripts along with the production of alternative protein forms might be a critical event in the development of MDS.

Myelodysplastic syndromes**0207****GENE EXPRESSION PROFILING OF CD34+ CELLS UNDERGOING HEMATOPOIETIC DIFFERENTIATION CAN DETECT SPECIFIC TRANSCRIPTIONAL PROGRAMS ALTERED IN BOTH, LOW- AND HIGH-RISK MYELODYSPLASTIC SYNDROMES**W.K. Hofmann¹, M. Komor², S. Gueller², O.G. Ottmann², C.D. Baldus¹, E. Thiel¹, D. Hoelzer²¹University Hospital Benjamin Franklin, Berlin, Germany;²University Hospital, Frankfurt/Main, Germany

The accumulation of molecular defects of the hematopoietic stem/progenitor cells is a hallmark of myelodysplastic syndromes (MDS). To detect alterations within the transcriptional program in MDS derived CD34⁺ cells during lineage-specific differentiation, CD34⁺ bone marrow cells were selected from healthy individuals (n=3) and patients with low-risk (IPSS, n=3) or high-risk (n=4) MDS and stimulated *in vitro* with EPO, TPO or G/GM-CSF to induce lineage-specific differentiation. Lineage-determined cells were harvested and if necessary purified by immunomagnetic beads at days 4, 7 and 11 for gene expression profiling. Gene expression was analyzed by oligonucleotide microarrays (HG-U133A, Affymetrix, Santa Clara, CA, USA). The experiments were done in triplicates for each of the time points and each of the conditions. First, we identified 260 genes with a significant expression pattern associated with normal lineage-specific differentiation. These continuously up- or

down-regulated genes are considered to be part of a specific genetic program of normal hematopoietic cells during lineage-specific differentiation. The course of expression of selected genes was confirmed by real-time polymerase chain reaction for all time points. In MDS, 57% of these genes showed a different expression from the normal transcriptional pattern. Thirteen of 24 genes up-regulated during normal erythropoiesis were oppositely expressed in MDS containing putative new erythro-specific genes like two GTPase activator proteins, RAP1GA1 and ARHGAP8, which regulate small Rho GTPases. Fourteen of 22 continuously up-regulated genes during normal granulopoietic development displayed a significantly different expression in MDS containing the putative candidate desmocollin 2, a gene which is involved in intercellular cell-adhesion. Delta-like 1 (DLK1) is known to be overexpressed in stem cells from patients with myelodysplastic syndrome. The role of DLK1 in normal hematopoiesis is still not defined. We found DLK1 with increasing expression during normal megakaryopoiesis but reverse expression during megakaryopoiesis in MDS. Interestingly, in erythropoiesis from both, high- and low risk MDS we found overexpression of Bladder cancer overexpressed (*BLOV1*) and Apoptosis inhibitor 5 (*API5*, which acts as a cellular survival factor by inhibiting apoptosis after growth factor withdrawal). These genes are not expressed in normal erythropoiesis. Furthermore, we identified the gene for a novel v-maf-like protein F, Maff-like (v-maf: musculoaponeurotic fibrosarcoma oncogene homolog F) to be significantly down-regulated exclusively in low-risk MDS. Maff belongs to a basic leucine-zipper (bZIP)-transcription factor family normally involved in multiple physiological processes including hematopoiesis and stress responses. Our data provide the first comprehensive transcriptional analysis of differentiating human CD34+ cells derived from normal individuals compared to MDS. It gives new insights to understand the alteration of differentiation and proliferation of MDS derived CD34+ cells.

0208

LA-DR2 B1* 1602 IS OVEREXPRESSED IN GREEK PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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HLA typing in myelodysplastic syndromes (MDS) provides insights into autoimmune mechanisms implicated in the pathogenesis of the disease. HLA-DR2 and its serologic split DR15 have been recently reported to be overexpressed in low-risk MDS patients in different ethnic groups and have been associated with a favorable response to immunosuppressive therapy. In this study we used the PCR-SSO molecular HLA typing for the HLA-DR2 B1*1501, *1502, *1601 and *1602 alleles in a group of MDS patients of Greek origin in order to investigate genetic and disease associations. In Greece, DRB1*1601 is more frequently expressed (22,4%) in the general population than DRB1*1501 (11,4%) and DRB1*1502 than DRB1*1602 (4,9% and 3,7% respectively), as previously reported from the HLA molecular typing of 246 healthy unrelated Greek volunteers, used as controls. 55 MDS patients of various subtypes were studied, that is 21 RA, 9 RARS, 8 RAEB, 6 RAEB-t/sAML, 6 CMML and 5 hypoplastic MDS patients. Statistical analysis was performed with the chi-square test. A significant overexpression was detected for the DRB1* 1602 allele, found at 10,9% of the MDS patients as a whole and at 13,4% of the low-risk RA and RARS subtypes ($p < 0,025$ and $P < 0,018$ respectively). Differences in the expression of DRB1*1501, *1502 and *1601 alleles were not significant between the MDS patients and controls, although DRB1 *1501 expression among the RA and RARS patients (23% vs 11,4% in the general population) showed a

trend to statistical significance ($p = 0,06$). It is noteworthy that the HLA-DRB1 *15 and *16 alleles were expressed in all MDS patients with a hypocellular bone marrow and in 5/9 patients with sideroblastic anemia. We conclude that HLA-DR2 B1*1602 is significantly overexpressed in Greek patients suffering from myelodysplastic syndromes. Larger numbers of patients are needed to establish ethnic differences of HLA-DR2 splits overexpression in this heterogeneous disease and to elucidate its association with the efficacy of immunosuppressive treatment.

0209

EXPRESSION OF THE PRION-LIKE PROTEIN DOPPEL IN BLAST CELLS OF ACUTE MYELOID LEUKEMIAS AND MYELODYSPLASTIC SYNDROMES: COMPARISON WITH NORMAL BONE MARROW CELLS

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The PRND gene, located on human chromosome 20pter-p12, 27 Kb downstream from the prion protein gene, encodes for the Doppel protein (Dpl), that has many biochemical and structural properties in common with the prion protein. Both prion protein and Dpl are associated with neurodegenerative disease by elusive mechanisms. Dpl is about 179 amino acid residues long, it is characterized by an α -helical conformation, and it is presented on the cell surface by a glycosylphosphatidylinositol anchor. Whereas prion protein is ubiquitous, Dpl is expressed during embryogenesis and in adult testis and does not seem to be required for prion disease progression, but appears to have an essential function in male spermatogenesis. No data are available on Dpl expression in hematopoietic cells. Since preliminary studies have demonstrated significant levels of Dpl expression in several types of cancer, we investigated the Dpl distribution in normal bone marrow cells, in leukemic cell lines and in bone marrow cells from patients with acute leukemia and myelodysplastic syndrome (MDS), in order to evaluate its possible ectopic expression in such disorders. Molecular and immunocytochemical studies were carried out on the human leukemic lines HL-60 and K562 and on bone marrow samples from 16 normal controls, 44 patients with acute myeloid leukemia (AML) and 63 patients with MDS (21 RA, 9 RARS, 14 RAEB, 7 RAEB-t and 12 CMML). A goat polyclonal antibody raised against a peptide mapping near the aminoterminal of Dpl of human origin (Santa Cruz Biotechnology, Inc.) was used for immunocytochemistry and Western blotting. Normal samples were negative or showed very weak expression in rare immature cells (median 1%); Dpl expression was restricted to CD34+ cells (71-82% positive) and down-regulated in stem cell differentiation. Dpl was detected in both cell lines and in most AML and MDS cases, with median percentages of positive blasts respectively of 13.5% (IQR 6.5-20%) and 16% (IQR 10-26%). Interestingly, in some pathological samples Dpl was ectopically localized in the cell cytoplasm and showed abnormal electrophoretic patterns. Quantitative RT-PCR revealed variable mRNA levels in almost all AML and MDS cases, but barely detectable levels in normal bone marrow ($p = 0,001$). These differences were confirmed by *in situ* hybridization. PRND expression was higher in advanced compared with early MDS ($p = 0,01$), but Dpl levels did not predict disease progression. In AML there was no correlation between Dpl levels and clinical or laboratory findings. Induction of HL-60 cells into granulocyte differentiation by ATRA was associated with down-regulation of Dpl expression. In conclusion, for the first time the expression of PRND has been demonstrated in human bone marrow cells. Its overexpression in leukemic and dysplastic cells could be explained by the immaturity or deranged differentiation of the transformed cells; anyway, the differential Dpl distribution in AML and MDS versus healthy subjects makes it a possible leukemia-associated antigen with important diagnostic and therapeutic applications. On the other hand, the Dpl expressing HL-60 and K562 cell lines may provide a useful model to study protein function and gene regulation.

0210**CLINICAL RELEVANCE OF FLT3 EXPRESSION IN MYELODYSPLASTIC SYNDROMES (MDS): CORRELATIONS WITH PROGRESSION TO AML AND RESPONSE TO TREATMENT**

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An internal tandem duplication (ITD) or a point mutation of the FLT3 gene occurs in one-third of MDS patient and is associated with progression to AML. Since ITDs and point mutations are the most common mechanisms through which the leukemic cell increases its FLT3 expression, we correlated this molecular datum with clinico-hematological features and disease outcome in 20 MDS patients. In particular, we checked whether FLT3 expression was increased in 6 MDS patients who progressed to AML and whether it was correlated with response to treatment. Our patients were examined at diagnosis and during the follow-up. They were 12 males and 8 females; their median age was 57 years (range 16-74). According to FAB classification 5 were diagnosed as refractory anemia with ringed sideroblasts (RARS), 11 as refractory anemia (RA) and 4 as refractory anemia with excess of blasts (RAEB). Fourteen patients showed a normal karyotype, three a del (20q), one a del (5) (q13q33), one a del (5) (q23q33) and one a del (12p). Blast cell percentage was 0-5% in sixteen patients, 6-10% in two and 11-20% in two. According to IPSS, eleven patients were classified as low-risk, six as intermediate-1 risk and three as intermediate-2 risk. FLT3 relative quantification was obtained through a real-time polymerase chain reaction (PCR) which employed SybrGreen I as DNA-binding fluorescent dye. Standard curve for real-time quantification was obtained by serial dilution of total RNA isolated from mononuclear cells collected from a patient affected by AML, exhibiting FLT3 ITD and elevated expression of FLT3 mRNA. Gene expression was calculated by DDCT method. FLT3 levels were normalized to ABL and calibrated on a normal sample. On diagnosis 17 patients presented an FLT3 expression similar to that of the normal sample, while the remaining 3 expressed the FLT3 gene two-four times more than the normal sample. In these last 3 patients no correlation with clinico-hematological parameters was seen. After a median time of eighteen months (range 8-39) a progression to AML occurred in a total of 6 patients (2 RA and 4 RAEB). Three of them had already had elevated FLT3 expression on clinical diagnosis, the other 3 have developed a quick rise in the expression of the gene just on progression to AML. These 6 patients expressed FLT3 two-seventeen times more than the normal sample. Three of them were submitted to different courses of intensive chemotherapy. One patient did not achieve any response and maintained elevated FLT3 expression levels. The remaining two entered a complete remission (CR) which lasted twenty and eight months. In CR both patients presented FLT3 expression levels identical to those of the normal control; on relapse both of them showed a quick rise of FLT3 expression. The gene was expressed six and eight times in comparison to the normal control. In conclusion on clinical diagnosis FLT3 expression was elevated in 15% of our MDS patients and was correlated with no peculiar clinico-hematological features; it was significantly increased in 6 patients who progressed to AML and was correlated with response to treatment.

0211**THALIDOMIDE ANALOGUE CC5013 (REVLIMID) SELECTIVELY INHIBITS IN VITRO GROWTH OF THE MALIGNANT CLONE IN MYELODYSPLASTIC SYNDROME (MDS) PATIENTS WITH 5Q DELETION**

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The thalidomide analogue CC5013 (Revlimid) induce around

80% complete erythroid responses and cytogenetic remissions in patients with MDS and 5q deletion. The effect is considered to be mediated through accessory bone marrow cells. *Aims.* To assess the direct effect of CC5013 on hematopoietic cells from patients with 5q- syndrome. *Methods.* We incubated selected CD34+ bone marrow progenitors from six 5q- syndrome patients and three healthy controls with CC5013±Epo in a 14-day model for pure erythroblast differentiation. CC5013 was added at day 0 at a concentration of 10 μ M, and proportionally in all supplemented medium. Cell count and viability was checked every second day, and FISH and FACS analyses at day 0, 7, and 14. In healthy controls, CC5013 in concentrations up to 500 μ M had no inhibitory effect on mononuclear cells, and 10 μ M had no inhibitory effect on erythroblasts. However, in erythroblast cultures of 5q- patients, the clone with 5q deletion was significantly inhibited (60% lower fold increase at day 14, $p=0.03$), while the cytogenetically normal progenitors were not affected. Furthermore, CC5013 seem to decrease the proportion of GPA+ erythroblasts at day 14 (42% reduction, $p=0.42$). Our results indicate that CC5013 selectively inhibits *in vitro* growth of 5q- cells, while not affecting growth of cytogenetically normal cells, and that the effect may be mediated through decreased survival of mature erythroid precursors carrying the 5q deletion.

0212**PHARMACODYNAMIC STUDY OF 5-AZACYTIDINE**

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The natural history of myelodysplastic syndromes could be changed by treatment with 5-azacytidine (azacytidine), a cytotoxic drug with favorable toxicity profile. The present subcutaneous dosing schedule, 75mg/m² for 7/28 days is based on clinical experience rather than on pharmacodynamic studies. *Aims.* The aim of the study was to assess the pharmacodynamic properties of azacytidine by using the myeloid P39 cell line. *Methods.* P39 cells were incubated with 0.1, 0.5 and 1.0 μ M azacytidine daily for 24-72 hours, followed by 48 hours in drug-free medium. The effect of azacytidine on cell viability, proliferation, apoptosis, cell cycle status and DNA methylation of the surrogate gene, β -cadherin was studied. Azacytidine decreased cell viability and proliferation, increased apoptosis and affected cell cycle status in a dose-dependant manner. However, the length of the exposure time, 24, 48 or 72 hours, at doses between 0.5-1 μ M did not significantly affect any of these variables. Using first order exponential pharmacokinetic model, we showed that the effect of 1, 2 or 3 μ M over 24 hours did not differ from that of 0.5 - 1 μ M given over 48 to 72 hours. Moreover 24, 48 and 72 hours of 1 μ M azacytidine exposure resulted in the same degree of hypomethylation, measured after 24 hours incubation in drug-free medium. *Conclusions.* Although these experiments must be repeated in models using primary MDS progenitors, they indicate that the dosing schedule of azacytidine could be adjusted and simplified, and that optimal cellular effect could be achieved with less than seven consecutive days of exposure.

0213**FAMILIAL HYPOCELLULAR MYELODYSPLASTIC SYNDROME DUE TO AN INHERITED MUTATION IN EXON 2 OF GATA-1 GENE**

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Dysplasia of erythroid, granulocytic and megakaryocytic lineages is the diagnostic hallmark of myelodysplastic syndromes (MDS). Transcription factors, including the GATA-1 protein,

play a key role in controlling the cellular proliferation and differentiation of hematopoietic stem cells. Inherited missense mutations in exon 4 of *GATA-1* have been found in some families, leading to a variable degree of macrothrombocytopenia with or without abnormalities in the erythrocyte lineage. Acquired mutations in exon 2 of *GATA-1* have been found in Down syndrome patients with transient myeloproliferative disorder (TMD) or megakaryoblastic leukemia (AMKL). These mutations prevent the synthesis of the full length *GATA-1*, but allow the synthesis of a smaller form of the protein (*GATA-1s*). To describe a novel inherited mutation, in exon 2 of the *GATA-1* gene, which allows only the synthesis of *GATA-1s* in members of a family, who present anemia and neutropenia. **Methods.** and **Results.** a male patient, 19 years old, was referred to our hospital due to anemia since the age of 4 years. Peripheral blood counts: RBC: $3.0 \times 10^6/\mu\text{L}$, HGB: 2.9g/dL, HCT: 8.3%, MCV: 94.4fl, MCH: 32.7pg, Retic: 0.6%, WBC: $2.6 \times 10^3/\mu\text{L}$, neutrophils: $1.0 \times 10^3/\mu\text{L}$, and PLT: $250.0 \times 10^3/\mu\text{L}$. The peripheral blood film demonstrated anisocytosis, macrocytosis, poikilocytosis, and neutrophils with pseudo-Pelger-Huet anomaly. The bone marrow aspirate and biopsy showed moderate hypocellularity with trilineage dysplasia. The presumptive diagnosis of hypocellular MDS was established. Karyotype: 46,XY. DEB test: negative. CFU-GM: 54 colonies/mL (normal range: 0.5×10^4 to 1.0×10^4). Ivy bleeding time: 9'20" (normal range: 2'15" to 9'30"), ristocetin-induced platelet aggregation test: 0.82 mg/mL (normal range: 0.7 to 1.2), platelet aggregation to $3 \mu\text{M}$ adrenaline: absent (normal: present), and platelet aggregation to $3 \mu\text{M}$ ADP: second wave absent (normal: present). The number of platelets expressing GPIX glycoprotein as well as those expressing GPIb, GPIIb, and GPIIIa were similar in the patient and controls (98.6 vs 99.5%, 98.5 vs 98.7%, 98.0 vs 119.0%, and 98.8 vs 99.4%; respectively). No significant differences in the mean fluorescence indexes (MFI) for GPIX (1,214.0 vs 1,015.5), GPIb (154.0 vs 136.5), and GPIIb (100.0 vs 96.1) platelet contents were found in patient and controls. In contrast, the MFI for measurement of GPIIIa was slightly lower in patient than in controls (1,538.6 vs 1,928.1). Hypocellular MDS and similar laboratory findings were also identified in 6 additional males of the same family. Hemizygous and heterozygous 332G/C mutations in exon 2 of *GATA-1* were found by sequencing PCR products in 7 males and in 4 phenotypically normal females of the family, comprising three generations. Only *GATA-1s*, probably resulting from alternative splicing of exon 2 was identified by RT-PCR and sequencing in affected males. In heterozygous females and normal members, both isoforms of the protein were found. **Conclusions.** these results indicate that the inherited 332G/C mutation in *GATA-1* is associated with a clinical and hematological picture similar to that of MDS syndrome in contrast with the other mutations in exon 2 observed in Down syndrome, which are associated with AMKL or TMD.

Financial support: FAPESP/CNPq/CAPES

0214

SURVIVIN EXPRESSION, APOPTOSIS AND PROLIFERATION IN CHRONIC MYELOMONOCYTIC LEUKEMIA

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Survivin is an inhibitor of apoptosis of the IAP gene family, that has a role both in counteracting apoptosis and in regulating cell division. It is overexpressed in all the most common human solid tumors *in vivo*. In neoplastic patients survivin expression has been associated with reduced tumor cell apoptosis *in vivo*, enhanced proliferative activity, accelerated rate of recurrence and shortened survival. Recently, we detected survivin in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) bone marrow cells; in MDS, we observed a tendential inverse correlation between survivin and apoptosis levels, whereas survivin expression was independent of the proliferative rate. Chronic myelomonocytic leukemia (CMML) is a

clonal disorder of a bone marrow stem cell characterized by the association of myelodysplastic and myeloproliferative features. Abnormalities in the regulation of the myeloid pathways for cellular proliferation, maturation and survival are the most important pathophysiological mechanisms. We analyzed the expression of survivin in bone marrow cells from patients with CMML to evaluate possible abnormalities in comparison with normal controls and other myelodysplastic and myeloproliferative syndromes, and to investigate a possible correlation between survivin expression and altered apoptosis, as measured by TUNEL technique, or altered proliferation, as evaluated by MIB-1 immunostaining. We also evaluated whether abnormalities in survivin expression were associated with peculiar laboratory and clinical findings. Survivin was detected by an immunalkaline phosphatase method using a primary murine monoclonal antibody raised against human recombinant survivin (clone 8E2, NeoMarkers) on bone marrow smears from 27 patients with CMML (14 MDS-CMML and 13 MPD-CMML), 66 patients with MDS (26 RA, 14 RARS, 17 RAEB and 9 RAEB-t), 24 patients with AML, 19 patients with chronic myeloproliferative disorders (MPD) and 25 non hemopathic subjects. In normal samples survivin was never detectable, whereas it was expressed in almost all pathological samples. In CMML survivin levels higher (median 24%, IQR 14-36%) than in MDS (median 8%, IQR 5-15%) ($p=0.0000$) and AML (median 14.5%, IQR 3-23.5%) ($p=0.01$), but similar to those found in MPD (median 18%, IQR 8-34%) were observed. In CMML and MDS apoptosis was significantly higher compared to normal controls and all other subtypes of leukemias ($p=0.0000$). Proliferation did not differ significantly in normal controls, MDS and CMML; the lowest levels were observed in AML and MPD ($p=0.0004$). In CMML there was no correlation between survivin expression and blast cell percentage, apoptosis or proliferation. Survivin expression and apoptosis did not differ significantly in the CMML FAB or WHO subgroups, whereas proliferation was higher in the dysplastic subgroup and in the subgroup with less than 10% bone marrow blasts, and did correlate with survival. In conclusion, CMML, like MDS but differently from MPD, is a disorder characterized by high proliferation and apoptosis. Survivin overexpression, by disrupting the balance between cell proliferation/differentiation and apoptosis, may play an important role in the pathophysiology of CMML. The detection of its deregulated expression may be clinically important, since it may provide a useful tool for diagnosis and a possible target for experimental treatments.

0215

CHEMOTHERAPY ALONE VERSUS STEM CELL TRANSPLANTATION IN CHILDREN WITH JUVENILE MYELOMONOCYTIC LEUKEMIA IN POLAND

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Juvenile myelomonocytic leukemia (JMML) is a rare malignancy comprising 2.5-3% of all childhood leukemias, and 20-30% of all cases of myelodysplastic and myeloproliferative diseases in patients younger than 15 years. **Methods.** We performed a retrospective analysis of 92 children diagnosed as primary myelodysplastic syndrome in Poland between 1989 and 2003. **Results.** JMML was diagnosed in 21 children (23%), 5 girls and 16 boys, aged from 0 to 12 years (median 3,4). The most common cytogenetic abnormality in this group was monosomy of chromosome 7 (5 pts, 20%). Low dose single agent or combination drug cytoreduction was given to 4 patients according to the recommendation of the EWOG-MDS (6-mercaptopurine alone or in combination with cytosine-arabinoside). Transformation to acute myeloid leukemia occurred in 4 children usually within 12 months from diagnosis. The median time from

diagnosis to transformation was 163 days (range 22-660). 14 children underwent hematopoietic stem cell transplantations in three centers in Poland (from matched family donors - 3, matched unrelated donors - 3, partially matched related donors - 8). Splenectomy prior to the transplantation was performed in 8 children. 6 children are alive (29%). The overall survival rate was better in the transplanted group as compared to the chemotherapy group (36% vs 14%). Median survival time of not transplanted children with JMML is about 1 year. Low platelet count, age above 2 years and high HbF at diagnosis were the factors of poor prognosis. The role of splenectomy is uncertain. If there is no possibility for stem cell transplantation, splenectomy may increase the platelet number and reduce the need of platelet transfusion. In spite of high transplant-related mortality, the treatment of choice is hematopoietic stem cell transplantation-preferably from matched family donor-and should be performed at early stage of the disease. Without transplantation or in case of relapse, non-intensive chemotherapy may ameliorate physical suffering, reduce the pain from organ infiltrates and possibly diminish the need for transfusions.

0216

G-CSF ENHANCES MITOCHONDRIAL FUNCTIONING IN MDS

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The myelodysplastic syndromes (MDS) constitute a group of clonal bone marrow stem cell disorders characterized by ineffective haematopoiesis and a high risk for transformation to acute myeloid leukaemia (AML). MDS patients show pancytopenia in spite of high or normal proliferation in the bone marrow. It is most likely that excessive apoptotic cell death of bone marrow progenitor cells contributes to these cytopenias. Presently, there is no cure for MDS and all treatment strategies are so far based on palliative patient care. We have previously shown that treatment with granulocyte colony stimulation factor (G-CSF) and erythropoietin (Epo) reduces the number of apoptotic erythroid progenitor cells in the bone marrow, improves haemoglobin levels and reduces transfusion need in patients with low-risk MDS. Recent reports suggest that G-CSF blocks apoptosis at the mitochondrial level. In order to investigate the antiapoptotic effects of G-CSF in detail, the myeloid P39 cells, originally derived from a MDS patient, were treated with all-trans retinoic acid (ATRA) in the presence of G-CSF. ATRA was chosen since it is supposed to induce apoptosis via the mitochondrial pathway. ATRA induced differentiation and subsequently apoptosis. It affected mitochondrial functioning long before any signs of apoptosis occurred. ATRA induced mitochondrial alterations were characterized by diminished mitochondrial oxygen consumption as well as decreased calcium uptake by mitochondria leading to a lower mitochondrial matrix calcium concentration. Interestingly, the growth factor G-CSF, which is known to inhibit apoptosis in ATRA-treated myeloid cells, prevented mitochondrial deterioration. It partially restored respiration as well as the capacity of mitochondria to accumulate calcium. Mitochondrial alterations occurred early in ATRA-treated cells and were later followed by apoptosis. G-CSF prevented the early mitochondrial changes as well as later apoptotic manifestation suggesting that mitochondrial dysfunction is important step for ATRA-induced apoptosis. Apparently, a disturbed intracellular calcium regulation is a link between differentiation and apoptosis since Nifedipine, a plasma membrane calcium channel blocker, or EGTA-AM, a chelator of intracellular calcium, inhibited late apoptotic like the collapse of the mitochondrial membrane potential and the activation of caspases. Thus, the ability of ATRA and G-CSF to modulate mitochondrial respiration and intracellular calcium control are novel findings which help to give a better insight in their precise molecular mode of action. The restoration of mitochondrial functioning by G-CSF offers a new explanation for its anti-apoptotic function in the treatment of MDS.

0217

HSC TRANSPLANTATION FROM MATCHED OR MISMATCHED RELATED DONORS FOR MYELODYSPLASIA AND SECONDARY ACUTE LEUKEMIA: A SINGLE CENTER EXPERIENCE OF 62 CASES

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To offer allogeneic hematopoietic stem cell transplantation to all patients with secondary leukemia or myelodysplasia even when a matched donor is unavailable. Here we report the results of matched or mismatched HSC transplants in 62 patients with treatment-related leukemia or myelodysplasia which were performed consecutively in our center between January 1986 and December 2004. Median ages (range) were 44 (17-65) for the 21 matched recipients and 39 (7-62) years for the 41 mismatched. Leukemia was secondary to MDS in 45 and was radio-chemotherapy-related in 8. Ten patients (7 matched, 3 mismatched) with either smouldering leukemia secondary to refractory anemia (n=5) or RAEB-t(n=5) received transplantation as upfront therapy. Primary neoplasias were Burkitt lymphoma (1), gastric cancer (1), Ewing sarcoma (1), uterine leiomyosarcoma (1), brain cancer (1), Hodgkin's disease (1), breast cancer (2). Median time from leukemia diagnosis to transplant was 10 months (range 2-60). At transplant, 28 patients (8 matched, 20 mismatched) were in CR and 34 (13 matched, 21 mismatched) were in relapse. Cytogenetics were unavailable in 19 patients, normal in 18, abnormal in 25. TBI-based conditioning (14.4 Gy fractionated in matched or 8 Gy single fraction in mismatched) was used in 56 patients and a chemotherapy-alone based protocol in 6 patients who had previously been irradiated because of prior cancer. TBI was followed by thiotepa, rabbit ATG and cyclophosphamide in the first 12 patients or fludarabine in the others. Melphalan was given instead of TBI. GvHD prophylaxis consisted only of ex vivo T-cell depletion. *Results.* One mismatched recipient died on day +10. Primary engraftment was achieved in 39 mismatched recipients and in 20 matched. Acute GvHD occurred in 3 cases and chronic GvHD in 3 (extensive 2, limited 1). Infections were the most common causes of 23 non-leukemic deaths (CMV 2, HHV6 encephalitis 1, EBV-related lymphoma 2, bacteria 3, toxoplasma 2, candida 2, fusarium 1, pn. carinii 1, aspergillosis 1, mycobacterium tuberculosis 1). Other causes of death were GvHD (1), idiopathic pneumonia (3), MOF (1), cirrhosis (1) and rejection (1). Eleven patients relapsed (6 were in CR and 5 in relapse at transplant). Median follow-ups (ranges) of survivors (13/21 matched; 15/41 mismatched) were respectively 76 (7-232) and 57 (3-144) months. Probability of EFS for patients in CR was 0.60±0.18 and 0.53±0.11 respectively, after matched or mismatched transplants (p=0.37). For patients in relapse at transplant, EFS was 0.57±0.15 in the matched and 0.20±0.08 in the mismatched group (p=0.04). The high engraftment rate, the very low incidence of GvHD and the extremely good survival with a normal PS make HSC transplantation from either a matched or mismatched family member feasible for patients with secondary leukemia or myelodysplasia who urgently need a transplant.

0218**GENE EXPRESSION PROFILING OF BONE MARROW CD34⁺ CELLS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES WITH AND WITHOUT A DEL (5Q)**

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The myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic malignancies, characterized by blood cytopenias, ineffective hematopoiesis and hypercellular bone marrow. We have used Affymetrix microarray technology to determine gene expression profiles in bone marrow CD34⁺ cells of 40 MDS patients and 11 healthy controls. The study included 13 patients with RA, 11 patients with RARS and 16 patients with RAEB. Fourteen of 40 patients had a del (5q). CD34⁺ cells were isolated from bone marrow samples of MDS patients and controls using MACS magnetic cell separation columns (purity greater than 85%). Extracted total RNA was amplified using the Two-Cycle Target Labelling and Control Reagent package (Affymetrix). Biotin-labelled fragmented cRNA was hybridized to GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix), covering over 47,000 transcripts representing 39,000 human genes. Cell intensity calculation and scaling was performed using GeneChip Operating Software (GCOS) and data analysis using GeneSpring 7. Genes up-regulated by >2 fold in the majority of MDS patients include IFITM1, an interferon-inducible protein implicated in the control of cell growth, and FHL2, an adaptor/docking protein involved in integrin signalling pathways and highly expressed in several tumor cell lines. IFITM1 was up-regulated in 30 of 40 MDS patients. Up-regulation of DLK1, previously reported in MDS, was confirmed in 18 of 40 MDS patients. Genes down-regulated by >2 fold in the majority of MDS patients include Gravin/AKAP12 (a putative tumor suppressor gene), NR4A2 (a gene involved in cell transformation and apoptosis), ARPP-21, CD24 and MME. Gravin/AKAP12 was down-regulated in 35 of 40 MDS patients. Apoptosis is one of the hallmarks of MDS. TNFSF10, a member of the tumour necrosis factor gene superfamily which induces cell apoptosis via the Fas/FasL pathway, was shown to be commonly up-regulated in the CD34⁺ cells of MDS patients. The anti-apoptotic gene COX2, previously shown to be commonly down-regulated in the neutrophils of MDS patients by our group, was found to be down-regulated in the CD34⁺ cells of approximately one third of patients in this study. The results for several genes have been confirmed by real-time quantitative PCR (TaqMan). A set of 149 probe sets obtained applying a parametric t-test with multiple testing correction ($p < 0.05$) could discriminate MDS patients with a del (5q) from those without a del (5q). Fifty-six of the 149 probe sets mapped to chromosome 5q and their expression levels were lower in MDS patients with del (5q) than those without del (5q), suggesting that the deletion in patients with a del (5q) has a gene dosage effect. Several genes that showed significantly different expression levels could be assigned to specific biological functions. In particular we noted the deregulation of many signal transduction genes in patients with MDS. We have shown that certain functional group of genes are deregulated in patients with a del (5q). This study has identified many deregulated genes in the CD34⁺ cells of MDS patients that may be important in the molecular pathogenesis of this disorder.

This study has been funded by Leukaemia Research Fund.

0219**LARGE PROSPECTIVE ANALYSIS OF SEQUENTIAL CYTOGENETICS IN ELDERLY MDS PATIENTS TREATED WITH LOW-DOSE 5-AZA-2'-DEOXYCYTIDINE (DECITABINE)**

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Cytogenetic abnormalities occur in at least half the patients with MDS or acute myeloid leukemia. However, there is a paucity of studies systematically addressing chromosomal changes with specific treatment of MDS, particularly in patients without detectable cytogenetic abnormalities. *Aims.* We wished to systematically monitor the karyotypes of patients treated within 3 consecutive MDS studies of low-dose decitabine, in order to be able to address i. in patients with initially abnormal karyotype the rate of cytogenetic normalization, ii. in patients with initial or treatment-induced normal karyotype the rate of karyotypic evolution with continued treatment. *Method:* Among 120 patients enrolled on these trials and successfully karyotyped, 55% had abnormal cytogenetics, 45% no detectable abnormality. Patients were followed sequentially with cytogenetics repeated every 2 courses of treatment, with signs of progression, and at relapse after achieving a remission. Wherever possible, at least 10-20 metaphases were evaluated. Of 46/53 patients with both normal karyotype and serial cytogenetics, 37 (80%) did not acquire detectable cytogenetic aberrations with continued treatment. 9 patients (20%) acquired clonal chromosomal abnormalities (5 all abnormal, 4 mosaic) during follow-up. 4/9 abnormalities were temporary and only normal metaphases were detected during the latest follow-up studies. 8/9 abnormalities were gains or losses of entire chromosomes, with monosomy 7 (alone or in combination with other changes) observed in 3 patients. Median time to first abnormal karyotype was 14 months from treatment start (range, 3-35). Of 67 patients with initially abnormal karyotype, 47% had IPSS high risk cytogenetics, either with chromosome 7 abnormality (n=6) or > 3 abnormalities (n=26). Major cytogenetic responses were noted in 24/67 patients (36%), with 15/32 (47%) in the high-risk group. Among patients with complex karyotype, the presence of chromosome 7 and/or 12 abnormalities was frequent in the cytogenetic responders but not the non-responders. After relapse, the majority of patients relapsed with the identical clone, whereas chromosomal evolution or a different clone were seen infrequently. In decitabine-treated patients with initial or treatment-induced normal karyotype, the rate and pattern of cytogenetic evolution is not higher than described in MDS cohorts not receiving specific treatment, arguing against induction of chromosomal instability. The rate of cytogenetic conversion in patients with initially abnormal karyotype is particularly high in patients with poor-risk cytogenetics, prompting the choice of poor-risk cytogenetics as the unifying inclusion criterion in the ongoing, prospective randomized European phase III MDS trial of low-dose decitabine.

0220**SECONDARY MYELODYSPLASTIC SYNDROMES IN CHILDHOOD: A DIAGNOSTIC SURVEILLANCE PROTOCOL**

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Myelodysplastic syndromes (MDS) in childhood are rare and heterogeneous clonal disorders of hematopoiesis with high risk of evolution to acute myeloid leukaemia (AML). They represent about 10% of all pediatric malignancies and can be idiopathic or secondary to a pre-existing bone marrow disorder or previ-

ous therapy (therapy related MDS, t-MDS). MDS diagnosis in childhood may be difficult and may require prolonged observation. **Aims.** In our Institution a surveillance protocol for early detection of secondary MDS has been applied to patients (pt) at risk: pt with bone marrow failure syndromes (both congenital and acquired) and those who received chemo and/or radiotherapy. **Methods.** Morphological analysis on peripheral blood, marrow aspirate and biopsy; immunophenotypic clustering by flow cytometry; standard cytogenetic test; FISH and biomolecular analysis (AML translocations, number of copies of *WT1* gene); hemopoietic progenitors assays (clonogenic assays performed in standard conditions in the absence of stimula, with increasing concentration of growth factors); GPI-linked proteins. Since 2002 circulating CD34+ cell count and apoptotic rate were evaluated by flow cytometry (CD34 and annexin V expression, absolute count evaluated by an ISHAGE-derived method). Patients with bone marrow failure syndromes: 14 Fanconi anemia (FA), 1 Kostmann syndrome, 2 dyskeratosis congenita, 3 Blackfan Diamond anemia, 2 pure red cells aplasia and 19 acquired aplastic anemia (AA) were monitored every 6-12 months. Among 212 children with malignancies receiving chemotherapy (especially alkylating agents) and/or radiotherapy, 9 were investigated at time of unexplained cytopenia. **Results.** MDS secondary to bone marrow failure syndromes: among FA, 3 out of 14 developed MDS/AML. 1 out of 19 AA developed RAEB-t 27 months after diagnosis. t-MDS: 1 out of 6 acute promyelocytic leukaemia t(15;17) pt developed chronic myelomonocytic leukaemia 36 months after diagnosis; 1 out of 59 Hodgkin disease developed RAEB-t 16 months after auto-BMT; 1 out of 88 pt with acute lymphoblastic leukaemia followed during maintenance treatment and off therapy developed an RAEB 20 months after diagnosis; 1 out of 59 pt with primary brain tumour receiving chemo and/or radiotherapy developed RAEB-t. In FA patients bone marrow aspirate allowed MDS diagnosis. In all the other patients bone marrow biopsy was necessary for diagnosis. The most frequent cytogenetic abnormality in our MDS series was monosomy 7 (3/8). In all valuable MDS patients a high peripheral blood CD34+ cell count with a low apoptotic rate has been found. In the patient with AA who developed RAEB-t, the CD34+ flow cytometric pattern appeared four months before MDS bone marrow morphological alterations. **Conclusions.** MDS diagnosis in childhood is often difficult, particularly in the early phases, and may require prolonged observation and repeated bone marrow biopsies. In our experience, the association of cytogenetic analysis, bone marrow biopsy and simultaneous evaluation of circulating CD34+ cell count and apoptotic rate is a sensitive and reliable diagnostic approach.

0221

DIFFERENT CLIP (CLASS II-ASSOCIATED INVARIANT CHAIN PEPTIDE) EXPRESSION ON CD7+ AND CD7- HEMATOPOIETIC PRECURSOR CELL SUBPOPULATIONS IN LOW-RISK MDS

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Effective tumor antigen presentation by MHC class II antigens to CD4⁺ T helper cells is crucial for immunosurveillance of acute myeloid leukemia. MHC class II tumor antigen presentation can be prohibited by the persistent presence of CLIP1. Investigation of the role of immunosurveillance in the progression of low risk MDS to AML. **Methods.** We evaluated 25 patients treated with a standardized regimen of epoëtin-beta (NeoRecormon) and filgrastim (Epo/G-CSF). Bone-marrow samples were taken at 0, 3, 6 and 12 months and analyzed by FACS. Hematopoietic stem cells were analyzed for aberrant markers and antigen presenting (HLA class I and II, CLIP) molecules. CD34⁺ cells of 7 patients showed aberrant CD7 expression, enabling to separate malignant from normal stem cells. Analysis revealed comparable HLA class I and II expression, but malignant CD7⁺ stem cells had significant higher CLIP expression than CD7⁻ and normal CD34⁺ cells (mean relative CLIP amount

290*10⁻³ vs 4*10⁻³, $p=0.001$ (CD7- (Figure 1)) and vs 11*10⁻³, $p=0.004$ (normal CD34⁺). Treatment with Epo/G-CSF diminished relative CLIP amount 5.5 times (median), indicating better antigen presenting capacity during therapy. **Conclusions.** These data indicate that in low-risk MDS, different stem cell subpopulations can be identified with varying immunogenicity profiles. It is hypothesized that CD7⁺/CLIP⁺ blasts escape immunosurveillance and grow out to CD7⁺ positive AML (until now demonstrated in one patient) and that therapy with Epo/G-CSF rendered stem cells more susceptible for the immune system by lowering the amount of CLIP. The increased immunogenicity could enable the immune system to control the disease and prohibit progression to AML.

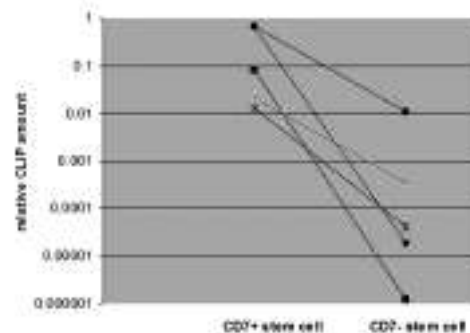


Figure 1. Different CLIP amount on CD7+ and CD7- stem cells

0222

DELETION OF TUMOR SUPPRESSOR GENES (TSGS) AND CLIVICAL CORRELATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS)

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The deletion of genomic sites harboring TSGs is known to be a powerful prognostic indicator in various chronic hematologic malignancies. However, the clinical significance of TSGs loss in MDS has not been thoroughly investigated. To summarize our experience on the incidence and essential clinical correlates of TSGs deletions in MDS, providing preliminary results from the study of 27 patients. **Methods.** The study included 17 men and 10 women (median age 74; range 38-84 years) with a documented diagnosis of MDS. The distribution of FAB subtypes was RA in 13, RARS in 3, RAEB in 6, RAEB-T in 4 and CMML in 1 case. According to the IPSS stratification, 5 patients were characterized as *low-risk*, 16 as *intermediate-risk* (11 as INT-1 and 5 as INT-2) and 6 as *high-risk* patients. The diagnostic bone marrow smears were studied with fluorescence in-situ hybridization (FISH) for deletions of chromosome regions 9p21 (p16/p14 and p15 genes), 9q34, 12p13 (TEL gene), 13q14 (RB1 gene D13S319 locus) and 17p13 (p53 gene). **Results.** A deletion in at least one of the chromosome regions or loci studied was detected in 10 patients (37%). In 6 of them, more than one region locus was lost, making up a total of 20 deletions. The commonest finding was loss of the 17p13 region (5 cases; two of them homozygous), followed by 9p21 loss (5 cases; one of them homozygous). Overall, the presence of TSGs deletions was associated with complex karyotype, high IPSS score and risk of death (regardless of leukemic progression). Interestingly, deletion in at least one of the TSGs studied was found in 5 of the 17 patients presenting with normal karyotype. In 3 of these patients, the MDS progressed to acute leukemia. Two of the 5

patients died at one and six months from diagnosis of the MDS, without leukemic conversion. *Summary/Conclusions.* Deletions of TSGs are not uncommon in MDS. Interestingly, we have found that certain TSGs, such as the p16/p14 and p15 genes at 9p21, the loss of which is mostly involved in the initiation or progression of lymphoid neoplasms, are also frequently deleted in MDS. Overall, TSGs deletion in this small group of patients is apparently associated with other adverse biological and clinical features and poor outcome. Therefore, these preliminary observations justify the expansion of the study in a larger patient cohort, in order to clarify if the loss of certain TSGs is an independent prognostic factor in MDS and, thus, may be of help in the initial diagnostic approach and risk stratification of the patients.

Myeloma - Biology I

0223

GAINS ON CHROMOSOME ARM 9Q REPRESENT A NOVEL AND INDEPENDENT MARKER OF ADVERSE PROGNOSIS IN MULTIPLE MYELOMA PATIENTS RECEIVING UPFRONT HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Genomic aberrations represent important prognostic markers in many hematological cancers. In multiple myeloma (MM), chromosome 13q and 17p deletions (13q-, 17p-) have emerged as important outcome predictors that indicate a dismal prognosis. Other chromosomal abnormalities have been discussed as prognostic markers in this disease but came not out as independent variables when they were tested in a multivariable fashion. However, the complexity of genomic rearrangements and the clinical heterogeneity seen in malignant plasma cell disorders argue against 13q- and 17p- as the sole genomic change of prognostic relevance. The significance of chromosome arm 1q, 9q, and 11q extra copies-three frequent genomic imbalances in MM-is undetermined. *Methods.* 90 patients (pts.) treated with one or two cycles of high-dose chemotherapy (HD-CTX) followed by autologous stem cell transplantation (ASCT) at a single center were analyzed by tri-color FISH and five DNA probes mapping to chromosome bands 1q21, 9q34, 11q25, 13q14, and 17p13. A multivariable analysis (Cox proportional hazards regression model) including genetic and clinical variables was performed. The most frequent aberrations in the present series were (in order of decreasing prevalence): +1q (n=39/78, 50.0%), +9q (n=38/78, 48.7%), 13q- (n=42/90, 46.6%), and +11q (n=39/85, 45.9%). 17p- was identified in only 3 out of 88 patients (3.4%), while +17p were present in 13 out of 88 patients (14.8%). The median follow-up time was 37 months (m) and the median event free survival (EFS) and overall survival (OS) time from first ASCT of the entire cohort was 26 m and 71 m, respectively. Multivariable analysis including genetic aberrations, β_2 -microglobulin, albumin, LDH, Salmon/Durie stage, and age at time of diagnosis revealed +9q and 13q- as the only independent predictors of EFS ($p=0.003$ and $p=0.01$, respectively). The mEFS in patients with 13q- was 19.0 m and 20.7 m in patients with +9q. In patients with concurrent +9q and 13q-, mEFS was only 12.2 m. In patients lacking these two abnormalities, mEFS was not reached. OS was not significantly influenced by any genetic or clinical variable in our series, most likely due to effective salvage treatment after relapse. *Conclusions.* +9q represents a novel and independent marker of adverse prognosis in MM. A single FISH experiment applying two DNA probes allows easy and rapid assessment of outcome in patients with malignant plasma cell disorders.

Supported by grants from the Deutsche José Carreras Leukämie-Stiftung (DJCLS-R04/04), the Deutsche Krebshilfe (70-3899-Li I), and the Wilhelm Sander-Stiftung (No. 2002.098.1) to P.L.

0224

DETECTION OF THE OPG/TRAIL COMPLEX IN AN IN VITRO OSTEOCLASTOGENESIS MODEL DERIVED FROM HUMAN MULTIPLE MYELOMA-BONE DISEASE

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Multiple myeloma (MM)-bone disease results from enhanced bone resorption related to increased recruitment and activity of osteoclasts (OCs) and low bone formation. Osteoclastogenesis is regulated by a complex signaling system, that involves receptor activator of nuclear factor- κ B (RANK), receptor activator of nuclear factor- κ B ligand (RANKL) and osteoprotegerin (OPG), all belonging to the Tumor Necrosis Factor (TNF) family. The aim of our study was to investigate a mode of regulation of osteoclastogenesis in MM-bone disease, entailing T cells and secreted factors. Unstimulated and unfractionated peripheral blood mononuclear cells (PBMCs) isolated from 32 MM patients with or without bone disease, and parallel T cell-depleted cultures were used as *in vitro* osteoclastogenesis models. In addition, unstimulated and unfractionated PBMC cultures from 32 controls with nonneoplastic disease without any skeletal involvement were also established. Our results showed that the OCs derived from PBMCs of the patients with MM-bone disease spontaneously developed and displayed a longer survival in a T cell-dependent way. Otherwise in the parallel T cell-depleted cultures, the addition of macrophage-colony stimulating factor (M-CSF) and RANKL was necessary to promote the formation of OCs, that however did not exhibit a longer survival. PB fresh T cells isolated from the patients with MM-bone disease overexpressed RANKL, OPG and TNF-related apoptosis inducing ligand (TRAIL), also found in large amounts in the culture media of PBMCs from the same patients. In addition, the OPG/TRAIL complex was detected by immunoprecipitation with an anti-TRAIL mAb in the T cell lysates and culture media of PBMCs from the patients with MM-bone disease. The interaction between OPG and TRAIL can explain the persistence of osteoclastogenesis in our system, despite the high OPG levels. The block of the anti-osteoclastogenic effect of OPG by TRAIL was further supported by the addition of functional anti-TRAIL mAb, causing a dose-dependent inhibition of spontaneous osteoclastogenesis, that recurred after exogenous RANKL. The OCs developed from the PBMCs of the patients with MM-bone disease expressed a T cell-modulated balance of death and decoy TRAIL receptors, whose ratio results critical for OC sensitivity to TRAIL-mediated apoptosis. In particular, these OCs overexpressed TRAIL decoy receptor DcR2 in the presence of T cells, and death receptor DR4 in the T cell-depleted cultures. In conclusion, our results highlight that T cells from the patients with MM-bone disease support *in vitro* the spontaneous formation of OCs with longer survival, involving the OPG/TRAIL interaction and the unbalanced OC expression of TRAIL death and decoy receptors.

0225

IMMUNOGLOBULIN LIGHT CHAIN EXPRESSED AND NON-EXPRESSED REPERTOIRE IN MULTIPLE MYELOMA (MM)

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Immunoglobulin light chain (IGK/IGL) rearrangements were analyzed in a series of 42 patients with κ -MM and 36 patients with lambda-MM. In κ -MM, 42 IGKV-J transcripts were amplified by RT-PCR; 40/42 transcripts were rearranged in-frame (IF), while 2/42 were out-of-frame (OF) and harbored stop codons.

Additional, non-expressed IGKV-J rearrangements were identified by DNA-PCR in 8/42 patients (16%); 2/8 were rearranged in-frame and carried somatic mutations. The most frequent IGKV genes in expressed IGKV-J rearrangements were 1-33/1D-33, 1-39/1D-39, 3-15, 3-20 and 4-1. The average homology of expressed and non-expressed IGKV-J rearrangements was, respectively, 93.4% and 98.5%. In κ -M^a, 38 IGLV-J transcripts were detected by RT-PCR; 36/38 were IF (expressed rearrangements), while 2/38 were OF (non-expressed rearrangements). The most frequent IGLV genes in expressed IGLV-J rearrangements were 3-1, 3-21, 2-14, 2-23, 1-47 and 1-40. The average homology of expressed IGLV-J rearrangements was 93.5%. In κ -M^a, sixteen IGLV-J rearrangements were amplified by DNA-PCR in 14/36 patients; two patients harbored double non-expressed IGKV-J rearrangements. Five non-expressed IGKV-J rearrangements carried mutations; 3/5 were IF. The IGKV4-1 gene predominated among non-expressed IGKV-J rearrangements either in κ -^{aa} or κ -^{aa} (7/24 rearrangements, 29%). Rearrangements of the IGK locus involving the κ deleting element (KDE) were also studied by DNA-PCR. In κ -M^a, IGKV-KDE and JKI-KDE rearrangements were amplified in 9/42 and 6/42 patients, respectively; based on PCR results, both IGK loci were rearranged in 15/42 κ -MM cases. In κ -M^a, IGKV-KDE and JKI-KDE rearrangements were amplified in 13/36 and 25/36 patients, respectively. Taking IGKV-J, IGKV-KDE and JKI/KDE rearrangements together, 32/36 κ -MM cases had at least one rearranged IGK allele; furthermore, PCR evidence for rearrangement of both IGK alleles was obtained in 15/36 lambda-^{aa} cases. Analysis of the distribution of somatic mutations by the multinomial probability model provided evidence for selection by classical, T-dependent antigen in 34/40 IGKV-J expressed rearrangements, 2/4 IGKV-J non-expressed rearrangements and 30/36 IGLV-J expressed rearrangements. In conclusion, IGKV/IGLV repertoire in MM appears to have important differences compared to the repertoire of normal B cells. The inactivation of potentially functional IGKV-J junctions by secondary rearrangements is compatible with receptor editing processes. The identification of mutated, non-expressed IGKV-J rearrangements could be interpreted either as indicative of secondary rearrangements in peripheral B cells or as a result of a 'bystander mutagenesis' process, whereby non-expressed rearrangements are mutated without selection for expression of a functional IG. Finally, in most M^a cases, the IG light chains play an active role in the antigen selection process.

0226

A MODEL FOR CLONAL EVOLUTION OF MULTIPLE MYELOMA BASED ON INTERPHASE CYTOGENETICS

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The modeling of tumorigenesis as a sequence of genetic changes is a challenging problem. So far, in multiple myeloma (MM) clustering analysis has been performed using results of conventional cytogenetics only. Two major subgroups were repeatedly described: hyperdiploid and hypodiploid MM. In view of the limitations of conventional cytogenetics in MM, interphase fluorescence *in situ* hybridization (FISH) was applied to delineate MM subgroups and their clonal evolution as a basis for further biological and prognostic evaluation. The study included 81 newly diagnosed MM patients (pts). Mean age was 61 years (range, 38-83). The distribution according to the clinical stage of disease (Salmon&Durie) was as follows: I (8 pts); II (12pts); III (61 pts). There were 48pts with IgG monoclonal protein, 20pts with IgA, and 12pts with secretion of κ/λ light chains. Non-secretory MM was diagnosed in 1pts. Median values of $\kappa/2$

microglobulin and albumin were 3,4 mg/L (range 1,3-53,6), and 36g/L (range 20-50), respectively. Application of the International Prognostic Index revealed the following distribution of scores: 1 (25pts); 2 (37pts); 3 (19 pts). Interphase FISH was applied on CD138-selected bone marrow cells (median purity, 95%) with 10 specific probes for chromosomes 1q21, 6q21, 8p12, 9q34, 11q23, 13q14.3, 17p13, 22q11 and 2 commercial dual-color fusion probe sets for the translocations t(11;14) (q13;q32.3) and t(4;14) (p16;q32.3). A copy number score (CS) was calculated for each patient by subtracting the number of probes expressing losses from the number indicating gains. Clustering analysis based on Kendall's τ coefficient and statistical modeling of oncogenetic tree based on maximum likelihood estimation were applied to elaborate the associations and biological order of cytogenetic aberrations. Per patient, a median of 5 probes (range, 1-10) displayed aberrant signal numbers. Additional copies were most frequently found for chromosomes 15q22, 19q13, 9q34, 11q23, and 1q21. Common losses were observed for 13q14.3, 17p13 and 22q11. Predominance of gains or losses was quantified by a copy number score (CS) for each patient. Two peaks (CS=+3, and CS=0) were found by plotting patient numbers over CS values, corresponding to hyperdiploid and non-hyperdiploid MM. Cluster analysis revealed four major branches: (i) gain of 9q, 15q, 19q, and/or 11q; (ii) deletion 13q and t(4;14); (iii) t(11;14); and (iv) gain of 1q. Statistical modeling of an oncogenetic tree indicated as early independent events: (i) t(11;14); (ii) gain of 15q/9q and/or 11q as a pathway putatively leading to the hyperdiploidy; (iii) deletion 13q followed by t(4;14) as a pathway putatively leading to the non-hyperdiploidy; and (iv) in agreement with the clustering analysis, gain of chromosome 1q21 separated early from the pathway leading to non-hyperdiploidy. Aberrations of 17p13, 22q11, 8p12 and 6q21 were found as subsequent events. MM with gain of 1q was found as a subentity with significantly higher κ -2-microglobulin and lower hemoglobin levels, indicating a poor prognosis. Clustering of interphase FISH results using a comprehensive probe set, and statistical modeling of an oncogenetic tree, give evidence for the existence of distinct subgroups of MM with possible different clinical outcome.

0227

THE BIOLOGICAL EFFECT OF STROMAL CELL-DERIVED FACTOR (SDF)-1 A LPHA ON MYELOMA CELLS

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Multiple myeloma (MM) is a progressive disease characterized as the expansion and accumulation of malignant plasma cells in bone marrow (BM). In BM myeloma cells show heterogeneity in myeloma patients, such as immature (MPC-1-) and mature (MPC-1+) types, and the morphology is also different. As mature myeloma cells closely contact to BM stromal cells, survival and proliferation of mature myeloma cells are likely dependent on stromal cells. Myeloma cells are found in close contact with the BM microenvironment, where adhesion molecules, cytokines and chemokines play a key role in the regulation of their proliferation, survival and lodgment. Stromal cells produce and secrete interleukin-6 (IL-6) a growth factor for myeloma cell *in vitro* and *in vivo* and stromal cell-derived factor (SDF)-1a, one of the CXC type of chemokines. To clarify the pathogenesis of MM and to identify the molecular target for MM treatment, it is important to understand the molecular mechanism of the survival and proliferation of myeloma cells. In this study we investigated the biological effect of SDF-1 on myeloma cell survival, proliferation and chemotaxis. In the present study we found that mature myeloma cells expressed

CXCR4 a receptor of *SDF-1*, more than immature primary myeloma cell and myeloma cell lines. *SDF-1a* induced the chemotactic activity of myeloma cells *in vitro*, also induced the phosphorylation of *ERK*^{1/2}, *AKT*, *I κ B* and supported the survival of myeloma in a serum free condition. Inhibition of *ERK*^{1/2} or *AKT* activity abolished the effect of *SDF-1* on the cell survival of myeloma cell lines. Flow cytometry showed that *SDF-1a* induced the nuclear accumulation of *NF- κ B* and expression *NF- κ B* target genes, such as several anti-apoptotic genes and *CXCR4* gene. Furthermore, a *NF- κ B* inhibitor, parthenolide suppressed the effect of *SDF-1a*-induced myeloma cell survival and the *CXCR4* expression. This study suggests that activation of *NF- κ B* by *SDF-1a* is an important pathway to inhibit apoptosis, promote proliferation, and induce chemotactic activity of myeloma cell by the enhanced expression of *CXCR4* gene.

0228

IN MYELOMA, SURFACE EXPRESSION OF CD130 CORRELATES WITH SURFACE EXPRESSION OF VEGF AND WITH INTRACELLULAR LEVELS OF THE PROLIFERATION MARKER, KI67

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Multiple myeloma (MM) is characterised by progressive accumulation of malignant plasma cells (myeloma cells) in the bone marrow (BM). It has been hypothesised that, during disease progression, CD45⁺ myeloma cells differentiate into CD45⁻ cells with higher proliferation rate and worsening prognosis. Myeloma cell expression of CD130, the signal transducing protein of IL6 receptor, and of CD56 may also be associated with poor prognosis. Vascular endothelial growth factor (*VEGF*) and its receptors have also been implicated in MM pathogenesis. To correlate surface expression of CD45, CD130, CD56 and *VEGF* and intracellular levels of cell proliferation marker, Ki67 and of anti-apoptotic protein, bcl-2 in myeloma cells. Salmon and Durie stage, disease status, CBC, serum creatinine and beta 2 microglobulin (β 2MG) and plasma cell% and morphology in BM were also recorded. *Methods.* BM aspirates were taken from 21 consecutive MM patients (2 stage I, 2 stage II, 15 stage III disease, 2 smouldering MM; 14 previously treated) and 1 MGUS. Immunophenotyping was performed by triple colour flow cytometry (Coulter EPICS-XL) on BM plasma cells, identified by reactivity with CD38PE and CD138PC5 and by SS versus FS scattergram. Positivity for surface antigen expression was defined as > 20% of cells staining positive for the antigen. *4.Results.* Median% (with range) of plasma cells positive for surface expression of CD45, CD130, CD56 and *VEGF* was 2.4% (0%-100%), 50% (16.5%-93.2%), 5.2% (0-100%) and 41.2% (11.3-94.7%). There was a positive correlation between% plasma cell expression of *VEGF* and of CD130 ($r=.493, p<0.05$). CD45 negative myeloma cases had higher *VEGF* mean fluorescent intensity (MFI) than CD45 positive cases ($p=0.04$). CD130 positive myeloma cases had increased% intracellular Ki67 levels than CD130 negative cases ($p=0.0035$). There was also a strong negative correlation between% intracellular Ki67 levels and% BM plasma cells ($r=-0.665, p<0.01$). Myeloma cells with plasmablast morphology had lower% surface CD56 ($p=.0148$). *5.Summary/conclusions.* Myeloma cell expression of CD45, *VEGF* and CD130 have been compared for the first time. Malignant plasma cells with absent or low expression of CD45 had higher intensity expression of *VEGF*. CD130+ MM cases had increased surface expression of *VEGF* and increased intracellular Ki67. The possibility that *VEGF* may contribute to the poor prognosis of CD130+ and/or CD45- myeloma needs further investigation. We also confirm the association of plasmablastic morphology with CD56- MM.

0229

CYCLOOXYGENASE-2 (COX-2) IS FREQUENTLY EXPRESSED IN MULTIPLE MYELOMA (MM) AND IS AN INDEPENDENT PREDICTOR OF POOR OUTCOME

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A perturbed microenvironment with secretion of inflammatory cytokines is typical of MM. Prostaglandins (pg) are implicated in inflammation and angiogenesis and play a role in the pathogenesis of several solid malignancies. Expression of *COX-2*, the key enzyme of pg synthesis in inflamed tissues, is common in many of these cancers and plays a major role in their development. Moreover, it often acts as a poor prognostic indicator. Despite a large amount of data concerning *COX-2* expression in solid tumors, few data are currently available in hematological malignancies. In MM there are several biological, epidemiological and clinical considerations suggesting a potential involvement of the pg pathway. Aim of this study is to verify the involvement of *COX-2* in MM and to assess its prognostic role. Patients and methods. *COX-2* expression has been assessed by western blotting (WB) as previously described (*Du Bois RN et al., Gastroenterology, 1996*). Our positive control was the *COX-2* positive cell line HT-29, while bone marrows (BM) from 15 healthy donors were our negative controls. We assessed a panel of 159 samples obtained by 148 patients with plasma cell dyscrasias. Twenty-one samples belonged to subjects with MGUS, 97 to patients with MM at diagnosis, and 30 to patients with relapsed/refractory MM. In 11 patients, samples taken at different treatment phases were available. To confirm WB findings and to demonstrate that *COX-2* expression occurs in malignant plasma cells immunohistochemistry (IC), and flow cytometry for *COX-2* were also performed in 42 and four patients, respectively. Finally, *COX-2* expression has been assessed in BM cells from four *COX-2* positive patients following selection for the CD138 antigen using the Miltenyi cell separation system. *COX-2* expression at the mRNA level has also been assessed by real time quantitative PCR. *Results.* The 15 normal BM were *COX-2* negative. In contrast, *COX-2* expression was noticed in 9.5% of MGUS, 31% of MM at diagnosis and 46.6% of MM at relapse. *COX-2* expression was prognostically relevant in terms of PFS at diagnosis (14 vs 36 months, $p<0.001$) and in terms of OS both at diagnosis (28 vs 52 months, $p<0.05$) and at progression/relapse (18 months vs not reached, $p<0.001$). *COX-2* expression was unrelated to disease stage, BM plasmacytosis, creatinine, Hb levels and β 2 microglobulin and acted as an independent prognostic factor in multivariate analysis (PFS, HR 7.11, 95% CI 2.8-17.9, $p<0.0001$; OS, HR 2.36, 95% CI 1.16-4.79, $p<0.05$). IC, cell separation and flow cytometry studies indicate that *COX-2* expression is related to the malignant plasma cell population. *COX-2* mRNA was also overexpressed in patients showing increased *COX-2* protein expression. *Conclusions.* a) *COX-2* is frequently expressed in plasma cell dyscrasias; b) *COX-2* expression is more frequent in advanced disease phases; c) *COX-2* expression is an independent predictor of poor outcome. Future studies are required to verify whether *COX-2* might be clinically useful as therapeutic target in MM.

0230**THE NEW HISTONE DEACETYLASE INHIBITOR ITF2357 IS A STRONG INDUCER OF APOPTOSIS IN MULTIPLE MYELOMA CELLS**A. Rambaldi¹, C. Micò¹, L. Cuppini¹, G.M. Borleri¹, F. Leoni², P. Mascagni², A. Salvi³, M. Galli⁴, J. Golay⁵, A.M. Barbui⁵, M. Introna¹¹Ospedali Riuniti, Bergamo, Italy; ²Italfarmaco, Cinisello Balsamo, Italy; ³Ospedali Riuniti, Bergamo, Italy; ⁴Ospedali Riuniti, Hematology Division, Bergamo Italy; ⁵Ospedali Riuniti, Hematology Division, Bergamo, Italy

Multiple myeloma (MM) is an incurable disease characterized by neoplastic proliferation of plasma cells and drug resistance to conventional therapies. Several studies demonstrate that aberrant acetylation/deacetylation of histone proteins is an integral part of growth arrest, differentiation and apoptosis in many tumors. Various agents such as the histone deacetylase inhibitors (HDACIs) modify histone status and are being examined for their potential therapeutic use in the treatment of numerous malignancies and in particular of MM. To determine the cytotoxic and anti-proliferative activity *in vitro* of a novel hydroxamic acid-based HDACI, ITF2357 (Italfarmaco, Cinisello Balsamo, Italia), in multiple myeloma in comparison to SAHA, the prototypic hydroxamic HDACI. The cytotoxic effect of ITF2357 and SAHA was assessed on 9 human myeloma cell lines and 6 freshly isolated MM samples using the Alamar Blue dye, annexin-propidium iodide staining and FACS analysis and standard cell cycle analysis. Patients' MM cells were purified by positive selection using anti-CD138 antibody and magnetic beads (Miltenyi). Clonogenic assays were performed by plating cells in methylcellulose and counting colonies after 21 days. ITF2357 had a strong cytotoxic activity in 7/9 MM cell lines, with IC₅₀ ranging from 0.1 to 0.2 μM. Only two cell lines were somewhat more resistant but still responded with an IC₅₀ of about 1 μM. SAHA tested in parallel on the same cell lines showed an IC₅₀ of 1 microM or above in all cases. Apoptosis induced by ITF2357 started to be observed at 24 hours but was best measured at 48 hours. Similarly to MM cell lines, ITF2357 had also more potent cytotoxic activity compared to SAHA against freshly isolated purified MM samples at both 24 or 48 hours of culture, with an IC₅₀ ranging from 0.1 to 0.8 microM. Clonogenic assays on human myeloma cell lines further confirmed the capacity of ITF2357 to completely abolish colony growth at lower concentrations than SAHA. Cell cycle analyses are being performed at different time points to determine whether a cell cycle block occurs before induction of apoptosis. These results demonstrate the efficacy of ITF2357 in inducing apoptosis of both freshly isolated MM samples and cultured MM cell lines, with a 2-10 fold higher potency compared to SAHA. They provide the framework for future phase I studies of ITF2357 in relapsed MM patients.

0231**MOLECULAR CLASSIFICATION OF MULTIPLE MYELOMA: A DISTINCT TRANSCRIPTIONAL PROFILE CHARACTERIZES PATIENTS EXPRESSING CCND1 AND NEGATIVE FOR 14Q32 TRANSLOCATIONS**L. Agnelli¹, S. Bicciato², M. Mattioli¹, S. Fabris¹, D. Intini³, D. Verdelli¹, L. Baldini³, F. Morabito⁴, V. Callea⁵, L. Lombardi¹, A. Neri¹¹Ospedale Maggiore IRCCS, Milan, Italy; ²Dip. Proc. Chim. - Univ. Studi, Padova, Italy; ³U.O. Ematol. ¹ - Osp. Maggiore, Milan, Italy; ⁴U.O. Ematol. A.O. 'Annunziata', Cosenza, Italy; ⁵Dip. CTM, A.O. 'B.M. Morelli', Reggio Calabria, Italy

The deregulation of CCND1, CCND2 and CCND3 genes represents a common event in multiple myeloma (MM), being at least one of them deregulated in almost all MM tumors. A recently proposed TC classification (1) grouped MM patients into five classes on the basis of their cyclins D expression profiles and the presence of the t(11;14), t(4;14), t(14;16) and t(14;20)

translocations, involving the immunoglobulin heavy-chain locus (IGH) at 14q32 locus and deregulating CCND1, FGFR3/WHSC1, MAF and MAFB genes, respectively. *Aims.* The aim of this study was to provide insights into the potential role of D-type cyclins and IGH translocations in the molecular classification of MM. Fluorescence *in situ* hybridization was used to investigate the cyclins D loci arrangements, and to detect the main IGH translocations and the chromosome 13q deletion. The cyclins D expression levels obtained by high-density oligonucleotide microarray analysis of purified plasma cells from 50 MM cases and the molecular characteristics were used to stratify the samples into the five TC classes. The microarray cyclins D expression data were validated by real-time quantitative PCR (Q-RT-PCR) and the concordance of the expression levels obtained by the two different methods was statistically verified. A multi-class classification analysis was performed on the gene expression data and used to identify the transcriptional fingerprints of the 5 TC groups. We identified 112 probe sets as characterizing the TC1, TC2, TC4 and TC5 groups, whereas the TC3 samples showed heterogeneous phenotypes and no marker genes. In particular, in the TC1, TC4 and TC5 groups we identified the molecular signatures associated to the primary IGH translocations target genes, while the TC2 group, which showed extra copies of the CCND1 locus and neither IGH translocations nor the chromosome 13q deletion, was characterized by the over-expression of 30 genes, mainly involved in protein biosynthesis at translational level. Among the most specifically modulated transcripts in the TC2 group we identified a novel gene containing a BTB/POZ domain, typical of many zinc finger transcription factors and associated with transcriptional repression activity. A meta-analysis performed on two publicly available MM datasets validated the identified gene expression signatures. Finally, we showed a very good concordance between the D-type cyclins expression levels as assessed by microarray and Q-RT-PCR analyses, thus suggesting Q-RT-PCR of highly purified plasma cells populations a suitable approach to the stratification of MM patients. *Conclusions.* Our data contribute to the understanding of the molecular and biological features of distinct MM subtypes. The identification of a distinctive gene expression pattern in TC2 patients may improve risk stratification and indicate novel therapeutic targets.

1. Hideshima T, et al. *Advances in biology of multiple myeloma: clinical applications.* Blood 2004;104:607-18.**0232****TRANSCRIPTION REPRESSION ACTIVITY IS ASSOCIATED WITH THE TYPE I ISOFORM OF THE MMSET GENE INVOLVED IN THE T(4;14) IN MULTIPLE MYELOMA**K. Todoerti¹, D. Ronchetti¹, L. Agnelli¹, R. Carbone², S. Castellani¹, S. Marelli¹, S. Minucci², L. Lombardi¹, A. Neri¹¹Ospedale Maggiore IRCCS Univ. di Milano, Milan, Italy; ²Istituto Europeo di Oncologia, Milan, Italy

The WHSC1/MMSET and FGFR3 genes are deregulated in multiple myeloma as the result of the t(4;14) (p16.3;q32) chromosomal translocation occurring in about 15% of the patients. The lack of FGFR3 expression in about one third of t(4;14) patients, has suggested a relevant role of MMSET as the gene target of the translocation. As a result of alternative splicing or transcription initiation events, MMSET normally encodes several putative isoforms, whose functional activities are still largely unknown. Although MMSET involvement in transcriptional regulation processes has been suggested on the basis of the presence of specific protein domains (SET, HATH, PHD and/or HMG) known to be involved in chromatin remodelling and in genes silencing mechanisms, no experimental data have yet been produced concerning this aspect and its biological role. *Aims.* Our aim was to provide insights into the transcriptional activity of the three main isoforms of the gene (MMSET I, MMSET II and RE-IIBP) and in their biological effects on proliferation in transfected cells. *Methods.* cDNA isoforms were subcloned in a

myc-tagged vector and transfected in HeLa or 293T cells. The subcellular localization was assessed by immunofluorescence analysis, and their transcriptional activity assayed by a luciferase test. The histone methyltransferase activity was investigated *in vitro*. Growth or apoptosis rate of transfected cells were evaluated using the *WST1* and *TUNEL* assay, respectively. **Results.** *MMSET I* and *MMSET II* were localized in the nucleus, whereas RE-IIBP isoform showed cytoplasmic and nucleolar staining. *MMSET I* was able to repress the transcriptional activity of a TK promoter in a dose-dependent manner, but neither *MMSET II* or RE-IIBP isoforms had effect in our system. In particular, the analyses of *MMSET I* deleted constructs suggested that the HATH domain, the HMG box and the N-terminal region of the protein are involved in the transcriptional repression activity. Interestingly, *MMSET I* transcriptional activity is at least partially dependent on the interaction with HDACs, as trichostatin A was able to reduce its repressional activity; furthermore co-immunoprecipitation analyses *in vitro* indicated that *MMSET I* recruits specifically HDAC1 and mSin3b, whereas no interaction with *HDAC2* or *HDAC4* was observed. Notably, neither *MMSET II* nor *RE-IIBP* displayed the predicted histone methyltransferase activity. Finally, *MMSET* isoforms did not affect growth or apoptosis rate in transfected 293T cells. **Conclusions.** Our results support the general hypothesis that *MMSET* may act as a transcription regulator, suggest that different functional activities could be associated with distinct isoforms, and contribute to our understanding of the functional roles of deregulated *MMSET* transcripts.

0233

EXPRESSION OF ADHESION MOLECULES ON PLASMACYTOMA

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Multiple myeloma (MM) is a plasma cell neoplasm characterised by disease dissemination at presentation with plasma cells appearing in multiple sites in the bone marrow. Solitary plasmacytoma (SP) presents as a single, localised plasma cell tumour in either bone or less commonly soft tissue. Cellular adhesion molecules (CAMs) have been implicated in the pathogenesis of many tumours. Although studies have reported on CAM expression in MM, there is no data in human SP. It may be that differential expression of CAMs by SP may explain why such tumours remain localised. To study adhesion molecule expression in SP/MM and attempt to identify molecules that may be responsible for the contrasting disease presentations of SP and MM. **Method.** After ethical approval had been sought and granted, archival biopsies were obtained from patients known to have either solitary plasmacytoma with no evidence of myeloma (n=14) or MM at presentation (n=11). Two-micron sections were cut from archival histological blocks. Sections were stained for expression of the following known adhesion molecules: CD138, CD56, CD44, CD54, CD31, CD29, CD28, CD106, CD49d (a4) and CD49f (a6) by indirect immunoperoxidase staining using standard methods. Antibodies were selected based on published literature indicating known, or suspected, expression in MM. Results are shown in Table 1, expressed as the mean percentage of positively stained plasma cells.

For statistical analysis positive staining was scored in 6 groups 0%, 0-10%, 10-30%, 30-50%, 50-70% and 70-100% expression. Slides were examined blindly. Statistical analysis was carried out using the Wilcoxon Rank Sum Test with Bonferroni adjustment, only *p* values below 0.005 were considered significant. No significant difference was found in the majority of CAMs investigated except for CD49d (a4) *p*= 0.0006. **Conclusions.** We have confirmed the expression of adhesion molecules known to be present on myeloma plasma cells and demonstrate that these CAMs are also expressed at a similar level by plasma cells in solitary plasmacytoma. However, CD49d (the α 4 subunit of the β 1 integrin (VLA-4)) showed a significantly lower level of expression in plasmacytoma than on MM plasma cells. The VLA-4 (CD49d/CD29) integrin is expressed on MM plasma cells and reported to be instrumental in the pathogenesis of

MM. *In vitro* studies have shown binding of VLA-4 to its ligands (fibronectin and VCAM-1 (CD106)) in bone marrow stroma promotes colonisation of plasma cells in the bone marrow, prevents apoptosis and enables cytotoxic resistance. As much of the cell adhesion in MM is VLA-4 dependant, the lower expression of CD49d (a4) on plasma cells of plasmacytoma could explain the localized nature of this disease and why there is reduced trafficking to the bone marrow. Up regulation of this integrin may be associated with the progression of SP to MM that occurs in the majority of patients. Our results allow a better understanding of the biological differences in these two medical conditions and indicate that down regulation of VLA-4 may be a useful therapeutic avenue in MM.

	Plasmacytoma	Multiple Myeloma
CD138	85	83
CD56	48	62
CD44	71	36
CD54	57	39
CD31	53	73
CD29	45	34
CD28	78	83
CD106	38	49
CD49d	8	45
CD49f	27	59

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0234

OSTEOPROTEGERIN AND SOLUBLE RANKL CONCENTRATIONS IN SERUM AND BONE MARROW OF MULTIPLE MYELOMA PATIENTS

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Multiple myeloma (MM) plasma cells secrete factors that activate osteoclasts resulting in the induction of osteolytic lesions. Recent studies suggest that MM triggers osteoclastogenesis by disrupting the balance between the receptor activator of NF- κ B ligand (RANKL), which has been shown to play a key role in normal osteoclast development, and osteoprotegerin (OPG), its natural antagonist. Therefore in this study we analyzed the concentrations of bone marrow (BM) and serum OPG and sRANKL in MM patients at diagnosis and also under treatment. Determinations of BM and serum OPG and sRANKL concentrations were performed in 133 MM patients and 42 healthy subjects by means of ELISA method using Osteoprotegerin ELISA and sRANKL ELISA kits (Biomedica, Vienna). **Results.** In the whole group of MM patients, OPG concentrations in particular patients ranged from 40 to 445 pg/mL with a mean concentration of 118 \pm 68, median 101 pg/mL while in healthy age- and sex-matched controls OPG levels ranged from 43 to 159 pg/mL, mean 82 \pm 26, median 81 pg/mL (*p*=0,0001). OPG serum levels were higher in MM patients with renal failure and patients with hypercalcaemia as compared with patients with normal renal function and patients with normal serum calcium concentrations (*p*<0,001; *p*=0,0,04, respectively). In the myeloma group serum OPG levels correlated with age (like in control group) and β 2-microglobulin serum concentrations ($r=0,36$; *p*<0,01 and $r=0,26$, *p*<0,01, respectively) and did not correlate either with the presence of osteolysis or with stage of disease. In BM of MM

patients OPG concentration ranged from 40 to 397 pg/mL with a mean level 123 ± 72 and median 103 pg/mL. There was a correlation between BM and serum OPG concentrations ($r=0,83$; $p<0,001$). The sRANKL serum levels in the group of MM patients were 19 ± 53 pg/mL and in healthy subjects $4,5 \pm 3,0$ pg/mL ($p=0,42$). In MM patients OPG and sRANKL serum levels were similar at diagnosis and in plateau phase of disease. Myeloma BM sRANKL concentration ranged from 0 to 130 pg/mL with a mean level 24 ± 26 and median 21 pg/mL. There was a correlation between BM and serum sRANKL concentrations ($r=0,7$; $p=0,000$). Median values of the sRANKL/OPG ratio for BM and serum of MM patients were 0,14 and 0,11, respectively. Median value of the sRANKL/OPG ratio for serum of controls was 0,11. In 20% of MM patients serum OPG levels are elevated and it may be a compensative reaction in relation to increased bone destruction. Significantly increased OPG concentrations in MM patients with renal failure may be related to its decreased elimination. There is not statistically significant association between sRANKL serum and BM levels and the main clinical and laboratory parameters of the disease such as stage, presence of osteolytic lesions, calcemia, monoclonal protein isotype, survival. Determinations of BM and serum sRANKL/OPG concentration ratio seem to not present clinical value.

0235

FIRST-LINE THALIDOMIDE-DEXAMETHASONE THERAPY IN PREPARATION FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN YOUNG PATIENTS WITH SYMPTOMATIC MULTIPLE MYELOMA

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Remarkable antimyeloma efficacy, reported to be about 30% with thalidomide alone and 50% with added dexamethasone in advanced and refractory multiple myeloma (MM), provided the basis for recent clinical trials aimed at investigating the role of these drugs as first-line therapy for patients with symptomatic MM. Aim Thalidomide-dexamethasone therapy was given in young patients (< 61 years) with previously untreated symptomatic multiple myeloma. The study protocol was approved by the national medical ethical committee and a written informed consent was obtained from the patients. The aim of this study was to assess the efficacy and toxicity of this combination as first-line therapy. During first-line therapy, thalidomide and dexamethasone were administered for 75 days (200 mg/day) and 3 months, respectively. The monthly dose of dexamethasone was 20 mg/m²/day for 4 days, with cycles repeated on days 9 to 12 and 17 to 20 on the first and the third month of therapy. After first-line therapy, a collection of PBSCs was performed in all patients. **Results.** Between May 2003 and February 2005, 65 patients were evaluated for response and toxicity after first-line thalidomide-dexamethasone therapy. On an intent-to-treat basis, the overall response (>or= minimal response) rate was 84%, including 24% of patients who obtained a complete remission. Three patients (4.6%) presented a disease progression during treatment (one of them died). Grade 1-2 toxicity: constipation (20%), neuropathy (10%). Grade 3-4 toxicity: infections (12%), deep-vein thrombosis (6%). Six patients (9.2%) required thalidomide discontinuation because of toxicity. Sixty two patients (95%) proceeded to PBSC mobilization and yielded a median number of 8×10^6 /kg CD34⁺ cells. First-line thalidomide-dexamethasone therapy is effective and relatively well tolerated in young patients (< 61 years) with symptomatic multiple myeloma. This combination does not affect PBSCs mobilization, and may provide an oral alternative to vincristine-doxorubicine-dexamethasone.

0236

THE SHORT SCHEDULE OF DCEP IS MORE FEASIBLE THAN THE INFUSIONAL SCHEME, MAINTAINS THE SAME EFFICACY IN MOBILIZING STEM CELLS, AND SHOWS AN OVERLAPPING TOXICITY

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High-dose melphalan with peripheral blood stem cell rescue represents today the standard therapy for young multiple myeloma (MM) patients. DCEP (dexamethasone, cyclophosphamide, etoposide, and cisPlatin) is an effective regimen for peripheral blood stem cell (PBSC) mobilization in MM patients with low hematological and extrahematologic toxicity. To compare the efficacy and toxicity of two different schedules of DCEP regimen. From 2000 to 2004, 152 consecutive patients aged less than 65 years, enrolled in a high-dose program, with comparable characteristics, were treated with two different schedules of the DCEP scheme. From January 2000 to December 2003, 106 patients (group I) were mobilized with the infusional-DCEP (Dexamethasone 40 mg/day i.v. in days 1-4; and 4 days continuous infusion of daily doses of Cyclophosphamide 400 mg/m²; Etoposide 40 mg/m²; cisPlatin 10 mg/m²). On the contrary, from January 2004, 46 patients (group II) were mobilized with short version of DCEP (DCEP-short) which consists of oral Dexamethasone 40 mg/day on days 1-4, Cyclophosphamide 700 mg/m²/day e.v., Etoposide 100 mg/m²/day e.v., cisPlatin 25 mg/m²/day in a total 8 hours infusion, for 2 days. In both groups, granulocyte colony stimulating factor (G-CSF 5 mcg/Kg/day) was started 48 hours after the end of chemotherapy till the end of PBSC collection. The median number of CD34⁺ cells was higher in group II (6.98×10^6 cells/kg) with respect to group I (5.29×10^6 cells/kg) but the difference was not significant ($p=NS$). The incidence of patient failing mobilization was low in the two groups, with no statistical difference: 7 patients (6.9%) after infusional-DCEP vs 3 patients (6.5%) after DCEP-short ($p=NS$). The number of patients collecting 2×10^6 CD34⁺ cells/Kg was slightly higher in group II (3 patients, 4.6%, after DCEP-short vs 2 patients, 3.2%, after infusional-DCEP) without reaching a statistical significance ($p=NS$). Thus, in the two groups, most of the patients collected an adequate number of CD34⁺ cells for the autotransplant procedure ($\geq 4 \times 10^6$ cells/Kg): 76.4% after infusional-DCEP and 79% after DCEP-short ($p=NS$). The incidence of grade III WHO neutropenia (neutrophil count <1000/mm³) was higher in group II than in group I but without reaching any statistical significance: 12 patients (11%) after infusional-DCEP vs 11 patients (25%) after DCEP-short ($p=NS$). However the number of patients requiring hospitalization for severe infection was low in both groups: 5 patients (4.7%) after infusional-DCEP vs 2 patients in the DCEP-short group (4.5%). Even if the incidence of thrombocytopenia (platelet <100000/mm³) was higher in II group (14 patients, 30%, in DCEP-short group vs 7 patients, 6%, after infusional-DCEP; $p=0.009$), there were not hemorrhagic events in both groups and no need for platelet support. **Conclusions.** DCEP-short is a good mobilizing regimen, sharing the same characteristics with the infusional-DCEP: high mobilizing efficacy, low toxicity with the advantage of an outpatient management.

0237**THE EFFECT OF RENAL IMPAIRMENT AND DIALYSIS ON SERUM FREE LIGHT CHAIN MEASUREMENT**G.P. Mead¹, S.D. Reid², P. Cockwell³, P. Hewins³, A.R. Bradwell¹¹University of Birmingham, Birmingham, UK;²The Binding Site Ltd., Birmingham, UK;³University Hospital, Birmingham, UK

Nephelometric serum free light chain (FLC) measurement is more sensitive than serum or urine electrophoresis for diagnosing and monitoring patients with light chain myeloma and AL amyloidosis. Many of these patients will have renal impairment during the course of their disease and, as FLC are cleared from the serum by glomerular filtration, this will affect serum FLC concentrations. The normal range for the κ/λ ratio has also been determined and deviations outside of this can be used to identify monoclonal FLC production. The aim of this study was to investigate the influence of renal function on serum FLC by measuring concentrations in patients with chronic renal failure (CRF) and those undergoing dialysis. *Methods.* We studied 106 patients attending general renal and low clearance clinics, 40 undergoing regular haemodialysis and 11 having continuous ambulatory peritoneal dialysis. Serum samples were collected from all patients; for hemodialysis patients immediately before and after dialysis. FLC and cystatin C were assayed nephelometrically and results were compared with previously published normal range data. Forty-seven percent (50/106) of patients with CRF (predialysis) had concentrations of both κ and lambda FLC greater than the normal range and the FLC concentration correlated with glomerular filtration rate (GFR; calculated by MDRD criteria): GFR vs log κ FLC, $R^2=0.574$ and GFR vs. log λ FLC, $R^2=0.549$. The correlation with cystatin C was stronger with R^2 of 0.778 and 0.727, respectively. There was also an increase in the average κ/λ ratio in patients with elevated FLC concentrations (mean 0.95 in chronic renal failure patients and 0.63 in normals). Patients treated by haemodialysis had elevated concentrations of both FLC but measurements immediately after dialysis showed that concentrations had fallen during dialysis (mean fall of 60% for κ and 37% for λ). Patients undergoing continuous peritoneal dialysis had elevated serum FLC concentrations similar to the other patient groups. Patients with impaired renal function showed increased serum concentrations of both κ and λ FLC and these correlated (inversely) with calculated GFR. The increase in the mean κ/λ ratio in these patients is explained by the change in clearance mechanisms from glomerular filtration (which clears dimeric free κ more slowly) to general pinocytosis (which clears κ and λ FLC equally). It might be possible to correct for this difference using cystatin C or other measures of GFR. The observation that haemodialysis removed significant amounts of FLC from the serum was unexpected and could be important for the treatment of light chain disease. The timing of FLC measurement, relative to dialysis, should be considered when monitoring patients with light chain multiple myeloma or AL amyloidosis.

0238**ANTI-ANGIOGENIC EFFECT OF BORTEZOMIB (VELCADE®) IN PATIENTS WITH MULTIPLE MYELOMA**

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Bortezomib (VELCADE®) is a proteasome inhibitor, which has been proven to be very effective for the treatment of refractory/relapsed multiple myeloma (MM). Although the mechanism of cell death via bortezomib is likely multifactorial, myeloma cell seems particularly sensitive to inhibition of the nuclear factor κ B pathway. In vivo preclinical studies on mice have shown that bortezomib also has an anti-angiogenic activity. *Aims.* The aim of this study was to investigate whether bortezomib has an anti-angiogenic effect in patients with MM. *Patients/Methods.* We studied the effect of bortezomib on angio-

genesis in bone marrow biopsies and serum samples of nine patients with MM. The patients studied (6M/3F; median age: 58 years) had received more than 4 lines of treatment previously, and had relapsed prior to bortezomib administration. Six patients had IgG MM, while one patient had IgA, one non-secretory and one light-chain MM. Bortezomib was given at a dose of 1.3 mg/m², iv, in 3-week cycles, on days 1, 4, 8, and 11 of each cycle, for at least 8 cycles. Microvessel density (MVD) was assessed in bone marrow trephine biopsies before treatment and after 4 and 8 cycles of treatment. Furthermore, the trephines were stained with commercially available monoclonal antibodies to VEGF and VEGF-receptors 1 and 2 (Flt-1 and Flk-1, respectively). Additionally, we evaluated the levels of vascular endothelial growth factor (VEGF) and angiogenin in patients serum using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, USA) before treatment and then after every cycle for eight cycles. *Results.* After bortezomib administration 5/9 patients achieved a partial response (PR), one had a complete response (CR), two had a minimal response (MR), and one experienced progression of the disease. In 6/9 patients there was a decrease of the MVD. More specifically, in 3/5 patients with PR and the two patients with MR there was a significant decrease in the MVD after four cycles of treatment (1.6, 2.9-, 2.4-, 2.2-, and 1.4-fold decrease of MVD, respectively). In the patient who achieved a CR there was a 1.8-fold decrease in MVD, after four cycles of treatment. In 4 patients further reduction of MVD was noticed after 8 cycle of treatment. All patients were positive for VEGF, Flk-1 and Flt-1 in bone marrow biopsies before treatment and continued to remain positive after bortezomib administration. There was a significant reduction in angiogenin levels by cycle 4 (mean±SD: 364±94.7 ng/mL vs. 557.5±154.9 ng/mL at baseline; $p=0.028$). This difference continued to remain significant after 8 cycles of treatment ($p=0.015$). On the contrary, there was no change in the levels of VEGF at cycles 1, 4, and 8. In three of the patients who responded, there was a good correlation between the serial estimations of paraprotein and angiogenin levels. *Conclusions.* These results suggest that bortezomib may exert part of its anti-myeloma effect through anti-angiogenic mechanisms. Further investigations are required to determine whether bortezomib is cytotoxic for endothelial cells or interferes with the autocrine and paracrine pathway of VEGF action by reducing the tumour burden.

0239**INTERMEDIATE DOSE OF THALIDOMIDE IN COMBINATION WITH DEXAMETHASONE AND ZOLEDRONIC ACID IS EFFECTIVE IN REFRACTORY/RELAPSED MYELOMA, IMPROVES ABNORMAL BONE REMODELLING AND REDUCES MARROW ANGIOGENESIS**E. Terpos¹, D. Mihou², R. Szydlo³, K. Tsimirika², E. Voskaridou⁴, M. Politou⁵, C. Karkantaris¹, A. Rahemtulla⁵, M.A. Dimopoulos⁵, K. Zervas²¹25¹ General Airforce Hospital, Athens, Greece; ²'Theageion' Cancer Centre, Thessaloniki, Greece; ³Imperial College London, London, UK; ⁴Thalassaemia Centre, Athens, Greece; ⁵University of Athens Medical School, Athens, Greece

The combination of thalidomide/dexamethasone is very effective in refractory/relapsed multiple myeloma (MM). The mode of action of thalidomide in myeloma is not fully understood. The receptor activator of nuclear factor- κ B ligand (RANKL)/osteoprotegerin (OPG) pathway and osteopontin (OPN) are crucial for the activation/differentiation of osteoclasts and correlate with myeloma bone disease. *Aims.* The aim of this study was the evaluation of the effect of intermediate doses of thalidomide and dexamethasone on disease course, bone remodelling and angiogenesis in patients with refractory/relapsed MM. We studied 35 patients, who received thalidomide at a dose of 200 mg/daily. Dexamethasone was given at a dose of 40 mg/daily for 4 days every 15 days until maximal response and then at 40 mg/daily for 4 days monthly. All patients were also given zoledronic acid, monthly. We measured, pre-, 3 and 6 months post-treatment markers of osteo-

clast activation [*sRANKL*, *OPG*, tartrate resistant acid phosphatase isoform-5b (*TRACP-5b*), markers of bone resorption [C-telopeptide of collagen type-I (*CTX*), markers of bone formation [bone alkaline phosphatase (*bALP*), osteocalcin (*OC*), and C-terminal propeptide of collagen type-I (*CICP*), and *OPN*. Furthermore, serum levels of vascular endothelial growth factor (*VEGF*), basic-fibroblast growth factor (*b-FGF*), tumor necrosis factor- α (*TNF- α*), which have angiogenic potential, and interleukin-6 (*IL-6*), *IL-1 α* , soluble *IL-6* receptor (*sIL-6R*), and transforming growth factor- β (*TGF- β*), which are involved in the disease biology, were measured before treatment and then every 2 weeks for 8 weeks. Microvessel density (*MVD*) was evaluated in marrow biopsies before and after treatment. **Results.** Before the administration of thalidomide/dexamethasone, patients had increased levels of *sRANKL* ($p=0.008$), *OPG* (<0.001), *sRANKL/OPG* ratio ($p=0.01$), *TRACP-5b* (<0.0001), *CTX* ($p=0.001$), *OPN* ($p=0.023$), and *CICP* ($p=0.01$), while they had reduced levels of *bALP* ($p<0.0001$) and *OC* ($p=0.001$) compared with controls ($n=30$). The pre-treatment ratio of *sRANKL/OPG* correlated with the extent of bone disease ($p=0.04$). Treatment administration resulted in a significant reduction of *sRANKL* ($p<0.0001$), *sRANKL/OPG* ratio ($p<0.0001$), *TRACP-5b* ($p<0.0001$) and *CTX* ($p=0.001$). Markers of bone formation, *OPG*, and *OPN* did not show any significant alteration. Changes of *sRANKL/OPG* ratio correlated with changes of both *CTX* and *TRACP-5b*. Pretreatment levels of *MVD*, *VEGF*, *b-FGF*, *IL-6*, *sIL-6R* were increased in the patients compared to controls. There was a strong correlation between *MVD* and plasma cell infiltration in the bone marrow ($p<0.0001$). Thalidomide plus dexamethasone produced a significant reduction of *MVD*. However, an increase in serum levels of *VEGF*, *b-FGF*, *IL-6*, *sIL-6R* was observed post-treatment even in responders. *TNF- α* , *TGF-beta*, *IL-1 α* did not differ between patients and controls and remained unchanged during the study. Thalidomide/dexamethasone was given for a median time of 10 months and the median follow-up period was 22 months. Response rate was 65.7%. Median survival was 18 months. β_2 -microglobulin, type of response, and *IPS* staging predicted for survival, while *sRANKL/OPG* ratio had a borderline predictive value for survival. **Conclusions.** These results suggest that the combination of intermediate dose of thalidomide with dexamethasone is very effective in patients with refractory/relapsed MM, reducing *MVD* and improving bone remodelling through the reduction of *sRANKL/OPG* ratio.

0240

INCREASED SERUM LEVEL OF INTERLEUKIN-15 IN MULTIPLE MYELOMA AND NON-HODGKIN'S LYMPHOMA PREDICT GOOD CLINICAL RESPONSE FOR THE TREATMENT

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IL-15 is produced by multiple tissues (placenta, skeletal muscle, monocytes, macrophages, lung, heart, kidney, bone marrow stromal cell lines). *IL-15* is the critical factor for NK cell development, acting through the *IL-15R α* , *IL-15* can also induce the proliferation of normal and malignant B cells. NK cells express surface receptors that regulate NK cell activation and lysis of target cells. They are likely to be important for the control of some cancers. B-cells lymphoproliferative disorders (non-Hodgkin's lymphomas, nHL and multiple myeloma, MM) constitute an extremely heterogeneous group of lymphoproliferative neoplasms in both histopathological and clinical presentation with often unpredictable response for the treatment. **Aims.** The aim of this study was to investigate the serum level of interleukin-15 in multiple myeloma (MM) and non-Hodgkin's lymphoma (nHL) patients. Forty MM and 37 nHL patients were studied. Clinical data are showed in the Table 1. Patients were studied before any chemotherapy. The effect of chemotherapy (CHOP for nHL and VAD for MM) after 4 courses was analyzed. The control group consisted of 15 age-matched normal donors.

Table. Clinical data and results.

MM	
Mean age/ Sex	Stage acc
63.9 years/ M/F	

The serum level of *IL-15* was measured in MM and nHL patients and normal donors. *IL-15* levels were measured by enzyme linked immunoassay - ELISA (IBL, Hamburg, Germany) following the manufacturer instruction. Analyses and calibrations were carried out in duplicate, intra- and interassay variations were within the range by the manufacturer. The data were analyzed using CH test. *IL-15* was detected in 10 out of 40 MM, in 10 out of 37 nHL patients and in 1 out of 15 in control group. We have demonstrated that patients with detectable level of *IL-15* have better overall response for the treatment that patients with undetectable level of *IL-15* ($\chi^2=17,0$; $p<0,0001$). *IL-15* is essential for survival of natural killer cells that are responsible for lysis of neoplasm cells. Increased serum level of interleukin-15 in multiple myeloma and non-Hodgkin's lymphoma may predict response for the treatment.

0241

PROGNOSTIC IMPACT OF THE EXPRESSION OF HLA-I AND IL6R CHAINS ON CLONAL PC AND THE SERUM OF MULTIPLE MYELOMA PATIENTS

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Recently, it has been shown that clonal plasma cells (PC) from different types of monoclonal gammopathies (MG) display distinct phenotypes consistent with an increased antigen-presentation and T-cell costimulation in MG of undetermined significance that deteriorates in malignant conditions. Despite this, the exact clinical significance of variations in the expression of most of these molecules remains largely unknown. **Aims.** In the present study we explore the clinical impact of both soluble and surface levels of several molecules involved in the interaction between clonal PC and the bone marrow (BM) immunological microenvironment in multiple myeloma (MM) **Methods.** Both the cellular and soluble levels of β_2 microglobulin, HLA-I, CD126, CD130, CD40 CD86 and CD95 were analyzed in a group of 29 MM patients at diagnosis using flow cytometry and ELISA techniques, respectively. The levels of each marker were correlated with other disease characteristics and overall survival. Our results show an adverse prognostic impact for patients with lower expression on BMPC of the HLA-I ($p=0.09$) and β_2 -microglobulin ($p<0.05$) antigen-presenting molecules, the CD126 ($p<0.001$) and CD130 ($p<0.05$) IL6R chains, and CD95 ($p=0.009$). Likewise, patients showing higher soluble levels of these molecules, except CD126, tended to have a shorter survival ($p=0.09$). Such prognostic impact was paralleled by an association with other specific adverse prognostic factors. Interestingly, upon considering the ratio between the soluble and PC expression of each molecule, an increased adverse prognostic impact was observed for HLA-I ($p<0.001$) and β_2 -microglob-

ulin ($p=0.01$) but not for the other molecules. Neither CD86 nor CD40 expression on BMPC showed prognostic value. Summary and conclusions: Overall, our results show that different imbalances between cellular and soluble levels of HLA-I, IL6R and CD95 molecules exist among MM patients being associated with a different disease outcome.

0242

THALIDOMIDE, DEXAMETHASONE AND PEGYLATED LIPOSOMAL DOXORUBICIN (THADD) IN ELDERLY/RELAPSED MULTIPLE MYELOMA (MM) PATIENTS

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MM patients who relapsed after standard or high-dose chemotherapy and those older than 65 years have limited treatment options. We combined thalidomide and dexamethasone with pegylated liposomal doxorubicin because of its favourable pharmacodynamic and toxicity profiles besides its cytotoxic and antiangiogenic activities in MM. In March 2003 we started a phase II, prospective, multicenter study with the aim to assess the response rate and the toxicity of the combination thalidomide 100 mg/day (continuous), dexamethasone 40 mg days 1-4 and 9-12 of each month and pegylated liposomal doxorubicin 40 mg/sqm on day 1. ThaDD regimen was administered every 4 weeks for 4-6 courses to patients with relapsed MM (rMM) or newly diagnosed MM older than 65 years (ndMM). For eligible patients transplantation was planned. All patients received antithrombotic and antimicrobial prophylaxis with warfarin (0.25 mg/day) and ciprofloxacin, respectively. SWOG criteria were used for definition of response and toxicity was graded according to the WHO criteria. At January 2005, 71 patients have been enrolled and 59 were evaluable. Twentyseven patients older than 65 years had ndMM whereas 32 had rMM. These latter had received a median of 2 prior treatments (range 1-6) and 14 patients had relapsed after high-dose therapy. Overall, median age was 71 years (range 41-82), 40 patients (68%) were classified as having intermediate-high risk disease according to IPI-MM and 33% of patients had unfavourable cytogenetics. Overall response rate was 88% (30% CR, 10% VGPR, 39% PR and 9% MR) whereas 4 patients (7%) had progressive disease. There were 3 early deaths (5%) occurring within the first months of treatment. Out of 27 ndMM older than 65 years, 9 (33%) achieved a CR, 5 (18.5%) a VGPR and 8 (30%) had PR. Regarding the 32 patients with rMM, 9 (28%) had CR, 1 (3%) VGPR and 15 (47%) achieved a PR. With a median follow-up of 12 months, projected 2-years OS, PFS and EFS were 73%, 50% and 42%, respectively. EFS at 2 years was 57% in elderly ndMM and 37% in rMM. Totally, we administered 253 courses of ThaDD combination. No patients required a treatment reduction but 13 patients delayed therapy for infections and 2 (3%) choose to discontinue it because of septic shock and pulmonary embolism, respectively. Grade 3-4 neutropenia and thrombocytopenia occurred in 18% and 5% of patients, respectively. Palmar-plantar erythrodysesthesia grade 2 was observed in 3 patients (5%) whereas 2 patients (4%) developed severe mucositis. Thirteen patients (22%) experienced constipation, 18 (30%) fatigue and 7 (12%) tremors less or equal grade 2 WHO. Peripheral neuropathy beyond grade 1 was not noted whereas 5 patients (8.5%) had symptomatic venous thromboembolic events (2 pulmonary embolism). Grade 3-4 fever occurred in 13 patients (22%) and included 9 FUO, 3 pneumonia and 1 septic shock. However, no patients died because of infection. This preliminary results suggest that ThaDD combination yields a high and good quality response rate both in elderly de-novo and relapsed MM patients. Toxicities have resulted easy to manage, being infections and DVT the main problems.

0243

PROGNOSTIC IMPACT OF CYCLOOXYGEASE-2 (COX-2) IN MULTIPLE MYELOMA

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A perturbed microenvironment with secretion of inflammatory cytokines is typical of multiple myeloma (MM). Prostaglandins (PGs) are important mediators in inflammation and angiogenesis and play a role in the pathogenesis of several solid tumors. Cyclooxygenase (COX)-2, the key enzyme of PG synthesis, is implicated in progression of some of these cancers. *Aims.* Despite considerable data concerning COX-2 expression in cancer, only limited information is available in hematological malignancies. Several biological, epidemiological and clinical considerations, however, suggest the involvement of COX-2 in MM. *Methods.* The protein expression of COX-2 was immunohistochemically evaluated in formalin-fixed, paraffin-embedded samples from 58 patients with primary diagnosis of MM. 54 samples were obtained from BM, while four samples were obtained from extramedullary lesions. Time to progression and a variety of clinicopathological features were evaluated using Kaplan-Meier method and Cox regression model. In addition, COX-2 expression was evaluated by staining bone marrow from healthy donors and in five patients with MGUS, monoclonal gammopathy of unknown origin. Thirty-one (53%) samples expressed COX-2, of which 29 were obtained from BM, while the remaining two samples were from extramedullary lesions. All five normal BM samples tested and samples from MGUS disorder stained negative for COX-2. Positivity for COX-2 was unrelated to stage, clinical and molecular features of disease. However, COX-2 expression appeared to be of prognostic relevance: at diagnosis the median time to progression was 16 months in COX-2 positive and 31 months in COX-2 negative patients ($p<0.001$). COX-2 is frequently expressed in MM and correlates with shorter progression free survival.

0244

ARSENIC TRIOXIDE (TRISENOX®) IN MULTIPLE MYELOMA (MM): AN ADJUNCTIVE ROLE FOR ASCORBIC ACID (AA)

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Preclinical studies have shown that arsenic trioxide induces apoptosis in malignant cells from several hematological diseases, including MM. Arsenic trioxide is primarily active through two major mechanisms: 1) specific activation of proapoptotic pathways and 2) activation of downstream apoptotic pathways through the generation of reactive oxygen species (ROS) and mitochondrial membrane depolarization. In MM cells, constitutive activity of transcription factors NF- κ B correlates with resistance to chemotherapy. Arsenic trioxide inhibits NF- κ B activity through its direct inhibition of the NF- κ B inhibitor I κ B and can restore apoptotic signaling in chemoresistant cells. Arsenic trioxide-induced apoptosis through the generation of ROS is modulated by the glutathione (GSH)-dependent redox system: malignant cell lines that are sensitive to arsenic trioxide have consistently low levels of GSH, while arsenic-resistance is associated with increased GSH levels. Agents that down-regulate intracellular levels of GSH, such as L-buthionine- (S,R)-sulfoximine (BSO) and ascorbic acid (AA), potentiate the apoptotic effect of arsenic trioxide *in vitro*. Several phase I/II studies have shown that arsenic trioxide has efficacy in MM as a single agent. Based on the described prelini-

cal data, a phase I/II trial was initiated using AA to modulate intracellular GSH levels and increase the efficacy of arsenic trioxide in MM patients. The phase I component of this trial showed that AA does not alter the pharmacokinetics of arsenic trioxide, and that elevated AA blood concentrations correlate with decreased intracellular GSH levels in peripheral blood cells. Interim analysis of the subsequent phase II shows an impressive improvement in total response rate (RR) compared to arsenic trioxide as a single agent (see Table).

	N	CR	PR	MR	SD	Total Disease Control
ATO	48	0 (0%)	2 (4%)	12 (25%)	10 (21%)	24 (50%)
ATO + Dex	11	0 (0%)	0 (0%)	2 (18%)	6 (55%)	8 (73%)
ATO + AA	17	0 (0%)	4 (24%)	5 (29%)	8 (47%)	17 (100%)
ATO + Dex + AA	21	2 (9%)	6 (29%)	NA	10 (48%)	17 (86%)
ATO + Mel + AA	17	0 (0%)	3 (18%)	6 (35%)	4 (24%)	13 (77%)

RR was 53% (N=17), with the remaining patients having SD. Similarly, the addition of AA to a combination regimen including arsenic trioxide and dexamethasone (Dex) also dramatically improved the RR compared to the combination of arsenic trioxide and Dex. Building on these observations, the combination of arsenic trioxide + AA was subsequently shown to enhance the efficacy of melphalan, even in melphalan refractory patients, and these data became the basis for a multicenter Dex-free phase II trial. The combined data from these studies demonstrate that ATO+ AA is an active combination in patients with MM, and should be further explored in combination with other agents.

0245

A PHASE 1 STUDY OF BORTEZOMIB (VELCADE®) IN COMBINATION WITH LENALIDOMIDE (REVLIMID™) IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Bortezomib and lenalidomide have demonstrated activity in relapsed and refractory multiple myeloma (MM). Both agents inhibit proliferation and angiogenesis and promote apoptosis of MM cells (*Anderson Cancer 2003;97:796*). These agents exhibit immunomodulatory effects and prevent secretion of cytokines by reducing interactions of MM cells with the bone marrow microenvironment. In phase 2 trials, bortezomib has induced durable responses with manageable toxicity in heavily pre-treated patients with MM. Lenalidomide has also produced durable responses in relapsed and refractory patients with MM, including those who received prior bortezomib. Lenalidomide and bortezomib do not appear to have overlapping toxicities and appear to have synergistic apoptotic effects *in vitro*. These findings suggest that this regimen may result in improved activity in MM. In phase 1 trials, the maximum tolerated doses (MTD) of bortezomib and lenalidomide were 1.3 mg/m² (twice weekly) and 25 mg/day, respectively. *Aims*. A phase 1 dose-escalation trial was initiated to determine the MTD and activity of bortezomib with lenalidomide in patients with relapsed and/or refractory MM. *Methods*. Eligible patients were enrolled in 3-patient cohorts to receive bortezomib 1.0 or 1.3 mg/m² in combination with lenalidomide 5, 10, 15, or 20 mg/day, for a total of 8 cohorts. Bortezomib was administered on days 1, 4, 8, and 11, and lenalidomide on days 1–14 of a 21-day cycle. Toxicity was assessed using NCI CTC criteria version 3.0. Dose-limiting toxicities (DLT) included grade ≥3 nonhematologic toxicity, grade 4 hematologic toxicity (grade 4 neutropenia lasting ≥ 5 days and/or neutropenic fever were DLT) other than thrombo-

cytopenia, and platelet count ≤10,000 on > 1 occasion despite transfusion. Evaluation of response was assessed using Bladé criteria at the end of cycle 2 and subsequent cycles. *Results*. Twelve patients with MM have been enrolled into cohorts 1–4. MTD was not reached in the first 3 cohorts. Nine have been treated, including 3 with relapsed and 6 with relapsed and refractory disease. The median number of prior therapies was 3 (range, 1–9). Five patients had undergone prior SCT; 7 had received thalidomide; 4 had received bortezomib. With a median of 6 cycles completed, patients have tolerated bortezomib 1.0–1.3 mg/m² and lenalidomide 5–10 mg/day without DLT. Grade 4 neutropenia occurred in 2 patients for < 5 days, and grade 3 thrombocytopenia was observed in 4 patients. No other significant toxicities have been reported. Nine patients are evaluable for response. In cohort 1 (bortezomib 1.0 mg/m², lenalidomide 5 mg/day), 3 of 3 patients achieved MR. In cohort 2 (bortezomib 1.3 mg/m², lenalidomide 5 mg/d), 2 of 3 achieved PR, and one had MR. In cohort 3 (bortezomib 1.0 mg/m², lenalidomide 10 mg/d), 2 of 3 had MR, and one had SD. *Conclusions*. Bortezomib and lenalidomide can be combined at active doses, and promising activity has been seen. Further investigation is ongoing to determine the MTD. Enrollment of an additional cohort of 10 patients at the MTD is planned for further evaluation of this regimen in relapsed and/or refractory MM.

Non-Hodgkin's lymphoma - Clinical I

0246

IS CT SCAN STILL NECESSARY FOR STAGING IN HODGKIN (HD) AND NON-HODGKIN'S LYMPHOMA (NHL) PATIENTS IN THE PET/CT ERA?

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Recently, PET/CT systems have been introduced into clinical practice and offer advantages including shorter image acquisition time, improved lesion localization and better diagnostic and staging accuracy. *Aims*. To assess the clinical impact of fused PET/CT data on staging and patient management in HD and NHL. *Methods*. 103 consecutive patients with NHL (n=68) and HD (n=35) were assessed, provided they had CT and PET/CT done at diagnosis. Staging and treatment decisions were obtained by two different clinical panels (A and B). Three comparisons were carried out in an attempt to assess the added value of each modality: CT vs. PET/CT; CT vs. CT + PET/CT; PET/CT vs. PET/CT + CT. *Results*. 1. NHL patients: Compared to CT, disease was upstaged by PET/CT in 37% and 31% (mostly in stages I and II) and downstaged by PET/CT in 7% and 1% by panels A and B, respectively. These were statistically significant differences for both panels ($p < 0.005$). Significant differences were also found between staging based on CT vs. CT + PET/CT for both panels ($p < 0.005$). A significant difference between staging based on PET/CT vs. CT and PET/CT was found according to panel B ($p = 0.005$) but not according to panel A. The changes in the therapeutic approach appropriate for early stage vs. advanced stage vs. 'watch and wait' varied according to CT vs. PET/CT (12% and 22%) CT vs. CT + PET/CT (13% and 22%) and PET/CT vs. CT + PET/CT (3% and 7%) as determined by panels A and B, respectively. 2. HD patients: Compared to CT, disease was upstaged by PET/CT in 23% and 31% and downstaged by PET/CT in 17% and 14% by panels A and B, respectively. However, these changes were not statistically significant. As for NHL, upstaging by PET/CT vs. CT was evident mostly for stages I and II. No significant difference was found between staging based on CT alone vs. CT + PET/CT for panel A but a significant difference was found by panel B ($p = 0.0023$). No significant differences were found between staging based on PET/CT vs. CT + PET/CT for both

panels. The treatment strategy was altered by both panels based on CT vs. PET/CT in 33% and 29% of the patients; in 32% and 23% according to CT vs. CT plus PET/CT and in 23% and 9% of patients according to PET/CT vs. CT + PET/CT. *Summary.* The addition of PET/CT to CT changed the management decisions in close to 20% of NHL and 30% of HD patients, mostly in early stages of disease. Thus, we recommend that it be added only in patients already defined as early stage disease by an initial diagnostic CT. On the other hand, adding CT to PET/CT changed the treatment approach only in 5% of NHL and 15% of HD patients. Thus, PET/CT performed as the initial staging procedure may well obviate the need for additional diagnostic CT in the majority of patients.

0247

RITUXIMAB MAINTENANCE THERAPY IN CD20+ B-CELL NON-HODGKIN'S LYMPHOMA - FIRST INTERIM RESULTS OF A PROSPECTIVE RANDOMIZED PHASE II STUDY

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Clinical and pharmacokinetic data suggest that the effect of rituximab could be improved by prolonged exposure to the drug. To test for this hypothesis we performed a prospective randomized trial of rituximab maintenance therapy in patients with CD20+ B-cell Non-Hodgkins-Lymphoma. After completion of standard treatment patients were randomized to either observation or maintenance therapy with rituximab (375 mg/m²) every 3 months for 2 years. Patients with aggressive lymphoma were enrolled if they had achieved a complete response (CR) after initial treatment. Patients with aggressive lymphoma with residual tumor mass were examined with positron emission tomography (PET) and qualified for randomization if PET showed no signs of tumor activity. Patients with indolent lymphoma qualified for the study if at least a partial response (PR) was achieved. So far 55 patients (pts) with CD20+ B-cell Non-Hodgkins-Lymphoma were enrolled in this trial. Histological subtypes included diffuse large cell lymphoma (25 pts.), follicular lymphoma (12 pts.), mantle cell lymphoma (9 pts.), primary mediastinal lymphoma (5 pts.), marginal zone lymphoma (1 pt.), Burkitt's lymphoma (1 pt.), primary intestinal lymphoma (1 pt.) and unclassified B-cell lymphoma (1 pt). No severe adverse events were observed during rituximab maintenance therapy. To date, all patients in the rituximab maintenance treatment group are in continuous clinical remission. Importantly, in the observation group without rituximab 4 patients (2 diffuse large cell lymphoma, one mantle cell lymphoma, one follicular lymphoma) have relapsed. We conclude that rituximab maintenance therapy is feasible, safe and well tolerated in patients with CD20+ B-cell Non-Hodgkins-Lymphoma and might protect against tumor relapse. Patient recruitment for this study is ongoing.

0248

SINGLE DOSE PEG-FILGRASTIM FOLLOWING CISPLATIN-CONTAINING REGIMENS IS A GOOD MOBILIZER OF PERIPHERAL BLOOD STEM CELLS (PBSC) IN RELAPSED /REFRACTORY LYMPHOMA

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Background. Therapeutic serum levels of G-CSF are maintained over a period of 2 weeks following the subcutaneous injection of a 6 mg single dose of pegfilgrastim. However, systematic data on the ability of pegfilgrastim to mobilize stem cells after chemotherapy are scarce. *Aims.* We evaluated the efficacy of a single fixed 6 mg dose of pegfilgrastim after salvage therapy with cisplatin-containing chemotherapy regimens for mobilizing peripheral blood stem cells in resistant/ relapsed

aggressive lymphoma patients. *Methods.* Between July and December 2004 eleven aggressive lymphoma patients (3 Hodgkin's lymphoma, 8 non-Hodgkin's lymphoma) were treated with chemotherapy cycles containing cisplatin (DHAP: dexametasona, cisplatin, cytarabine, or IPAD: idarubicin, dexametasona, cisplatin, cytarabine). They received the first day after the end of chemotherapy (day +1) a 6 mg single dose of pegfilgrastim. Duration of grade 4 of neutropenia, adverse events, time to neutrophil and CD34+ cell recovery were recorded. *Results.* Following the administration of either DHAP or IPAD regimens 10/ 11 pts were able to harvest a median of 8.4×10^6 /CD34+ cells (range 3.6-27) after 11 days (median, range 9-14). In this group of patients a single apheresis procedure was sufficient to obtain a median of 16×10^6 CD34+/kg body weight (range 5.4-27) in 8/11 pts. Grade 4 neutropenia was present in all pts with a media duration of 3 days (range 1-7). Pegfilgrastim determined mild bone pain as only adverse event. Three patients have been autografted so far with pegfilgrastim-mobilized $3.6-5.4-18 \times 10^6$ cells /kg respectively and all of them showed a rapid and sustained engraftment after high-dose chemotherapy. *Summary.* In conclusion, a high number of PBSC was obtained by a single 6 mg dose of pegfilgrastim following chemotherapy in relapsed/refractory lymphoma patients after a median of 11 days from stimulation. The harvest permitted a successful autologous transplantation in 3 patients.

0249

RITUXIMAB IMPROVES OUTCOME IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA. A SINGLE CENTRE RETROSPECTIVE STUDY

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Several studies (GELA 98.5, MINT) have shown survival benefit for combination of chemotherapy (CHT) plus rituximab (R) in patients with diffuse large B-cell lymphoma (DLBCL) but results of other studies are not so convincing (ECOG 4494 trial). *Aims.* retrospective analysis of 94 patients (pts) with newly diagnosed DLBCL treated in our institution between January 2001 and November 2004 with efficacy evaluation of rituximab. *Methods.* 94 pts with DLBCL, median age 58.5 years (range, 19-85) were evaluated. Initial stage I/II/III/IV was found in 27/33/17/19 pts, IPI 0-1 in 32 pts (34%), IPI 2-5 in 62 pts (66%), respectively. Elevated LDH was present in 73%, extranodal involvement in 64%, bulky disease >7cm in 36% of cases. The first-line regimen CHOP (cyclophosphamide, vincristine, doxorubicine, prednisone) +/- R was used in 64% of pts. 21 younger pts under 65 years (22%) with high risk (AA IPI 2-3) were consolidated by high dose chemotherapy with autologous stem cell transplantation. We compared the subgroup of pts treated with CHT+R to pts treated with CHT only. These two subgroups were comparable according to IPI. Fisher's exact test and log rank test were used for statistical analysis. *Results.* 75 pts (80%) were eligible for evaluation as of December 2004 - 32 pts (67%) in CHT+R group and 43 pts (93%) with CHT only. uCR+CR were achieved in 84% of pts. after the first-line therapy. 97% pts. achieved uCR+CR in group with CHT+R as compared to 74% in the group with CHT only (p=0.01). Subsequent relaps or progression were observed in 20 pts (27%) - in 17 pts (39%) in CHT group in comparison to 3 pts (9%) in CHT+R group (p=0.007). Median time to relaps or progression was 10 months (mo), range 6-33. Early relaps (i.e. within 12mo) was seen in 23% in CHT group vs.6% in CHT+R group (p=0.05). Second line therapy induced 7 CRs and 2 PRs (ORR 53%). 80% of pts are alive at the time of analysis (December 2004) - 94% in CHT+R group and 67% in CHT group (p=0.001). 78% of pts remian in CR, actuarial 2-year survival is 86% and actuarial progression free survival (PFS) is 60%. Pts treated with CHT+R had longer PFS (p=0.007) and EFS (p=0.05) compared to pts with CHT group. Median of follow-up is 15mo (range 2-48) - 11.5mo in CHT+R group (range 2-45m) vs. 25.5mo (range 3-48mo) in CHT group. *Conclusions.* DLBCL is a potentially curable disease. 84% of our patients achieved uCR+CR after first-line therapy.

Rituximab in combination with CHT significantly improved achievement of CR, reduced frequency of relapse (including early relapses) and progression and prolonged PFS and EFS. Response rate in the second-line therapy was 53%. These results confirm that CHT+R is now standard treatment option for all patients with DLBCL.

0250

CYSTEINE PROTEASES AND THEIR ENDOGENOUS INHIBITOR CYSTATIN C IN PATIENTS WITH HEMOBLASTOSES AND MICE WITH EXPERIMENTAL LYMPHOMAS

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Cathepsins B and L and their endogenous inhibitor cystatin C participate in processes of tumor growth, vascularisation, invasion and metastasis. Alterations in the expression at mRNA and proteins levels, as well as in the activity and trafficking of cysteine proteases and their inhibitors, have been found to correlate with malignancy of various human tumors. The results of clinical investigations on cysteine cathepsins and their endogenous inhibitors in human tumors have shown that these molecules are highly predictive for the length of survival and may be used for assessment of risk of relapse and death for cancer patients. *Aims.* We investigated cathepsins B and L activity and cystatin C concentration in serum of patients with Non-Hodgkin's lymphoma, Hodgkin's lymphoma and in serum, tumor tissue, liver and spleen of mice with sensitive and resistant variants of lymphosarcoma LS before and after treatment. *Methods.* Cathepsins B and L activity was measured by immunofluorescent method (Kirchke H., Barrett A.J., 1987), using Z-Arg-Arg-NMCA and Z-Phe-Arg-NMCA as substrates, respectively. Serum cystatin C concentration was measured by enzyme-linked immunosorbent assay (KRKA, Slovenia). *Results.* We observed that serum cathepsins B and L activity decreased in patients with Non-Hodgkin's lymphoma and advanced stages of Hodgkin's lymphoma. Serum cystatin C concentration increased in these patients. The lower cathepsins activity and higher cystatin C concentration were revealed in advanced stages of diseases to compare with earlier ones, in patients with B-symptoms (fever, night sweats, loss of body weight) to compare with patients without them. The patients with high-grade lymphoma had lower cathepsins activity to compare with low-grade one; the patients with mixed cellularity variant of Hodgkin's lymphoma had lower activity of these enzymes to compare with nodular sclerosis. Patients with haemoblastoses, achieving remission after following chemotherapy, had higher cathepsins activity and lower cystatin C concentration before treatment to compare with patients with tumor drug resistance and unfavorable prognosis. After successful treatment cathepsins B and L activity and cystatin C concentration returned to normal value. In experimental study we revealed that cathepsins B and L activity in serum, tumor tissue, liver and spleen and serum cystatin C concentration of untreated mice with resistant variant of lymphosarcoma LS was significantly lower than their activity in mice with sensitive variant. Successful treatment resulted in increasing of cathepsins activity in both variants of lymphosarcoma LS, however, the data for treated mice with resistant variant were lower to compare to treated mice with sensitive variant. After treatment serum cystatin C concentration increased to normal value in mice with sensitive variant; there were no changes of cystatin C level in mice with resistant variant of lymphosarcoma LS. *Conclusions* According clinical and experimental findings cysteine proteinases cathepsins B and L and their endogenous inhibitor cystatin C are proposed to be the marker for cancer diagnosis and prognosis. Besides that, they may serve for definition of advance and activity of tumor process and success of following chemotherapy effect. kotelkin@online.nsk.su

0251

GASTROINTESTINAL INVOLVEMENT IN MANTLE CELL LYMPHOMA (MCL) A CLINICOPATHOLOGIC STUDY

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To investigate the clinical, endoscopic and microscopic involvement of the GI tract in a prospective series of MCL. *Methods.* 12 patients with MCL have been prospectively and consecutively entered in a staging workup that included upper and lower endoscopy of the GI tract. Multiple biopsies of the stomach and colon were taken from pathologic mucosa and also from macroscopically normal mucosa. Specimens were assessed immunohistochemically, with FISH and PCR. *Results.* Only 1 patient presented with GI symptoms at diagnosis. *Endoscopy:* Upper GI: mild erythema in 4 cases (33%), ulcer in 1 (8%) and macroscopically normal in the remaining patients (58%). Lower GI: mild erythema in 1 case (8%), polyps in 3 (25%) and macroscopically normal in the remaining patients (67%). As a whole, 7 patients had upper or lower endoscopic findings. *Pathology:* Upper GI: 9 cases (75%) had microscopic infiltration and MCL was confirmed in 8 (67%). Lower GI: 11 cases (92%) had lymphoid infiltrates and MCL was confirmed in 67% of cases. Microscopic features and immunohistochemistry according to endoscopic findings are shown in Figure 1

	Endoscopy	Lymphoid infiltrates or nodules	CD20 / CD5 ⁺	Cyclin D1 ⁺
Upper GI	Abnormal	100%	100%	100%
	Normal	63%	50%	53%
Lower GI	Abnormal	100%	100%	100%
	Normal	29%	50%	53%

All positive cases for CD20 and CD5 were positive for cyclin D1. FISH analysis with the BCL-1/IgH probe (Vysis) and PCR studies are still under evaluation. In global, all patients had microscopic involvement of the GI tract by MCL. Even a patient with localized MCL in the tonsils had microscopic involvement of the stomach. *Conclusions.* In our series, GI involvement by MCL was detected in all patients. All patients with endoscopic abnormalities had infiltration by MCL at the microscopic level. In half of the patients with normal endoscopy, GI tract involvement could be demonstrated at the microscopic level. Immunohistochemistry with CD20, CD5 and cyclin D1 was more efficient than FISH as a diagnostic tool in this setting.

0252

ANEMIA AS PROGNOSTIC FACTOR FOR SURVIVAL OF ELDERLY PATIENTS WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMAS

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Aggressive non-Hodgkin's lymphoma (NHL) in elderly patients may share a severe outcome with a shorter survival because of some unfavorable prognostic factors. Although anemia is not included as individual parameter in International Prognostic Index, recent literature data suggest that baseline anemia is an important poor prognostic factor for NHL outcome. The aim of this study was to assess the value of anemia as prognostic factor for survival of elderly aggressive NHL. *Patients and methods.* We studied 134 patients (69 males, 65 females) older than 60 years of age (ranges 60-76) with aggressive NHL treated between 1995-2004 with conventional CHOP chemotherapy (6 monthly cycles) in a single institution. By histology, 73.13% were diffuse large B-cell lymphomas, 9.70% immunoblastic, 4.47% lymphoblastic, 2.98% anaplastic, 1.49% mantle cell, and 8.20% peripheral T-cell lymphomas. 19.40% of patients were stages I-II, 42.53% stage III, and 38.05% stage IV.

The baseline and post chemotherapy incidence of anemia, therapeutic response, early relapse, and survival were evaluated. **Results.** Baseline anemia was present in 35 patients (26.11%) with hemoglobin (Hb) level below 10 g/dL in 20.89% of cases, and below 8 g/dL in 5.22% of cases. In 4.47% of cases a Coombs positive hemolytic anemia was found. By stage, anemia was present in 15.38% of stages I-II, 19.29% of stage III, and 39.21% of stage IV patients. 31 of 35 anemic patients (88.57%) were in stages III-IV of disease. After completion of 6 cycles of chemotherapy, a complete response (CR) was reached in 54.47%, and a partial response in 42.53% of cases; 2.98% of patients were unresponsive. CR rate was higher in patients without anemia (57.57%) than in cases with anemia (45.71%). After 3 cycles of chemotherapy, the CR was reached in 61.40% of CR patients without anemia and in 31.25% of those with anemia and CR ($p < 0.005$). 68.75% of cases with anemia reached a CR after 6 cycles of treatment. At that moment, 35 of initially non-anemic patients (35.35%) were found with anemia (22.22% with Hb < 10 g/dL, and 13.12% with Hb < 8 g/dL). Six months after treatment completion, 6.25% of cases without anemia and 22.85% of cases with baseline or post chemotherapy anemia were in relapse. Relapse rates at 12 months were 23.43% and 59.99%, respectively. A 3-year survival rates were 57.81% of patient without anemia, 34.28% of patients with baseline anemia ($p < 0.005$), and 45.71% of cases with post chemotherapy anemia ($p > 0.005$). 5-year survival rates of these groups were 29.68%, 8.57%, and 17.14%, respectively. In anemic patients having concomitant high abnormal level of LDH, a 3-year survival rate was lower (23.80%) than in cases with normal baseline LDH value (50%). Anemia is an important prognostic factor in elderly aggressive NHL. Both, baseline and post chemotherapy anemia are poor prognostic factors influencing outcome and survival of patients. Correction of anemia could improve the survival rate and the quality of life of patients over 60 year of age with aggressive NHL.

0253

TREATMENT TOXICITY AND LMB-89 PROTOCOL MODIFICATIONS IN CHILDHOOD B-CELL LYMPHOMAS (B-NHL) - A REPORT OF THE POLISH PEDIATRIC LEUKEMIA/ LYMPHOMA STUDY GROUP (PPLLSG) E.

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Treatment toxicity and LMB-89 protocol modifications in children with B-NHL has been investigated. The patients (pts) were treated in 10 oncohematological centers of PPLLSG in 1993-2005 years. 146 children with B-NHL were included into the analysis. **Methods.** The diagnosis was based on histomorphological and immunophenotyping investigations. Treatment intensity was adapted to 3 risk groups (A,B,C), according to LMB-89 protocol. Treatment toxicities were established according to WHO Toxicity Staging. **Results.** 5% pts were classified to gr. A, 82% to B and 13% to C. Complete remission (CR) was in 134 of 146 (92%) pts: 8 (100%) in gr A, 113 (94%) in B and 13 (68%) in C. A total of 565 cytostatic cycles were applied: 16 in gr.A, 428 in B and 121 in C. 7 pts had primary surgery complications (6-alive, 1-early death). COP used in 138 pts. 28% (39/138) of them had COP complications (44% tumor lysis syndrome, 18% anuria, 18% intestinal occlusion, 15% haemorrhage, 5% v. cava superior syndrome). Myelosuppression was especially after COPADM 2 (gr.B) and CYVE 2 courses. FUO was noticed after 31% and 40% of gr B and C courses, respectively. Mucositis WHO grade > 2 mainly occurred in the

COPADM courses and was probably due to the combination of HDMTX and doxorubicin. Severe infections were observed after 14% of cycles. 81 treatment modifications were performed; most of them presented as MTX dose reduction. A total of 10 pts (7%) relapsed: 8 with late RC, achieved after CYM/CYVE 2 (6 cases) or after CYM/CYVE1 (2 cases) and 2 with RC, after induction therapy. 4/146 (2,7%) toxicity-related deaths were observed: 0 in gr A, 2,5% in B and 5,2% in C (1-St.aureus sepsis+varicella, 1- peritonitis+acute renal failure, 1-multiorgan failure+myelosupresion, 1-lungs failure). EFS was 0, 85 for all pts. The EFS of group A was 1,00, group B 0,74 as compared with 0,54 of group C ($p=0,00395$) The EFS were 1,00 and 0,89 for children, started chemotherapy < 48 h and > 48 h after primary surgery, respectively ($p=0,25303$). The EFS were 0,94 and 0,84 for children with < 21 and > 21 days interval between COPAD (M)1 and COPAD (M)2, respectively ($p=0,07251$). **Conclusions.** Higher EFS and overall survival of B-NHL could be achieved thanks to an improvement of supportive care (adequate blood product substitutions, regular infection specific prophylaxis, MTX therapy monitoring amelioration) for therapy toxicity elimination (compared with previous study). Most of relapses are observed in children with late RC, but not in pts with chemotherapy modifications. Interval between primary surgery and chemotherapy onset < 48 h may be considered as a marker of better clinical outcomes in childhood B-NHL. Interval between COPADM1 and COPADM2 > 21 days delays RC achievement and fails to improve overall survival. Use of drugs, preventive uratic nephropathy, could explain the decreased percentage of mortality related to tumor lysis syndrome.

0254

CLINICAL IMPLICATIONS OF EXTRANODAL VERSUS NODAL PRESENTATION OF DIFFUSE LARGE B-CELL LYMPHOMA-A STUDY OF 238 CHINESE PATIENTS IN A REGIONAL HOSPITAL IN HONG KONG

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Clinical difference between extranodal and nodal NHL remains inconclusive, partly due to the controversial definition of primary extranodal lymphoma (PEL), and partly due to the heterogeneity of histological subtypes. As diffuse large B-cell lymphoma (DLBCL) constitutes the largest subtype of NHL and the incidence of PEL is known to be higher among Asians than Caucasians, our study aims to compare the clinical course and treatment outcome of PEL against nodal DLBCL in Chinese patients. Patients and methods Clinical records of all adult Chinese patients with biopsy-proven DLBCL managed in a regional hospital in Hong Kong over a 10-year period were reviewed.

Demographics	Male.
	Median age [range]
	Age > 60
	IPI HI-H
	Stage III-IV
	B symptoms.

Clinical staging was according to the Ann Arbor system based on physical examination findings, bone marrow biopsy results, and computer tomography/gallium scan results. Patients were

defined as having PEL when their disease was confined to one or more extranodal sites and with no (or only minor) nodal involvement. Waldeyer's ring was considered as an extranodal site; spleen and bone marrow as nodal. Standard chemotherapy regimens (CEOP or ProMACECytoBOM) were delivered to eligible patients with good performance status-three to four courses followed by involved field radiotherapy for early stages; six to eight courses for patients in advanced stages. Radiotherapy (RT) was delivered for localized disease and bulky disease post-chemotherapy. *Results.* From July 1995 to June 2004, 248 Chinese patients with DLBCL were identified. Ten patients presenting with disseminated disease (extranodal and nodal disease on both sides of diaphragm) were excluded from analysis. Of the remaining 238 patients, 139 (58%) had PEL and 99 (42%) nodal disease. Their clinical features were shown below.

For DLBCL, the incidence of PEL is higher among Chinese than Caucasians. In addition, they tend to present with less advanced disease and lower LDH level than those with nodal disease. More PEL patients required only RT as definitive treatment than patients with nodal disease. There is no other statistically significant treatment outcome difference between the two groups in terms of remission rate, mortality rate and overall survival.

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0255

FOLLICULAR LYMPHOMA IN EARLY STAGE: NATURAL HISTORY AND PROGNOSTIC FACTORS IN A SERIES OF 48 PATIENTS WITH LONG-TERM FOLLOW-UP

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Most patients with follicular lymphoma (FL) present in advanced stages and are currently deemed to be incurable with standard treatments. Although the outcome of patients with localized disease is much better, whether these patients may be cured is still a matter of debate. *Aims.* To analyze the clinical features and outcome of a series of patients with FL in early stages with a long follow-up. Forty-eight patients (25M/23F; median age: 50 years) consecutively diagnosed with FL in Ann Arbor stage I (25 cases) or II (23) at a single institution with a median follow-up of 9.5 years. Main biological and clinical characteristics at diagnosis, including FL International Prognostic Index (FLIPI), treatment and response were assessed and analyzed for prognosis. The histologic subtypes were: FL type I, 20 cases (42%), type II, 24 (50%), type III, 3 (6%), unclassifiable, 1 (2%). Distribution according to FLIPI was: low risk, 36 cases; intermediate risk, 5. Treatment mainly consisted of combined chemotherapy (CHOP in 34 cases) plus involved-field radiotherapy in 26 cases. Forty patients (89%) achieved complete response (CR), 3 (7%) partial response (PR), and 2 (4%) were non-responders; the remaining 3 patients did not receive therapy. No initial variable predicted CR achievement. 57% of the patients in CR eventually relapsed, with a risk at 10 years of 46%. Serum b2 microglobulin >1.6 mg/L and intermediate-risk FLIPI predicted relapse. Histologic transformation was observed in 6 patients, with a 10-year risk of transformation of 13%. Twelve patients have died during follow-up, in two cases due

to unrelated causes. Overall survival (OS) at 10 years was 79%. FLIPI was the sole variable with predictive value for OS. *Conclusions.* Although the majority of patients with localized FL achieve CR, the relapse rate is high. FLIPI is of prognostic interest in these patients.

0256

FOLLICULAR LYMPHOMA (FL), CHRONIC HEPATITIS C AND TYPE 2 DIABETES MELLITUS (T2DM): IS THERE A LINK?

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FL is the 2nd most frequent non-Hodgkin's lymphoma (NHL) after diffuse large B-cell lymphomas and represents 20-25% of all NHL. The role of hepatitis C virus (HCV) infection in the pathogenesis of NHL is controversial. A high prevalence of HCV infection in patients (pts) with NHL has been reported in Italy (10-35% of cases in several studies) and Japan. By contrast, several studies in Northern Europe and Canada have not found any increased prevalence of HCV in B-cell NHL, suggesting a possible geographic variation. The HCV infection is characterized by a preferential chronic evolution with mild to severe liver disease, including cirrhosis and, in lesser proportion, hepatocarcinoma. Out of these complications, HCV is frequently reported to complicate extrahepatic manifestations including B-cell NHL, autoimmune thrombocytopenia and T2DM. A possible link between HCV infection and T2DM has been suggested. *Aims.* We evaluated the association between HCV and T2DM in 59 pts with previously untreated FL seen at our Institution between January 2002 and December 2004. Patients and *Methods.* Nine pts (15%) were HCV+ and 10 (17%) were affected by T2DM. Among the 9 FL-HCV+ pts (5 male and 4 female), 6 (67%) displayed T2DM. The median age was 68,3 years (range 59-82 years); CSII one pt(11%), CS III 3 pts (33%), CS IV 5 pts (56%); G1 2 pts (22%), G2 3 pts (33%), G3 4 pts (44%); 4 pts (44%) with extra nodal involvement and 5 pts (56%) with nodal involvement; only one patient(11%) with bulky disease; BM-positive in 3 pts vs BM-negative in 6 pts (33% vs 67%); FL International Prognostic Index (FLIPI) score: low risk in no pts, intermediate risk in 2 pts (22%) and poor risk in 7 pts (78%). *Results.* Our preliminary data showed that the prevalence of T2DM was significantly higher in pts with FL-HCV+ than in controls (pts with FL-HCV-): 67% vs 8%, respectively (Chi-square: 18.649; p <0.000). In addition, there was a high percentage of poor risk pts (78%) in the former group. *Conclusions.* Pts with FL-HCV+, irrespective of cirrhosis, have a significantly increased prevalence of T2DM compared to pts with FL-HCV-. This prevalence is much higher than observed in the general population. Case-control, population-based case-control and hospital-based case-control studies are needed to confirm our preliminary observation.

0257

RESULTS OF TREATMENT OF ADVANCED CUTANEOUS LYMPHOMA WITH PEGYLATED LIPOSOMAL DOXORUBICIN, BLEOMYCIN, VINBLASTINE AND DACARBAZINE

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Primary cutaneous lymphomas (PCL) in the majority of histologic variants present an indolent behaviour and a good prognosis with a prolonged survival. In a very small subset of patients, PCL is aggressive at the onset and in patients who are resistant or have relapsed after repeated traditional topic or sys-

temic therapies an advanced disease is more frequently observed. In these patients no therapy is capable of inducing a stable remission of the disease. The efficacy and low toxicity of Pegylated Liposomal Doxorubicin as a single agent in second-line therapy of T-Cell PCL was recently demonstrated by Wolina U. *Aims*. In our study we tested the safety and efficacy of Pegylated Liposomal Doxorubicin (Caelyx®) in the treatment of advanced PCL, in association with three drugs of proven effectiveness in nodal lymphoproliferative and other primary cutaneous neoplastic disorders: Bleomycin, Vinblastine and Dacarbazine (CBVD). From February 2003 to January 2005 we observed 19 consecutive patients with advanced PCL (17 cutaneous T-cell lymphomas, 2 cutaneous B-cell lymphomas). Patients received CBVD therapy, Pegylated Liposomal Doxorubicin, Bleomycin (Caelyx®) 12mg/m², Bleomycin 10 mg/m², Vinblastine 6 mg/m², Dacarbazine 375 mg/m² at days 1 and 15, that was administered intravenously (i.v.) every 4 weeks for 6 cycles. Before the treatment patients were submitted to a complete staging of disease including TC-scan, bone marrow biopsy, and immunophenotyping of peripheral blood cells. *Results*. The response to the treatment has been evaluated in 12 patients who completed six cycles of therapy or presented a Progression of Disease (PD) during the treatment. In fact, at this writing, six patients are still in treatment, and one patient with a CR at the fourth cycle suspended therapy for recruitment of autologous hemopoietic stem cells. Three out of 12 evaluated patients (25%) had a PD: two patients with Sezary Syndrome (SS) after two CBVD cycles and one patient with Anaplastic Large-Cell Lymphoma (ALC) CD30+ evolved from a SS after four cycles. Nine of the 12 patients (75%) obtained a Complete Response (CR) with the disappearance of cutaneous and nodal lesions and systemic symptoms (itching, fever, weight loss). One of the patients in CR presented a relapse at the seventh month. One patient died due to a cerebrovascular accident in the 10th month of CR. Seven out of 9 patients are in CR after a median observation of 11 months (range 5 -21 months). The CBVD therapy was well tolerated. No patient presented cardiac or mucosal toxicity, nor alopecia or palmoplantar erythrodermia. Two patients presented neutrophils count below 500/μl and 1 of them had a severe bacterial pneumonia which was resolved with i.v. antibiotics. Our experience demonstrates that the CBVD association is an effective and safe therapy for advanced PCL. The CR rate and duration, at this moment, are comparable to that reported in patients with similar characteristics. A longer observation and a larger number of patients are needed to confirm our results.

0258

PRIMARY MEDIASTINAL (THYMIC) LARGE B-CELL LYMPHOMA (PMLBCL): EXCELLENT OUTCOME WITH THE COMBINATION OF RITUXIMAB-CHOP (R-CHOP) AND RADIOTHERAPY (RT)

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CHOP combination chemotherapy is not a satisfactory approach for PMLBCL. Given the young age of most of these patients, MACOP-B or even chemotherapy (CT) with consolidation high dose therapy with autologous stem cell support (HDT-ASCT) have also been used. However there is no established optimal treatment for PMLBCL. Recent data have shown that R-CHOP is superior to CHOP in patients with diffuse LBCL, so that it is now the standard of care for patients >60 years old. However its precise role in younger patients, and particularly those with PMLBCL, is not well established yet. *Aims*. The evaluation of the efficacy of R-CHOP±RT in PMLBCL and the comparison of this approach with CHOP±RT, administered

to historical controls. Between 1994-2004, 46 patients with PMLBCL were treated in two participating centers. R-CHOP displaced CHOP in the treatment of PMLBCL at a given time-point in each center. Thus 15 consecutive patients who received R-CHOP, were compared to 31 consecutive historical controls, who had been treated with CHOP prior to that point. *Results*. The median age of the patients was 31 years (17-58) and 32/46 (70%) were females. Age-adjusted IPI was ≥2 in 40% and 42% of patients who received R-CHOP and CHOP respectively ($p=0.90$). All individual IPI parameters as well as B-symptoms were also well balanced between the two groups. The complete response (CR) rate was 100% for R-CHOP±RT vs 61% for CHOP±RT ($p=0.009$). No relapse has been recorded after R-CHOP, while all relapses after CHOP occurred within 22 months from diagnosis. The 3-year failure free survival (FFS) was 100% vs 47±9% for patients who received R-CHOP±RT vs CHOP±RT ($p=0.005$). Within the subgroup of patients with L/LI risk IPI the corresponding 3-year FFS rates were 100% vs 61±11% ($p=0.059$), while they were 100% vs 26±13% ($p=0.02$) among patients with HI/H risk IPI. The 3-year event free survival (EFS) for all patients was 93±7% vs 47±9% ($p=0.02$). The 3-year overall survival was 93±7% vs 47±9% ($p=0.27$), while the 3-year lymphoma specific survival was 100% vs 67±9% ($p=0.049$). *Conclusions*. R-CHOP and RT provided impressive results with no failures among 15 patients. In comparison to CHOP-treated historical controls, highly significant differences in favor of R-CHOP were recorded in terms of CR and FFS rates. EFS and lymphoma specific survival were also improved. Based on these results we continue to treat PMLBCL patients with R-CHOP and RT. The need for more aggressive strategies is therefore questionable.

0259

SOLID NEOPLASMS AND HCV INFECTION IN NON-GASTRIC MARGINAL ZONE B-CELL LYMPHOMA OF MALT

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To define the clinical features, the incidence of solid neoplasia and the pattern of HCV infection in non-gastric MALT lymphoma. Patients: We studied 121 patients with a confirmed histology of marginal zone B-cell lymphoma of MALT according to the WHO classification, who presented with a clinically prevalent non-gastric extranodal site of disease. The primary site of lymphoma was: orbit and ocular adnexa (24), Waldeyer's ring (21), skin and subcutaneous tissue (20), parotid gland and minor salivary glands (22), lung and pleura (9), breast (4), liver (3), small bowel (3), thyroid gland (1), colon (1), female genital tract (1), multiple mucosal sites (12). Median age at diagnosis was 64 years (23-86) (77 F, 44 M). Most pts. (95%) had not B symptoms and a good performance status (93%). Forty-nine pts had stage I disease (40%), 12 stage II (10%), 4 stage III (3%) and 56 stage IV (47%). Bone marrow was involved in 43 (36%), and peripheral blood in 7 (6%). Thirteen pts (12%) had spleen enlargement (11 with bone marrow involvement) and 45 (37%) nodal involvement (24 loco-regional only). Seventeen (14%) had hemoglobin values <11 g/dL, 4 (3%) had platelet count <100,000/μL, 14 (12%) abnormal serum LDH levels, 24 (20%) had a little monoclonal component (14 IgM, 8 IgG, 2 IgA). An autoimmune background was present in 10 pts (9%). HCV-serology was positive in 34 of 100 valuable pts (34%). All HCV-

positive pts showed a single extranodal site and seven had nodal disease. Nineteen pts (56%) had active chronic hepatitis: 5 had liver biopsy demonstrating signs of liver damage; circulating HCV-RNA was detectable in 14; ultrasound scans of abdomen showed signs of liver damage in 11; cryoglobulins were present in 3. Six pts were HbSAg-positive (2 co-infection with HCV). An history of solid neoplasia was present in 20 pts (18%) (14 F, 6 M): previous in 13, concurrent in 3, subsequent in 4 (breast 7, endometrium 3, thyroid 2, lung 2, small bowel 1, colon 1, salivary glands 1, skin 1, prostate 1, stomach 1). In 2 patients the site of cancer and lymphoma was the same (breast and lung). First-line therapy consisted of CHOP-like regimens in 33%, single alkylating agent in 26%, local radiotherapy in 11%, surgical resection in 10% while 20% were followed without therapy. A CR or PR was obtained in 50% and 16% respectively. After a median follow-up of 2.3 years, median OS was 10.1 years and median EFS 1 year. In univariate analysis features significantly associated with longer OS were: a single extranodal site ($p=0.00001$), localized disease ($p=0.006$), normal serum LDH ($p=0.0009$), absence of nodal involvement ($p=0.0001$), and HCV-positivity ($p=0.049$). In multivariate analysis only multiple extranodal sites had a negative influence on OS ($p=0.03$). *Conclusions.* This survey on a multi-centre series of non-gastric MALT lymphomas shows an evident association with HCV infection and solid cancer and that the frequent dissemination at presentation with multiple extranodal sites and/or nodal involvement is characterized by a worse prognosis.

Non-Hodgkin lymphoma - Clinical II

0260

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER: A CASE SERIES OF A TRANSPLANT CENTER

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Post-transplant lymphoproliferative disorders (PTLD) are frequent and serious complications: the incidence ranges from 2 to 6 percent and the mortality ranges from 40 to 70 percent. To analyze PTLD in our Hospital. One thousand and eight-hundred fifty-three solid organ transplants were carried out between 1988 and 2002: kidney 981, heart 402, liver 364, lung 106. We report 20 patients (1.1%) who developed a PTLD: kidney 8 (0.81%), heart 7 (1.74%), liver 1 (0.27%) and lung 4 (3.77%). There were 17 men and 3 women. The mean time between organ transplantation and PTLD was 53 months (5-129). The mean age at transplantation was 45 years. Sixteen cases received a triple immunosuppressive regimen with cyclosporine or tacrolimus and sirolimus or azathioprine or mycophenolate and steroid. Eleven patients suffered acute rejection during the first month after transplantation. They received therapy with a high dose of steroids. Three patients suffered chronic rejection. The mean age at PTLD was 55 years. The PTLD presented extranodal masses in seven cases: the involved organs were the gastrointestinal tract in six cases and solid organ transplantation in one case. The diagnosis was performed by biopsy: 17 (81%) NHL (DLBCL 13, Burkitt L 1, MALT L 1, lymphoblastic T L 1, anaplastic L 1); HD 2; polymorphic PTLD 2. Among 21 cases, 14 cases presented stage I-II disease: 10 NHL with low IPI, 2 HD, and 2 polymorphic PTLD. Six cases presented stage III-IV disease with high IPI. Epstein Barr Virus (EBV) was detected in 11 of 16 patients tested. The mean time between grafting and EBV-positive PTLD was 35 months and the mean time between grafting and EBV-negative PTLD was 104 months. Immunosuppression was reduced in 18 cases but only the polymorphic disease had a prolonged complete remission. The 2 HD were

treated with chemotherapy in one case and radiation in the other. They achieved complete remission. Among 10 NHL low IPI, local therapy (surgery and/or radiation) was used in 6 cases, and it was combined with rituximab in 3 of them. Monotherapy with rituximab was used in 2 cases and only the immunosuppression was reduced in the rest. Among 6 NHL with high IPI, 4 cases were treated with chemotherapy and 2 of them were combined with rituximab. Two cases died before the start of the treatment. All NHL I-II with low IPI achieved complete remission but 1/3 died because of complications related to the treatment and 1/3 relapsed. All of the NHL III-IV with high IPI died with a mean time of 5 months. The event-free survival was 38% in our series. The mean follow-up was 40 months (4-69). Low IPI ($p<0.0023$) and limited stage ($p<0.003$) were statistically significant in survival unlike the other series. The associated EBV was not statistically significant. NHL I-II with low IPI can progress and non-aggressive regimens can be ineffective. HD had a good prognostic in our series. Reducing the immunosuppression was sufficiently effective in the polymorphic varieties.

0261

INTENSIVE CHEMOTHERAPY WITH CODOX-M-IVAC FOR BURKITT'S LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Burkitt's lymphoma (BL) is one of the highly aggressive non-Hodgkin's lymphomas that is so rapidly growing that it may be fatal within months if not treated promptly. To evaluate the efficacy of intensive chemotherapy with 4 cycles of alternating CODOX-M-IVAC (cyclophosphamide, vincristine, doxorubicin, methotrexate, cytarabine, etoposide, ifosfamide) and intrathecal prophylaxis (Magrath I, 1996). Patients older than 60 and with other related complications received dose adjusted treatment. We review 16 patients (10 adults, 6 children) with BL treated at our centre between 1997 and 2003, with intensive-dose chemotherapy regimen. The median patients' age was 39 years (range, 5-72). Five patients were older than 60, and 3 were HIV positive. The international prognosis index (IPI) at diagnosis was high in 12 patients. LDH was higher than 500u/L in all patients, 10 patients had hyperuricemia and 3 patients had renal impairment. Seventy-five percent of the patients had stage IV, 8 patients had marrow involvement and 2 patients had central nervous system (CSN) disease. Massive disease with Bulky-mass was described in 12 patients (75%), abdominal presentation being the most frequent (9), 15 involved extranodal sites (11 with more than one area). Five patients were diagnosed in the lymph nodes or masses, 6 in pleural or ascites effusions, 4 in other sites and 1 in bone marrow specimen. Therapy-related toxicities (TRT) following the first cycle of chemotherapy treatment were tumor lysis syndrome (37%), severe mucositis (68%), bacterial infections (50%) and acute renal failure (18%). Three patients died during the treatment, one older than 60 with cardiac complications due to tumor lysis syndrome, and 2 HIV positive patients with severe mucositis and sepsis. Patients evaluated (13): 76% (10) achieved durable complete remission (CR) with a median follow up of 24 months. Four evaluated patients older than 60 years: two patients had durable CR and two patients had relapse and died. One patient died because of an accident. Intensive, short-duration chemotherapy with CODOX-M-IVAC is an effective regime for patients younger than 60 and children (HIV negative) with EFS and OS rates of 100%. Patients older than 60 years have more often relapsed and found with refractory disease with OS rates of 40% ($p=0.0132$). Patients with HIV infection died because of their immunosuppression status and therapy-related toxicities ($p=0.016$). Bone marrow involvement at diagnosis was a prognosis factor in this group with poor rates of EFS and OS ($p=0.039$).

0262

VALIDATION OF PIT PROGNOSTIC MODEL ON A NEW COHORT OF PATIENTS WITH A CENTRALLY REVIEWED DIAGNOSIS OF PERIPHERAL T-CELL LYMPHOMA, UNSPECIFIED (PTCL-U)

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The prognosis of PTCL is very poor, with a 5-year overall survival ranging between 25 and 40%, despite aggressive, polychemotherapy or high-dose chemotherapy followed by ASCT. Recently, we published a new prognostic score, called PIT (Prognostic Index for PTCL-U), based on a retrospective survey of 385 PTCL-U cases recorded in the archives of Intergruppo Italiano Linfomi (IIL) (Gallamini: *Blood* 103, 2474; 2004). Validation of the prognostic impact of PIT model on a new cohort of PTCL-U patients consecutively admitted in 21 IIL centers that were not included in the former study and whose histological diagnosis was centrally reviewed. We reviewed the records of 66 patients enrolled in IIL clinical trials between January 1990 and December 2003. Minimal requirements were PTCL-U histology and a complete set of clinical data including, age, IPI and PIT parameters, systemic symptoms, bulky disease, chemotherapy regimen, treatment response, duration of the follow-up and status at the last follow-up. Paraffin-embedded blocks of the patients were sent to Bologna referral center for histological review. The diagnosis was checked on morphological grounds by two blinded observers and supported by immunohistochemistry and molecular analysis, if needed. The clinical characteristics of the patients were the following: 40 males and 26 females, the mean age was 59.3 years, and 58% were aged more than 60 y., non-ambulatory (ECOG 2-4) performance status (PS) in 15%; 23% were anemic, 59% presented with systemic symptoms. Bone marrow trephine biopsy was positive in 26%, 20% had 2 or more extra nodal sites, 48% had abnormal LDH values, stage III-IV was recorded in 74% of the pts. Treatment was antracycline-based in 61%, high-dose chemotherapy plus ASCT in 18%, and other therapy in 21%. After a median follow-up of 42 months (3-152), 71.2% of the patients were died. Both PIT (four risk categories) and simplified PIT (low-risk, PIT 1-2 and high risk, PIT 3-4) were able to classify patients in significantly different risk categories ($p=0.01$ and $p=0.008$ respectively). The 5-yr survival were 60.0%, 20.6%, 17.8% and 10.9% for PIT, 34.7% and 13.2% respectively for simplified PIT. **Conclusions.** In this study we were able to confirm the prognostic role of the PIT model. Since only four very simple clinical variables are needed for the score, we suggest to use this model for clinical studies on PTCL-U.

0263

PRELIMINARY RESULTS OF DOSE ADJUSTED-EPOCH PLUS RITUXIMAB (DA-R-EPOCH) FOR UNTREATED HIGH-RISK AGGRESSIVE LYMPHOMAS. PILOT STUDY IN A SINGLE CENTRE

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Poor risk aggressive non-Hodgkin's lymphoma have a short EFS and OS when treated with conventional chemotherapy schedules. High dose therapy followed by stem cell rescue has been attempted with disparate results. Based on Wilson et al. schedule, a pilot study has been conducted to assess the efficacy and toxicity of DA-R-EPOCH in patients with IH/H IPI score diffuse large B-cell (DLBCL) and follicular large (grade 3) B-cell (G3FL) lymphomas. **Methods.** Since August 2002 to November 2004, 23 untreated patients (pts) were enrolled in the study. All pts had LDH serum levels over the normal range. The regimen consisted on 6 cycles every 21 days of Rituximab (day 1) fol-

lowed by DA-R-EPOCH as reported by Wilson VH et al.¹ **Results.** Characteristics are: median age 62 years (range: 26-71), 10 M and 13 F; 16 (70%) DLBCL and 7 (30%) G3FL; 16 (70%) CS IV and 12 (52%) B symptoms; bone marrow involvement 9 (39%); ≥ 1 extranodal involvement 18 (78%); PS ≥ 2 in 16 (70%); 38% high b2microglobulin levels; Hb < 100 g/L in 11 (48%) and serum albumin < 3 g/dL in 7 (30%); high age adjusted IPI 14 (61%). Of 20 patients evaluated for response 15 achieved CR/CRu (75%) and 3 PR (15%) with an overall response rate of 90%. Additional radiotherapy was used in 5 pts. At a median follow-up of 15 months (range: 1-29) estimated 2-year EFS and OS is 67% and 66% respectively. After 119 cycles administered: grade 3-4 mucositis was observed in 7 courses (6%), grade 3-4 neuropathy in 1 (<1%) and neutropenic fever in 18 (15%). **Conclusions.** DA-R-EPOCH is a feasible approach and as effective as other more intensive chemotherapy schedules in patients with high or intermediate-high IPI score aggressive lymphomas (DLBCL and G3FL) with acceptable toxicity. Accrual and follow-up continue.

1. Wilson WH, Grossbard ML, Pittaluga S, et al. *Blood* 2002;99:2685-93.

0264

INCIDENCE AND RISK FACTORS FOR CENTRAL NERVOUS SYSTEM MANIFESTATION IN ELDERLY PATIENTS WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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Manifestation of central nervous system (CNS) disease in patients with aggressive non-Hodgkin's lymphoma (NHL) during or after first line treatment is associated with a poor prognosis. There is no general consensus regarding prophylactic treatment in this patient category, particularly in the elderly. **Aims.** To define the incidence and risk factors for CNS manifestation in a large cohort of elderly (>60 years) patients with aggressive NHL and no CNS disease at diagnosis. **Methods.** This Nordic study included a total of 455 previously untreated aggressive NHL patients with clinical stage II - IV disease with no CNS involvement at diagnosis (*Blood* 2003;101:3840). The large majority had diffuse large B cell lymphoma. Patients (median age, 71 years; range, 60-86 years) were randomised to receive CHOP (doxorubicin 50 mg/m²) or CNOP (mitoxantrone 10 mg/m²) with or without G-CSF (5 μ g/kg on day 2 until day 10-14 of each cycle every 3 weeks; 8 cycles). Intrathecal methotrexate was given prophylactically to patients with lymphoma bone marrow involvement and in patients with testicular, orbital, sinus and epidural sites of presentation. At this time point information is available for 428/455 patients. After a median observation time of 8 years in surviving patients 31/428 (7.2%) developed CNS disease. Risk factors for CNS disease and outcome will be presented. **Conclusions.** A significant proportion of elderly patients with advanced aggressive NHL and no CNS manifestation at diagnosis develop CNS disease despite prophylactic measures in clinically defined high risk patients.

0265**THE DETECTION OF EARLY CARDIOTOXICITY IN ADULT PATIENTS TREATED FOR AGGRESSIVE LYMPHOMA WITH CHOP REGIMEN**

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The cardiotoxicity of anthracyclines is a serious problem in oncological treatment. *Aims.* The authors conducted a one-year prospective study to determine whether CHOP regimen (cyclophosphamide, doxorubicin, vincristin, prednisone) used in the treatment of aggressive non-Hodgkin's lymphoma is associated with the presence of an early impairment of cardiac function. The authors examined prospectively 47 pts. (27 male and 20 female) aged 49±14 years who were treated with CHOP regimen. Rest echocardiography was performed at baseline and one-year control. Cardipulmonary exercise test was performed only at the one-year control examination. We have calculated ejection fraction (EF), parameters of diastolic function, myocardial performance index (MPI), and pVO₂ was measured. The cumulative dose of doxorubicin 277±42 (300mg/m²) was given. 30% of patients had the risk factors of cardiotoxicity before the cure. The baseline EF was 64±5% (65%) and decreased to 62±5% (64%) ($p<0.001$) after the treatment and to 58±7% (57%) at the one-year control ($p<0.0001$). 23% of pts exhibited the drop in EF >10% during the follow-up, 43% revealed pathologically increased value of MPI >0.55, and 47% impaired diastolic function compared to the baseline values, respectively. 21% of patients exhibited a decrease of pVO₂ < 20 mL/kg/min, and 17% pVO₂ < 80% of the reference value, respectively. None of patients developed signs of heart failure. Multivariate analysis showed, that advanced age >60 years (OR=2.07; $p<0.05$) and the presence of risk factors (OR=3.82; $p<0.03$) have been best predictors of cardiotoxicity. *Conclusions.* The results show that early subclinical cardiotoxicity was frequent in patients receiving the CHOP regimen. The value of described changes for the development of heart failure has to be assessed during prospective follow-up.

The study was supported by grant of Ministry of Health of Czech Republic No. NC/7354-3.

0266**BORTEZOMIB (VELCADE®) IN THE TREATMENT OF RELAPSED CLASSICAL HODGKIN'S DISEASE**

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Bortezomib is a selective proteasome inhibitor that has demonstrated clinical activity with manageable toxicity in the treatment of patients with multiple myeloma as well as different types of non-Hodgkin's lymphoma. The safety and activity of bortezomib as therapy for patients with Hodgkin's disease are unknown. Our group recently found that bortezomib exhibited significant activity against Hodgkin's disease in preclinical experiments (Zheng *et al. Clin Cancer Res.* 2004;10:3207). Bortezomib inhibited cell proliferation and promoted apoptosis of 4 Hodgkin's disease-derived cell lines in a dose- and time-dependent manner. This *in vitro* activity was independent of I β B gene status. Furthermore, bortezomib sensitized these cell lines to chemotherapy and TRAIL. *Aims.* Based on these encouraging findings, we conducted a clinical trial to determine the safety and activity of bortezomib in the treatment of patients with relapsed classical Hodgkin's disease. *Methods.* Patients with measurable, classical Hodgkin's disease who had relapsed after ≥ 2 prior treatment regimens, including prior autologous stem cell transplantation, were eligible for this study. A platelet count > 50,000/ μ L, ANC > 1,500/ μ L, bilirubin < 2 mg/dL, creatinine < 2.5 mg/dL, and no CNS involvement with Hodgkin's disease or evidence of HIV infection were also required.

Bortezomib 1.3 mg/m² was administered intravenously on days 1, 4, 8, and 11 of 21-day cycles in an outpatient setting. Evaluation of bortezomib activity was determined following 3 cycles of therapy. Treatment of up to a maximum of 6 cycles was allowed, if disease progression was not observed after cycle 3. Administration was delayed in patients with a platelet count < 30,000/mm³ on the day of treatment. *Results.* Thirteen patients, including 8 men and 5 women, have been enrolled in this trial. The median age is 31 years (range, 21–74). All the patients relapsed following a median of 5 prior treatment regimens (range, 2–7). Twelve patients relapsed after autologous stem cell transplantation. The median baseline platelet count was 126,000/ μ L (range, 66,000–339,000/ μ L). Twelve patients have received one or more doses of bortezomib and are assessable for treatment-related toxicity. Bortezomib was reasonably well tolerated, with the majority of toxicities characterized as low grade. Two patients developed grade 3 dyspnea, and one patient had grade 3 neutropenic fever. The most common hematologic toxicity was progressive thrombocytopenia that often necessitated treatment delays. Four of 12 patients had a nadir platelet count of < 30,000/ μ L during the first cycle. One partial remission was observed in a patient who was also receiving low-dose prednisone, and one patient achieved a minimal response, with improvement in PET scan activity. *Conclusions.* These preliminary data demonstrate encouraging clinical activity of bortezomib, with manageable toxicity in patients with relapsed classical Hodgkin's disease. A trial of bortezomib administered alone for one cycle followed by ESHAP in patients with Hodgkin's disease after first relapse will be conducted to evaluate the activity of bortezomib in combination with chemotherapy in this disease.

0267**FOLLICULAR NON-HODGKIN'S LYMPHOMA WITH LOW-LEVEL OF BCL2/IGH+ AT DIAGNOSIS: FAST AND COMPLETE MOLECULAR RESPONSE IRRESPECTIVE OF FLIPI, FOLLOWING CHLORAMBUCIL - RITUXIMAB COMBINATION THERAPY**

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The achievement of a molecular response (bone marrow and peripheral blood clearance of BCL-2/IgH+ positive neoplastic cells) seems to be associated to a longer EFS and might prolong OS in follicular NHL patients. Therefore aggressive chemotherapy including high-dose schedule is used to treat such patients even as first line treatment. Rituximab has become a well-established treatment for NHL patients and is able to induce molecular response in about 30% of the cases when used as single agent. The addition of Rituximab to chemotherapy increases the clinical and molecular remission rate and to prolongs the time to treatment failure. Between November 2001 and June 2004, 24 consecutive newly diagnosed follicular (grade 1 or 2) NHL patients, median age 54 years (range, 30 to 74 years) were treated with oral Chlorambucil 6mg/m² administered daily for 6 consecutive weeks in association with standard 4-weekly Rituximab (375 mg/m² i.v). Eight weeks later, responding patients received a consolidation therapy with Chlorambucil 6mg/m² daily for 2 weeks every month (4 cycles) plus Rituximab (375 smg/mq i.v) once/monthly. BCL-2 translocation on peripheral blood and bone marrow was evaluated by quantitative and qualitative PCR at diagnosis, and by qualitative PCR after the induction and maintenance therapy and 3 monthly during the follow-up. According to FLIPI, 10 (42%) patients were defined at low risk, 9 (37%) at intermediate risk and 5 (21%) at high risk. At diagnosis, bone marrow involvement was present in 9 (37%) of cases. Ten patients had a positive BCL-2 translocation in bone marrow and in peripheral blood, whereas 2 patients presented a positive PCR in the peripheral blood only; eleven out of 12 patients had a low level (<100 pg) of BCL-2/IgH+ cells by quantitative PCR. All patients completed the planned treatment without any delay or dose reduction and were fully assessed for clinical and molecular response. 23/24 patients obtained an

objective response with a high rate of CR (87%). After a median follow-up of 27 months (3.5-86.6 months), two patients relapsed at 6.7 and 19.7 months respectively. All 12 BCL2 positive patients at diagnosis obtained a clearance of BCL-2 translocation both in bone marrow and in peripheral blood and are in CR after a median follow-up of 26 months (8.0-36.0 months). Our regimen is safe and able to induce clinical and molecular response in follicular NHL patients even in those at intermediate and high risk according to FLIPI. This combination therapy seems to induce a complete and durable molecular response in patients with low level of BCL2/IgH cells at diagnosis.

0268

TREATMENT WITH THE PROTEASOME INHIBITOR BORTEZOMIB IN PATIENTS WITH RELAPSED OR REFRACTORY MANTLE CELL LYMPHOMA: PRELIMINARY RESULTS OF THE PINNACLE STUDY

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Bortezomib (VELCADE®, Millennium Pharmaceuticals, Inc.) is the first proteasome inhibitor used in humans, with activity in multiple myeloma (approved as third line) and also evidence of activity as a single agent in relapsed, refractory non-Hodgkin's lymphoma (NHL), with especially encouraging activity in mantle cell lymphoma (MCL) (Goy et al. *J Clin Oncol.* 2005;23:667-675; O'Connor et al. *J Clin Oncol.* 2005;23:676-84). The objective of this multicenter phase 2 study is to evaluate the activity of bortezomib in patients with MCL. Patients with relapsed or refractory MCL (maximum of 2 prior therapies) were eligible for enrollment. Bortezomib was given at 1.3 mg/m² by intravenous bolus on days 1, 4, 8, and 11 of a 21-day cycle for 4 cycles beyond complete response (CR) or up to 1 year unless stopped because of progression or toxicity. Response was initially assessed by the investigators using the International Workshop criteria with a scheduled Central radiology review to determine final response. **Results.** 115 patients from 33 sites in the United States and Europe were enrolled from June 2003 through January 2005 in this 3-stage, phase 2 study. Among the 48 patients evaluable for response at the second stage, the median age was 66 y and 15% were females. Other baseline characteristics included International Prognostic Index ≥ 2 in 88%, lactic dehydrogenase above normal in 40%, Karnofsky performance score $\geq 70\%$ in 92%, the presence of ≥ 1 extranodal site in 77%, 2 prior lines of therapy in 57% of patients. The median time interval from diagnosis to enrollment in the study was 2.3 y (range, 0.2-9.0 y). The median number of cycles administered was 4 (range, 1-17). With a median follow-up of 6.2 mo, a response (CR + unconfirmed CR [CRu] + partial response) was observed in 19 of 48 patients (40%), 6 of them being CR/CRu (6%). Assessment of the median time to progression, duration of response, and survival are ongoing. Bortezomib was well tolerated, and the adverse event profile was manageable. Serious adverse events were reported in 34% of 102 patients; however, only 21 of the 46 events were considered treatment-related by the investigator. Vomiting, abdominal pain, dehydration, and asthenia were the most common bortezomib-related serious adverse events. A nadir platelet count of less than 25,000 cells/ μ L was reported in only 9.3% of patients. As seen in MM, thrombocytopenia was cyclical, decreasing during treatment and recovering to baseline by the start of the next cycle.

Conclusions. The activity of bortezomib in MCL was encouraging, with an overall RR of 40% in this international, multicenter study. These promising results support continued evaluation of bortezomib as a new therapeutic option for patients with MCL. The accrual goal of this study is 152 patients, and an update of all results will be presented at the meeting.

0269

A STUDY OF PEGYLATED LIPOSOMAL DOXORUBICIN IN PATIENTS WITH CUTANEOUS LYMPHOMA

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The therapy of advanced or refractory Cutaneous T-cell lymphomas (CTCL) is often unsatisfactory. Pegylated liposomal doxorubicin (PEG-DOXO) has favourable pharmacodynamic and toxicity profiles; recently the first data on the efficacy and safety of PEG-DOXO for patients affected by CTCL have been published. **Aims.** Our study was aimed to evaluate the efficacy and the toxicity of PEG-DOXO in the therapy of advanced or relapsed CTCL. Besides 17 patients affected by CTCL, we have considered even one patient affected by a Cutaneous B-cell lymphoma (CBCL). The primary outcome measure of our study was the overall response (OR) rate; the secondary ones were the side effects and the clinical benefit (i.e. time to treatment failure TTF). **Methods.** Eighteen patients (5 female, 13 male) aged 29-84 (median 67 years) were treated with PEG-DOXO 20 mg/m² administered intravenously every 3-4 weeks for 2-8 cycles (median 4 cycles). According to TNM classification of *Mycosis Fungoides*, among the nine patients with MF, 1 patient was in stage IB; 2 patients in stage IIA; 5 patients in IIB (3 of these were affected by the folliculotropic MF) and 1 patient in IIIA. As for the other nine patients, 2 of them had a MF evolving towards large cell transformation respectively CD30+ CTCL and CD30- CTCL; 3 patients had Sezary Syndrome (SS); 3 patients had primary CTCL other than MF. One patient was affected by a primary cutaneous marginal zone B-cell lymphoma. PEG-DOXO pretreatment included: the association \square -IFN+PUVA or retinoids in 4 patients; radiotherapy in 5 patients; polychemotherapy (1-5 lines of treatment) in 10 patients; topical/systemic steroid in 2; one patient hadn't been pretreated. Among all patients, 28% presented cutaneous patches, 11% plaques, 44% tumours and 17% had erythroderma. **Results.** Seven patients (39%) achieved a complete response (CR); 8 patients achieved a partial response, resulting in an overall response (OR) rate of 83% (15 patients). The follow-up after the onset of the treatment with PEG-DOXO was 3-38 months. Overall Survival (OS) was 26.4 \pm 9.9 months; Time to Treatment Failure (TTF) was 11.87 \pm 2.13 months. Our results showed that the median Relapse Free Survival (RFS) was not reached; the estimated 38-month OS and TTF are respectively 38% and 40%; as for RFS after 36 months of treatment, 63% patients were free from relapse. The reasons for 'failure' in 7 patients (39% of cases) were imputable to relapse (3 patients), toxicity (1 patient) or non response (3 patients: stable disease, progression, voluntary suspension). Toxicity due to treatment was accurately monitored; PEG-DOXO was successfully tolerated; adverse effects were observed in 5 patients (27.8%); they were temporary and mild; only in one patient grade IV toxicity (neutropenia) occurred. Two patients dropped out, one because of a capillary leakage syndrome and another patient because of voluntary suspension. PEG-DOXO shows, compared with other chemotherapeutic regimens, a significantly high clinical activity and a good safety profile even in advanced and pretreated patients.

0270

ACTIVITY OF CHLAMYDIA PSITTACI-ERADICATING ANTIBIOTIC THERAPY IN MALT-TYPE LYMPHOMAS OF THE OCULAR ADNEXA (OAL): A POLYMERASE CHAIN REACTION (PCR)-BASED PROSPECTIVE STUDY

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Some bacteria have been associated with lymphomagenesis and regarded as targets for new therapeutic approaches. Antibiotic therapy results in the regression of bacterium-related MALT-type lymphomas arising in the stomach, skin, ocular adnexa, and bowel. Regression of *Helicobacter pylori*-negative gastric MALT lymphomas treated with antibiotics has also been reported. Studies assessing these issues in OAL do not exist. **Aims.** To assess tolerability and anti-lymphoma activity of *Chlamydia psittaci* (Ch.ps.)-eradicating therapy in OAL. To analyze activity according to the Ch.ps.-DNA expression in lymphoma tissue samples. **Methods.** Eighteen OAL patients were treated with Ch.ps.-eradicating therapy (doxycycline 100 mg, bid, orally for three weeks), at diagnosis in 8 cases and after relapse in 10. All patients had measurable disease: unilateral lymphoma in 12 cases, bilateral in four, and orbital lesion plus cervical lymphadenopathies in two. Ch.ps.-DNA expression was investigated by multiplex touchdown enzyme time-release (TETR) PCR in lymphoma and PBMC samples [Ferreri *AJM et al. JNCI* 96:586, 2004]. PCR products were sequenced to confirm specificity. Patients with PBMC carrying Ch.ps. DNA were assessable for eradicating activity. The 16 patients with a follow-up of at least 3 months were evaluable for response, which was assessed after one, three and six months and every six months afterwards. Median follow-up after doxycycline was 8 months (range 1-36). **Results.** OAL samples of nine (50%) patients carried Ch.ps.-DNA (PCR+ve); four (44%) of them carried DNA also in the PBMC. PBMC from the nine patients with PCR-ve lymphoma were negative. All patients completed doxycycline treatment with excellent tolerability; one month after doxycycline, chlamydial DNA was no longer detectable in the PBMC of all 4 assessable patients. Among the 16 evaluable patients, objective response was complete (CR) in three cases, partial (PR) in five, minimal (<50%; MR) in two (ORR= 63%); four patients had stable disease (<6 months), and two patients experienced progressive disease (<3 months). Response duration ranged between 3 and 34 months. None of the responders experienced relapse. Objective response was observed in six (3 CR, 2 PR, 1 MR) of the 8 evaluable PCR+ve patients and in four (3 PR, 1 MR) of 8 PCR-ve patients (ORR= 75% vs. 50%, Fisher exact test, $p=0.36$); median follow-up was 26 and 7 months, respectively. ORR was similar between patients treated at diagnosis or relapse (71% vs. 56%, $p=0.63$), and among patients with lesions in pre-irradiated or not irradiated areas (50% vs. 67%, $p=0.61$). The two patients with regional lymphadenopathies achieved CR after doxycycline. **Conclusions.** Ch.ps.-eradicating therapy is fast, cheap and harmless. Doxycycline is able to induce durable regression of OAL, mostly in patients with PCR+ve lymphoma. Responses observed in PCR-ve OAL might suggest that alternative infectious agents sensitive to doxycycline (other *Chlamydiae*) are associated with these lymphomas or that, in some cases, Ch.ps.-DNA load is too low to be detected by TETR-PCR. The adoption of more sensitive and specific PCRs is in progress, and activity of doxycycline in OAL patients will be assessed soon in a large, international phase II trial.

0271

SEVERE NON HAEMOLYTIC ANEMIAS IN FLUDARABINE-TREATED PATIENTS AFFECTED BY LOW GRADE NON-HODGKIN LYMPHOMA

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Fludarabine as single therapeutic agent or in combination with cyclophosphamide or mitoxantrone is at present an excellent option to treat indolent lymphomas and chronic lymphocytic leukemia. Several studies have demonstrated the efficacy of oral fludarabine, which is a formulation pleasing for the patient. However, several side effects are reported in literature: the main toxicities are increased infectious risk and myelosuppression, causing, sometimes, severe anemia, neutropenia and thrombocytopenia. Autoimmune anemias and thrombocytopenias have been also reported. **Methods.** and **Results.** From July 2003 to February 2005 we treated 36 patients affected by low grade non Hodgkin's lymphoma/CLL - like with oral fludarabine (40 mg/m²/d for 5 days on a 28 - day cycles for a maximum of 6 courses). At this time 26 patients have completed their schedule treatment; 5 patients have stopped the treatment for toxicity; 5 patients are still on treatment. We have observed 5 cases of severe myelotoxicity presenting as unexpected severe non haemolytic anemia needing prolonged transfusional support in the majority of cases (4/5 patients). Table 1 reports patient characteristics at start of fludarabine treatment and at anemia occurrence. Four patients had received fludarabine as front line therapy, while one patient was in relapse 7 years after a CHOP regimen. After a variable number of fludarabine courses, patients showed signs of myelotoxicity. Reticulocyte counts were not increased and the Coombs test was negative in all patients. Bone marrow aspirate showed aspects suggesting a myelodysplastic syndrome with trilineage dysplasia (hyper- or hypoplastic bone marrow with erythroid hyperplasia, dysgranulopoiesis and increased number of small non - lobulated megakaryocytic cells without excess of blasts) in absence of bone marrow involvement by clonal lymphocyte. Iron load was normal in all cases. Prolonged transfusional support was necessary for 4 patients and oral prednisone treatment was started in all cases. Patient n.4 received rHuEPO 10,000 U.I. s.c 3 day week with good response.

Table 1.

Patient	Sexi	Age	Stage	Previous treatment	HB (g/dl)		ANC (/ μ L)		PLT (/ μ L)	
					pre-treat.	post-treat.	pre-treat.	post-treat.	pre-treat.	post-treat.
1	F	69	IV A	No	11,9	6,7	2240	2000	61000	38000
2	F	69	IV A	No	12,5	5,0	4500	1100	140000	25000
3	M	79	IV A	No	9,7	5,5	2500	1800	120000	69000
4	M	58	II A	No	15,6	8,7	3600	1000	200000	38000
5	M	78	IV A	CHOP	11,7	7,4	3200	600	229000	13000

After a median time of 10 weeks from the last course of fludarabine, a slow haematological recovery occurred that allowed a chemotherapy change in 3 cases. **Conclusions.** In conclusion, oral fludarabine is an effective agent to treat indolent lymphomas with an overall response rate superior to 70% and complete remission rate in 30% of cases. However, severe myelotoxicity may occur, limiting its use and in some cases preventing the completion of treatment. Thus, a careful monitoring of blood counts is necessary in all patients receiving oral fludarabine, particularly in older patients, often affected by clinically significant co - morbidities. When anemia occurs, all other causes of anemia must be checked: in particular, autoimmunity, iron deficiency, bone marrow involvement or myelosuppression must be evaluated. At the present, there are no predictive factors of myelotoxicity that could help in preventing fludarabine - associated severe cytopenia.

0272**SINGLE INJECTION PEGFILGRASTIM EFFECTIVELY SUPPORTS THE DELIVERY OF DOSE-DENSE CHEMOTHERAPY: A COMBINED ANALYSIS OF THREE PHASE 2 TRIALS**

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Pegfilgrastim has been proven effective when used from the first-cycle for neutropenia induced by standard (21-day) chemotherapy in patients with haematologic and solid tumours (Holmes 2002, Green 2003, Vose 2003). Similar results have been reported in phase 2 pegfilgrastim studies utilizing 3 dose-dense (14-day) chemotherapy regimens: CHOPR-14 in NHL (Lopez ASH 2004), BEACOPP-14 in Hodgkin's disease (Engert ASH 2004), and ACE-14 in SCLC (Pirker ASCO 2004). Although these studies cover 3 tumour types and used different chemotherapy regimens with different therapeutic objectives, they share identical endpoints: to measure the delivery of full chemotherapy doses on schedule (FDOS), neutropenia, and adverse events. *Aims.* A combined analysis of the 3 studies was conducted to evaluate the impact of pegfilgrastim on the ability to deliver chemotherapy in 14-day treatment cycles. *Methods.* Patients with aggressive B-Cell NHL, high-risk Hodgkin's disease, and extensive SCLC received up to 6 cycles of CHOPR-14, up to 8 cycles of BEACOPP-14, and up to 6 cycles of ACE-14 chemotherapy, respectively. Patients in all 3 studies treated with pegfilgrastim received a single dose of 6 mg and were included in this combined analysis. The endpoints of the studies included proportion of chemotherapy cycles given at FDOS, absolute neutrophil count(ANC) profiles, and safety. A cycle was considered *on schedule* if it started within 17 days after the start of the previous cycle, and at *full dose* if a dose of >75% was administered for each myelosuppressive agent. The number of cycles delivered at FDOS were measured and compared with the results from the individual studies. Safety analysis was conducted in all studies. Due to differences in primary disease and co-morbidity characteristics of the patients from each study, safety results in this combined analysis were focused on the incidence of adverse events with a known association to prolonged neutropenia or to pegfilgrastim treatment. *Results.* A total of 100 patients received pegfilgrastim treatment and were included in the analysis (32 patients on CHOPR-14, 41 patients on BEACOPP-14, 27 patients on ACE-14). Individual study results are reported (Table).

Regimen	Number of Cycles at FDOS/ Total Number of Cycles	Percent Cycles Delivered at FDOS
CHOPR-14	175/188	93%
BEACOPP-14	238/294	81%
ACE-14	107/121	88%

The combined analysis of data from all studies shows that a single injection of pegfilgrastim per cycle of chemotherapy allows 86% (520 of 603) cycles to be delivered at FDOS. Bone pain of any severity was reported in 33 (33%) patients overall. The overall safety profile was similar to that already established with pegfilgrastim or daily filgrastim. *Summary/conclusions:* Pegfilgrastim was effective and well tolerated in 14-day dose-dense chemotherapy regimens with a consistently positive influence on the delivery of full doses of chemotherapy on schedule and regardless of chemotherapy regimen or tumour type.

0273**HCV-RELATED INDOLENT NON-HODGKIN LYMPHOMAS: EFFECT OF ANTIVIRAL THERAPY**

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An association between Hepatitis-C Virus (HCV) infection and Lymphomas has been demonstrated by several studies from Italy and other country. A possible pathogenic role of the viral infection on lymphoma development is supported by experimental results. The possibility of obtaining clinical and instrumental response of the hematological disease following antiviral treatment with Interferon±Ribavirin has been shown in limited series and isolated cases. *OBJECTIVES* We report a monocentric experience on 7 patients affected by HCV-related indolent non-Hodgkin lymphomas treated with Interferon±Ribavirin. *Results.* 7 patients were treated: 2 of them affected by Small Lymphocytic Lymphoma/CLL, 3 by Lymphoplasmocytoid Lymphoma, 1 by Marginal Zone-Splenic Lymphoma and 1 by MALT-type Lymphoma. Median age was 56.4 years (range 33-77 years) with 3 males and 4 females. Treatment consisted of Pegylated -IFN (Peg-IFN) at the dose of 80 mg/week in 4 cases and -2-IFN at the dose of 3 MU x 3/week in 3 cases; in 2 patients associated treatment with IFN+Ribavirin was performed. Treatment duration ranged from 6 to 18 months. A virological response, evaluated by HCV-RNA negativization was observed in 5 patients while 1 patient did not respond and 1 could not be evaluated and discontinued treatment due to lymphoma progression. Tansaminase elevation was present before treatment in 5 patients. A hematological response was observed in 6 patients (3 CR and 3 PR). All patients who achieved a virological response had also a hematological response. *Discussion.* Antiviral therapy can induce complete or partial hematological response in HCV-related indolent lymphomas of different histologic subtypes.

Quality of life**0274****PSYCHOGENIC REACTIONS AT THE PATIENTS WITH LEUKEMIA AFTER BONE MARROW TRANSPLANTATION**

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Background. There are anxiety and depressive disorders described more often among psychogenic reactions at the patients with leukemia. Thus the situation of bone marrow transplantation (BMT) can be considered, on the one hand, as in itself significant distressing factor, and with another - as a stage of treatment (additional distressing factor) of oncohematological patients which at moment of BMT have already long enough history of disease and its intensive therapy. *Aims.* To specify the typology of psychogenic reactions at the patients with leukemia after BMT. *Methods.* 39 patients with hematological malignancies (AML (n = 9), ALL (n = 11), CML (n = 19)) have been surveyed by the clinical method. There are psychogenic reactions (non affective and affective ones) as well as psychogenic disorders at mentally ill (schizophrenic reactions) were possible to allocate among the mental disorders at our patients. *Results.* Non affective psychogenic reactions were submitted with neurotic reactions with prevalence of 'denying' (in this case patients deny not disease as it is, but only those aspects which have the menacing sense) (7 cases) and anxiety-phobic reactions (14 cases). Affective reactions at the sampled patients were submitted with psychogenic anxious hysterodepression which distinctive feature at patients with leukemia is rather superficial expressiveness of

actually affective disorders and prevalence of hysteriform disorders, in some cases comparable on to a level with hysterical psychoses (9 cases) and the mixed affective conditions (4 cases). It was observed 5 cases of development of psychogenic reactions at mentally ill. Such reactions were characterized by anozognoy toward to medical disorder and were characterized by discharged (egodistonic) attitude to the fact of leukemia. Considered reactions were observed at patients with distinct attributes of schizophrenic process both at a stage of stabilization, and during active current of process. *Conclusions.* It is possible to consider BMT as the powerful provoking factor of formation of psychogenic reactions at patients leukemia.

0275

USE OF PERCUTANEOUS KYPHOPLASTY AND VERTEBROPLASTY FOR PAINFUL VERTEBRAL BODY FRACTURES IN PATIENTS WITH ONCO-HAEMATOLOGICAL DISEASES

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Vertebral fractures are the most important source of morbidity in patients with multiple myeloma (MM) and one of the most disabling effects of a prolonged corticosteroid therapy for haematological patients (pts). Non-operative treatments such as cytotoxic drugs and radiotherapy (in patients with MM) or analgesic medications and biphosphonates (in all pts) are increasingly used but none of these agents are effective in relieving pain in all cases. Surgical management generally involves vertebrectomy and reconstructions with PMMA (polimetilmetacrylate) bone cement, but this technique is not suitable for the treatment of patients with multifocal vertebral lesions. Another treatment for vertebral fracture is the vertebroplasty that involves the percutaneous injection of PMMA into a fracture vertebral body; however this procedure does not reexpand a collapsed vertebra, it can just reinforce and stabilize a fracture and it is often related to leakage of PMMA through cortical defects, with epidural compression of neural elements. A recent modification of vertebroplasty is the percutaneous balloon kyphoplasty (PBK): under general anesthesia, a 13-gauge needle is introduced through a small dermatotomy and advanced to the posterior aspect of each pedicle along its superolateral cortex; the needle is directed medially and caudally through the pedicle; then using a hand-mounted drill, bilateral channels are created to reach the posterior tract of VB. Through the channel a high-pressure balloon is introduced and inflated to reduce the VB back to its original height; the cavity so obtained is subsequently filled with the PMMA. The balloon inflation and the PMMA filling were performed under fluoroscopy vision. *Methods.* Between March 2003 and November 2004 nine (9) patients underwent percutaneous kyphoplasty (3/9) and vertebroplasty (6/9): 6 pts were affected with MM and 3 pts were affected with Non Hodgkin Lymphoma (NHL), with vertebral fractures after corticosteroid therapy. In the group of pts with MM, the median age was 72 years (range 70-83), the average number of vertebral fractures per patient were 2 (range 1-4) and the pts with multiple fractures underwent 2 treatments. In the group of pts with lymphoma, the median age was 65 years (range 60-73) and they presented an isolated vertebral fracture. *Results.* In MM group of pts, the average of pre-treatment Karnofsky performance status was 40 (range 30-60) and the average of VAS (the pain score, with points subjectively assigned by patients in a range: 0 = absence of pain, and 10 = maximum pain) was 9 (range 8-10): after treatment Karnofsky grade and VAS were 80 (range 50-90) and 2 (range 2-5), respectively. In NHL group before treatment, the average of Karnofsky performance status grade was 50 (range 50-60) and the VAS was 8; after treatment the average of Karnofsky grade and VAS were 90 and 2, respectively. *Conclusions.* In our experience, Kyphoplasty and vertebroplasty were effective in relieving pain and improving the quality of life of the pts. The safety and efficacy of this procedure needs to be evaluated in a larger number of patients.

0276

FATIGUE INTERFERENCE WITH QUALITY OF LIFE (QOL) IN HEMATOLOGICAL MALIGNANCES

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Fatigue is one of the most distressing symptoms in patients with hematological malignances (HM). It is obvious that the population of patients with HM is heterogeneous in terms of clinical characteristics. However, the information on fatigue frequency and severity in HM subgroups is lacking. In addition, the effect of fatigue severity level on a patient's QoL is under-reported. Understanding of fatigue interference with a patient's QoL provides the basis for better management of patients with HM. The goal of this research was to study fatigue prevalence and severity in patients with HM and to investigate the relationship of QoL impairment to fatigue severity levels. Patients and *Methods.* 157 patients with HM (male/female 67/90; mean age 59.01, SD - 16.9) were enrolled in the study: indolent non-Hodgkin lymphoma (NHL) - 35 (22.3%), aggressive NHL - 34 (21.7%), chronic lymphocytic leukemia (CLL) - 25 (15.9%), multiple myeloma (MM) - 24 (15.3%), Hodgkin's disease (HD) - 20 (12.7%), chronic myelogenous leukemia (CML) - 19 (12.1%). SF-36 and Brief Fatigue Inventory were used for patient-reported outcome assessment. QoL impairment was evaluated on the basis of Integral QoL Index (calculated by the Integral profile method). The following grades of QoL impairment as compared to a population norm (PN) were used: mild (25% decrease from a PN), moderate (25-50% decrease), severe (50-75% decrease) and critical (>75% decrease). To compare with PN a gender- and age-adjusted sample of healthy controls from the normative population data was used. For statistical analysis ANOVA was used (p-level <0.05). *Results.* Moderate (4-6) and severe (7-10) grades of fatigue were observed in 68.0% of CLL, 62.5% of MM, 52.9% of aggressive NHL, 52.6% of CML, 40.0% of HD, and 14.2% of indolent NHL patients. Moderate and severe fatigue resulted in critical QoL impairment in 66.1 and 72.7% of patients, respectively. Half of the patients with mild (1-3) fatigue (30.6% of the sample) had no or mild QoL impairment. The majority of patients with no fatigue (22.9% of the sample; 65.7% - indolent NHL, 20.6% - aggressive NHL, 15.0% - HD, 5.3% - CML, 4.2% - MM, and 4.0% - CLL) exhibited no or mild QoL impairment. The Integral QoL Indices differed significantly depending on the grade of QoL impairment. *Conclusions.* The vast majority of patients with CLL and MM, half of the patients with aggressive NHL and CML, and 40% of HD patients experience severe and moderate fatigue, which in most cases results in critical QoL impairment as compared to a population norm. The majority of patients with indolent NHL have no fatigue and in doing so exhibit no or mild QoL impairment. Assessment of fatigue severity and grading of QoL impairment is worthwhile to provide an adequate management of patients with HM.

0277

THE IMPACT OF NUTRITION HABITS AND SOCIOECONOMIC STATUS ON IRON DEFICIENCY AND RELATED ANEMIA IN CHILDHOOD: A RETROSPECTIVE STUDY IN 3,100 CHILDREN

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Iron deficiency is one of the commonest medical conditions encountered in children worldwide. It is a well-recognized cause of childhood anemia and has been linked to impairment of mental and motor development. The aim of the present study was

to evaluate the prevalence of iron deficiency (ID) and iron deficiency anemia (IDA) in children residing in Northern Greece, and to assess its possible association to family socioeconomic status and nutrition habits, such as breast-feeding, home-cooked meat and fast-food consumption. For this purpose, 3,100 children aged between 8 months and 15 years were evaluated. Their socioeconomic status was determined according to the Hellenic National Center of Social Research, based on the father's profession, the mother's profession and the place of residence (urban-semiurban). Although all participating children resided in presumably *developed* regions of Northern Greece, that meaning that they had access to medical and nutritional counseling, the incidence of ID and IDA was considerable (14% and 2.9%, respectively). The results differed considerably between the various age groups, iron depletion occurring predominantly in children of younger age groups ($p < 0.001$), with a greater incidence in children aged between 8 months and 2 years old (ID 34.1%, IDA 16.1%). Both ID and IDA presented with a higher prevalence in semiurban compared to urban regions ($p < 0.001$). The father's profession was a significant factor only in children residing in semiurban regions, while the mother's profession was of statistical importance ($P < 0.001$) between all categories of professions, with a greater significance in children residing in semiurban regions. Out of the three factors determining the socioeconomic status, the place of residence was the most significant in relation to the development of iron depletion in the children studied. Moreover, the multivariate regression model for the incidence of either ID or IDA revealed as independent ($P < 0.001$) favorable factors breast-feeding, consumption of home-cooked meat and non-consumption of fast-food meals. A significant correlation was found between the mothers profession and the ratio of home-cooking compared to fast-food consumption ($P < 0.001$, Pearson test). The results of our study show that the problem of iron deficiency remains prevalent in Northern Greece, mainly affecting the vulnerable toddler group. Since early detection and treatment are of the utmost importance in order to assure adequacy of iron intake, especially during the first 24 months of life, nutritional education should be provided to all families not only by pediatricians but also by government agencies as well as the media. Furthermore, since children may belong to groups presenting educational and cultural barriers, special screening programs should be initiated in high-risk populations.

0278

AML TREATMENT IN ELDERLY PATIENT: A THERAPEUTIC AND PHARMACOECONOMIC DILEMMA

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Which is the best treatment for AML in elderly patients is still debated. Actually there are three main options: supportive treatment (ST), conventional chemotherapy (CC) and low dose chemotherapy (LDC). *Aims.* of this study is to define the best therapeutic and pharmacoeconomic approach in elderly AML. This is a retrospective nonrandomized study. A cost analysis on 15 patients hospitalization was performed. The monthly cost of hospitalisation or specific care was calculated dividing the global expense of hospitalisation in each group of treatment, for the sum of survival months of patients of considered group. *Results.* We present a two center study. 21 patients (12F/9M), median age 72 years (R 65-80), were treated as follows: 9 with CC, 9 with LDC, 3 with ST. 14 patients presented comorbidity, 13 had PS 0-1, 8 had secondary leukaemia and M2-M4 were the most represented FAB subtypes. The most frequent comorbidities were diabetes (7pts), second neoplasms (5pts) and ischemic cardiopathy (4pts). Global median survival for all patient, without regard for the treatment received, was 3 months (R1-10). Median survival was 5 months for patients treated with CC, 3 for LDC and 1 for ST. Median hospitaliza-

tion was 1 month for ST (R0.5-1), 2 months for CC (R1-8) and 1 month for LDC (R0.2-2). Monthly cost of hospitalisation was q5100 for ST, q3700 for CC and q1500 for LDC. The antibiotic expense was higher in ST (q2900/month vs q1100/month in CC vs q700/month in LDC), but transfusion and chemotherapy expense were higher in CC (q1200/month vs q900/month in ST vs q400/month in LDC). Erythropoietin use didn't reduce transfusional expense (q1200/month vs q700/month in patients without erythropoietin). G-CSF administration wasn't effective in antibiotic expense reduction (q1800/month vs q1100/month in patients without G-CSF). New drugs (Mylotarg and Gleevec) increase significantly chemotherapy costs (q900/month vs q400/month) and hospitalisation expense (q4100/month vs q2300/month). *Summary/conclusion.* In conclusion ST seems to be the less effective option for survival, hospitalization length and sanitary expense. Instead LDC seems to be an economic and effective option especially if performed in outpatient setting. Nevertheless these data need further confirmation on a larger patient cohort.

0279

HAEMATOLOGY AND PALLIATIVE CARE - AN INTEGRATED APPROACH

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Background. The National Institute for Clinical Excellence (NICE) (1) has recommended a more integrated system of care for patients with haemato-oncological disorders. It is recognised that palliative care can contribute to improving quality of life, throughout the patients illness and can help to manage symptoms and support carers and families. In Ayrshire (in West Scotland, UK) there has been excellent co-operation between hospital and community palliative care teams and the haematology team caring for patients with the full spectrum of haematological disorders. *Methods.* and *Results.* 82 referrals were made to the HPCT between 2000-2004, this represented 52 patients. There were many multiple referrals. 21 males age range 37-88 years, mean 68 years and 31 females age range 50-94, mean 74 years. Diagnosis - Acute leukaemia 15 (14 AML, 1ALL), M. myeloma (MM) 14, Non-Hodgkins lymphoma (NHL) 16, Chronic Lymphatic Leukaemia (CLL) 3 and Others 4. Reason for referral were symptom control 29, psychological support 31, Hospice Specialist Nurse support at home 31, Hospice transfer 4. Some patients were referred for more than one reason. Outcomes following referral were-drug changes 29, patient/family support 22, Hospice specialist nurse support at home 35 and again there may be more than one. Of 52 patients referred, 48 have died, 1 was transferred to long term care and 3 continue to receive support at home. In the UK it has been suggested that fewer patients with hematologic malignancies die at home or in a hospice compared to patients with other solid tumours (2). In our series, the place of death was hospital 29 (60%), home 10 (21%), Hospice 7 (15%), unable to determine 2 (4%). These figures are very similar to those in a previous UK study (2). There is often a perceived difficulty in deciding when patients should be referred to the palliative care services. We looked at the time between referral to palliative care and death. There was a marked difference depending on the underlying disease. In AML 31 days (mean) 14 days (median), NHL 13 weeks (mean) 3 weeks (median), MM 9.5 months (mean) 5 months (median). In view of this marked variation we looked at haematological parameters and serum albumin to see if these might be indicators of poor prognosis, but none is a sensitive marker. *Conclusions.* We believe it is greatly to the patients benefit if haematology and palliative care work together. It is essential that there is an opportunity for informal discussion of patients, so that referrals can be made in a timely way and care can be shared between the two disciplines. A referral to palliative care should not mean that the important therapeutic relationship between the patient and the haematology team is lost-instead the relationship is strengthened by extra support and expertise.

0280**ECONOMIC ANALYSIS OF A DOMICILIARY PROGRAM OF SUPPORTIVE AND PALLIATIVE CARE FOR PATIENTS WITH HEMATOLOGICAL MALIGNANCIES**C. Cartoni¹, G. Brunetti¹, G.M. D'Elia¹, G. Morano¹, M. Breccia¹, M. Gentile¹, A. Nastri², M.G. Marini², F. Mandelli¹, P. Martino¹, R. Foa¹¹*Polinclinico Umberto I, ROME, Italy;* ²*ISTUD - Ist. Studi Direzionali, Milan, Italy*

The implementation of home care programs for the hematologic patients requires the assessment of the specific costs of the domiciliary setting. *Aims.* To analyze the use of resources and the global costs of the services provided at home of the patients, according to their phase of disease. *Methods.* During 2 yrs, 185 patients were assisted at home to provide: - supportive care, for cytopenic pts. early discharged (ED) from the hospital, after the chemotherapy; - palliative care, for pts. with different life expectancy: < 3 months in terminal phase, (TE); > 3< 6 months in advanced phase (AD); > 6 months in chronic phase (CH). The cost drivers evaluated concerned: 1) health care providers and coordination/support team; 2) materials and medicines; 3) laboratory; 4) transfusional support. In 40 cases indirect costs (for the patient and the family) have been also evaluated. Mean monthly costs (MMC) of home care are expressed in q and have been compared with national Diagnostic Related Groups (DRGs) scale charges for hematological diseases and with district fares for palliative home care for patients with oncological diseases. *Results.* Use of resources: among the 4 groups of pts., ED and TE pts. required the higher mean monthly No. of home visits (24.1 and 23.2, respectively) and of transfusions (6.8 and 6.22); they were assisted for a median No. of 19 days and 22.8 days, respectively. Costs: The MMC of pts. was affected by the following variables: disease status, transfusional needs, type of hematological disease. CMM for TE pts (q 4.232,82) and ED pts. (q 3.986,57) resulted higher than for AD pts. (q 2.304,04) and CH pts (1488,43 q). With the exclusion of CH groups, these costs exceed the district fares for home care of oncological pts. The highest CMM, for pts. with acute leukemia and > 4 transfusion/mos. was (q 11.080), resulted lower than the highest corresponding DRG. The MMC sustained by the families was q 2.027,72. *Conclusions.* From the perspective of the purchaser/provider of health services, costs of home care for some categories of hematological pts. are lower than those of hospitalization, although superior to the current national fares for home care programs. From the perspective of the health care as a whole, the economic burden charged to the patients and their families must be taken into account in the costs evaluation.

0281**PAIN AT THE INJECTION SITE: RESULTS OF A CROSS-OVER STUDY COMPARING EPOETIN BETA AND DARBEPOETIN ALFA ADMINISTERED SUBCUTANEOUSLY IN HEALTHY VOLUNTEERS**B. Coiffier¹, F. Berthou², P. Fiorentini³, F. Montestruc³¹*Centre Hospitalier Lyon-Sud, PIERRE-BENITE, France;*²*Hôpital Nord, PRIEST EN JAREZ, France;* ³*F. Hoffmann-La Roche, NEUILLY-SUR-SEINE, France*

The subcutaneous (SC) route of administration is recommended for the use of Erythropoiesis-Stimulating Agents (ESAs) in anaemic cancer patients. Pain at the injection site may reduce the compliance of the ESA in these patients. *Methods.* This single-blind, randomised cross-over study, conducted in 40 healthy volunteers, aimed to evaluate the pain at SC injection site of two commercially available ESAs in Europe, epoetin beta (EPO-B) and darbepoetin α (DA). After SC administration of 0.9% saline (SAL) (0.3 mL), subjects were randomised to receive identical volumes (0.3 mL) and equivalent doses of either EPO-B or DA of 6000 IU and 30 μ g, respectively. After one week of wash-out, subjects crossed over to receive the other study drug. A verbal scale ranging from no pain (0) to severe pain (5) and a 10-cm ungraduated Visual Analogue Score (VAS) (0 = no pain, 10 =

maximal pain) were used to evaluate pain. *Results.* Of the 40 healthy volunteers included (mean age 29.9 \pm 10.5 yrs; men 47.5%), 37 completed the study. Data from the intention-to-treat population were analysed and were expressed as median (inter-quartile). Immediately after injection, median verbal pain score was 0 (0.0–1.0) for EPO-B, 2 (0.0–3.0) for DA and 0 (0.0–1.5) for SAL ($p < 0.0001$, EPO-B vs DA; signed rank test). Also, immediately after injections VAS measured 0.5 (0.0–1.5) for EPO-B, 2.0 (1.4–4.2) for DA and 0.4 (0.0–2.3) for SAL ($p < 0.0001$, EPO-B vs DA; signed rank test). Fewer subjects taking EPO-B (5.4%) reported pain as moderate-to-severe immediately after injection compared with those taking DA (37.5%) or SAL (12.5%). Of the 37 completers, 12 (32.4%) reported moderate-to-severe pain with DA and no pain or low pain with EPO-B. There were no volunteers who had moderate-to-severe pain with EPO-B and no pain or low pain with DA ($p < 0.0001$ EPO-B vs DA, McNemar test). One hour after injection, no significant difference was observed between pain intensity induced by SC injection of EPO-B, DA or SAL. Median pain (measured using VAS) immediately after injection of DA was higher (3.2, 1.3–5.1) in the group who had switched from EPO-B ($p = 0.18$, Wilcoxon test). SC injections of EPO-B, DA and SAL were generally well tolerated in the subjects completing the study. *Conclusions.* Subcutaneous injection of epoetin beta is no more painful than saline and is significantly less painful than subcutaneous injection of darbepoetin alfa. Pain associated with subcutaneous darbepoetin alfa is more pronounced after switching subjects from subcutaneous epoetin beta.

0282**DON'T CALL US SURVIVORS! RESULTS OF A SELF-HELP GROUP EXPERIENCE FOR YOUNG PATIENTS WITH HODGKIN'S (HD) AND NON-HODGKIN'S (NHL) LYMPHOMA**

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Given the great improvement of clinical results in most fields of oncohematology, it is likely that new psychosocial end-points and human needs might come out from those patients who expect to be long survivors or even cured. AIMS To understand what catching cancer in a significant period of life for professional and family planning has meant for a cohort of young patients with lymphoma and what emotional and cognitive changes they have played to cope with such an experience. Preliminarily, patients with HD and NHL <45 years old who were treated at our department were invited to take part in a focus group guided by a psychologist and an hematologist in order to agree on main psychosocial items on which they focused their interest. In a second phase, the items were discussed with them in depth during four consecutive meetings. Every meeting was recorded, transcribed and analyzed using an human science approach with the purpose of tracing specific patterns of understanding. Contents were then categorized in five areas: self, health, family, social aspects and coping strategies. Only those conclusions with full agreement by all participants are reported. Six of ten patients (2 female and 4 male, median age 35,5 yrs; 4HD and 2 NHL; 1 still under treatment and 5 off therapy with a median follow-up of 27,6 months accepted our invitation and went through all meetings. Concerning self, the strongest feeling was an addiction to react against disease constraints in order to maintain an autonomous life without changing their targets. Greater sensitivity toward other people suffering was also mentioned. Patients' main concern about health was fear of recurrence of disease, of pain and personal suffering as well as of thoughts of death; the feeling of being dissected and catalogued as well as the presence of physical long-term consequences such as early menopause and loss of fertility were also reported. Concerning their relation within the family a significant difficulty in supporting their parents and their children was underlined. However, while a feeling of guilt with respect to their parents for making them worried is specially bothersome, the presence of children is viewed as a great resource. Concerning social aspects, patients underlined that

despite maintaining their occupation was difficult during treatment, it was still viewed as an important help for coping with the disease. Further problems were finding a new job or obtaining a life insurance. Coping strategies included deleting of negative thoughts, constrains to maintaining of normal life style, the continuous search of family and social supporto such as a continuous and frequent contact with their doctor. When a young patient has the potential of becoming a long survivor from an oncologic disease, participation to groups is most advisable in order to focus on relevant psychosocial needs that might be overlooked otherwise. All patients have agreed to participate in further groups in order to introduce new patients.

0283

INTERVIEWING PATIENTS WITH HEMATOLOGICAL MALIGNANCIES FOR STRESS-IS THERE A NEED, IS THERE A CONNECTION?

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A number of studies have indicated a possible role of stress in the genesis and progression of malignant diseases, however no definitive conclusions have been drawn regarding hematological malignancies. Increasing incidence of malignant lymphoma and leukemia noted during the last decade at our Department, as well as the available data describing the influence of stress on the neuroendocrine response and the immune system, raised interest for investigating the frequency, severity and types of stress events in our patients 3-12 months prior to diagnosis. A standardized questionnaire helped patients to identify traumatic events like war, different types of accidents and natural disasters, assaults, torture, rape, imprisonment, life-threatening situations or previous illness, death of close friends or family members, etc. Among 168 interviewed patients, 61 patients had non-Hodgkin lymphoma, 32 chronic lymphocytic leukemia, 25 multiple myeloma, 21 chronic myeloproliferative disorder, 19 Hodgkin disease, and 10 patients had acute leukemia. A total of 90 patients (54%), suffering from any of these malignant diseases, experienced stress prior to diagnosis (NHL - 59% pts, CLL-50% pts, MM-48% pts, CMPD-57% pts, Hodgkin-52% pts, and AL-40% pts). 36 patients (21%) had one serious stress event, 30 pts (18%) two stress events, three stress events were recorded in 15 patients (9%), and 10 patients (6%) had four stress events prior to diagnosis. Although the recorded percentages seem impressive, further comment and analysis of these and similar results and possible mechanisms affecting the immune system of distressed patients should be conducted - leading to the design of a longitudinal interdisciplinary study including a sufficient number of healthy participants, monitored for psychosocial, biochemical, genetic, hormonal and other parameters.

0284

HEALTH-RELATED QUALITY OF LIFE OF PATIENTS WITH RELAPSED MULTIPLE MYELOMA RECEIVING BORTEZOMIB VERSUS HIGH-DOSE DEXAMETHASONE IN THE APEX TRIAL

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In a large international randomized phase 3 trial (APEX) of 669

patients with relapsed multiple myeloma, bortezomib demonstrated significant improvement in time to progression, response, and survival over high-dose dexamethasone. In this multicenter study, grade 3 and 4 toxicities were comparable in the two treatment arms. Data regarding health-related quality of life were prospectively collected during the study. *Aims.* To evaluate the impact of treatment with bortezomib versus high-dose dexamethasone on health-related quality of life of patients with relapsed multiple myeloma in the APEX trial. *Methods.* EORTC QLQ-C30 and FACT/GOG-NTX, two health-related quality of life instruments, were administered prior to therapy (baseline), as well as at 6, 12, 18, 24, 30, 36, and 42 weeks after initiation of therapy, or until discontinuation of treatment with bortezomib or high-dose dexamethasone. Modified intention-to-treat analysis was used. The Global Health Status scale of the EORTC QLQ-C30 was the a priori primary end point for these statistical analyses. The other scales and symptom scores served as secondary end points. The Hochberg-Benjamini method was used to adjust for multiple comparisons and is reflected in the presented P values. All analyses were performed using generalized estimating equations of multiple imputed datasets. *Results.* Adequate health-related quality of life data were collected and analyzed for 598 of 642 patients; 44 patients were excluded due to a lack of baseline or follow-up data. Patients in the two treatment arms exhibited comparable baseline demographic and clinical characteristics, NTX scores, and, for the most part, EORTC scores. Due primarily to early closure of the trial and disease progression in several patients, substantial data (from 12.5% at week 6 to 75.6% at week 42) were not available. Extensive statistical analyses were undertaken to correct for potential bias related to the missing data. Compared with high-dose dexamethasone, bortezomib was associated with a significant improvement in the primary end point of Global health (P = 0.0005), as well as the secondary end points of Physical, Role, Cognitive, and Emotional Functioning (adjusted P values < .05), and the symptom scales of Nausea, Dyspnea, Sleep, Diarrhea, and Financial Impact (adjusted P values < 0.05). High-dose dexamethasone was not found to be superior over time to bortezomib for any health-related quality of life end point examined. Other alternative methods that adjusted for potential informative censoring, such as the Sun and Song and the Pattern-Mixture model analyses, further supported these conclusions.

0285

QUALITY OF LIFE IN HEMOPHILIC PATIENTS: COMPARISON BETWEEN PATIENTS WITH INHIBITORS AND PATIENTS WITHOUT INHIBITORS

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Health-Related Quality-of-Life (HRQoL) is one of the most important outcome measures in hemophilia care: modern treatment has prolonged patients' life expectancy and is now focused on improving their HRQoL. The development of inhibitors has represented one of the most challenging complications of hemophilia treatment, compromising the effectiveness of treatment with clotting factor concentrates, increasing the risk of limb- and life-threatening bleeding, severe arthropathy, physical disability and mortality. Comparison of HRQoL in adult hemophilic patients with and without inhibitors. *Methods.* HRQoL of patients with inhibitors was evaluated in the Cost Of Care Inhibitors Study (COClS) [Gringeri et al, Blood 2003] and that in patients without inhibitors was evaluated in the Cost Of Care of Hemophilia Study (COCHE). Patients were recruited at the Italian Hemophilia Centers and were administered a battery of questionnaires: results of two generic instruments, EuroQoL-5D and Short Form-36, are reported. *Results.* Fifty patients with inhibitors (98% were high responders, with peak titre > 5 BU) returned HRQoL questionnaires: the median age was 35 year (15-64), all the patients had hemophilia A, 94.2% severely

affected. The enrolled patients without inhibitors were 232, with median age 34 (18-74), 86.5% with hemophilia A, 73.4% severely affected. The mean±SD score of SF-36 Physical Component Summary score was 42.3±10.4 (median 43.5, 14.3-59.8) in patients without inhibitors, and 36.9±10.7 (median 35.2, 15.2-55.0) in those with inhibitors. The mean±SD Mental Component Summary score was 45.8±8.5 (median 46.8, 16.3-62.6) in patients without inhibitors, and 50.2±11.8 (median 52.8, 15.5-68.1) in those with inhibitors. With EuroQoL-5D, 77.8% vs 78.0% patients (without and with inhibitors, respectively) reported any problems in the dimension *pain/discomfort*, 64.0% vs 66.0% reported problems in *mobility*, 43.9% vs 38.0% reported problems in *anxiety/depression*, 40.3% vs 54.0% reported problems with 'usual activities', 16.9% vs 34.0% reported problems with *self-care*. EQ-Visual Analogue Scale had a mean±SD of 66.2±18.4 (median 70.0, 9-100) in patients without inhibitors, and a mean±SD of 65.5±16.2 (median 66.0, 30-95) in those with inhibitors. The mean±SD utility score was 0.7±0.2 (median 0.7, from -0.2 to 1) in both the groups. Discussion Hemophilic patients have an impaired HRQoL compared to the general population. General HRQoL in patients with inhibitors is not different from that of patients without inhibitors, i.e. they have a poor physical but a relatively good emotional perception of health.

0286

THE COST OF CARE OF HEMOPHILIC PATIENTS: COMPARISON BETWEEN PATIENTS WITH AND PATIENTS WITHOUT INHIBITORS

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Background. Modern hemophilia management has prolonged patients' life expectancy and is now focused on reducing arthropathy and improving HRQoL. The health care of hemophilic patients adsorbs a large amount of resources. This situation becomes extreme when patients develop inhibitors that compromise the mainstay of treatment with clotting factor concentrates. **Aims.** Comparison of cost of care for hemophilic patients with and without inhibitors. **Methods.** Cost of care of patients with inhibitors was evaluated in the Cost Of Care Inhibitors Study (COCIS) [Gringeri et al, *Blood* 2003] and that in patients without inhibitors was evaluated in the Cost Of Care of Hemophilia Study (COCHE). These are two multicentre, longitudinal (the observational period was of 18 months in the COCIS and 7-8 months in the COCHE), ambispective (i.e. retro- and prospective) studies, involving adult moderate and severe patients sequentially enrolled at the Italian Hemophilia Centres. Information on demographics, clinical data, resource absorption, and quality of life was collected. This analysis pertains on estimate of cost of treatment with clotting factor concentrates, from the perspective of the Italian National Health Service. All costs are expressed in €/person/month (year 2002 for COCIS, 2004 for COCHE). Results from COCHE are referred to data collected during the recruitment visit (time horizon = 4 months before enrollment). Fifty-two patients with inhibitors were enrolled in the COCIS study: median age 35 years (15-64), 100% with hemophilia A, 94.2% with severe hemophilia, 98% high responders (peak titre > 5 BU). The patients without inhibitors, enrolled in the COCHE study, were 232: median age 34 years (18-74), 86.5% with hemophilia A, 68.3% with severe hemophilia. Patients with inhibitors reported 0.60 total bleeding events/patient/month (0.46 in joints, 0.12 in muscles, 0.02 in other sides). Patients without inhibitors reported 1.89 total events/patient/month (1.47 in joints, 0.75 in muscles, 0.27 in other sides). Most patients with inhibitors (71.0%) were treated with recombinant activated factor VII (rFVIIa), 46.7% were treated with activated prothrombin complex concentrates (aPCC), 25.0% received high doses of human plasma-derived and 5.8% received recombinant factor VIII (FVIII) concentrates. Patients without inhibitors were on treatment

with recombinant (59.1%) or plasma-derived (40.9%) factor VIII/IX concentrates. Patients on prophylaxis were 32.8% and those on-demand regimen were 67.2%. Total cost of care with concentrates was 17,725 and 7,804 €/person/month in patients with and without inhibitors, respectively. Cost for prophylaxis of patients without inhibitors was 15,224 €/patient/month and cost for treatment on-demand was 4,221 €/patient/month. The incremental cost per bleeding avoided of prophylaxis vs on-demand treatment in patients without inhibitors was 8,990 €. Cost for prophylaxis is almost 2.6 times higher than cost for treatment on-demand in patients without inhibitors. Treatment of hemophilic patients without inhibitors cost 56% less than treatment of patients with inhibitors. In particular, patients without inhibitors on prophylaxis cost 14% lower and those on-demand regimen cost 76% lower than patients with inhibitors.

0287

EVALUATION OF HEALTH RELATED QUALITY OF LIFE (HR-QOL) AND PREFERENCES ON ANTICOAGULANT TREATMENT IN PATIENTS RECEIVING VITAMIN K ANTAGONISTS (VKA)

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VKA is indicated for a number of conditions and are frequently prescribed as long term treatment. There are a number of characteristics that can potentially induced dissatisfaction and reduced HR-QoL. Therefore in the development of new therapeutic strategies is very important to take in to consideration Patients' preferences. To evaluate Health-Related Quality of life (HR-QOL) in patients receiving VKA and to establish patients' preferences on different anticoagulant treatment options. **Methods.** Fifty-nine consecutive patients receiving VKA (35 male; age range 37-80 years) were enrolled. The more frequent indications for VKA treatment were atrial fibrillation and venous thromboembolism. Each patient was matched by age and sex with 4 controls from a database of a population based naturalistic prospective survey. The EuroQoL (EQ-5D) was used to evaluate HRQOL in both groups. EQ-5D is a self-administered generic standardized questionnaire that provides a simple descriptive profile and a single value for health status by a visual analogue scale (VAS). VAS is a thermometer-like scale in which the respondents self-rated their health status from 0 to 100 (100=best status). EQ-5D comprises 5 dimensions of health: mobility, self-care, usual activity, pain/discomfort and anxiety/depression. In addition to this a conjoint analysis exercise was administered. Patients had to choose between two different scenarios in 9 pair-wise comparisons. The attributes considered had previously been selected using an ad-hoc questionnaire administered to a sample of 20 patients and 6 physicians. The following 6 attributes were selected: cost of treatment for the patient (€ 0 vs. €15 vs. € 75/month), pharmaceutical formulation (tablets once daily vs twice daily administration vs once sub-cutaneous weekly injection), frequency of monitoring (every 2 weeks vs every 1 month vs every 6 months), interactions with drugs or food (attention required vs not required), dose adjustment (required vs not required), minor bleeding (few vs. no). **Results.** Usual activities resulted more frequently impaired in patients than controls ($p=0.048$). No significant differences were reported in mobility, self care, pain/discomfort and anxiety/depression. The figures obtained using VAS to assess the global health status were: 71.8 (SD, ±17.3) in patients treated with VKA and 73.9 (SD, ±15.1) in controls ($p=0.168$). Regarding preferences, all attributes considered, excluding dosage modification, tested important to respondents. Patients' preferences were (in decreasing order): tablets once daily administration, once monthly visit, no minor bleeding, no attention required to interactions with other drugs or food. The variable 'cost' was a significant determinant in patients' choice. **Conclusions.** Perception of impairment in usual activities was more frequently reported among patients on VKA treatment than in

matched controls. However, the overall perception of health status was not significantly different. In fact preferences of patients on VKA treatment seem to mirror most attributes of the already ongoing treatment. Patients followed by an anticoagulation clinic are confident in their treatment and they are probably reluctant to make changes. Different settings, i.e. patients starting anticoagulation, may have different attitudes.

0288

DISEASE STATE AND QUALITY OF LIFE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES: A REVIEW OF INSTRUMENTS, DISEASE BURDEN, AND PREDICTIVE FACTORS FOR OUTCOMES

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Despite increased interest, few studies have examined the role of health-related quality of life (QOL) assessments in evaluating myelodysplastic syndromes (MDS) treatment outcomes or synthesized the research findings. *Aims.* To review and evaluate available QOL instruments in MDS, understand the QOL burden of the condition, identify MDS disease characteristics that influence QOL outcomes, and provide recommendations for future research. *Methods.* A systematic literature review was performed to identify QOL studies performed in MDS patients. The search included medical and QOL databases, Internet, and manual searches. The search was restricted to studies published in English from 1995 to 2005. *Results.* Fifty-three abstracts were located; 13 relevant studies were selected for detailed review. The literature review revealed that four QOL scales were employed: the Functional Assessment in Cancer Therapy-Anemia (FACT-An), European Organization of Research and Treatment of Cancer QLQ-C30 (EORTC), Quality of Life-E (QOL-E), and Short Form-36 Health Survey (SF-36). Each demonstrated acceptable validity in MDS patients. The FACT-An, QOL-E, and SF-36 had good internal consistency with Cronbach's α coefficients for subscales ranging from 0.92 to 0.94, 0.72 to 0.88, and 0.75 to 0.94, respectively. The FACT-An and the EORTC QLQ-C30 instruments showed appropriate responsiveness to change in health due to treatment in prospective randomized trials. Given the availability of an additional anemia module to the questionnaire, the FACT-An was shown to be a particularly relevant tool to measure QOL in MDS. Two studies quantified the QOL burden of MDS compared to the general reference population. Statistically significantly ($p < 0.05$) worse QOL was observed in the following domains: fatigue, physical functioning, role physical, vitality, social functioning, emotional functioning, and global health. Cognitive functioning was least affected by MDS. None of the studies stratified QOL differences according to the MDS disease sub-group categories or to different International Prognostic Scoring System (IPSS) risk scores. However, four studies established a link between transfusion independence and better QOL. Irrespective of the type of QOL questionnaire used, all studies demonstrated that QOL was significantly ($p < 0.05$) better in transfusion-independent patients, and this remained the case even after controlling for haemoglobin level. Six studies examined the impact of treatment on QOL in MDS patients. Improvements in clinical status, such as achieving partial or complete response, were associated with improved QOL. Changes in overall QOL were mostly linked to improvements in the fatigue, dyspnea, physical, and social functioning QOL sub-scales. *Conclusions.* MDS leads to significant QOL burden compared to similarly aged adults in the general population. QOL is an important endpoint in the research of new MDS treatments, and several validated instruments are available for use. Research evidence on the clear link between transfusion dependency and impaired QOL suggests a potentially important role for new treatments aiming to achieve higher transfusion independence in MDS patients.

0289

BURDEN OF BONE MARROW TRANSPLANTATION (BMT) ON CAREGIVERS

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Only a few studies have approached the burden of BMT on caregivers. Caregiver Reaction Assessment (CRA) is an instrument designed to assess the psychosocial aspects of caring, but to our knowledge, it has never been used in the setting of BMT. Objective of the study The aim of this study was to examine the variables that predict caregiver burden in four subsets of severe illness and to evaluate the indirect costs of a severe illness, particularly in the BMT setting. *Methods.* A prospective evaluation with CRA was conducted on caregivers of BMT, solid tumours, neurological and dementia patients. Between November 2003 and June 2004, 67 caregivers who assisted for at least 6 months patients undergoing BMT (N=20) or affected by cancer (22), severe neurological illness (21) or dementia (4) were analysed using the CRA questionnaire, which consists of 4 negative dimensions and one positive subscale (disrupted schedule, financial problems, lack of family support, loss of physical strength and self-esteem). The questionnaire was translated into Italian and German language and cultural and social variables were introduced outside the CRA in order to evaluate local factors. Statistical analysis was performed using the Kruskal-Wallis test for the univariate analysis, and a cluster analysis for the multivariate analysis. *Results.* The 5 factors of CRA are not significantly correlated with age, occupation, duration or first experience of caring. Caring lasting 6 to 12 hours/day was associated with a lower satisfaction ($p < 0.001$) in comparison to caring for more than 12 hours or less than 6. BMT caregivers have a significant higher self-esteem ($p < 0.0001$) and lower consequences on the physical status ($p < 0.001$). The cluster analysis identified the BMT setting for higher self-esteem and higher invested time in comparison with the other groups. Comment The CRA questionnaire seems to be useful in the setting of BMT to identify the psycho-social burden of caring. In this hematological setting self-esteem was higher and the social-psychological burden was lower, the more time was invested. This potential curative procedure and the characteristics of these patients may reduce the psychosocial stress of caregivers in comparison to other severe illnesses.

0290

VALIDATION OF THE HAEMOPHILIA-SPECIFIC QUALITY OF LIFE QUESTIONNAIRE FOR ADULT PATIENTS WITH HAEMOPHILIA (HAEM-A-QOL)

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Haemophilia and its treatment impact on the quality of life of hemophilic patients. Quality of Life (QoL) is one of the most important patient-based outcome criterion. The impact of the health condition and the effects of single treatment strategies (i.e. on-demand treatment, prophylaxis) on patient well-being can be described assessing health-related quality of life (HR-QoL). For the adequate assessment of HR-QoL validated instruments are necessary. While haemophilia-specific questionnaires for children exist (Haemo-QoL: v. Mackensen et al., 2004; CHO-KLAT: Young et al., 2004), no validated instrument for adults is up to now available, even though some developmental work has already started internationally. A multi-centre study coordinated by the University of Hamburg in cooperation with the University of Milan was performed in haemophilia centres in Italy. This validation study was sponsored by an unrestricted educational grant of BAXTER. *Aims.* This study was designed

to develop a disease-specific HR-QoL measurement for adult patients with haemophilia (Haem-A-QoL) and to validate the Haem-A-QoL in Italy. *Methods.* The study consisted of three phases: a) a qualitative phase including focus groups (patients, physicians/nurses), item generation, expert ratings (patients, physicians) and item formulation, b) a feasibility testing including feedback and cognitive debriefing and c) psychometric analysis including reliability and validity testing. *Results.* Focus groups with semi-structured interviews were performed in 32 patients from 6 Italian haemophilia centres. In addition physicians and nurses were asked the same questions. Patients and physicians/nurses considered similar dimensions important for HR-QoL (physical health, social interaction, dependence, future, work). Aspects mentioned in the focus groups were compared with items of the Haemo-QoL questionnaire-a disease-specific questionnaire for haemophilic children-in order to have a core instrument available both for children and adults. Based on focus group results a draft version of the questionnaire was formulated and given to patients and physicians in order to evaluate the items concerning importance and relevance for haemophilia and comprehensibility. The preliminary version of the Haem-A-QoL was revised (omitting and rewording of items) considering the results of expert ratings. The revised version consisted of 50 items pertaining to 11 domains. The revised Haem-A-QoL was given to more than 100 adult haemophiliacs in Italy in order to test the feasibility and to analyse the psychometric characteristics of the questionnaire. The mean completion time was 14 minutes and the questionnaire was well accepted by patients. Psychometric testing revealed satisfactory to good characteristics for reliability (Cronbach's α ranging from .66 - .93) and validity. Testing of convergent validity showed high correlation of the Haem-A-QoL with the SF-36, analyses for discriminant validity revealed significant differences between clinical subgroups such as 'severity' and 'infections'. *Conclusions.* The development of a disease-specific HR-QoL instrument requires multiple steps and the involvement of different experts (patients, physicians, nurses) in order to have a standardised and valid questionnaire available. The newly developed Haem-A-QoL is well-accepted by adult haemophiliacs and showed quite satisfactory psychometric characteristics. The questionnaire will be translated now in other languages in order to include the Haem-A-QoL in international studies.

0291

HOW IS LIFE INFLUENCED BY DISEASE AND TREATMENT IN PATIENTS UNDERGOING STEM CELL TRANSPLANTATION?

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Individual measures of quality of life (QoL) have been initiated to overcome the possible limitations of predetermined measures. The Schedule for the Evaluation of Individual Quality of Life- Direct Weighting (SEIQoL-DW) is such an instrument. In personal interviews individuals are asked to nominate the most important areas in life, both positive and negative, crucial for QoL. The nominated areas are rated regarding satisfaction and weighted to capture the importance of each selected area. The Swedish version has been extended with a disease-specific module, which evaluates what areas in life that are influenced by disease and to what extent. *Aims.* The aim of the study was to evaluate individual QoL in patients with malignant blood disorders undergoing autologous stem cell transplantation (SCT) and the patient's view of what impact the disease has had on life using the Swedish version of the SEIQoL-DW. *Methods.* Thirty-three patients (20 men and 13 women) with malignant blood disorders listed for autologous SCT entered the study consecutively. Patients were evaluated the week before start of high dose treatment, and six and twelve months after SCT. Participants were interviewed with the

Swedish version of the SEIQoL-DW to assess QoL. In addition patients were asked to fill out a standardized measure evaluating functional status, the Sickness Impact Profile (SIP). *Results.* The most commonly nominated areas (>50% of patients) important in life were family, health concerns and relations to others. The areas influenced by disease were diverse health problems (fatigue, physical limitations, psychosocial impact), view of life and oneself, family, relations to friends and work. The areas in life influenced by disease were rated as more troublesome and functional status (SIP) as poorer the week before SCT compared to six and twelve months after. Overall QoL was rated as good at all three occasions. *Summary/conclusion:* Health problems, relationships to family and friends and work-life are affected by disease the first year following SCT. Despite this, general QoL is rated fairly high in patients with malignant blood disorders following SCT. lena.wettergren@pubcare.uu.se

0292

DEVELOPMENT OF A DISEASE-SPECIFIC QUALITY OF LIFE QUESTIONNAIRE FOR CHILDREN AND ADOLESCENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP-QoL)

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Background. Quality of life (QoL) assessment is considered an important outcome measure in medicine describing patients well-being and function, evaluating treatment intervention effects and analysing quality and cost of care. Disease-specific measures are especially developed for QoL assessment in patients with a specific health condition providing a clear pattern of their symptoms or impairments. QoL assessment in children with coagulation disorders is a new area. Recently the Haemo-QoL questionnaire was developed for children with haemophilia. For children with Idiopathic Thrombocytopenic Purpura (ITP) no validated disease-specific instrument is available at the moment. Barnard et al. (2003) developed an ITP-specific questionnaire for children in Canada, which has not been validated yet and translated into other languages. *Aims.* Development and validation of a disease-specific questionnaire for children with ITP of two age groups (4-7 years, 8-16 years) and their families. *Methods.* The development of the ITP-QoL consists of three phases: a) a preparatory phase including literature search, adaptation of the haemophilia-specific questionnaire (Haemo-QoL) for children with ITP (ITP-QoL) and focus groups with children and parents in order to find additional aspects important for their health-related QoL; b) a developmental phase including implementation of additional aspects in the ITP-QoL found in the preparatory phase, translation of the newly developed ITP-QoL from English into Italian, German and Swedish and constructing proxy versions for parents; c) a pilot testing phase including filling in the questionnaire by children with acute and chronic ITP and their parents, feasibility testing of the ITP-QoL, cognitive debriefing session concerning comprehensibility and relevance of items and retesting the questionnaire after one week. In addition physicians are asked to evaluate the questionnaire concerning importance and comprehensibility of items with respect to different age groups. Clinical data are obtained as well in order to define type of disease and treatment strategies for further subgroup analysis. Data analysis are performed with regard to the psychometric properties of the newly developed questionnaire. For the analysis of reliability internal consistency as well as re-test reliability are calculated. Validity is inferred from confirmative correlation between new and standard questionnaires and discriminative between clinical subgroups. *Results.* Dimensions of the haemophilia-specific Haemo-QoL questionnaire were considered important as well for children with ITP. Items in the respective domains were adapted and reformulated for ITP. Additional dimensions were included in the ITP-QoL such as 'complaints due to treatment' and 'hospital and staff' as well as questions

concerning the received treatment. Two age group versions were constructed as an interview version for young children (aged 4-7 years) and a self-report version for older children (aged 8-16 years), two proxy versions were constructed for parents. The preliminary version of the ITP-QoL consisted of 22 and 80 items respectively to the two age groups pertaining to 8 and 12 domains respectively (treatment, complaints due to treatment, bleedings, feelings, view, family friends, perceived support, other persons, sport and school, dealing, hospital and staff). *Conclusions.* The ITP-QoL is currently pilot-tested in children and adolescents with ITP in Sweden, Italy and Germany.

0293

QUALITY OF LIFE IN PATIENTS WITH HAEMOPHILIA - FIRST RESULTS OF THE ESCHQOL STUDY

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Background. Chronic diseases such as haemophilia affect the every-day-life of patients and its treatment might impact on patients health-related quality of life (hrQoL). While hrQoL assessment is considered an important outcome criterion in clinical trials, only recently it became as well of interest in the field of haemophilia. With hrQoL assessment the impact of the health condition on the patient as well as the effects of treatment strategies can be described. In a prospective cohort study, funded by the European Commission, more than 1500 patients (4 years of age or older) with haemophilia are investigated in 22 European countries concerning clinical, quality of life and health economic outcomes (ESCHQoL Study). *Aims.* Aim of this paper is to describe first hrQoL results from the ESCHQoL study of patients with haemophilia across countries and treatment options. *Methods.* The ESCHQoL Study consist of a pilot testing phase and a field testing phase including baseline and follow-up observation after a 6-month period. Patients (children, adults) with haemophilia are asked to complete a comprehensive questionnaire concerning socio-demographic, psycho-social and health economic information and to fill in a diary during the 6-months period. Three different age group versions of the questionnaire are available for children, an interview version for young children (aged 4-7 years) and self-report versions for children (aged 8-12 years) and adolescents (aged 13-16 years). Their parents fill in a proxy version of those questionnaires respectively to childrens age groups. HrQoL is assessed with generic (children: KINDL, GHQ; adults: SF-36) and disease-specific questionnaires (children: Haemo-QoL; adults: Medtap). In parallel, clinical information is obtained from physicians. *Results.* In the pilot testing 70 patients (41 adults, 29 children) were included from seven European countries (Italy, Germany, Romania, Hungary, Sweden, U.K., France). Significant differences in hrQoL were found across countries and concerning treatment schemes. Significant differences were found in hrQoL between prophylactic and on-demand treatment in the dimensions 'physical functioning' and 'emotional role' of the SF-36. *Conclusions.* Differences between countries and treatment options were found in the pilot testing for the hrQoL of haemophiliacs which have to be examined in the field study. Preliminary results underline the importance of the aim of the ESCHQoL Study: to compare QoL outcome of haemophilia care in Europe in order to recommend future improvements.

0294

ASSESSMENT OF QUALITY OF LIFE IN PRIMARY IMMUNE DEFICIENT PATIENTS

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Background. The primary immunodeficiency disorders (PID) are abnormalities in development and maturation of the immune system and result an increased susceptibility to infection. Individuals with PID may experience frequent infection, which limit their ability to perform their activities. They may have a significant limitation on their physical and psychological well-being secondary to their illness. *Aims.* To investigate comparison between quality of life in primary immune deficient children and healthy ones a cross-sectional study was designed. *Methods.* 26 patients completed the PEDQOL (Pediatric Quality Of Life) questionnaire to evaluate their quality of life and were compared with healthy children. The impact of demographic and disease-related variables was examined in PID population. *Results.* Children with PID had lower PEDQOL scores in all dimensions compared with healthy ones (P Value<0.001) and patients with hummoral immune deficiency had better quality of life (P Value=0.04) than other PIDs. Increasing age was negatively associated with certain aspect of PEDQOL. (P Value=0.03) We found that male sex was significantly associated with worse than quality of life (P Value<0.001) and bigger family was related to worse quality of life. (P Value=0.04) Quality of life was higher in patients with completed vaccination than others. (P Value=0.04) other variables were not major predictors of quality of life. *Conclusions.* Children with PID have significantly worse PEDQOL than the healthy ones, that is indicating more attention to early diagnose and treatment or prevention of PID. Better quality of life in hummoral deficiency may be cause of good therapy for them. Advanced age and male sex were associated with worse physical wellbeing and emotional scores that may be reflect to have greater decline in ability to perform physical tasks related social activity secondary to their diseases or complications.

0295

A COST-EFFECTIVE SCREEN FOR μ -THALASSEMIA MINOR IN CHILDREN OF A SELECTED POPULATION CAN LEAD TO THE BIRTH PREVENTION OF μ -THALASSEMIA MAJOR

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Background. μ thalassemia major is a very severe and cronic autosomal recessive gentic illness that has a significant morbidity and a shortened life span. Due to paucity of clinical signs, individuals with the carrier state, μ thalassemia minor, are most often misdiagnosed. In populations with a high inter-marriage rate and high birth rate and individuals affected by this condition, many individuals of the extended families are known or suspected to have the carrier state, μ thalassemia minor. The aim of this study was to screen individuals within populations at-risk for thalassemia minor and to implement a prevention plan of thalassemia major. *Methods.* 381 complete blood tests from children between 1-18 years of age were collected during a period of 6 months in the town of Lakia, (10,000 inhabitants, 49% children). Individuals with blood samples with microcytosis (MCV of < 75 fl) were further analyzed by hemoglobin electrophoresis (HbEF) for markers of μ thalassemia minor (hemoglobin A2> 3.5%). Samples were sent for mutation analysis. Identified families with thalassemia minor individuals were offered genetic counseling with and emphasis on young couples planing parenthood. Early chorionic villy sampling in pregnancy was offered to couples with the minor. *Results.* Thirty five percent(133) of the children were anemic (corrected for age and

gender) 93 children (24.4% of samples) had MCV values <75 fl. Fifty-four children had a HbE^f sent and 20 individuals (5.2% of the study) belonging to 6 previously unknown families were diagnosed with α thalassemia minor. None of the 6 most common mutations in this population were found in these individuals. In the post-study period, 5 abortions of thalassemia major fetuses were performed and only one new child with this condition was born. No sibling of this individual was identified with thalassemia minor in the population studied. 14.9% of the population had ferritin values compatible with iron deficiency anemia, 5 had thalassemia minor as well. **Conclusions.** A cost-effective screening study for α thalassemia minor can be carried out in populations with known affected individuals, and is recommended to avoid the birth of any individual with α thalassemia major. The prevalence of iron deficiency anemia was found to be similar to that found in children from lower socioeconomic classes. A program to prevent and treat iron deficiency and other anemias is warranted. The previously unknown families carry a mutation different from those described in the target population. amoser@bgu.ac.il

Stem cells - Biology and therapy I

0296

ROLE FOR IFN- γ IN THE IMMUNOMODULATORY ACTIVITY OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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Background. Mesenchymal stem cells (MSCs) inhibit the proliferation of HLA-unrelated T lymphocytes to allogeneic stimulation, but the mechanisms involved are not fully understood. However, this effect is operational *in vivo*, as it has been shown recently that third party haploidentical MSCs can be safely infused to treat severe acute GvHD refractory to conventional immunosuppressive therapy. **Aims.** We have studied which lymphocyte subsets may be affected by human MSCs and which mechanisms may be involved in the onset of MSC inhibitory effect. **Methods.** MSCs have been generated from bone marrow aspirates of healthy donors, recruited after informed consent, and expanded in complete DMEM medium (15% FCS). MSCs have been characterized by immunophenotype and *in vitro* multilineage differentiation. The inhibitory effect of MSCs has been studied on CD4⁺, CD8⁺, CD4⁺CRTH2⁺ T cells, NK cells, and B cells isolated from peripheral blood, using assays of proliferation, CFSE labelling and BrdU uptake, Transwell \mathcal{A} experiments, evaluation of apoptosis by annexin V, flow-cytometry analysis of cytokine synthesis at intracellular level, blocking antibodies against IFN- γ receptor and Fas Ligand, and competitive inhibitors of the IDO pathway. **Results.** MSCs suppress the proliferation of both CD4⁺ and CD8⁺ T lymphocytes, as well as of NK cells, while they do not have effect on the proliferation of B lymphocytes. MSCs inhibit the capacity of T lymphocytes and NK cells to proliferate, without affecting the expression of cell activation markers, inducing cell apoptosis or mimicking or enhancing the activity of T regulatory cells. The suppressive activity of MSCs is not contact-dependent and requires the presence of IFN- γ produced by activated T cells and NK cells. Accordingly, even activated B cells become susceptible to the suppressive activity of MSCs in the presence of exogenously added IFN- γ . IFN- γ produced by activated T cells or NK cells induces MSCs to suppress in turn their proliferation by enhancing their indoleamine 2,3-dioxygenase activity. The suppressive activity of MSCs is completely abrogated by the addition of an anti-IFN- γ receptor (IFN-gR) mAb.

Conclusions. These findings allow to suggest that some disorders which are primarily mediated by T helper 1 cells, such as GvHD, might improve with the *in vivo* infusion of MSCs because of the activation mediated by T cell-derived-IFN- γ of the immunomodulatory properties of MSCs, which in turn inhibit T- and NK cell proliferation. In addition MSCs could have a role in controlling IFN- γ -dependent B cell disorders such as some autoimmune diseases.

0297

DIFFERENTIATION INTO ENDOTHELIAL CELLS FROM TWO SUBTYPES OF ADHESION CELLS IN A CORD BLOOD

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Background and Aims. A subset of leukocytes in adult bone marrow, peripheral blood, and cord blood functions as endothelial progenitor cells that incorporate into the vasculature in animal models of neovascularization. These cells were CD34⁺ and VEGFR-2⁺ (vascular endothelial growth factor receptor 2) cells. On the other hand, several reports have described that CD14⁺ cells also differentiated into endothelial cells. The basic mechanisms by which differentiation proceeds are still unclear. This study analyses the *in vitro* differentiation of CD34⁺ and CD14⁺ cells in cord blood. **Methods.** Mononuclear cells (MNCs) of cord blood were cultured on fibronectin (FN) coated culture plates in EBM-2 medium containing 5% fetal bovine serum, vascular endothelial growth factor, and basic fibroblast growth factor. After 7 days, phenotypes and morphology of adherent cells were analyzed. After sorting two subtypes of adherent cells cultured for 7 days, which were different cell size, each subtype cell was cultured on FN coated culture plates in EBM-2 medium. Their differentiation was followed using cytological and immunohistochemical techniques. **Results.** During MNCs were cultured on FN coated plates, two subtypes of adherent cells, which were different cell size, appeared within 7 days. Large size cells (B type cells) were temporary seen until 3 weeks. On the other hand, small size cells (A type cells) were continuously seen. Whereas both type cells expressed CD31, CD45, and VEGFR2, A type cells only expressed CD34 and B type cells strongly expressed CD14. Thus, the phenotype of these type cells was different. However, these both type cells differentiated into endothelial cells during the culturing on FN coated plates for 3 weeks after isolation of each type cells. Therefore, these endothelial cells differentiated from each type cells expressed CD31, CD34, CD144, VEGFR2, and von Willebrand factor and not to CD14 and CD45. To determine whether the growth of A and B type cells were different, the number of each type cells was measured weekly. B type cells were grown more than A type cells. **Conclusions.** It was possible to have carried out differentiation into endothelial cells from two different subtype cells of cord blood. The one-type cells were mainly CD34⁺ cells and the other type cells were mainly CD14⁺ cells.

0298

THE 'ENDOTHELIAL PROGENITOR CELLS' (EPC) SHARE PHENOTYPICAL MARKERS WITH CELLS OF THE MONOCYTIC-MACROPHAGE CELL LINEAGE

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Introduction. The presence of circulating Endothelial Progenitor Cells (EPC) in adult humans has been recently suggested and its implication in neovascularization is under intensive investigation. In the present study we have attempted to iden-

tify the EPC, which have colony forming capacity within 7 days of culture, and look for their immunophenotypic profile. *Material and Methods.* 42 healthy donors were analysed. Median age was 38 years and M/F ratio was 19/23. Cells were obtained from bone marrow (n=7), steady-state PB (n=20), apheresis products (n=4) and buffy-coat products (n=9). EPC were obtained by culturing MNC in IMDM, 20% fetal calf serum, penicillin-streptomycin, VEGF and b-FGF. At day 7 the colonies were counted and immunophenotype and immunohistochemistry studies were carried out on them. Sequential studies were performed on days +14, +21 and +35. The following primary antibodies were analysed by immunohistochemistry in an Opti-max Plus equipment: CD45, vWf, Lysozyme and CD31. Phenotypical characterization was performed using the following monoclonal antibody combinations (FITC/PE/PerCP/APC): -/-/CD45/CD34; CD31/CD133/CD45/CD34; CD105/CD4/CD45/CD34; CD15/CD13/CD45/CD33; CD14/CD16/CD45/CD34; cytoplasmic control/CD14/CD45/CD34; lysozyme/CD14/CD45/CD34. Data acquisition was performed in a FAC-Scalibur flow cytometer (BDB) and the analysis performed with the paint-a-gate program (BDB). Von Willebrand gene expression was analysed by real-time RT-PCR according to the Taq-Man[®] Gene Expression Assays protocol. HUVEC were used as positive control for vWF. Monocytes were isolated from buffy-coat products and growth in the same conditions as EPC until day +21. *Results.* The mean number of EPC colonies at day 7 was significantly higher in BM (813±695) than in steady-state PB (21.2±2.5), while mobilised PB displayed intermediate values (272±274). Immunohistochemistry analysis of these colonies showed that they were positive for CD45, CD31 and lysozyme and negative for vWf. Phenotypical analysis showed that they were positive for CD4, CD13, CD14, CD31, CD33, CD45 and lysozyme, weak positive for CD15 and CD105, and did not express CD16 or CD133. This phenotypic profile remained unchanged in all time-points analysed. By contrast, monocytic cells didn't form colonies at day 7 of culture but cord-like structures could be seen in 7 out of 9 cases. Flow cytometry analysis showed that the immunophenotype of cultured monocytes at day +21 was identical to that pre-cultured monocytes and both were similar to those obtained with EPC. The expression of V-cadherin was weak on monocytes, but after 12h of adhesion monocytes became positive and we observed that these cells had a weak VE-cadherin expression, which strongly increased after the adhesion procedure. PCR analysis showed small amounts of vWF transcripts in EPC as well as in monocytes and its expression increased after the culture in presence of VEGF. *Comments:* Our results in both cell subtypes suggest that the so called EPC and monocytic cells share similar markers, including some specific marker such as lysozyme or VE-cadherin.

0299

POSSIBLE INVOLVEMENT OF BONE MARROW MESENCHYMAL STEM CELLS IN THE PATHOPHYSIOLOGY OF RHEUMATOID ARTHRITIS

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Background. There is evidence suggesting that the bone marrow (BM) may be involved in the pathophysiology of rheumatoid arthritis (RA) by providing abnormal mesenchymal stem cells (MSCs) in peripheral blood that gradually replace the normal synovial cells in the affected joints. In favour of this hypothesis are previous data from our Laboratory suggesting abnormal support of haemopoiesis by patient long-term BM culture adherent stromal cells. *Aims.* To probe further the role of BM in the pathophysiology of RA by evaluating the quantitative, molecular and functional characteristics of BM MSCs in RA and exploring their osteogenic and chondrogenic potential. *Methods.* BM mononuclear cells (BMMCs) from RA patients (n=5) and healthy controls (n=10) were isolated from BM posterior iliac crest aspirates and MSCs were expanded according to established protocols. MSCs were characterised by flow cytometry

(CD45RA⁻, CD105⁺, CD144⁺, CD90⁺, CD73⁺) and their adipogenic, chondrogenic and osteogenic capacity. For the quantification of MSCs in the BMMC fraction at day-0, we performed a limiting dilution assay by culturing 100-10000 BMMCs in 96-well culture plates and enumerating the positive for colonies wells after 6-weeks of culture. For the evaluation of the functional characteristics of MSCs we studied (a) their clonogenic potential using a standard colony forming unit-fibroblast (CFU-F) assay and enumerating the CFU-Fs/100 MSCs plated through passages (b) their proliferative potential time-course by using the MTT assay and evaluating the cell doubling time ($2^n = \text{cells counted}/\text{cells plated}$) in each passage. We also studied the gene expression level of pro-inflammatory cytokines involved in RA including IL1 β , TNF α , IL6, IL8, and IL15 and also of genes regulating cytokine expression such as the wnt/fz family members by means of RT-PCR. Finally, we quantitated the chondrogenic (collagen II and aggrecan), osteogenic (alkaline phosphatase) and adipogenic (PPR α and AP2) potential of RA MSCs at the gene expression level by RT-PCR. *Results.* We found that the number of MSCs at day-0 did not differ significantly between RA patients (2.6±1.9/10,000 BMMCs) and controls (2.5±2.0/10,000 BMMCs). The immunophenotypic characteristics of MSCs did not differ between patients and controls. Compared to healthy controls, however, patient MSCs displayed impaired CFU-F forming potential (F165=21.747, P <0.001; passage 1-5), impaired proliferative capacity in the MTT assay (F156=57.989, P <0.001 at passage 1) and increased cell doubling time (F148=58.509, P <0.001; passage 1-7). Patient MSCs at passage 2 displayed increased IL1 β , TNF α , Wnt5A expression compared to controls suggesting a genotype similar to that of the synovial fibroblasts. The chondrogenic and adipogenic potential of patient MSCs at passage 2 did not differ from the respective of the controls in terms of collagen II and aggrecan expression and PPR α and AP2 expression, respectively. The osteogenic potential, however, quantitated by the alkaline phosphatase expression was found decreased in the RA patients studied. *Conclusions.* BM MSCs in RA display defective clonogenic and proliferative potential, defective osteogenic potential and abnormal expression of IL1 β , TNF α , Wnt5A. These data raise the possibility that abnormal BM-derived MSCs in RA may contribute or sustain the joint damage in the disease.

0300

CYTOKINE-INDUCED EXPRESSION OF HOMING RELATED ADHESION MOLECULES ON CD34⁺ CORD BLOOD STEM CELLS AND CELL ENGRAFTMENT IN NOD/SCID MICE

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Adult human haematopoietic stem cells have a higher plasticity than previously suspected. That was demonstrated with respect to their differentiation into organ specific cell types. Stem cell homing, engraftment and tissue repair is a multistep process and little is known about the mechanisms that determine homing and transdifferentiation process of stem cells. Stem cell differentiation and organ regeneration are controlled by growth factors, cytokines and chemokines. In vitro, homing-related characteristics, cell cycle phase, proliferation rate, expression of adhesion molecules and the interfilament proteins vimentin and cytokeratin 8/18 on CD34⁺ cord blood cells were examined by flow cytometry and immunohistochemistry. Untreated cord blood derived CD34⁺ cells are mainly in the G0/G1 cell cycle phase, expressed adhesion molecules CD11a, CD18, CD49d, CD31, CD54, CD44, CD62L on a low level, were vimentin positive and negative for hepatocyte specific marker cytokeratin 8/18. The stem cell factor (SCF) stimulated cell proliferation, increased the S- and G2/M cell cycle phase as well as the expression of the integrin adhesion molecules CD11a, CD18 and CD49d. The expression of cytokeratin 8/18 was increased and vimentin remained unchanged. Hepatocyte growth factor (HGF) did not induce stimulation of cell prolifer-

ation and expression of the adhesion molecules, a decrease of vimentin expression was associated with an increased expression of cytokeratin 8/18. Using NOD/SCID mice, blood distribution, homing and engraftment of naive and cytokine-stimulated CD34+ cord blood cells were studied in different organs at various time points, as measured by FACS analysis and human-specific PCR. Kinetics of stem cell distribution in blood revealed a fast elimination of human cells from mouse blood 20min after application. In parallel, an increase of engrafted stem cells in a time-dependent manner in different mouse organs, preferentially in bone marrow and spleen was registered. SCF preincubated CD34+ cells showed no significant additional effects on engraftment in organs of mice. Chemically (CCI4) induced liver injury increased the number of engrafted stem cells in the liver (2fold). Additionally, HGF i.p. administration stimulated further the engraftment of untreated and SCF-treated stem cells in the bone marrow (28% human cells with HGF versus 18% in control) and spleen. Further experiments are intended to elucidate the potential of adult stem cells for organ regeneration after cancer or degenerative diseases.

0301

EFFECT OF PTH ON HEMATOPOIESIS IN HUMAN AND MURINE LONG-TERM BONE MARROW CULTURES

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Expansion of hematopoietic stem cells (HSC) is frequently needed for efficient repopulation after HSC transplantation. Stimulation of HSC proliferation with growth factors *ex vivo* is accompanied by their differentiation into more mature precursors rather than HSC expansion. Parathyroid hormone (PTH) that regulates calcium metabolism might influence osteoblast functions, particularly in view of their being an important part of hematopoietic stem cell 'niche'. Long-term bone marrow culture (LTBMC) capable to support long-term maintenance of HSC was used for investigation of possible mechanisms of HSC expansion without differentiation. The effect of PTH treatment on hematopoietic precursor kinetics in LTBMC was studied. Rat or human synthetic PTH (1-34) was used in concentration 10⁻⁷M since the initiation of LTBMC for the whole period of cultivation (10 weeks). Cell number, CFU-C (CFU-GM, BFU-E), CAFC and LTC-IC production were periodically measured. Gene expression was detected by semi-quantitative RT-PCR. PTH treatment decreased total cell production in murine culture only. The number of CFU-C in murine culture treated with PTH was 1,2-1,7 fold lower during first 6 weeks; at 10 weeks CFU-C number increased up to 6 fold versus control cultures. During 9 weeks in human LTBMC treated with PTH the number of CFU-C gradually increased from 1,1 to 2,25 fold. The increase of CAFC 28-35 concentration ranged from 1,3-1,8 fold after 3 weeks to 7 fold after 10 weeks in culture. The survival of CAFC 28-35 in 2-step culture on PTH-treated and untreated irradiated adherent cell layers (ACL) differed in human and murine cultures. Unlike in murine, in human system the number and 1-5 days survival rate of CAFC-35 on ACL treated with PTH was 2-3 fold higher than on untreated ones. To prove that the stromal and not the hematopoietic cells are main target of PTH the bone marrow cells were seeded on irradiated ACLs treated with PTH for 4-6 weeks. Both in human and murine cultures the expansion of early and late hematopoietic precursors was higher on the PTH-treated ACL. The analysis of hematopoiesis-related gene expression revealed the increase of Bmi-1, Notch and Jagged-1 in the PTH treated LTBMC for the first 3 weeks in culture only. The revealed ability of PTH to increase the number of early hematopoietic precursors in LTBMC might be investigated for expansion of different populations of HSC.

0302

KINETICS OF MOBILIZATION OF PROGENITOR STEM CELLS INTO PERIPHERAL BLOOD IN THE ACUTE CORONARY SYNDROME

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Background. Adult stem cells may contribute to myocardial regeneration after ischemic injury. Previous studies have shown a mobilization of progenitor stem cells into peripheral blood in patients with ST elevation acute myocardial infarction in comparison with healthy control subjects. The aim of this study was to assess the dynamics and magnitude of the mobilization of progenitor stem cells into peripheral blood in relation to the degree of necrosis and the inflammatory response across the spectrum of the acute coronary syndrome. **Methods.** Twenty-seven patients with acute coronary syndrome; 9 with ST elevation myocardial infarction (STEMI), 10 patients with non-ST elevation myocardial infarction (NSTEMI), and 8 patients with unstable angina (AI) were enrolled. The percentage of progenitor stem cells (CD 34+, CD 133+ and CD 117+) in peripheral blood was assessed using a flow cytometer (Facsan - BD) on admission, daily during the first week, at days 14, 21 and 30 after the event. Data are expressed as the absolute number of cells per ml. (median and range), related to the absolute leucocyte number. Correlation coefficients were established using Spearman's Rho test. **Results.** Mobilization of progenitor stem cells occurs since the first day from the onset of symptoms, peaking on day 4th, and is detectable until 1 month. No significant differences were documented between patients with STEMI, NSTEMI and AI in relation to the maximum absolute number of CD34+ : 4.12 [3.18-6.84]; 3.90 [3.36-6.16]; 5.60 [3.25-7.03], p=0.89, CD 133+ : 4.20 [2.12-7.52]; 4.01 [3.14-5.23]; 3.20 [2.24-4.50], p=0.92 and CD117+ : 5.29 [3.72-7.29]; 4.89 [3.32-5.89]; 5.16 [3.20-7.49], p=0.83. The correlations between these parameters and the degree of necrosis (determined by the maximum troponin T levels) and inflammatory response (determined by the maximum C reactive protein levels) were non significant. **Conclusions.** Mobilization of progenitor stem cells into peripheral blood occurred in similar amounts in the different clinical presentations of acute coronary syndrome, though no correlation with the amount of myocardial damage and the degree of inflammation was found. These data suggest that progenitor stem cell mobilization response to myocardial damage is not sufficient to prevent left ventricular remodelling.

0303

CONDITIONING REGIMENS IN STEM CELL TRANSPLANTATIONS FACILITATE HOMING BY INCREASING BONE MARROW SDF-1 THROUGH INDUCTION OF SDF-1 GENE TRANSCRIPTION

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Background. Stromal Derived Factor-1 (SDF-1) is a key regulator of engraftment. The clinical relevance of SDF-1 is suggested by the relation between *in vitro* migratory capacity of CD34-positive cells towards SDF-1 and engraftment after stem cell transplantation (SCT). For better insight in engraftment after SCT clarifying the determinants of SDF-1 is therefore important. **Aims.** We investigated SDF-1 responses after chemotherapy and irradiation in allogeneic SCT patients and *in vitro* using the murine stromal M210B4 cell line. **Methods.** SDF-1 protein levels were measured in conditioned medium (CM) of the M210B4 cell line, in peripheral blood (PB) plasma and bone marrow (BM) plasma by ELISA. The migration supportive capacity of CM was tested by allowing CD34-positive cells to migrate over fibronectin coated transwells for 4 hours. Furthermore, sdf-1

gene expression was investigated by means of a quantitative PCR using mRNA from M210B4 cells or from buffy coats of peripheral blood and bone marrow cells. **Results.** Both non-myeloablative and myeloablative regimens induced a significant ~3-fold increase in SDF-1 protein levels in BM and a ~1.5-fold increase in PB SDF-1 protein levels (n=5, both p<0.05), thereby clearly increasing SDF-1 BM-PB gradients (p<0.05). A corresponding increase in sdf-1 mRNA copies (r=0.92) was observed, indicating that induction of sdf-1 gene expression is involved in the conditioning regimen-induced increase in SDF-1 protein levels. Different conditioning regimens were found to diversely affect SDF-1 protein levels and sdf-1 gene expression. Accordingly, *in vitro* experiments showed a significant ~2-fold increase in SDF-1, both after irradiation and cytarabine treatment of M210B4 cells, which resulted in significant increased migration of CD34-positive cells. Moreover, the increase in SDF-1 after irradiation and chemotherapy was found to be dose and time dependent. Summary/conclusions Conditioning regimens increase BM SDF-1 levels through induction of gene transcription, thereby facilitating homing. Interestingly, the variation in increase of SDF-1 after different conditioning regimens and *in vitro* data indicate that homing efficiency may be improved by reconsidering the optimal moment of stem cell infusion and the type of conditioning.

0304

STEM CELL MOBILIZATION WITH PEGFILGRASTIM VS. FILGRASTIM IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES: CORRELATION OF MOBILIZATION EFFICACY AND ADHESION MOLECULE EXPRESSION

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Background. A single dose of Pegfilgrastim (6 mg; Neulasta, Amgen, USA) has been proven safe and highly efficient in stem cell mobilization after chemotherapy in patients with hematologic malignancies. It is known that the crosstalk between adhesion molecules, bone marrow microenvironment, and cytokines facilitates the multi step process of stem cell mobilization from bone marrow to peripheral blood. Investigating healthy stem cell donors we found a negative correlation of the extent of VLA-4 and LFA-1 with mobilization efficacy. **Aims.** The present study should evaluate if CD34-positive cells of patients with malignancies show differences in the expression of adhesion molecules (VLA-4, L-selectin, LFA-1, PECAM-1, CD44), of chemokine receptor CXCR4 and of G-CSF receptor according to mobilization efficacy. In addition, the expression of the mentioned antigens was compared after Pegfilgrastim (39 pts.) and Filgrastim (62 pts) mobilization. **Methods.** We investigated 101 aphereses with a 4-color flow cytometric procedure: CD34/CD45 combined with two different adhesion molecules in each sample. The quantitative (mean fluorescence intensity) and qualitative (%) antigen expression was assessed. Patients were divided into three groups according to peripheral CD34-positive cell count at the day of apheresis: $\leq 30/\mu\text{L}$ (poor mobilizer; 41 pts.), 31-100/ μL (standard mobilizer; 29 pts.), $> 100/\mu\text{L}$ (good mobilizer; 31 pts.). **Results.** First, evaluating all 101 aphereses together the quantitative antigen expression was significantly higher in poor vs. good mobilizing patients for VLA-4 (GeoMean: 66 vs. 48; p<0.0001), LFA-1 (23 vs. 16; p=0.0349). In contrast, CD44 showed a significantly lower expression in poor vs. good mobilizing patients (814 vs. 940; p=0.0223) and L-selectin a trend in the same direction (264 vs. 326; p=0.0697). Second, considering the percentage of antigen positivity, only L-selectin expression was significantly different between poor and good mobilizing patients (91% vs. 95%, p=0.0077). VLA-4, PECAM-1, and CD44 were expressed in 100% of CD34+ cells independent of mobilization capacity. Finally, comparing mobilization regimen, adhesion molecule expression revealed to be comparable in poor and standard mobilizers with respect to Pegfilgrastim or Filgrastim application. But within the good mobilizers the quantitative expression of PECAM-1 and the

qualitative expression of L-selectin was significantly lower after Pegfilgrastim compared to Filgrastim mobilization. **Conclusions.** Our analyses suggest that in patients with hematologic malignancies a higher VLA-4 and LFA-1 expression is associated with a reduced mobilization efficacy whereas a higher L-selectin expression is observed in good mobilizers. The expression of most tested antigens was comparable after Pegfilgrastim and Filgrastim mobilization. Only PECAM-1 and L-selectin expression seemed to be regulated differently in the good mobilizers group.

0305

DIFFERENTIAL SUBCELLULAR LOCATION OF CD91 (LDL RECEPTOR-RELATED PROTEIN) IN LYMPHOCYTE SUBSETS

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Background. The LDL receptor-related protein (LRP, also known as CD91) is a ubiquitously-expressed receptor involved in the endocytosis of circulating and matrix-bound components. In addition, LRP has been demonstrated to associate with and to control the function of other receptors that display a cell-restricted expression patterns. For instance, we have recently shown that LRP forms a functionally active complex with beta2-integrin adhesion molecules at the surface of monocytes and granulocytes (Spijkers et al. (2005) Blood 105:170). **AIM:** Although the presence of LRP in cells of the myeloid-lineage has been well recognized, little is known regarding the expression and function of LRP in lymphocyte cells. The present study was designed to analyze the expression pattern of LRP in various lymphocyte cells: CD4+ and CD8+ T-lymphocytes, B-lymphocytes and natural killer (NK)-cells. **Methods.** Various lymphocyte-subsets were isolated using specific microbeads-conjugated antibodies and MACS separation. LRP mRNA was detected by RT-PCR. LRP protein was analyzed using Western blotting, confocal microscopy and flow cytometry. **Results.** In each of the isolated lymphocyte cells (CD4+ and CD8+ T-lymphocytes, NK-cells and B-lymphocytes) LRP mRNA as well as LRP protein could be detected, as analyzed by RT-PCR and Western blotting, respectively. Confocal microscopy and FACS analysis demonstrated that LRP was predominantly localized at the cell surface of virtually all NK-cells and B-lymphocytes. In contrast, LRP appeared to be located in intracellular pools in CD4+ and CD8+ T-lymphocytes. We considered the possibility that LRP could be translocated to the cell surface upon stimulation of these cells. This was first tested by stimulating T-lymphocytes using interleukin-2 and phytohemagglutinin. Interleukin-2 and phytohemagglutinin stimulation resulted in a transient increase of cell-surface-located LRP protein. After 48 h, the number of T-lymphocytes containing LRP at the cellular surface had increased from 2% till 12%, which was followed by a gradual decline to baseline levels in the subsequent 5 days. In a second approach, changes in LRP-surface expression were monitored in a mixed-lymphocyte reaction. Incubation of peripheral blood mononuclear cells derived from two different donors resulted in a near immediate translocation of LRP to the cell-surface in 60-70% of the T-lymphocytes. By using isolated cells, we found that this translocation occurred exclusively in the presence of monocytes. **Conclusions.** Our data clearly show that CD4+ and CD8+ T-lymphocytes, NK-cells and B-lymphocytes contain LRP mRNA and protein. However, there is a dissimilar localization of LRP protein in these cells. LRP is located at the cell surface in NK-cells and B-lymphocytes, whereas LRP is present intracellularly in T-lymphocytes. The observation that LRP can be rapidly recruited to the cellular surface in T-lymphocytes upon interaction with non-autologous monocytes may point to a role of LRP in the pathophysiology of transfusion-related complications, such as graft-versus-host disease.

0306**THE INFLUENCE OF ADMINISTRATION ORDER OF BUSULPHAN AND CYCLOPHOSPHAMIDE ON DENDRITIC CELLS IN STEM CELL TRANSPLANTATION MOUSE MODEL**C. Nilsson¹, J. Forsman¹, Z. Hassan¹, M. Abedi-Valugerdi¹, C. O'Connor², H. Concha², M. Jansson¹, R. Tehrani¹, M. Hassan¹¹Karolinska Institutet, STOCKHOLM, Sweden; ²University of Dublin, DUBLIN, Ireland

Busulphan (Bu) in combination with cyclophosphamide (Cy) is used frequently as a preparative regimen for patients undergoing stem cell transplantation (SCT) for both malignant and non-malignant disorders. However, the treatment related toxicity is still the dose-limiting factor. The order in which busulphan and cyclophosphamide is administered may influence both the adverse effects and the therapeutic efficacy of Bu/Cy regimen and also the amount of dendritic cells. Host dendritic cells can stimulate CD4+ donor cells and cause GVHD. *Aims.* In the present study, we investigated the effect of the administration order of Bu/Cy on the myeloablative-, immunosuppressive effect and the recovery of dendritic cells and their origin in a mouse model. *Methods.* Female balb-C mice were divided in two groups, the first group received liposomal busulphan (15 mg/kg/dayx4) followed by Cy (100 mg/kg/dayx2) while the second group was given Bu/Cy in reversed order. At the day of transplantation (day-0) donor (8-10 weeks old males) marrow cells (sca1) were isolated from femurs, separated and infused via the tail to the recipients (6x10⁵ cells per mouse). Mice were sacrificed during the conditioning and up to 30 days after the treatment. *Results.* No significant difference was observed in the myeloablative effect between both treatment groups. The immunosuppressive activity as expressed as CD3+/CD19+ and CD4+/CD8+ was similar for both treatment schedules. The cytokines (IL-2, TNF- α and IFN- γ) detected at day 0 were lower when Cy was given first compared to that observed when Bu was given first. CD11C+ from donor origin was significantly higher from day +1 until day +9. The administration of Cy prior to Bu resulted in significantly higher CD 86+ cells between day 0 and day +5 compared to that found in mice received Bu prior to Cy. Dendritic cell origin was detected using fluorescence in situ hybridization (FISH) by labelling Y-chromosome (from the donor). The labelled cells were counted as the percent of 500 cells from each animal. *Summary.* we conclude that Cy-Bu treatment has resulted in lower levels of cytokines that most probably can be less harmful for the new stem cells, faster engraftment and higher ratio of donor dendritic cells. This treatment strategy may be a promising strategy to reduce transplantation related complications.

Thrombosis and thrombophilia I**0307****TISSUE FACTOR (TF) MRNA EXPRESSION PATTERN IN HUMAN NEUTROPHILS. EVIDENCE FOR A NEUTROPHIL-DERIVED TF**

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Background. Tissue factor (TF), apart from its key role in thrombosis, is currently believed to be implicated in both infection and inflammation. In addition, an alternative splice of TF (asTF) that is soluble and is implicated in thrombus formation and propagation has been recently described. Neutrophils represent the first line defense against bacterial infections, and play an important role in innate immunity; however a great inconsistency exists regarding neutrophils' TF expression and generation. *Aims.* To investigate the ability of neutrophils to

express TF and asTF, as well as their potential contribution in the circulating TF. *Methods.* We studied the TF mRNA expression pattern by amplifying the entire coding sequence of TF using semi-quantitative RT-PCR. Freshly separated peripheral blood neutrophils, originated from 12 healthy individuals, were examined before and after stimulation with LPS and interferon α (IFN α). Immunocytochemistry was performed in order to confirm the RT-PCR findings. In addition, a modifying Prothrombin Time (PT) was performed using supernatant of unstimulated and stimulated neutrophils, in order to estimate the thrombogenic activity. *Results.* Seven out of 12 healthy subjects showed no TF and asTF mRNA expression in neutrophils under normal conditions. Two individuals expressed both TF and asTF mRNA, while in the remaining three subjects only asTF mRNA expression was observed. Stimulation with LPS and IFN α resulted in strong induction of TF and asTF expression in all individuals, with a shift towards greater asTF expression. Functional assays demonstrated the thromboplastic activity of neutrophils' supernatant after stimulation with IFN α . *Summary/conclusions:* Our findings indicate that unstimulated neutrophils from healthy individuals present a diversity of TF and asTF expression. This expression can be increased dramatically after *in vitro* stimulation with LPS or IFN α , and the supernatants derived from stimulated cells have TF thromboplastic activity. The *in vitro* induction of TF and asTF from neutrophils, under certain conditions, suggest their potential contribution in circulating TF.

0308**STUDY OF GENETIC RISK FACTORS FOR CARDIOVASCULAR DISEASE IN THE IRANIAN POPULATION BY MEANS OF REVERSE-HYBRIDIZATION TESTSTRIPS**C. Oberkanins¹, S.H. Amini², A. Ghamari³, K. Kahrizi³, H. Puehringer¹, H. Najmabadi³¹ViennaLab Labordiagnostika GmbH, VIENNA, Austria; ²Karimnejad Genetic Center, TEHRAN, Iran; ³Soc Welfare Rehab Science University, TEHRAN, Iran

A number of genetic and environmental risk factors have been found or suspected to predispose to cardiovascular disease (CVD), the term collectively used for disorders of the heart and blood vessels. We have developed a reverse-hybridization assay (CVD StripAssay) for the rapid and simultaneous detection of twelve candidate CVD risk factors (Factor V Leiden, Factor V R2, Prothrombin G20210A, Factor XIII V34L, beta-Fibrinogen -455 G-A, PAI-1 4G/5G, GPIIIa L33P, MTHFR C677T, MTHFR A1298C, ACE Ins/Del, Apo B R3500Q, Apo E2/E3/E4). The test is based on multiplex PCR and hybridization to a test-strip presenting a parallel array of allele-specific oligonucleotide probes for each mutation. We have applied these teststrips to investigate the prevalence of CVD risk mutations among 208 asymptomatic Iranians from different regions and ethnic groups. The allele frequencies of mutant Factor V Leiden (1.2%) and Prothrombin G20210A (0.5%) in our cohort were below previously published figures on the population of Tehran (2.7% and 1.5%, respectively; Zeinali et al. 2000). Mutant MTHFR C677T (24.8%) and Factor XIII V34L (14.2%) occurred less frequently than among Europeans, but exceeded the much lower frequencies known from India and most of Asia. The prevalence of mutant MTHFR A1298C in our study population (41.8%), however, was remarkably high. Apo E2 (4.6%) and E4 (5.8%) alleles were observed in relatively low frequencies compared to population studies in Europe and the USA. Our comprehensive population data should represent a valuable basis for further investigation on the contributions of genetic CVD risk factors in Iran.

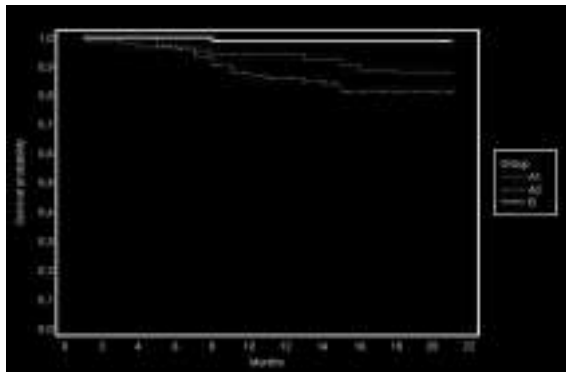
0309

RESIDUAL VEIN THROMBOSIS ASSESSMENT ESTABLISHES THE OPTIMAL DURATION OF ORAL ANTICOAGULANTS IN PATIENTS WITH IDIOPATHIC OR PROVOKED DEEP VEIN THROMBOSIS: A RANDOMIZED, CONTROLLED TRIAL

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The use of individual parameters, such as Residual Vein Thrombosis (RVT), for deciding the optimal duration of oral anticoagulants (OA) has not been yet evaluated. Aim. In this study we prospectively investigated patients with a first episode of DVT using RVT for assessing the duration of OAT. During 3 years, 252 patients with DVT of the lower limbs (either idiopathic or provoked) were evaluated. *Methods.* At the 3rd month of OA, patients were classified as having or not RVT, as previously described (Siragusa et al., *Thromb Haemost* 1993;538:1435). Patients with RVT were randomly assigned to continue OA for 1 year (group A1), or to stop it (group A2). In patients without RVT, OA was discontinued (group B).



All patients were followed-up for additional 12 months after OAT discontinuation. *Results.* 218 patients completed the study; the incidence and timing of combined events (recurrent thrombosis and major bleeding) is reported in the figure. *Conclusions.* This investigation shows that patients without RVT maintain a low risk of recurrences even in absence of OA, no matter the type of DVT (either idiopathic or provoked).

0310

THROMBOPHILIA IN WOMEN WITH UNEXPLAINED FETAL LOSS

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Recent investigations highlight the potential role of thrombophilia for determining fetal loss.

Aim. The purpose of this study was to investigate the possible role of inherited and acquired thrombophilia in women with unexplained abortions and intrauterine fetal death. *Methods.* We studied 49 women with a first episode of unexplained late fetal loss (fetal death after 20 weeks or more of gestation) and 48 women who had one or more normal pregnancies and no late fetal losses. All the women were tested for the common tests for acquired and congenital thrombophilia. The results are shown in the table. The presence of LAC, Factor V Leiden, Factor II mutation, combined defect and hyperhomocysteine levels (fasting and post-methionine loading) determined an increased

risk of fetal loss in cases when compared to controls; this was statistically significant. *Conclusions.* The presence of acquired and congenital thrombophilic factors is associated with an approximate tripling of the risk of late fetal loss.

	Patients	Controls	Odds Ratio (95% Confidence Interval)	p value
No. of patients	49	48		n.s.
Mean age, y	28.6±7.4	27.8±7.8		n.s.
Thrombophilia				
LAC	30%	2.1%	4.9 (1.7-13.19)	<0.04
Other (Fibrinogen C and S, AT, A-PCC)	4.28%	0%		
FV Leiden	12.2%	4.2%	2.9 (1.1-4.7)	<0.04
FII mutation	16.3%	6.3%	2.6 (1.2-4.0)	<0.04
MTHFR C677T	49%	35.4%	1.4 (0.6-2.0)	n.s.
MTHFR A1298C	34.2%	27.5%	1.9 (0.2-1.6)	n.s.
Combined defects	32.7%	4.28%	7.8 (2.2-3.4)	<0.05
Fasting homocysteine levels (mean±SD in µmoles per liter)	14.8±14.5	9.8±7.5		<0.05
PostL homocysteine levels (mean±SD in µmoles per liter)	34.2±16.5	11.5±6.4		<0.05

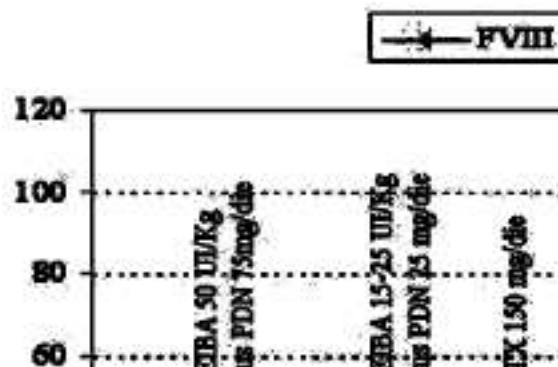
0311

SUCCESSFUL TREATMENT OF ACQUIRED HAEMOPHILIA WITH FEIBA® AND RITUXIMAB®

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Few evidences are present on the best treatment for managing patients with acquired haemophilia. The efficacy of activated prothrombin complex concentrate (FEIBA®) for stopping severe bleeding has been tested but a concern regards the risk of thrombosis. Immunotherapy is often unable to eradicate auto-antibodies against FVIII; the role of Rituximab® (an anti-CD20 monoclonal antibody) in this setting is still under investigation. We report a case of a patient with unstable angina who developed severe bleeding episodes (hematoma and hemoarthrosis) due to acquired hemophilia. *Methods.* FEIBA® was administered at a dosage from 20 to 50 UI/Kg accordingly to the bleeding episodes; steroids and cyclophosphamide were administered, but unsuccessfully. *Results.* Rituximab® was therefore initiated at standard dosage (375 mg/m² i.v. once weekly for 4 consecutive weeks) determining the lack of auto-antibodies after 8 months (figure). Our case highlights the role of FEIBA® to control bleeding without increasing the risk of thrombosis, even in this patient with unstable angina. Therapy with Rituximab® achieved a full response after 8 months, a period of time longer than previously (1-3 months) reported.



0312**FACTOR VIIIa REGULATES SUBSTRATE DELIVERY TO THE INTRINSIC FACTOR X-ACTIVATING COMPLEX**

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The intrinsic factor X (fX)-activating complex is composed of the enzyme factor IXa (fIXa), the cofactor factor VIIIa (fVIIIa) and the substrate factor X (fX) assembled on a negatively charged phospholipid (PL) surface. The exact mechanisms of assembly of the fX-activating complex remain insufficiently understood. *Aims.* Our study was aimed to explore formation of intermediate membrane-bound binary complexes of fIXa, fVIIIa, and fX in the course of assembly of the fX-activating complex and their roles in its functioning. Using flow cytometry, we analyzed the equilibrium binding of coagulation factors (individually and in their various combinations) to 0.8- μ m synthetic phospholipid vesicles (25/75 phosphatidylserine/phosphatidylcholine) in order to detect and quantitate formation of binary complexes, and subsequently analyzed their effects on the rate of fIXa catalysis. The binding of fluorescein-labeled factors demonstrated that fVIII (fVIIIa), fIXa-EGR, and fX bind to 32700 ± 5000 (33200 ± 14100); 20000 ± 4500 ; and 30500 ± 1300 binding sites per vesicle with apparent Kd of 76 ± 23 (71 ± 5), 1510 ± 430 , and 223 ± 79 nM, respectively. fVIII binding was not affected by the presence of either fIXa or fX. In its turn, fVIII at 10 nM induced appearance of additional high-affinity sites for fIXa (1810 ± 370 , 20 ± 5 nM) and fX (12630 ± 690 , 14 ± 4 nM), and fX at 100 nM induced appearance of high-affinity sites for fIXa (541 ± 67 , 23 ± 5 nM). Studies of the kinetics of the fX binding to PL vesicles revealed that the presence of fVIII (a) led to a 4-fold increase of the fX affinity thus confirming effective formation of the high-affinity fVIII (a)/fX complex on the PL surface. In the fIXa generation assay, the apparent Michaelis constant of fX activation by fIXa was a linear function of fVIIIa concentration, with the slope of 1.00 ± 0.12 and intrinsic Km of 8.0 ± 1.5 nM. By titrating both fVIIIa and fX in parallel binding and fIXa generation assays, we demonstrated a positive correlation between the fIXa generation and the fX binding to fVIIIa. This suggested that formation of fVIII (a)/fX complex regulates fX activation, most likely due to the 'anchoring' role of fVIII (a) which provides additional binding sites for fX on the PL surface. To test whether fX binds to membrane-bound fVIII (a) directly (*free substrate model*) or via initial interaction with the PL membrane (*bound substrate model*), we titrated phospholipid concentration (10-1000 μ M), phosphatidylserine content (12.5-50%) of vesicles and fX at two fVIIIa concentrations (1.5 and 12 nM) in fIXa generation assay. These studies revealed that at high fVIIIa concentrations the delivery of fX to the fVIII (a)/fIXa complex follows the 'free substrate' model. *Conclusions.* Our study revealed effective formation of the high-affinity fVIII (a)/fX complex on the phospholipid membrane in the course of assembly of the fX-activating complex and suggested its role in regulating the rate of fIXa generation. Two fundamental functions were earlier ascribed to the cofactor fVIIIa in fIXa catalysis: (1) enhancement of the catalytic constant of the reaction and (2) increase of the amount of phospholipid-bound enzyme fIXa. Our data suggest an additional function of fVIII (a) in increasing the amount of phospholipid-bound substrate fX and its delivery to the fX-activating complex.

0313**PREVALENCE OF PROTHROMBOTIC INHERITED ABNORMALITIES AND CENTRAL VENOUS CATHETER-RELATED THROMBOSIS IN PATIENTS WITH HAEMATO-ONCOLOGICAL DISEASE**

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A central venous catheter (CVC) is commonly used for variety of indications. The benefit derived from these devices can be offset by thrombosis, which may be complicated by pulmonary embolism and CVC dysfunction. Often, thrombosis may force premature CVC removal and the need for anticoagulant treatment with its concomitant bleeding risk. *Aims.* In this prospective study, we assessed the incidence of CVC-related thrombosis in patients with haemato-oncological disease. We determined the contribution of prothrombotic inherited abnormalities in blood coagulation, to CVC-related thrombosis in these patients. *Methods.* This prospective study was conducted between May 2002 and April 2004 at the 'National Center for Bone Marrow Transplantation', Tunisia. CVCs were externalized, non-tunneled, polyurethane double lumen catheters (Arrows, Readings, USA). All CVCs were placed in the subclavian vein by infraclavicular approach, in the operating room. Catheters were inserted percutaneously, using the Seldinger technique. Before catheter's insertion, laboratory prothrombotic markers included the factor V Leiden, the prothrombin gene Gly20210A mutation, plasma antithrombin levels, and protein C and S activity. All patients, were systematically examined by ultrasonography just before, or < 24 hours after, catheter removal, and in case of clinical signs of thrombosis. Two radiologists performed the ultrasonography. *Results.* A total of 147 patients were included during the 24-month study period [76 Male, 71 Female, Median age: 28 years (4-60 years)]. Median duration of catheterization was 35 days (7-45 days). Five (3.4%) and three (2%) patients presented a protein C and protein S deficiency, respectively. Only one patient had an antithrombin deficiency (0.7%). Two patients (1.4%) were heterozygous for the factor V Leiden mutation. We observed a CVC-related thrombosis in 9 patients (6.1%). Two of the 9 patients presented a prothrombotic inherited abnormality (one factor V Leiden mutation and one protein C deficiency). The incidence of CVC-related thrombosis was higher in patients with a prothrombotic inherited abnormality (2/11 versus 7/136; $p=0.04$). *Conclusions.* Our results suggest that prothrombotic inherited abnormalities contribute substantially to CVC-related thrombosis in patients with haemato-oncological disease. In view of physicians' reluctance to prescribe prophylactic anticoagulant treatment in these patients, a priori determination of prothrombotic inherited abnormalities may form a basis to guide these treatment decisions.

0314**EXPERIMENTAL STUDIES ON THE RELATIVE EFFICACE OF HEPARIN-LIKE ANTI-COAGULANT FROM PLANT AS ANTITHROMBOTIC AGENT**

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Background Low molecular weight heparin (LMWH) shows anti-factor Xa and antithrombotic activities. A number of plants (Filipendula ulmaria and Paeonia suffruticosa) has heparin-like component of low molecular weight which are identical LMWH an animal origin is revealed. It is shown antithrombotic effect of plant anticoagulant from roots of a peony at chronic peroral injection. *AIMS.* The purpose of the present work consist in revealing ability plant heparin (PH) from Paeonia suffruticosa to prevent formation of fresh blood clots at intranasal administration PH. *Methods.* In the present work methods elec-

trophoretic, chromatographic and spectral analysis were used. The formation of blood clots was carried out on method Wessler with our updating - in isolated by metal clips a fragment vein jugularis injected thrombin with activity 3-5 sec (thrombin + stas of vessel) . PH was administrated intranasal to animals (albino rats) in daily doses 0.5 mg/kg of body weight within 3-5 days before formation of blood clots. Antithrombotic effect estimated on frequency of cases of thrombus formation and on weight of blood clots in 1 hour after thrombus formation. *Results.* When forming blood clots beside rats on background of the action of PH thrombus or were not formed or in case of their formation were small. So, quantity of cases of thrombus formation in experiment(on a background administrated PH) made 63 per cent from the control (administration 0.85 per cent solution of NaCl). The average weight of the formed blood clots in experiment made only 18-20 per cent from the control (100%). When the dose of PH was increased to 1.0 mg/kg, there was a fall in the incidence of thrombosis to 58 per cent which was not significantly different from the effect achieved with 0.5 mg/kg PH. If the dose of PH was increased to 2.0 mg/kg, there was minimal evidence of thrombus formation (< 10 per cent). Thus it has not been marked any collateral negative influences PH (for example, haemorrhagic action). *Conclusions.* The difference in antithrombotic effectiveness between 2.0 mg/kg of the PH and either the 0.5 mg/kg or the 1.0 mg/kg dose is highly significant. So low molecular weight heparin-like anticoagulant from peony can serve antithrombotic agent without side-effects.

0315

DETERMINATION OF CROSS-LINKED FIBRIN DEGRADATION PRODUCTS USING DD2-DD6 ANTI-D-DIMER MAB PAIR: A POSSIBLE SOLUTION OF D-DIMER STANDARD PROBLEM

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Background. D-dimer is a smallest and relatively stable fibrin degradation product. It is a marker of hypercoagulation and widely used especially to exclude deep venous thrombosis. Despite the widespread use of D-dimer test in clinical practice, it is not clear what is really measured by anti-D-dimer kits. A wide range of D-dimer precursors of different molecular weight can be expected to be present in plasma. It makes D-dimer not a proper standard for fibrin degradation products (FDP) determination if antibodies used have different specificity to D-dimer and to high molecular weight FDP. *Aims.* The aim of the work was to study the sensitivity of three MAb anti-D-dimer pairs to D-dimer and to FDP in plasma. *Methods.* D-dimer was produced from clotted fibrinogen by streptokinase induced plasmin digestion and subsequent chromatography purification. ELISA using anti-D-dimer MAbs DD1, DD2, DD3, DD4, and DD6 (Hytest, Finland) were performed. *Results.* The sensitivity of MAb pairs to D-dimer was determined as about 100 ng/mL using DD1-DD6, 200 ng/mL using DD2-DD6, and 25 ng/mL using DD3-DD4 systems. The titration curves of plasma as a source of high molecular weight FDP were compared with curves of purified D-dimer titration. It was found that titration curves had different slopes when DD1-DD6 or DD3-DD4 pairs were used. It means that these antibodies react differently with D-dimer and with high molecular weight FDP. In addition, the sensitivities of these pairs to D-dimer were higher than to high molecular weight FDP. At the same time, the titration curves of D-dimer and high molecular weight FDP were parallel when DD2-DD6 pair was used. It means that these antibodies have the same specificity to D-dimer and to high molecular weight FDP. *Conclusions.* Thus, D-dimer can be used as a standard for the determination of cross-linked materials in plasma using DD2-DD6 pair at least as 'D-dimer units'.

0316

VALUE OF ROTATION THROMBELASTOMETRY (ROTEG) IN PREDICTING THE RISK OF PARANEOPLASTIC VENOUS THROMBOEMBOLISM-A PILOT STUDY

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Background. Venous thromboembolism (VTE) is a frequent and well known complication of malignant disease. As it causes a considerable burden of morbidity and mortality, diagnostic methods, able to identify patients at high risk to develop VTE, are urgently warranted. Up to now no single laboratory-test has been introduced into clinical practice that would allow us to predict the risk of an individual patient to develop VTE and such might justify prophylactic anticoagulation. As the coagulation-cascade is a complex system involving many single factors and steps, measuring a single component might be inferior to performing a functional assay like ROTEG-analysis in identifying a hypercoagulable state. *Aims.* Determination of the ability of ROTEG-analysis to detect hypercoagulability in patients with metastatic malignant disease. *Methods.* 22 consecutive patients with newly diagnosed metastatic malignant disease (7 colorectal carcinomas, 7 non small cell lung cancers, 2 pancreatic carcinomas, 2 soft tissue sarcomas, 1 gastric carcinoma, 1 pharyngeal carcinoma, 2 adeno-carcinomas of unknown primary site) were analyzed by using ROTEG. These results were then compared with a control group of 50 healthy volunteers. *Results.* Looking at the ROTEG-analyses, two patterns of results could be observed. While 9/22 patients showed values within the range of our control group, 13/22 patients had pathological results on their ROTEG-tests. These patients had shorter clotting times (mean 496,8 sec. versus 578,2 sec.), shorter clot forming times (mean 132,1 sec. versus 273,4 sec.) and increased maximum clot firmness (mean 70,6 mm versus 51,8 mm) in the nativ NATEG-assay. Activation of the intrinsic coagulation system by INTEG-assay resulted in no difference concerning clotting time but clot forming time (mean 36,4sec. versus 64,1sec.) and maximum clot firmness (mean 71,9mm versus 59,8 mm) were substantially different. Exactly the same results were generated by the activation of the extrinsic coagulation system (EXTEG-assay). The mean clot forming times were 40,8sec versus 84,6sec.. The mean values for maximum clot firmness were 68,5mm and 61mm respectively. *Conclusions.* ROTEG-analysis seems able to define two different groups within a population of patients with metastatic malignant disease with one of them showing signs of hypercoagulability like shortened clotting time, clot forming time and increased maximum clot firmness. Whether these patterns of results can be used to predict the risk of VTE remains to be confirmed by larger prospective studies.

0317

RISK FACTORS FOR THROMBOPHILIA IN EXTRA-HEPATIC PORTAL VEIN OBSTRUCTION

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Information on the role of thrombophilia in extra-hepatic portal vein obstruction (EHPVO) is scanty. *Aims.* To assess the association between thrombophilia and EHPVO. *Methods.* Sixty-five patients with EHPVO, 500 with deep vein thrombosis (DVT) of the lower limbs and 700 healthy controls were referred for thrombophilia screening including the search for gain-of-function mutations in genes encoding coagulation factor V (factor V Leiden) and prothrombin (prothrombin G20210A); antithrombin, protein C and protein S deficiency; and hyperhomocysteinemia. *Results.* At least one abnormality in the thrombophilia screening was found in 40% of patients with either EHPVO or lower limb DVT and in 13% of controls, for odds ratios of 4.0 (95%CI 2.3-7.0) and 4.4 (95%CI 3.3-5.9), respectively. Statistically significant associations with EHPVO

were observed for the prothrombin G20210A mutation [odds ratio 8.1 (95%CI 3.8-17.5)] and the deficiencies of antithrombin, protein C or protein S taken together [odds ratio 4.5 (95%CI 1.1-18.0)]. The odds ratio for the prothrombin G20210A was approximately twice that for lower limb DVT. Patients with factor V Leiden had an odds ratio for EHPVO of 0.8 (95%CI 0.1-6.4) and for lower limb DVT of 7.5 (95%CI 4.4-13.0). The odds ratio for EHPVO in patients with hyperhomocysteinemia was 2.0 (95%CI 0.9-4.9). At variance with lower limb DVT, oral contraceptive use was not associated with an increased risk of EHPVO. Myeloproliferative disorders were diagnosed in 35% of patients with EHPVO. *Conclusions.* The risk for EHPVO is increased in the presence of thrombophilia due to the prothrombin G20210A mutation and to the deficiencies of the naturally occurring anticoagulant proteins, but not to factor V Leiden.

0318

THE PERFORMANCE OF IMMUNOTURBIDIMETRIC D-DIMER ASSAY IN OUTPATIENTS WITH SUSPECTED PULMONARY EMBOLISM (PE)

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Several studies have shown that D-dimer testing can reliably be used to exclude PE in outpatients. A number of D-dimer assays are available in clinical practice, but these assays have different sensitivities and specificities to detect thrombosis. Immunoturbidimetric D-dimer assays are rapid quantitative assays run on automated analyzers. One such assay used by many laboratories has not been sufficiently evaluated in clinical studies and its optimal cut-off value has not been clearly defined. *Aims.* to evaluate the performance and to determine the optimal cut-off value for the immunoturbidimetric latex agglutination D-dimer (Liatest, Stago) in outpatients referred for suspected PE, by a prospective outcome study. *Methods.* 495 consecutive patients referred to Østfold Hospital Trust - Fredrikstad, Norway, for suspected PE between Feb. 2002 and Dec 2003, were recruited to a clinical trial evaluating a decision based algorithm combining D-dimer and multi-slice computer tomography (MSCT). D-dimer was performed as a first step test. PE was considered absent in patients with D-dimer <0.4 mg/L if the clinical probability (CP) for PE was low or intermediate. Patients with normal D-dimer but high CP and patients with elevated D-dimer proceeded to MSCT.

Cut-off (mg/l)	0.3	0.4	0.5	0.6	0.7
Sensitivity	100%	100%	99%	96%	96%
Specificity	31%	36%	43%	48%	53.6%
NPV	100%	100%	99.3%	97.5%	97.7%
LR+	1.4	1.5	1.7	1.8	2.0
LR-	0	0	0.02	0.08	0.07

Thus, the results of follow-up alone served as the gold standard in patients with D-dimer ≤0.4 and low or intermediate CP and MSCT in addition to the follow-up results in the remaining patients. Patients with no PE were followed-up for 3 months to assess the 3-month thromboembolic risk. Sixty-three patients were excluded and the final cohort consisted of 432 patients. PE was diagnosed in 102 (23%) patients. 120 (28%) patients had D-dimer value <0.4 mg/L. The CP for PE was non-high in 103 patients and high in the remaining 17. Those with high CP (n=17) proceeded to MSCT; none had PE. The 3-month thromboembolic risk in patients with D-dimer <0.4 mg/L was 0% (95% CI 0-3.7); sensitivity 100%, specificity 38.8%, and negative predictive value (NPV) 100%. The effect of increasing the cut-off value on the performance is shown in the table. The 3-month thromboembolic risk for patient with D-dimer <0.7 was

0%. The prevalence of PE was 3.8%, 27% and 50% for the respective D-dimer intervals: 0.5-0.9, 1.0-1.9, ≥2.0. A 0.4 mg/L seems to be the optimal cut-off value for this assay. At that cut-off, the test safely ruled out PE regardless of the CP. The performance of the test is comparable to that reported for ELISA based D-dimer.

0319

A NORMAL D-DIMER AND A LOW CLINICAL PROBABILITY TEST SAFELY EXCLUDES VENOUS THROMBOEMBOLISM. A FOLLOW-UP D-DIMER ADDS NO FURTHER INFORMATION

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Venous thromboembolism (VTE), i.e. deep vein thrombosis (DVT) and pulmonary embolism (PE) are common conditions at the Emergency Room (ER). The diagnosis is often a challenge because the clinical signs and symptoms are not sufficient to establish or exclude the disease. Of patients presenting with symptoms suggestive of deep vein thrombosis only twenty-five percent have the disease. It is therefore needed to find a simple, reproducible diagnostic tool to find those patients with a low probability of having the disease. Earlier studies have shown that in patients with low clinical probability a normal D-dimer safely obviates the need for further radiological investigation. *Aims.* The aim of this study was to find out if a low clinical probability using Wells pre-test probability score together with a normal D-dimer safely exclude VTE in out-patients at our hospital and, if a follow up D-dimer test increases the sensitivity of this algorithm. *Methods.* 130 patients (68% women) presenting at the ER during February until October 2004 with suspected VTE and having a low pre-test probability score according to Wells and a normal D-dimer test (TinaQuant®) were included in the study. Exclusion criteria were an elevated D-dimer (i.e. >0.5 mg/L) and/or high clinical probability according to the Wells score. A follow-up D-dimer test was made 3-7 days after inclusion and if abnormal additional investigations were made to exclude VTE. All patients were contacted by phone by one of the study-doctors after 3 months. During the period 199 (57% women) patient were excluded, 21% of these had VTE. 109/199 had a D-dimer test made; none of the patient with a normal D-dimer had VTE. *Results.* There were 101 (78%) patients with suspected DVT and 29 (22%) with suspected PE in the study. 101/130 (78%) had a follow-up D-dimer, and in 13% of the patients the second test was elevated. None of these had VTE. 130/130 (100%) had a follow-up after 3 months, none of these patients reported any clinical signs of VTE giving this algorithm 100% sensitivity in excluding VTE. The study is ongoing and an intermediate result will be presented at the congress. A normal pre-test probability score according to Wells and a normal D-dimer safely exclude VTE. A follow-up D-dimer test adds no further information.

0320

PREOPERATIVE AND POSTOPERATIVE ANTITHROMBOTIC PROPHYLAXIS IN SURGICAL PATIENTS WITH INHERITED THROMBOPHILIA-OUR EXPERIENCES

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Inherited thrombophilia (IT) is defined as an abnormality of the clotting and/or fibrinolytic cascade which leads to a hypercoagulable state. IT is recognized as a major risk factor for venous thromboembolism (VTE). Each year in Poland approximately 60 000 person suffer from deep venous thrombosis (DVT) and approximately 20 000 people die of pulmonary embolism (PE). The aim of this study is to show our experiences in preoperative and postoperative antithrombotic prophylaxis in surgical patients with IT. From January 2000 to December 2004 in Department of General and Hematological Surgery in Warsaw 30 patients with IT (18 women, 12 men)

aged from 28 to 63 years (average 45, 4) were operated. Each of them had in history from 1 to 4 episodes of DVT and/or PE and they were examined for IT. Those patients have had following defects: in 15 cases factor V Leiden mutation, in 7 protein S deficiency, in 5 protein C deficiency, in 2 prothrombin gene G20210A mutation, in one case protein S deficiency and prothrombin gene G20210A mutation. In surgical patients with IT we have performed the following surgical procedures: 8 laparoscopic cholecystectomy, 5 abdominal hernioplasty, 3 inguinal hernioplasty, 6 excision varicose veins of the legs and 4 simultaneous excision of the crural ulceration with skin grafting, 3 excision of the popliteal cyst, 2 splenectomy, one resection of the haemorrhoidal varices and one appendectomy. All of surgical patients with IT before operation have taken chronically oral anticoagulant to keep INR between 2, 0-3, 0. Each of them has withdrawn oral anticoagulant 2 days before operation and in the same day they have received once a day half of the treatment dose of LMWH. In the day of operation half of the treatment dose of LMWH was divided into two equal parts. First part of LMWH was given 2 hours before surgical procedure and second 12 hours after operation. In the next days patients have received once a day half of the treatment dose of LMWH. In 4th day after operation there was added treatment with oral anticoagulant. When INR was between 2, 0-3, 0 during two following days LMWH was withdrawn. In all patients with IT intermittent pneumatic compression or graduated compression stockings was used during surgical procedures and later until the moment of full mobilization. *Results.* We did not observe any symptoms of DVT and/or PE in clinical examination in all operated patients with IT in 3-months follow up after surgical procedures. We used Doppler ultrasonography for detection of postoperative DVT. In none of patient with IT we did not detect episodes of DVT in this examination. We did not observe any intraoperative and postoperative hemorrhagic complications. Outcome measures were: clinically overt bleeding and the amount of postoperative blood loss. These results suggest that our model of preoperative and postoperative prophylaxis in patients with IT is safe and efficacious for the prevention of VTE.

0321

LOW DOSES OF STREPTOKINASE IN THE LOCAL TREATMENT OF AXILLARY SUBCLAVIAN VEIN THROMBOSIS IN PATIENTS WITH FACTOR V LEIDEN MUTATION

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Axillary subclavian vein thrombosis (ASVT) usually occurs in the place of junction of three large anatomical areas: neck, shoulder girdle, and thorax. This place is especially exposed to traumas that may induce inflammatory reaction. Additional anatomical factors such as: cervical rib, abnormal clavicle variant, callus around clavicle fracture, abnormal attachment of anterior scalene muscle may lead to external compression and thrombosis. Another causative factor may also be thrombophilia. Thrombosis following physical effort is defined as Paget-von Schroetter syndrome. In approximately 12% of cases the symptoms of pulmonary embolism are found. The most frequent symptoms of this syndrome are swelling and pain of upper limb. The patients are treated according to the rules approved for the lower limb vein thrombosis management. A complete resolution of occluded veins occurs in 15-30% of cases. Aim. The aim of our study was to assess the efficacy of local thrombolytic therapy with the use of low doses of streptokinase applied in local continuous infusion directly into the thrombus. Material. Two young women aged 19 and 26 years were observed with the signs and symptoms of ASVT, lasting 48 hours, caused by effort stress to the left shoulder girdle. The main symptoms were swelling and pain in the left upper limb. Method. Diagnosis of ASVT was confirmed by Doppler flow ultrasonography then the catheter was introduced through cephalic vein in the thrombus vicinity under phlebographic con-

trol and a continuous streptokinase (Streptase®) infusion (20 000 U.I./h for 48 hours) was started. Phlebography was performed after 24 and 48 hours to assess the treatment efficacy. Two hours after completing thrombolytic treatment the continuous infusion of standard heparine for 5 days was applied with simultaneous oral anticoagulant administration beginning from the second day. *Results.* In both patients a complete vein recanalisation was achieved; clinical symptoms also resolved during further treatment. The factor V Leiden mutation was later detected in both patients. Secondary anti-thrombotic prophylaxis was restarted and continued indefinitely. *Conclusions.* Our experience shows a possibility of administration of local thrombolytic therapy in patient with freshly diagnosed axillary subclavian vein thrombosis. It seems to us that this treatment may be safely applied in thrombophilic patients.

Thrombosis and thrombophilia II

0322

HAEMORHEOLOGIC ADVANTAGE OF SPINAL OVER GENERAL ANAESTHESIA IN NIGERIANS: A LABORATORY BASED EVIDENCE

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Altered hemorheology has been linked with regional and general anesthesia while a 12 percent reduction in fibrinogen concentration has been reported after spinal anaesthesia in Caucasians. It is therefore assumed that these changes is only attributed to Caucasians, hence there are no literatures on Africans especially Nigerians. We have therefore attempted to compare the effect of spinal and general anaesthesia on rheologic parameters of Nigerians, to ascertain its possible effects and any geographical variations. *Methods.* Rheological variables were assessed longitudinally at pre (before surgery and anaesthesia), Post 1hr, 24hr, 48hr and 72hrs in twenty surgical patients having different clinical conditions comprising of 13 spinal and 7 general anaesthetic subjects. They were compared with 10 apparently healthy individuals as controls. The parameters studied include plasma and whole blood viscosities (PV and WBV respectively), plasma Fibrinogen concentration (PFC), Euglobulin lysis time (ELT), hematocrit(HCT) and Erythrocytes sedimentation rate (ESR). Spinal anaesthesia recorded statistically reduced PV, WBV and PFC from 48th hr and sustained throughout the experimental duration, while ESR and ELT showed a significant increase as early as 24th hr post operation ($p < 0.05$ respectively). General anaesthesia showed a non significant decrease in all the parameters except ESR at 24th hr post operation ($p < 0.05$). Improved haemorheology coupled with hypofibrinolytic activity is a major underscore of spinal anaesthesia while general anaesthesia exhibited a stable rheologic indices. The use of spinal anaesthesia where possible is further emphasized.

0323

EFFECT OF THE MTHFR C677T POLYMORPHISM ON HOMOCYSTEINE LEVELS

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The polymorphism C677T in the gene for methylenetetrahydrofolate reductase (*MTHFR*) results in an amino acid substitution, alanine to valine. The mutation renders the *MTHFR* protein thermolabile. Thermolabile 5,10-methylenetetrahydrofolate reductase is a cause of mild hyperhomocysteinemia. High

serum homocysteine concentrations are suggested to be a risk factor for arterial and deep vein thrombosis. *Aims.* In this study, the effect of *MTHFR* C677T on serum homocysteine levels was investigated. *Methods.* 96 individuals aged 36.9 ±13.1 years (34 men/62 women, matched in age, 37.7±11.1 / 36.9±10.4 years, non-diabetics, without vitamin B12, or folate deficiency) were enrolled. *MTHFR* genotypes were analyzed using PCR amplification and digestion with restriction endonuclease *Hinf*I. Total homocysteine levels (tHcy) were determined by ACS:180@ Automated Chemiluminescence Systems (Bayer). Statistical analysis was performed according to genetic results as well as to gender in combination to genetic results. *Results.* Genotypes frequencies were 42.7% (41 total, 11 men, 30 women) for C/C, 46.9% (45 total, 20 men, 25 women) for C/T and 10.4% (10 total, 3 men, 7 women) for T/T. The frequencies of alleles were 0.66 for C allele and 0.34 for T allele. Total homocysteine levels (mean±SD, μmol/L) in C/C, C/T, T/T groups were 11.88±3.47, 13.43±5.06, 14.36±7.07 respectively. There was no statistical difference between the three mean values ($p>0.05$). The elevation of tHcy levels for C/T heterozygous genotypes and T/T homozygous genotypes compared to C/C homozygous genotypes was 1.55 μmol/L (13%) and 2.48 μmol/L (20.8%) respectively. The frequencies of alleles were 0.62 and 0.69 for C allele and 0.38 and 0.31 for T allele in men and women respectively. C/C homozygous and C/T heterozygous men have statistically higher tHcy concentrations than C/C homozygous and T/T heterozygous women [13.84±4.33 vs 11.16±2.85, $p=0.025$, and 15.64±5.4 vs 11.67±4.22, $p=0.005$, elevation: 2.68 μmol/L (24%) and 3.97 μmol/L (34%), respectively]. There was no statistical difference in tHcy levels between T/T homozygous men and women. In our study, polymorphism C677T of *MTHFR* gene seems to contribute to a mild elevation in serum tHcy levels, but without statistical significance. Therefore, men showed higher tHcy concentrations as compared to women. This may be the effect of acquired, lifestyle-related factors (smoking, nutrition, alcohol). Both *MTHFR* C677T polymorphism and serum homocysteine levels should be estimated in a thrombophilia investigation.

0324

THROMBOPHILIA IN INFLAMMATORY BOWEL DISEASES

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Patients with inflammatory bowel disease (IBD) are increased risk for thromboembolic complications. *Aims.* To investigate factor V Leiden G1691A, methylentetrahydrofolate reductase (MTHFR) C677T, and prothrombin G20210A mutation as thrombophilic factors in patients with IBD and without thromboembolism. *Methods.* Twenty-seven patients with 22 colitis ulcerosa (CU) and 5 Crohn's disease (CD), and 47 healthy were included to the study. The DNAs were obtained from peripheral blood by using pure polymerase chain kit. Factor V Leiden G1691A, prothrombin G20210A and MTHFR C677T mutations were investigated in DNA by using Light Cycle. *Results.* The heterozygote FV Leiden G1691A mutation was detected in 3 (11.1%) patients with IBS and 2 (4.2%) controls ($p>0.05$). Heterozygote prothrombin G20210A mutation was detected in 2 (7.4%) patients with IBS and none of controls ($p>0.05$). Heterozygote MTHFR C677T mutation was found in 10 (37%) patients with IBS and 15 (32%) controls ($p>0.05$). Homozygote MTHFR C677T mutation was detected in 4 patients (14.9%) with IBS and 3 (6.3%) controls ($p>0.05$). There was neither homozygote prothrombin G20210A and nor factor V Leiden G1691A mutations in both groups. *Conclusions.* We found that there was not relationship between IBD and these hereditary thrombophilic factors.

0325

VASCULAR ENDOTHELIAL PROGENITOR CELLS (EPC) AND EPC RELATED FACTORS OF HUMAN CORD BLOOD DERIVED FROM PRETERM INFANTS

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Bone marrow and peripheral blood of adults contains endothelial progenitor cells (EPCs) that participate in neovascularization. EPCs have an important role on vasculogenesis and they are reported to be enriched in cord blood (CB) of mature infant. The hematopoietic stem/progenitor cells are more enriched in umbilical CB of preterm infants than CB of term infants. But little is known about EPCs from human CB of preterm infants. We therefore compared EPCs derived from preterm infants with term infants. We also assessed the factors which mobilize EPC in CB. *Methods.* and *Results.* CBs were obtained in accordance with institutional guidelines including guardian's informed consent. We classified preterm group as gestational age less than 36 weeks (n=27) and term groups as more than 37 weeks (n=27). Mononuclear cells (MNCs) of CB were subjected to flowcytometry analysis to examine surface expression of CD34, CD133, VE-cadherin, VEGFR-2 (KDR), and VEGFR-3 (flt-4). Phenotypic analysis showed that CD133 (+) KDR (+) cells, which are thought to be EPCs, were significantly enriched in preterm group ($p<0.05$). To further examine the existence of EPCs in CB, MNCs were cultured on human fibronectin-coated plastic plates. The numbers of spindle-shaped attaching (AT) cells were counted under a phase-contrast microscope. AT cells which represent presumptive EPCs were more enriched in preterm CB ($p<0.05$), which was consistent with phenotypic analysis. ELISA assay showed that the concentrations of VEGF, stem cell factor (SCF) and stromal derived factor 1alfa (SDF) were elevated in the CB of preterm infants. In addition, CD34 (*) VEGFR3/flt-4 (+) cells, which are thought to be presumptive lymphatic EPCs, were also significantly enriched in preterm group ($p<0.05$). We also detected that the VEGF-C, the ligand of VEGFR3, was higher in preterm group ($p<0.05$). *Conclusions.* These data suggest that the frequency of EPCs in umbilical CB of preterm infants is higher than in CB of term infants. The high concentration of VEGF, SCF and SDF-1alfa seems to be associated with the enriched EPCs in preterm CB. Moreover, CB of preterm infants seems to be a much more robust source for not only vascular EPCs but also presumptive lymphatic EPCs.

0326

PREVALENCE OF FV G1691A, PT 20210A AND MTHFR C677T MUTATIONS AMONG NATIVE CRETAN CHILDREN

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The prevalence of thrombophilia mutations varies among different populations according to their genetic origin. *Aims.* The aim of this preliminary study was to evaluate the prevalence of Factor V G1691A (FV-Leiden), prothrombin G20210A and methylentetrahydrofolate reductase (MTHFR) C677T mutations among native Cretan children. *Methods.* Fifty one children were evaluated that were consecutively included in the study as they were admitted in the Dept of Pediatrics for common childhood diseases. The study was approved by the Hospital's Ethics Committee and consent was obtained from the patients' parents. One ml of peripheral blood collected in EDTA during the routine blood sampling at admission, was used in order molecular diagnosis for the presence of FVL, PT G20210A and MTHFR C677T mutation (FV-PT-MTHFR strip assay[®], Viennalab

Labordiagnostika GmgH, Austria) to be performed. The family history as regards maternal fetal loss, ischemic stroke, deep venous thrombosis or premature onset cardiovascular disease in any of the patient's parents or grandparents was recorded. The allele frequencies were estimated using the Hardy-Weinberg Equilibrium. **Results.** Thirty out of 51 children (58.8%) were positive for the presence of at least one mutation (95%CI=45.3-72.3%). Single carriers were 25 pts (49%) and combined carriers were 5 pts (9.8%). The carrier frequency (95%CI) was for FV Leiden 11.7% (3-20%), for PT G20210A 3.9% (0-9%) and for MTHFR C677T mutation 52% (39-66%). The corresponding allele frequencies were 5.9%, 2.9% and 34.3% respectively. Children positive for thrombophilia mutations had an increased probability of positive family history related to premature thromboembolic complications compared to children without thrombophilia mutations: 19 out of 30 positive children had a positive family history (63%) versus 6 out of the 21 negative children (28%) ($p < 0.05$). Additionally a maternal history of premature fetal loss was more often present in positive patients (7 out of 30) versus negative patients (3 out of 21), but the difference was not significant. **Conclusions.** In this preliminary study, FV Leiden, PT G20210A and MTHFR C677T mutations were common among native Cretan children and associated with a positive family history for premature cardiovascular or thromboembolic disease.

0327

THE ASSOCIATION BETWEEN FACTOR XIII VAL34LEU POLYMORPHISM AND EARLY MYOCARDIAL INFARCTION

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Factor XIII catalyses covalent bonds between fibrin monomers, thus increases the clot stability and resistance to fibrinolysis. Congenital FXIII deficiency is a rare disorder, which causes bleeding diathesis. Recently, G to T point mutation in codon 34 of exon 2, which encodes the Valin-Leucine change in the A subunit close to the thrombin activation site has been described. Factor XIII Leu34 allele has been reported as to be protective against myocardial infarction (MI) in some studies, although other studies have found no association. **Aims.** The aim of this study was to investigate the association of FXIII Leu34 allele with myocardial infarction. A hundred and thirty patients who had myocardial infarction before the age of 60 and 130 healthy control subjects were included in our study. Genomic DNA had been extracted from venous blood samples and a PCR method was used to genotype FXIII Val34Leu polymorphism. **Results.** The distribution of genotype frequencies came out to be different in MI cases (92.31% Val, 7.69% Leu) compared to controls (80.77% Val, 19.23% Leu; X², $p=0.0001$). Patient and control groups were separated into two age subgroups (18-50, 51-60). The difference between patient and control groups was not statistically significant in the 51-60 age subgroup. In the 18-50 age subgroup, the difference was extremely significant ($p < 0.0001$). Our findings support the hypothesis that VL polymorphism in factor XIII gene has a protective effect against MI, especially in younger patients.

0328

INCREASED FREQUENCY OF THROMBOTIC RISK FACTORS IN HEMOPHILIACS

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Hemophilic patients not only are born with their coagulopathy, but also carry their bleeding tendency throughout their

entire life. Therefore, it would be sensible to expect from these patients a high frequency of bleeding episodes. However, life itself proves that this is not the case, though the reason has not yet been explained. In fact, haemophiliacs bleed mainly under certain and specific circumstances (trauma, surgery, etc). Aim To bring some light in the prophylactic mechanism that seems to have developed. Material: We examined blood samples from 65 patients, 47 normal individuals- 29 men and 18 women with mean age 37 ± 12 and 58 hemophiliacs with mean age 42 ± 11 . **Methods.** 14 coagulation factors and natural anticoagulants were studied as well as the 3 mutations related to thrombophilia (FVLeiden, FIIIG20210A, and MTHFR C677T). Patients with levels of natural anticoagulants of less than 75%, or levels of coagulation factors of more than 135% were designated as having one or more risk factors for thrombotic events. We used Compact STA (STAGO) for measurement of natural anticoagulants level, and BCS (Dade-Behring) for measurement of coagulation factors level. Mutations were detected with classic RFLP PCR analysis. **Results.** There were 3 subjects with a single risk factor detected among the control group (2 with FV-Leiden, 1 with deficiency of FXII and 1 with elevated levels of FVIII). In contrast 50 from the 58 subjects in the haemophiliacs group were found to be carriers of one or more risk factors. More particular, low levels of PC were detected in 6 haemophiliacs, low levels of PS in other 6, low levels of AT in 4, of plasminogen in 2, while 3 haemophiliacs showed a significant increase in FII levels, 7 in FV levels, 6 in FVII levels, 5 in FIX levels, 1 in FXI levels. 13 were found with reduced levels of FXII, 12 with increased levels of vWF:RiCof activity and 20 with increased levels of vWF:Ag. Abnormal aPCr was detected in 10 haemophiliacs, and heterozygosity for FVLeiden mutation or prothrombin G20210A polymorphism in 13 haemophiliacs. 16 haemophiliacs were MTHFR677T homozygous, and 4 more were both FVLeiden heterozygous and MTHFR677T homozygous. Chi square criterion was applied to determine the statistic difference between the 2 groups and x² value of 24.05 ($p < 0.0002$) is considered to be statistically significant. It is worth noted that only 8 subjects were found to be risk factor free. 14 carried 1 risk factor, 14 carried 2 risk factors, 8 carried 3, 9 carried 4, and 5 carried 5 risk factors. The above results are in line with the observation that hemophilic patients bleed only after trauma, surgical intervention etc. It seems probably that during the million years of human life on earth one or more compensatory mechanisms have developed. These appear to play a significant role in the rate of bleeding episodes in haemophiliacs and may also be able to explain the increasing frequency of thrombotic events in these patients.

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ANTITHROMBIN-III LEVELS IN NEWBORNS WITH SEPSIS

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Hemostatic system in newborns differs from adults. Spontaneous thrombosis does not occur despite the natural anticoagulant levels are low at birth. But when some risk factors are added to the physiological anticoagulant and fibrinolytic hypofunction hemostatic balance easily moves to thrombosis in pathological cases in newborns. Fibrin formation occurs in microvascular bed with the systemic activation of coagulation and inflammation in sepsis. **Aims.** We aimed to search fibrinolytic system and its natural inhibitors in healthy newborns and with sepsis, and the effectiveness of antithrombin-III (AT-III) in newborns with sepsis. **Methods.** Patient group was consisted of 16 newborns with sepsis diagnosed with clinical (nutrition intolerance, respiratory distress, circulation failure, neurological signs, skin lesions, hypothermia and hyperthermia) and laboratory parameters (white blood cells and neutrophil indexes at whole blood count, thrombocytopenia, microbiological culture results, C-reactive protein (CRP), Interleukin-6 (IL-6), Interleukin-1b, and TNF- α levels). Control group consisted of 30 newborns. Protein C, protein S, AT-III, D-dimer, fibrinogen, pro-

thrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT) were controlled in whole study population at the beginning and, after a week in newborns with sepsis. **Results.** Absolute neutrophil and platelet counts, hemoglobin, hematocrit, CRP, IL-6 and TNF- levels were significantly different in newborns with sepsis from controls. Thus AT-III, protein C, protein S, D-dimer, fibrinogen, PT, aPTT, TT levels were evaluated for fibrinolytic system and natural inhibitors. Antithrombin-III levels were significantly lower in patient than control group. But when physiological low levels were encountered at newborn period AT-III levels were not as low as for replacement treatment. Hence AT-III treatment was not given. Protein C and protein S levels were significantly lower and, PT, aPTT and TT were prolonged. **Conclusions.** Antihrombin-III levels were significantly lower in newborns with sepsis when compared with control group. But these levels were not as low as to threat the newborn with sepsis by Antihrombin-III. More extensive studies must be enrolled before AT-III concentrates to take place at routine treatment in patients with sepsis. However they are potential therapeutic agents in this process.

0330**THE 4G/4G GENOTYPE OF TYPE-1 PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1) IS ASSOCIATED WITH INCREASED MORTALITY IN PATIENTS WITH SEPTIC SHOCK**

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Genetic polymorphisms may influence susceptibility, outcome and complications of sepsis. In septic shock, fibrinolysis impairment with fibrin deposition and thrombosis in the microvasculature may contribute to the prognostic. Type-1 plasminogen-activator-inhibitor (PAI-1) plays an important role on the regulation of the fibrinolytic system. High PAI-1 plasma concentrations are associated with many thrombotic disorders where fibrinolysis is impaired. The human PAI-1 gene contains a common insertion/deletion polymorphism (4G/5G) that determines a higher PAI-1 plasma levels in 4G/4G subjects, and consequently a fibrinolysis down-regulation, compared to 5G/5G carriers. **Aims.** To evaluate the influence of the fibrinolysis, including the 4G/5G PAI-1 gene polymorphism, in the susceptibility and outcome of septic shock. **Methods.** We evaluated 234 consecutive patients (mean age 62 years, 61% men) admitted in a Medical Intensive Care Unit(ICU) during 12-months period. Seventy-four (32%) patients had septic shock at ICU admission or in the first 48 hours. APACHE II, SAPS II, and SOFA scores were calculated at ICU admission, and thereafter on a daily basis. Tissue-type plasminogen activator (tPA) and PAI-1 levels were determined by ELISA. PAI-1 genotyping was carried out by RFLP-PCR. **Results.** No differences were found in the levels of tPA and PAI between patients with or without septic shock. The allele frequencies of PAI-1 4G/5G polymorphism were similar between patients with and without septic shock (0.53/ 0.47 and 0.48/ 0.52 respectively). The genotype distribution in patients with septic shock was: 4G/4G 20 patients, 4G/5G 38 patients and 5G/5G 16 patients. PAI-1 levels were significantly higher in 4G/4G patients compared with 4G/5G and 5G/5G patients ($p < 0.05$). The APACHE II, SAPS II and SOFA scores at ICU admission were similar among the PAI-1 genotype groups. However, shock septic patients with 4G/4G genotype had a greater worsening of APACHE II and SAPS II scores along their ICU stay ($p < 0.05$ compared with the other genotypes). Fifteen (20%) patients with septic shock died (8 4G/4G, 5 4G/5G and 2 5G/5G, $p = 0.03$). The probability to die in 4G/4G patients with septic shock was 40%, while in 4G/5G and 5G/5G patients was 13% in both (OR for 4G/4G: 4.5, 95% CI: 1.4-14.8) ($p = 0.03$). The 4G/4G genotype of PAI-1 is related to a greater mortality in patients with septic shock. PAI-1 4G/5G polymorphism does not increase the susceptibility to septic shock.

0331**HOMOCYSTEINE AND CEREBROVASCULAR ACCIDENT. IS THERE ANY RELATION?**

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High levels of tHCy in plasma, have been suggested by many authors, that are related with a high risk of coronary disease, cerebrovascular accident and phlebothrombosis. **AIM:** The aim of our study was to evaluate the levels of tHCy in relatively young patients, who suffered a transient ischemic attack (TIA) or stroke. One hundred and patients were examined (73 men and 27 women), that have been admitted in the hospital for a cerebrovascular accident(CVA). In case of TIA, the diagnosis was confirmed by the typical neurological symptoms, otherwise it was confirmed by brain CT-scan and/or MRI. We excluded all patients with other predisposing factors, like hypertension, hyperlipidemia and diabetes mellitus. A full cardiologic check up (with U/S and ECG) was normal and also the examination of carotids and vertebrobasal system (with Triplex) was negative. Mean age was 49,4 years old for men (range of age 7-66) and 48,7 for women (range of age 16-69). 250 apparently healthy volunteers blood donors were used as a comparison group. They were all men of mean age years old (range of age 23-55). tHCy was measured with Elisa method (Abbot-AxSYM). Patients and healthy volunteers were divided in two categories: those who had tHCy ≥ 15 $\mu\text{mol/l}$ and those with tHCy > 15 $\mu\text{mol/l}$.

Table.

	Women	Men	Blood donors
tHCy > 15 $\mu\text{mol/l}$	11 (15,3%)	16 (21,9%)	53 (18,4%)
tHCy level	8,9 \pm 2,82 $\mu\text{mol/l}$	10,8 \pm 2,89 $\mu\text{mol/l}$	12,9 \pm 1,9 $\mu\text{mol/l}$

No important difference was found in the homocysteine plasma levels between patients and healthy ones. There is a crucial need for full understanding of the possible relevance of hyperhomocystinemia with vascular disease. More studies are necessary, so that our therapeutic approach could be done in time and is beneficial as well.

0332**FACTOR XIII VAL34LEU POLYMORPHISM IS ASSOCIATED TO THE RISK TO DEVELOP THROMBOSIS IN PATIENTS WITH ANTIPHOSPHOLIPID ANTIBODIES AND HIGH FIBRINOGEN LEVELS**

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The effect of thrombophilic polymorphisms in the development of thrombosis in patients with antiphospholipid syndrome (APS) is not well known. The Leu allele of the Factor XIII (FXIII) Val34Leu polymorphism has been associated to a decreased the risk of both arterial and venous thrombosis in patients without APS. In addition, it has been described that in vitro fibrin structure may be modified by interactions between fibrinogen concentration and FXIII polymorphism. **Aims.** To study the association between the FXIII Val34Leu polymorphism and thrombosis in patients with antiphospholipid antibodies (APA). **Methods.** We included 172 consecutive patients with APA but without the known thrombophilic polymorphisms factor V Leiden and factor II G20210A: 83 with primary APS, 43 with APS secondary to systemic lupus erythematosus, 32 with systemic lupus erythematosus and APA but without APS (SLE), and 14 asymptomatic individuals with APA. FXIII

Val34Leu polymorphism was determined by allele-specific polymerase chain reaction (PCR). Fibrinogen concentration was measured by a coagulometric assay using thrombin (Clauss' method). *Results.* FXIII genotypes were the following: Val/Val in 102 patients (59.3%), Val/Leu in 60 patients (34.9%), and Leu/Leu in 10 (5.8%). FXIII Leu allele frequency was 23.3%, and no significant differences were found among the different groups (22.3% in primary APS, 23.3% in secondary APS, 21.9% in SLE, and 32.1% in asymptomatic individuals with APA). Fibrinogen concentration was 3.17 ± 1.11 g/L, without differences among the groups. A total of 82 patients (47.7%) had previous thrombosis (43 venous and 47 arterial episodes). FXIII Leu allele frequency was lower, but without statistically significant difference, in patients with thrombosis (20.6%) than in patients without thrombosis (25.7%). Fibrinogen levels were not different in patients with (3.30 ± 1.05 g/L) and without (3.06 ± 1.15 g/L) thrombosis. Patients were stratified according to fibrinogen levels: 122 patients had fibrinogen levels equal or lower than 3.50 g/L (44.3% with thrombosis) and 50 had fibrinogen levels higher than 3.50 g/L (56.0% with thrombosis). FXIII Leu allele frequencies were significantly lower ($p=0.03$) in thrombotic patients with high fibrinogen levels (8.9%) than in no-thrombotic patients with high fibrinogen values (27.3%) or than in patients with normal fibrinogen levels (with (27.8%) or without thrombosis (25.0%)). FXIII Leu allele has protective effect on thrombosis in patients with high fibrinogen levels (OR= 0.24; 95% CI= 0.06-0.93) but not in those with normal fibrinogen levels (OR= 0.88; 95% CI= 0.43-1.82). *Conclusions.* The FXIII Leu allele seems to have a protective effect in the development of thrombosis in patients with APA but only in those with high plasma fibrinogen values.

0333

A POSSIBLE ROLE FOR TISSUE FACTOR IN SEMINAL COAGULUM FORMATION?

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Studies of seminal tissue factor (TF) are few and mostly based on small numbers. It was suggested that TF may not participate in the seminal coagulum formation but recent identification of a number of haemostatic factors in semen justifies a re-evaluation of seminal TF. Objectives: Antigenic and functional seminal TF were compared with fertility status and the location of TF within semen evaluated by semen fractionation. *Material and Methods.* Semen was collected from sub-fertile (n=19), normal (n=33), semen donors (n=30) and vasectomy subjects (n=62), some fractionated into sperm, a prostasome-rich fraction and seminal plasma. Functional and antigenic TF levels were measured and related to conventional fertility parameters. Semen contains high concentration of functional and antigenic TF. Most TF was found in seminal plasma, but it was also detected in prostasomes and sperm fractions. TF antigen levels were higher in vasectomy subjects than sub-fertile, normally fertile, donor ($p=0.018$) and a 'pooled normal semen parameters' (PNSP) stratification [derived from a combination of measurements] ($p=0.06$). The sub-fertile group showed a wider variation than normal, donor or the PNSP subjects. Seminal TF antigen levels correlated significantly with sperm agglutination and morphology. Abnormal sperm morphology and antisperm antibodies associated with elevated TF antigen. *Conclusions.* Semen contains functional and antigenic TF at high concentrations. TF levels were appropriately associated with seminal parameters known to favour male fertility. A fully functioning clotting system probably exists in semen, so seminal TF may have some procoagulant role and be an influence on male fertility.

0334

THROMBOSIS AND OTHER CLINICAL MANIFESTATION OF HEREDITARY THROMBOPHILIA

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Most of the patients with thrombosis have no any recognizable hemostatic disorders associated with thrombosis. However, some patients have hereditary hypercoagulable states, which contribute to development of thrombosis and other clinical manifestation as miscarriages and fetal complications. The aim of this study is to investigate incidence, localization and trigger mechanisms of thrombosis, rethrombosis, as well miscarriages and treatment of patients with hereditary thrombophilia. We diagnosed hereditary thrombophilia in 141 patients, (64 male, 77 female) with deficiency of antithrombin in 14, protein C in 15, protein S in 9, and factor XII in 9, resistance to activated PC or factor V Leiden in 80, prothrombin 20210 mutation in 7, and polymorphism C677T of methylenetetrahydrofolate reductase (t-MTHFR) in 20 patients. However, 13 patients have two hereditary thrombophilias. Also acquired antiphospholipid antibody was detected in 11 patients with one inherited thrombophilia and in 2 patients with two hereditary thrombophilias. Our group consisted of highly selected patients: 101 with thrombosis, 15 with recurrent miscarriages, and 25 members of their families. Patients with different hereditary thrombophilia developed first thrombosis at the median age from 25 to 33 years and they had 200 thrombotic events with 10% of arterial thrombosis as transient ischemic attack (6) or stroke (6), myocardial infarction (6) and peripheral arterial thrombosis (2). Recurrent thrombosis occurred in 67/101 patients (57%) and 24 of them had 3 or more (3-5) thrombosis. Most of the patients developed venous thromboembolisms, principally of leg (55%) and pulmonary embolisms (16%), abdominal veins (12%) and others (7%). In 72 patients with hereditary thrombophilia, thrombotic event was associated with surgery, trauma or immobilization in 23, pregnancy or delivery in 27, malignancy in 6, infections in 5, oral contraceptives in 2 and disrupt of anticoagulant therapy in 1 patient. When diagnosis of hereditary thrombophilia was established all patients with two or more venous thromboembolic events were treated with long-term anticoagulant therapy and they had not recurrent thrombosis. Patients with arterial thrombosis treated with low doses of aspirin (75 mg). Two patients died (2.2%), one after surgery due to mesenteric thrombosis and other by stroke and myocardial infarction after delivery. Fifteen of 77 women (19%) with hereditary thrombophilia had 42 miscarriages (range 2-7). The 8 of them successfully delivered after the prophylactic treatment with low molecular weight heparin given during pregnancy and 4-6 weeks after. Twenty-five patients (18%) with hereditary thrombophilia, median ages 36 years, have no any event. In conclusion, prophylactic long-term anticoagulant therapy is effective in patients with hereditary thrombophilia and recurrent venous thrombosis. Usually, recurrent thromboses in patients with thrombophilia were associated with acquired risk factors. Prophylactic anticoagulant therapy during pregnancy, delivery, and 4-6 weeks after is recommended in woman with inherited thrombophilia and previously recurrence miscarriages or thrombosis.

0335**THE ASSOCIATION OF INFLAMMATORY RESPONSE WITH THE ENDOTHELIUM, PLATELETS AND THE COAGULATION SYSTEM IN PATIENTS WITH ULCERATIVE COLITIS**G.E. Pamuk¹, Ö. Vural², B. Turgut³, M. Demir², H. Amit², A. Tezel²¹Trakya University Medical Faculty, Edirne, Turkey; ²Trakya Medical Faculty, Edirne, Turkey

Some clinical studies had reported an increased incidence of thromboembolism in inflammatory bowel diseases, including ulcerative colitis (UC). *Aims.* In our study, we evaluated patients with UC which is a disease with a chronic inflammatory response and it produces a hypercoagulable state independent of the clinical disease activity. We investigated the association of the inflammatory response with activation parameters of the endothelium, platelets and the coagulation system in patients with UC. *Methods.* We included 18 UC patients (10 females, 8 males, mean age: 38.2) and 18 healthy control subjects (11 females, 7 males, mean age: 40.8) into our study. The patients' clinical features and endoscopic findings were recorded down; whole blood counts and acute phase parameters were also noted. The patients were divided into two according to the clinical activity index: 9 patients were taken as active and 9 patients as inactive. In all patients and control subjects, platelet P-selectin (CD62P) expression, platelet-monocyte complexes (PMC), platelet-neutrophil complexes (PNC), and platelet microparticles (PMP) were determined by flow cytometry (Coulter EPICS XL2, Beckman Coulter, Miami, USA). In addition, plasma E-selectin (CD62E ELISA Kit, Diaclone Research Besançon, France), thrombin-antithrombin complex (TAT) (Enzygnost TAT micro, Dade Behring Marburg GmbH, Germany), and serum sCD40L levels (Human sCD40L, Biosource International, Inc. Camarillo, USA) were determined by ELISA. *Results.* There was no significant difference between the sex distribution and mean ages of the groups ($p>0.05$). The parameters evaluated in active, inactive UC patients and in the control group are seen in the Table. PMC were significantly higher in active UC patients than in controls ($p<0.05$); PMP were significantly higher in controls than in inactive UC patients ($p=0.01$). Platelet CD62P expression was higher in both active and inactive UC patients than in the control group (p values < 0.01). In active UC group, E-selectin level was lower than in the inactive group; sCD40L was significantly higher than in the control group (p values < 0.05). In UC patients, the clinical activity index had a significant negative correlation with E-selectin level ($r=-0.68$, $p<0.01$); the endoscopic activity index had significant negative correlations with PMP ($r=-0.5$, $p<0.05$) and sCD40L level ($r=0.48$, $p<0.05$). The percentage of platelets expressing CD62P had a positive correlation with CRP level ($r=0.54$, $p=0.01$) and platelet count ($r=0.5$, $p<0.05$).

	Active UC	Inactive UC	Controls
ESR (mm/hr)	33.7±21.4	14±14.7	11±10.5
CRP (mg/dl)	2.5±4	1.45±2.8	0.5±0.6
Platelets (x10 ⁹ /l)	347±171	287±88	259±548
PMC (%)	60.5±58.3	43.6±23.8	24.7-10.2
PNC (%)	23±25.4	20.6±9.3	10±5.2
CD62P (%)	19.6±21.5	9.3±3.9	6.9±10.5
PMP (%)	3±0.7	2.8±0.7	3.5±0.5
TAT (µg/l)	5.9±8.1	21.4±30.5	9.3±5.6
E-selectin (ng/ml)	25.5±6.6	38.1±11.2	35.5±10.1
sCD40L (ng/ml)	5±1.4	3.9±2.3	2.9±1.7

Patients with pancolitis were not significantly different from others when all parameters were considered ($p>0.05$). In UC patients, increased platelet activation parameters (CD62P vs sCD40L) and increased PMC formation might be associated with the increased incidence of thromboembolic events during the course of the disease. It was interesting to find that PMP thought to have a role in thromboembolic events were decreased in UC patients: and, this might be because of the deposition of PMP as microaggregates in colonic mucosa.

0336**A PRELIMINARY STUDY ON THE FEASIBILITY OF EXTENDING THE INTERVAL BETWEEN PROTHROMBIN TIME TESTS FOR PATIENTS ON WARFARIN THERAPY**

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Anticoagulant therapy with vitamin K antagonists is a burden for most patients due to the need for frequent laboratory monitoring and dose adjustments. The ACCP Guidelines suggest for stable patients an interval of not more than 4 weeks between the tests for prothrombin time (Grade 2C). To evaluate in a routine clinical setting what proportion of patients could potentially be monitored with longer intervals. *Methods.* At the Hamilton General Hospital all patients monitored by the Thrombosis Service were reviewed 2 years retrospectively regarding the maintenance dose of warfarin. Patients monitored for longer than 3 months at the service were eligible for evaluation. If the most recent maintenance dose had been unchanged for at least 3 months back it was considered 'stable'. *Results.* Of 1316 patients treated with warfarin and monitored by us, 1100 (83.6%) had been followed for more than 3 months. Of the latter, 49.8% had stable maintenance dose for the past 3 to 24 months, 33.5% for at least 6 months, 21.1% for at least 9 months and 13.1% for 12 months or more. These patients had had occasional INR levels outside their therapeutic range (INR 2.5-3.5 for mechanical heart valves; 2.0-3.0 for the remainder), which were cosmetically corrected by skipping a dose or giving a higher dose on a single day. Most of the aberrances towards high INR levels were due to antibiotic therapy. There was no difference in the proportion of patients with different indications for anticoagulant therapy between the 'stable' and 'unstable' groups (51-52% mechanical valves, 29% atrial fibrillation, 10-14% venous thromboembolism). *Conclusions.* A substantial proportion of the patients on long-term treatment with warfarin have a very stable maintenance dose. Provided that the patients will contact the monitoring center for dose adjustments in case of new medication with an interacting drug, the interval between laboratory controls can probably be increased to once every 3 months. This could provide an improved quality of life for the patients and to some extent reduced costs for the health care system. The safety of this regimen should be investigated in a prospective randomised trial.

Transfusion, apheresis, granulocytes I

0337

HBV DNA DETECTION (LOW VIRAL LOAD) IN HBSAG EIA-NEGATIVE ANTI-HBC POSITIVE BLOOD DONORS

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Available data although not conclusive, strongly imply that there is an association between HBsAg-negative, anti-HBc-positive blood units and transfusion transmission of HBV. The objectives of our study is to detect the rate of HBV DNA positive, anti-HBc-positive, HBsAg EIA-negative, anti-HBs negative blood donors in Corfu island Greece, to estimate the viral load and calculate the rate of this potentially infectious HBV units relative to all blood donations. A total of 37150 allogeneic donations were collected at our unit and were all tested for HBsAg and for anti-HBc with AxSYM Abbott Laboratories, of which 116 (0,31%) were HBsAg positive and 1642 (4.41%) were anti-HBc positive with negative results on all other routine blood donor screening assays. (anti-HIV1 and 2, anti-HCV, anti-HTLV, serologic test for syphilis and elevate ALT). All HBsAg negative-anti-HBc positive donations were then tested for HBe Ag, anti-HBe, Core-M and anti-HBs (AxSYM Ausab, Abbott Laboratories), of which 805 were found anti-HBe positive, 469 (1,26%) anti-HBs negative (less than 10 IU/mL.) and 315 (0,84%) anti-HBs between 10-99 IU/mL. A total of 186 of 469 specimens eligible by our serologic criteria were tested by PCR. 50 anti-HBs negative specimens were PCR positive with estimated copy numbers 400-2000 copies per ml. PCR method was ORTHO and was considered positive if reactive with > 400 copies per ml. The detection rate of HBV DNA positive, anti-HBc-positive, anti-HBs negative, donations was 26,8% and the projected rate among all anti-HBc positive donations was 3.04%. The above results to estimate 1.34 HBV DNA positive, HBsAg-negative, anti-HBc positive, anti-HBs negative unit in every 1000 blood units. Anti-HBc screening detects a small number of potentially infectious HBsAg EIA-negative, HBV infected donors with low viral load. We consider anti-HBc and anti-HBs detection a simple and useful screening for blood donors which allows us further minimize the risk transfusion transmission of HBV.

0338

THROMBOTIC THROMBOCYTOPENIC PURPURA: REPORT OF TEN CASES TREATED AT ISTITUTI OSPITALIERI OF CREMONA

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Thrombotic thrombocytopenic purpura (TTP) is a microangiopathic disease characterized by schistocytic haemolytic anemia, thrombocytopenia, neurologic deficits; sometimes fever and renal failure may occur. Plasma exchange has evolved as the mainstay of therapy for acute episodes of TTP and has improved survival. Aim. To present the experience of Haematology Unit of the Istituti Ospitalieri of Cremona regarding this issue. Materials and methods. Our study includes 10 patients diagnosed as TTP between December 1988 and February 2005. Diagnosis was made on clinical manifestations, blood counts, peripheral blood morphology, hemostasis analyses. Data are summarized in Table 1. The analysis of our case series showed that TTP is a rare disorder with prevalence in women; it does not appear to be related to others diseases (only patients number 3 and 4 were under oral contraceptive pills); numerous sessions of plasma exchange with reinfusion of cryosupernatant plasma have been necessary to achieve a complete remission respect to those with fresh frozen plasma.

Table 1

Patient's	Age (y)	Sex
1 E	49	F
2 E	38	F

Plasma exchange lowered the mortality rate of TTP: in our experience the overall survival is 90%, however, patients should be followed after recovery because of high relapse risk.

0339

CLINICAL AND SEROLOGICAL PROFILE OF BLOOD DONORS WITH A POSITIVE DIRECT ANTIGLOBULIN TEST (DAT)

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A positive direct antiglobulin test occasionally occurs in normal blood donors, and is often discovered when the donor's red cells are found incompatible in a compatibility test. The incidence of a positive DAT was expected to increase since more sensitive techniques (gel test) were installed. The aim of our study was to examine whether DAT positive otherwise healthy donors presented any clinical or laboratory abnormalities. *Methods.* In the first 19.000 cross-matches last year (in 10 months) 38 were found incompatible due to DAT positivity of blood donors' red cells (0,02%). DAT positive [(1+)- (3+)] samples were only IgG positive in 32 cases, only C3d positive in 5 and IgM positive and C3d positive in 1 case. All blood donors were notified and thirty two of them responded to a request for a further sample. A complete blood count, a reticulocyte count, bilirubin (total, direct, indirect), transaminases, serologic immunological tests (ANA, anti-DNA, anti-ENA, RF, anticardiolipin antibodies), quantitative assessment of immunoglobulins, APTT and lupus anticoagulant were performed, as well as serologic tests for markers of viral infections. DAT and IAT were performed by gel test (ID-DiaMed) according to the manufacturer's instructions. DAT were performed with polyvalent and monovalent reagents (anti-IgG, -IgM, -IgA, -C3c, -C3d). The blood donors were also examined clinically. The donors who had positive immunological tests were referred to a rheumatologist for further investigation. *Results.* Among the thirty one blood donors eight had received medication the last 24 hours before blood donation, two had been vaccinated for hepatitis B recently, four presented signs of a viral infection soon after blood donation, three had evidence of an allergic condition, five had positive tests for anticardiolipin antibodies and ANA, two were positive for anticardiolipin antibodies only and two had a positive ANA test only. In six blood donors we did not find any abnormality that might be interrelated to DAT positivity. *Conclusions.* All blood donors with positive DAT should be requested to undergo further investigation. Some of them are possibly candidates to long medical follow-up, especially those with other immunologic abnormalities such as positive ANA and/or anticardiolipin antibodies. The eligibility of such donors for future donation of whole blood, platelets or plasma needs to be elucidated.

0340**HAEMOLYTIC REACTION DUE TO ANTI-JK3**

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Red blood cell alloantibodies directed against antigens of the Kidd system are notorious for causing delayed hemolytic transfusion reactions. The antibodies are formed because of pregnancy or transfusion. Blood donors with the red blood cell (RBC) phenotype Jk (a-b-) are extremely rare in the white population and exhibit a frequency of less than 0.1%. However, the rare phenotype Jk (a-b-) is more common in Polynesians (1.4%). Individuals with Jk (a-b-) phenotypes typically form anti-Jk3 with inseparable anti-Jka and anti-Jkb activity. Some Jk (a-b-) patients' sera may show an additional distinct anti-Jka or anti-Jkb component when examined with adsorption studies. Case report: A 63 years-old Caucasian female with a negative antibody screen, no prior history of transfusion, presented with gastrorrhagia. It is reported four pregnancies with no history of haemolytic disease of the newborn (HDN). On admission, her haemoglobin was 5.5g/dL. She was given 4 units of crossmatch-compatible RBC. On day 6 her haemoglobin was 12.1 g/dL, with a total bilirubin of 0.72mg/dL and lactate dehydrogenase of 141 U/L. On 10th day an unexpected fall in Hb (5.6 g/dL) occurred with an increase of bilirubin to 1.9 mg/dL and of lactate dehydrogenase to 1042 U/L. A new blood sample obtained for antibody screening and additional crossmatches showed a pan-agglutination and incompatible crossmatch. Anti-Jk3 antibody high titer was detected in the plasma by gel-test using Liss/Coombs cards (ID-Diamed). The DAT was negative and the antibody reacted equally with Jk (a-b+), and Jk (a-b+) panel cells (Jka:1/16384 and Jkb:1/16384). Other alloantibodies could not be excluded, because Jk (a-b-) cells are not available. She was started with erythropoietin-a (EPO), folic acid, Fe IV and high dose intravenous immunoglobulin (IVIG). The EPO was discontinued after four week of therapy when the haemoglobin was 12 g/dL. Two months later her haemoglobin was 13,5 g/dL and anti-Jk3 was present in the same titer. A year later her blood cell count was normal and the anti-JK3 was detected in a lessened titer (1/16). No additional distinct anti-Jka or anti-Jkb component was shown after two adsorptions at 37 °C using carefully selected phenotyped red cell compatible with patient's Rh, Fy, MNSs, Lu, Le system and Jka (+) and Jkb (-), but two additional alloantibodies anti-c and anti-E of low titer (1/4) were revealed. The rare anti-Jk3 alloantibody found in this case displayed the erratic nature of many Kidd system antibodies. Although anti-Jk3 may cause mild hemolytic disease of newborn, she did not have a history of HDN. Our patient was sensitized to a Kidd antigen during pregnancy, but showed no serologically detectable antibody until challenged with a massive transfusion following a gastrorrhagia. The use of EPO and high dose intravenous immunoglobulin succeeded to avoid transfusion with incompatible RBC unit.

0341**ASSOCIATION BETWEEN APPROPRIATE USE OF TRANSFUSION IN CHILDREN AND IN-HOSPITAL MORTALITY: A VENEZUELAN PROSPECTIVE COHORT STUDY**

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Blood transfusion risks have been studied extensively; but data regarding appropriate use of transfusion and survival on hospitalized children are unknown. The purpose was to determine the association between appropriate use of transfusion in children and in-hospital mortality. It was a prospective cohort study. Appropriateness of blood transfusion was evaluated

using American of Blood Bank Pediatric Hemotherapy. This research was carried out in Hospital Infantil 'Dr. Jorge Lizarraga', Valencia, Venezuela from January 2002 until September 2003. The main outcome measure was death during hospitalization. *Results.* 617 children (< 15 years old, male = 57%) were evaluated. Appropriateness was 81.2% (501/617). Overall in-hospital mortality was 9.8% (95% CI = 7.6% to 12.5%). For non appropriate transfusion group, incidence rate (IR) of in-hospital mortality was 7 per 1000 while IR for appropriate transfusion group 3 per 1000. IR ratio was 1.98 (95% CI = 1.05 to 3.6, $p=0.01$). Log-rank test was 0.01. Hazard ratio (HR) crude was 2 (95% CI = 1.2 to 3.5). In patients > 1 years old, HR was 4.4 (95% CI = 1.39 to 13.6, $p=0.01$); in patients < 1 years old, HR was 1.5 (95% CI = 0.79 to 2.9, $p=0.2$). *Conclusions.* Appropriate transfusion in children did appear to influence the risk of in-hospital mortality in this children population. Educational efforts addressing appropriate use of blood component should be increased. Further analysis of the association of transfusion and mortality is a need.

0342**GRANULOCYTE CONCENTRATES OBTAINED BY POOLED BUFFY COATS**

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Neutropenia may necessitate neutrophil (NE) transfusion, but the availability of granulocyte concentrates obtained by apheresis is limited. To obtain therapeutic doses of NE by pooling of buffy coats (BCs) derived from whole blood donation. Twelve AB0 identical BCs were pooled and centrifuged at 1200 g for 20 minutes (primary pools). To reduce the volume of the primary pools, the granulocytes were concentrated by a separation device (Compomat G4, Fresenius, Germany). 5 primary pools were pooled into a secondary pool to obtain a therapeutic NE dose. To assess the quality of the secondary pool, blood counts, pH-values, phagocytosis (PhagoTest, Orpegen, Germany) and oxidative burst (BurstTest, Orpegen, Germany) were measured instantaneously and after 24 hours of storage. Pooling of 60 BCs followed by centrifugation and separation resulted in a secondary pool with a NE dose of $3.5 \pm 0.6 \times 10^{10}$ meeting the European requirement of a therapeutic dose of 1×10^{10} NE for transfusion. Centrifugation and separation resulted in a product volume of 428 ± 29 mL which is below the quality requirement of 500 ml per unit. NE function and pH decreased after 24 hours of storage (see Table).

	Day 0	Day 1
Oxidative burst (%)	35.2 ± 19.5	25.0 ± 29.9
Phagocytosis (%)	69.5 ± 21.3	42.5 ± 22.9
pH	6.47 ± 0.16	5.48 ± 0.13

pH-values were > 6.0 in all pools at the day of preparation. *Conclusions.* Granulocyte concentrates with a therapeutic dose of NE were obtained by pooling of whole blood derived BCs. Centrifugation and separation of the BCs resulted in volume reduction of the product thereby avoiding volume overload of the recipient. BC derived granulocyte concentrates showed impaired NE function compared with apheresed NE for phagocytosis and oxidative burst at the day of preparation (Schwanke et al.; Transfusion Med 2005). Due to a loss of NE function and a decline of pH after 24 hours of storage immediate transfusion of BC derived granulocytes is recommended.

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FOCUS ON HOSPITAL TRANSFUSION IMPROVEMENT: EXPERIENCE FROM THE BETTER, SAFER TRANSFUSION (BEST) PROGRAME.M. Wood¹, K. Botting², J. Carnell³, T. Farrugia⁴, J. Forsyth⁵, M. Gaskell⁶, N. Gilby²

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Blood supplied for transfusion in Australia is now extremely safe. Many of the residual transfusion risks relate to clinical practice, including transfusion decision-making and product administration. The Better, Safer Transfusion (BeST) Program seeks to improve hospital transfusion practice and patient outcomes by focussing on transfusion appropriateness and safety. The program is an initiative of the local health department, the Department of Human Services, Victoria, Australia. It follows a three-year project (*Blood Matters*) which concentrated on training of transfusion nurses and improving clinical practice in university teaching hospitals. BeST aims to: (i) improve awareness & knowledge of transfusion practice within hospitals; (ii) implement appropriate and best practice for clinical decision-making and blood administration; (iii) develop and implement a state-wide haemovigilance system; and (iv) engage and support the private and rural hospital sectors. Key goals are to achieve practical & sustainable improvements that can be measured and evaluated. *Methods.* Expert working groups report to a multidisciplinary BeST advisory committee, supported by a secretariat (project officer & transfusion nurse). Working groups identify priority target areas & potential interventions and develop proposals. The advisory committee reviews proposals & progress and ensures coordination. Implementation occurs through local institutional, community and professional channels. *Results.* The program started in July 2004. For the working group on improving hospital awareness and knowledge of transfusion practice, activities to date have included: Advancing the transfusion nurse role by supporting development & delivery of the *Blood Matters* educational course as an entirely on-line certificate in transfusion practice, open to both internal & external applicants. Working with hospital quality and risk management groups (including transfusion committees) to promote better transfusion practice. Education for patients, including: liaison with consumer groups & hospital community advisory committees to promote transfusion awareness & information sharing; and developing a plain language brochure (print & electronic) with answers to frequently asked questions, including transfusion risks & benefits. Education for health professionals [medical, nursing, perfusionists, laboratory & clerical staff], including: developing key messages & suggestions for undergraduate & postgraduate training programs; working with hospitals to promote key messages & information about transfusion resources at new staff orientation & continuing education sessions; developing a *frequently asked questions* brochure for laboratory & clinical staff on transfusion administration (product storage & handling, infusion rates, indications etc.), both as a convenient reference, and to improve understanding of the activities of all personnel in the hospital transfusion process. More information on BeST activities is available at: <http://www.health.vic.gov.au/best>. *Conclusions.* Progress to date has been very encouraging. There has been enthusiastic engagement and uptake of BeST activities by patients, health professionals & educators. Transfusion nurse roles continue in most major institutions, and the on-line transfusion course will recommence in early March 2005. This program hopes to build on the success of previous work and create a truly sustainable model for promoting improvements in clinical transfusion practice.

0344

THE ROLE OF FLOW CYTOMETRY AND SYSMEX- ANALYSIS OF THE BEST TIMING OF LEUKAPHERESIS FOR COLLECTION OF PERIPHERAL BLOOD STEM CELLS (PBSC)

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The most important problem of successful PBSC collection is correct and fast decision when to time and start leukapheresis with the best results of harvest. There are two helpful methods at disposal. The first method - flow cytometric evaluation of CD34+ stem cells is very useful, precise but time-consuming, expensive and demanding. Therefore a new detection method of haematopoietic progenitor cells (HPC) on Sysmex XE-2100 analyzer seems to be needed in this field. Sysmex can detect the immature myeloid cells quickly as a method of stem cells screening in blood. *Results.* To investigate advantages and disadvantages of both methods we analysed data on PBSC collection procedures in our experimental group of patients. 23 patients with NHL were analysed, 11 men, 12 women. Median of age was 49 years (19-68 years old). We were interested in count of leukocytes before starting leukapheresis, the median was $9,2 \times 10^9/L$ (3,3-87,2). The median of HPC count analysed by Sysmex was 25,5 (10-707) and the median of count CD34+ cells by flow was $79,1 \times 10^9/mL$ (15,8-131,8). The harvest of these group of patients was successful, the median was $6,4 \times 10^6/kg$ (1,2-75). *Conclusions.* Our results and experience have proven that counting HPC on Sysmex can facilitate optimal time of PBSC collection beginning while saving time and cost. The minimal threshold for beginning leukapheresis is 10 HPC/mm³.

0345

HAEMOVIGILANCE IN THE NORDIC COUNTRIESO. Flesland¹, J. Jørgensen², T. Krusius³, J. Säfwenbergl⁴, S. Gudmundson⁵

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The five Nordic countries have different hemovigilance systems, but all systems are based on reporting being voluntary and anonymous, and the organisations are based on the profession of transfusion medicine, not the authorities. The systems are only in part financed by the authorities. A Nordic haemovigilance group is established to coordinate the work. Data collection and reporting Nordic coordination and cooperation is facilitated by harmonizing definitions, reporting systems and annual reports. Suggestions for improvement are shared to the benefit of all countries. All countries focus on the serious adverse events and reactions, incorrect/inappropriate blood transfusions, and *near misses* of transfusion. The systems also allow for reporting of less serious hazards during defined periods. Examples are; reports of all pre-analytical mistakes in one country during one year, and reports of all transfusion reactions during one year in another country. This data gives useful background information and this type of data collection can be repeated for a new period when needed, e.g. to validate the effect of improving the procedures related to transfusion. All Nordic countries also report on severe adverse events and reactions related to blood donation. This work has been of particular importance as the Blood Directive requires us to give correct information to Blood Donors about possible risks. Each country makes separate annual reports. Rapid Alert Reports At present reports are received from EHN, or other international or local sources. The need for national systems, or a common Nordic system, to distribute these reports has been discussed.

Suggestions for improvement Several suggestions for improvement has so far been put forward, including bacterial detection systems for platelet concentrates, simplification of the procedures bed-side at the start of transfusion, and identical labels and forms used for transfusion in all hospitals. Data from 2004 Table 1 shows data from Nordic countries on serious complications, serious risks and *near misses* of transfusion. Discussion Blood transfusion is not completely without risk. 200 reported events were analyzed. Approximately 1,7 million transfusions caused two deaths, one from TRALI and one from acute haemolytic transfusion reaction. There were 41 reports of the wrong component being transfused and 18 reports of blood given to the wrong patient

0347

RHESUS D POSITIVE PLATELET TRANSFUSIONS IN RHESUS D NEGATIVE IMMUNOSUPPRESSED PATIENTS: IS IT TIME TO STOP PROPHYLAXIS?

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Up to 19% of immunocompromised Rhesus (Rh) D negative patients receiving Rh positive platelet transfusions will develop Rh D antibodies. Modern platelet production methods typically result in platelet products with extremely low levels of red cell contamination and more recent alloimmunization rates are significantly lower. As a result, routine use of Rh D immunoglobulin (RhD Ig) in immunocompromised Rh D negative females with child-bearing potential who receive Rh incompatible platelet transfusions is not routinely recommended at our Cancer Centre. To assess whether our Rh D Ig policy was justified, based on a retrospective assessment of all Rh D negative haematology patients at our institution who received Rh D mismatched platelets on at least one occasion.

All patients had a malignant haematological disorder and were receiving immunosuppressive chemotherapy, radiotherapy or undergoing autologous peripheral blood stem cell transplantation. Method: Our Information Technology Service screened two hospital data bases to identify all patients over a seven year period (1998-2004) who were Rh D negative and had received Rh positive blood products. Subsequently each patient's electronic history was assessed to limit the enquiry only to transfusion of Rh mismatched platelets - patients were excluded if either Rh D positive red cells or Rh D Ig were administered. Each history was assessed for the duration of follow up of antibody screening, with a minimum of seven days required for inclusion in our analysed cohort. Antibody screening was performed using the IAT method at 37 degrees C (1998-2000) and the Diamed gel antibody screening technique (2001-04). Result: A total of 69 patients were identified through our computer search, with 18 excluded due to incomplete (less than 7 days) follow up with an antibody screen. One patient was excluded as she was given Rh D Ig following platelet transfusion, and two had anti-D antibodies demonstrable pre-exposure to Rh positive platelets, leaving 48 patients evaluable. Median age was 58 (22-79), with 26 males and 22 females included. Median number of Rh D positive units administered was 10 (3-140). Products included whole blood-derived random donor platelets (as individual units or pools), and single donor ABO- and HLA-matched apheresis platelet products with variable collection methodologies, red cell contamination, residual white cell count and plasma content. Median follow up from initial exposure to Rh mismatched platelets was 53 (7-689) days. No patient had documented anti-D antibodies following Rh D positive platelet administration. Conclusions. Our retrospective study supports the international literature on which our local guidelines were based. Routine prophylactic administration of Rh D Immunoglobulin in Rh D negative women of child-bearing potential with a malignant haematological condition requiring platelet transfusion is not recommended. This not only conserves a valuable product for appropriate use but also avoids unnecessary exposure for patients. Prospective studies are required in this patient population to confirm these recommendations.

SIMULTANEOUS SESSIONS

Stem cell biology

0348

HB-EGF/HER-1 SIGNALLING IN BONE MARROW MESENCHYMAL STEM CELLS: INDUCING CELL EXPANSION AND PREVENTING REVERSIBLY MULTI-LINEAGE DIFFERENTIATION

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Epidermal growth factor receptor-1 (*EGFR-1/HER-1/ErbB*) regulates proliferation and cell fate during epidermal development. HER-1 is activated by several EGF-family ligands including heparin-binding epidermal growth factor-like growth factor (HB-EGF), a mitogenic and chemotactic molecule that participates to tissue repair, tumour growth and other tissue-modelling phenomena, such as angiogenesis and fibrogenesis. In many of the processes in which HB-EGF is often involved, mesenchymal stem cells (MSCs), the precursors of different mesenchymal tissues, have a role. We have studied whether the *HB-EGF/HER-1* system is expressed in human MSCs and which role it plays in MSC biology. MSCs have been generated from bone marrow aspirates of healthy donors, recruited after informed consent, and expanded in complete DMEM medium (15% FCS). MSCs have been characterized by immunophenotype and *in vitro* multilineage differentiation. The expression of HB-EGF, HER-1 and HER-4 (the other receptor for HB-EGF) has been studied by flow-cytometry and RT-PCR. Then we have studied the short- and long-term effect of HB-EGF on MSC proliferation and multilineage differentiation by specific assays and differentiation-specific gene expression by quantitative RT-PCR. **Results.** We have found that MSCs normally express HER-1, but not *HB-EGF* or *HER-4*. Under the effect of *HB-EGF*, MSCs proliferate more rapidly and persistently, without undergoing spontaneous differentiation. This effect occurs in a dose-dependent fashion, and is specific, direct, long-lasting, comparable to other growth factors such as bFGF, and HER-1-mediated, as it is inhibited by anti-HER-1 and anti-HB-EGF blocking antibodies. The effect is tightly controlled because surface HER-1 is down-regulated after interaction with HB-EGF: this occurs rapidly but reversibly, because *HER-1* RNA is still synthesized and leads to the re-expression of surface HER-1 a few hours after HB-EGF removal from culture. By contrast, HER-1 expression is permanently lost during MSC differentiation into mesenchymal cell lineages. Moreover, HB-EGF reversibly prevents adipogenic, osteogenic and chondrogenic differentiation induced with specific media, by preserving MSC potential. **Conclusions.** This study provides the first evidence that HB-EGF/HER-1 signalling is mitogenic for MSCs and may prevent reversibly their differentiation, leading to self-renewing rather than differentiative cell divisions. The rapid *ex vivo* MSC expansion and down-regulation of their sensitivity to physiological differentiation agents could represent a valid alternative to other factors, such as bFGF, with an advantage in terms of MSC differentiation potential, *in situ* recruitment and proliferation, and therefore of *in vivo* transplant efficiency. It has to be investigated whether the HB-EGF/HER-1 signalling may contribute *in vivo* to maintain a broad, proliferating pool of undifferentiated MSC, thus ensuring the regenerative process or the efficient angiogenesis to neoplastic growth. The use of HB-EGF inhibitors could have a role in these conditions.

0349

HUMAN NKT CELLS ENHANCE HAEMOPOIESIS THROUGH RECOGNITION OF CD1D EXPRESSED IN HAEMOPOIETIC STEM CELLS

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Experimental evidence suggests that T cells regulate hematopoiesis. NKT cells, a small but powerful subset of regulatory T cells, secrete hematopoietic cytokines upon engagement of their TCR. Because of these properties, we postulated that NKT cells are involved in the regulation of hematopoiesis, and if so, their restriction element, the glycolipid-presenting, MHC-like CD1d antigen would be expressed on HSC. CD34+ HSC were purified from human mononuclear umbilical cord blood cells (HUCB) either by positive selection or by lineage depletion. Multi-colour flow cytometry and sorting of NKT cells and HSC were performed on a FACSCalibur and a FACS-Diva. CFC and LTC-IC assays were performed using standard protocols. IL-3, SCF and GM-CSF were quantified by a Quantikine immunoassay. α -galactosylceramide (aGC)-pre-activated NKT cells were co-cultured, with CD34+ cells at 10:1 ratio and then plated for CFC assays. NKT cell-depleted or -replete HUCB samples were plated for CFC or LTC-IC assays. Statistical analyses were performed by using either Wilcoxon Signed Ranks or Mann-Whitney U test. **Results.** One percent of CD34+ cells expressed CD1d (n=6, range 0.4-1.67%). Expression of both primitive (CD117, CD133, CD7, CD90) and lineage markers was similar in CD1d+CD34+ and CD1d-CD34+ cells. Moreover, 6.2% (1.9-10.5%) and 12.8% (10.1-16%) of CD1d+CD34+ cells were HLADR- and CD38- respectively, consistent with an immature HSC phenotype. Lin-CD1d+CD34+ cells displayed LTC-IC activity, although at a lower frequency than Lin-CD1d-CD34+ cells: 1/52.3 cells (60.2-34) versus 1/24.6 (35.6-20) respectively, (n=4). Similarly, CFC activity was detected in the CD1d+ fraction, again at lower frequency than the CD1d- fraction: 1 CFC/10.6 cells (26-7.7) vs 1/4.2 (5-3.8), respectively (n=4). Purified NKT cells were promptly activated when co-cultured with CD1d-expressing HSC in the presence of aGC but not in the presence of diluent. By contrast, very little activation was seen either in the presence of aGC or diluent when NKT cells were co-cultured with CD1d- HSC. Thus, CD1d+CD34+ cells are able to present antigen and activate NKT cells. Co-culture of pre-activated NKT with CD34+ cells enhanced the clonogenic capacity of the latter by 3-fold: 1 CFC/14 cells (21-10.4) in the presence of NKT vs 1/43 cells (55.5-37) without NKT (n=5; p=0.024). GM-CSF (256pg/mL) was detected only in the co-culture supernatants, whereas IL-3 and SCF were below the level of detection. In accordance with these results, NKT cell-depleted HUCB demonstrated 3-fold lower CFC activity compared to NKT cell-replete HUCB: 1/1120 cells (1/5000-1/420) vs 1/422 (1/2000-1/352) respectively (n=5; p=0.043). Similarly, LTC-IC activity was 2-fold lower in NKT cell-depleted compared to non-depleted samples: 1/2171 cells (1/3496-1/1591) vs 1/1110 (1/2910-1/650), respectively, (n=4, p=0.068). **Conclusions.** In summary, we have shown that i) CD1d is expressed in a subset of HSC with long- and short-term clonogenic ability, ii) CD1d+ HSC can activate NKT cells and c) CD1d-restricted NKT cells promote hematopoiesis. These findings reveal a novel link between hematopoiesis and the CD1d-NKT cell axis of immune regulation and set the scene for the study of the role of NKT cells in the processes of engraftment and rejection in HSC transplantation.

0350**FLT3 LIGAND IS IMPORTANT FOR BALANCED RECOVERY OF HEMATOPOIETIC FUNCTION WITHIN THE BONE MARROW NICHE AFTER STEM CELL TRANSPLANTATION**

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Flt3 ligand (FL) is an early-acting cytokine interacting with *flk2* tyrosine kinase receptors expressed by hematopoietic stem cells (HSCs). Our previous studies demonstrated that serum FL levels are specifically upregulated in patients undergoing myelo-suppressive conditioning prior to HSC transplantation. Elucidation of the FL role in hematopoietic recovery may, therefore, be of importance for understanding the cytokine microenvironment within the regenerating bone marrow (BM) niche after HSC-based therapies. We studied the mechanism of FL expression and its role in BM regeneration *in vivo* using mice with experimentally-induced myeloablation. Upon sublethal irradiation of wild-type (wt) mice, basal FL levels of 100-350 pg/mL increased up to 1600 pg/mL on day 4 of BM aplasia and returned to normal upon hematopoietic recovery on days 20-24. On day 4, membrane-bound FL was strongly upregulated on the surface of CD3⁺ cells, indicating that T cells produce FL during aplasia. However, the existence of other cellular FL sources was implicated by observation that in sublethally irradiated NOD/SCID and *Rag2^{Δc}* mouse mutants, lacking mature T cells, serum FL levels were equally elevated as in wt mice. To search for the main FL source, we employed *flk2*^{-/-} and *FL*^{-/-} mice. First, we demonstrated that basal FL levels in *flk2*^{-/-} mice are constitutively increased to 6500 pg/mL. The abnormally high levels of FL could be stably corrected to normal upon transplantation of wt BM. Conversely, FL levels were persistently elevated in wt mice which received *flk2*^{-/-} BM. These reciprocal transplantations between wt and *flk2*^{-/-} mice revealed that regulation of FL levels is intrinsic to donor BM cells. Further, when wt mice were transplanted with *FL*^{-/-} BM and subjected to secondary irradiation, high levels of FL were produced, thus suggesting that BM-resident non-hematopoietic cells rather than irradiation-sensitive hematopoietic cells are the FL source. HSCs (Lin⁻Kit⁺Sca1⁺) were present at similar frequencies in wt and *flk2*^{-/-} mice, indicating that upregulation of FL in *flk2*^{-/-} mice is not due to differences in HSC content, but the absence of *flk2* expression. Oral administration of *flk2* kinase inhibitor PKC412 (90 mg/kg daily for 2 weeks) lead to 2.5 fold increase in FL serum levels in the absence of peripheral or BM aplasia, further suggesting that regulation of FL levels is related to *flk2* signaling. To examine functional properties of *flk2*^{-/-} in comparison to wt HSCs, we generated mixed chimaeras by transplanting BM of both mouse strains at the 1:1 ratio. At 2 months, chimaeric peripheral blood consisted of up to 85% of wt and only 15% of *flk2*^{-/-} cells; moreover, the development of *flk2*^{-/-} lymphoid CD3⁺ and B220⁺ cells was impaired, indicating that repopulation with BM cells lacking the *flk2* receptor is less efficient and skewed. These results demonstrate that loss of HSCs due to hematopoietic injury, lack of *flk2*-expressing cells, or block of *flk2* signaling, provide a signal for FL release *in vivo* and that FL is important for a balanced differentiation of hematopoietic lineages within the regenerating HSC niche in the BM.

0351**GENE EXPRESSION PROFILING IDENTIFIES A UNIQUE SET OF GENES SPECIFICALLY EXPRESSED IN HUMAN HEMATOPOIETIC STEM CELLS FROM BONE MARROW, MOBILIZED PERIPHERAL BLOOD, UMBILICAL CORD BLOOD AND FETAL LIVER**

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Hematopoiesis is characterized by a well-regulated balance of self-renewal, differentiation, migration and homing of hematopoietic stem cells (HSCs). Although these characteris-

tics are commonly used to define stem cells, the mechanisms involved in the regulation of these HSC functions are only partly understood. Gene expression analysis of HSCs could help to reveal the molecular mechanisms underlying their biological properties. Therefore, we generated gene expression profiles of HSCs and hematopoietic progenitor cells (HPCs) from different human sources and determined the genes that were differentially expressed between these cell populations. *Methods.* Hematopoietic stem cell enriched CD34⁺CD38⁻ and the more differentiated CD34⁺CD38⁺ progenitor cell populations were isolated from human bone marrow (BM), mobilized peripheral blood (mPB), umbilical cord blood (UCB) and fetal liver (FL). cRNA was synthesized using 250-500 ng total RNA from each sample according to the Affymetrix small sample labeling assay. The gene expression profiles were evaluated using Affymetrix U133A oligonucleotide arrays containing 22,283 probe sets representing approximately 13,000 different human genes. *Results.* In total 7708 transcripts were found to be expressed in the HSCs from BM, mPB and UCB. Analysis of fetal liver samples is in progress. Comparative analysis of CD34⁺CD38⁻ versus CD34⁺CD38⁺ expression profiles revealed that the number of genes over- and underrepresented in HSCs varied from 222 to 943 for each individual tissue. However, comparison of these gene expression profiles to identify common HSC specific genes demonstrated 71 genes to be overrepresented and 41 genes to be underrepresented in HSCs of all three tissues. By performing this intersection analysis we have ruled out the tissue specific differentially expressed genes and generated a relatively small unique set of genes specifically expressed in HSCs of BM, mPB and UCB. These HSC specific genes, of which the majority was shown to be involved in transcription and cell signalling, may be important for HSC properties. A subset of the genes overrepresented in human HSCs was also found to be present in the murine HSC specific gene set published by Ivanova et al. (*Science* 2002;298:601-4). These evolutionary conserved genes are likely to be involved in key hematopoietic stem cell functions. This study reveals the differences between the transcripts associated with HSCs versus the more differentiated progenitor cells and yielded a unique list of common HSC specific genes that may contribute to a better understanding of the biological properties of HSCs. The data analysis of fetal liver derived HSC and HPC expression profiles is in progress. The complete list of genes specifically expressed in HSCs from human bone marrow, mobilized peripheral blood, umbilical cord blood and fetal liver will be presented at the meeting.

0352**CLONING AND CHARACTERIZATION OF A NOVEL HUMAN MYELOID-ASSOCIATED DIFFERENTIATION MARKER GENE FROM HUMAN BONE MARROW STROMA CELLS**Q. Wang¹, L. Li Nan², W. Xiaojian¹, Y. Hai¹, S. Wenji¹, Z. Lihuang¹, W. Jianli¹, C. Xuetao¹¹Zhejiang University, Hangzhou, China, ²Institute of Immunology SMMU, Shanghai, China

The hematopoietic lineage commitment is a very complex biological process, the molecular mechanism of lineage choice of multipotent progenitor cells remain largely unknown. Analysis of the genes involved in hematopoietic cell lineage commitment will provide some insights into the mechanism of hematopoietic differentiation and even contribute to elucidate the causes of many hematopoietic disorders. *Aims.* In the present study, we reported the molecular cloning and fundamental characterization of a novel gene shares high homology with mouse myeloid-associated differentiation marker (*Myadm*) gene from bone marrow stromal cells (BMSCs), thus designated as huBMSC-*Myadm*. The mRNA expression pattern, expression regulation in myeloid leukemia cells after PMA stimulation, cytolocalization of huBMSC-*Myadm* were investigated. *Results.* A full-length cDNA of 2080 bp isolated from BMSC cDNA library was predicted an ORF encoding 322 amino acids. The

deduced protein contains eight putative transmembrane segments. Monitored by fluorescence confocal microscopy, we demonstrated the membrane localization of huBMSC-Myadm. Northern blot analysis shows that huBMSC-Myadm mRNA is expressed in human spleen, lung, liver, testis, prostate, skeletal muscle, and peripheral blood leukocyte (PBL). RT-PCR analysis revealed that huBMSC-Myadm mRNA is selectively expressed in monocytes, monocyte-derived immature or mature dendritic cells (DCs), human BMSCs, and promyeloid or monocytic leukemia cell lines. Message was not detected in human CD4+ or CD8+ T lymphocytes or CD19+ B lymphocytes freshly isolated from PBMCs, nor in T cell leukemia cell lines and lymphocytic leukemia cell lines. HuBMSC-Myadm mRNA expression was significantly upregulated in acute promyelocytic leukemia HL-60 and chronic myelogenous leukemia K562 cell line after phorbol myristate acetate (PMA)-induced differentiation. **Conclusions.** Our study suggests that huBMSC-Myadm is selectively expressed in myeloid cells, and involved in the myeloid differentiation process and PMA-induced leukemia cell differentiation.

Myeloproliferative disorders

0353

FINAL REPORT OF A PHASE II TRIAL OF PEGYLATED INTERFERON A-2B THERAPY IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA. CLINICAL RESPONSES, EFFECTS ON PRV-1 EXPRESSION AND IMPACT ON QUALITY OF LIFE

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Interferon- α (IFN) is an effective treatment in myeloproliferative disorders. However, 21-25% of patients discontinue therapy due to side effects. **Aims.** We performed a phase II feasibility study of PegIntron[®] treatment in 22 PV and 20 ET patients, 20 females 22 males, median age 54 years (29-77). Inclusion criteria were a platelet count of $>400 \times 10^9/L$ in patients with symptoms or previous thrombosis ($n=26$) or $>1000 \times 10^9/L$ in asymptomatic patients ($n=16$). 15 patients had previously received therapy; anagrelide (7), hydroxyurea (6), busulfan (1), P³² (1). Median time from diagnosis to study 0.8 years (0.01-30.2). Initial dose was 0.5 $\mu g/kg$ once weekly. Treatment goal was CR defined as a platelet count $<400 \times 10^9/L$ in symptomatic patients and <600 in asymptomatic patients. If CR was not achieved within 12 weeks, the PegIntron dose was doubled. The dose was subsequently decreased to the lowest maintaining CR. QoL was investigated using QLQ-C30 and HAD. Neutrophil PRV-1 mRNA expression was analyzed by quantitative RT-PCR prior to and after therapy. **Results.** Mean baseline platelet count was $875 \times 10^9/L$, and 512, 448, 362, $352 \times 10^9/L$ at 3, 6, 12 and 24 months. No thrombotic or bleeding complications were observed. At 6 months, 29/42 patients (69%) had achieved CR, after a median of 83 days. Median dose at CR was 0.5 $\mu g/kg$. CR rate was not related to diagnosis or gender. At 6 months 4/14 responding PV patients required phlebotomy, compared to 20/22 before PegIntron. 4 patients discontinued therapy early due to side effects, another 9 were taken off study at 6 months (side effects 2, insufficient response 7). 20/29 patients main-

tained CR at 12 months, whereas 9 went off study. 13/20 patients have completed the planned 2-year treatment in CR, the remaining 7 are in CR at 22-23 months. Side effects were common, the majority WHO grades I-II; fatigue (76%), injection site reaction (64%), flu-like (64%), headache (56%), muscle pain (51%), insomnia (24%), hair loss (20%). However, only 4/13 patients treated for 2 years report any side effects at 2 years. QLQ-C30 revealed significant increases in fatigue, dyspnoea and diarrhoea at 6 months, but all parameters returned to baseline at 1 year. Thirty-five percent of patients reported depressive symptoms, but HAD-scales showed no evidence that PegIntron induces clinical depression or anxiety compared to baseline. *PRV-1* data are presently available for 11 *PRV-1* positive patients (5 PV, 6 ET). *PRV-1* expression is normalized in 1 PV and 1 ET at 24 months and in 2 ET patients at 6 months. Effects on splenomegaly and cytogenetics will be presented. **Conclusions.** PegIntron effectively reduced platelet counts in 29/42 patients. 20 patients are projected to maintain CR at 2 years with unaffected QoL, whereas 22 patients discontinued therapy. Reversal of *PRV-1* positivity noted in a subset of patients (not previously reported after hydroxyurea) suggests that PegIntron can have an effect on the malignant clone in PV and ET. IFN is a valuable therapeutic alternative in patients who tolerate initial side effects.

0354

IMATINIB IN FIP1L1-PDGFR α POSITIVE HEMATOLOGICAL DISORDERS ASSOCIATED WITH EOSINOPHILIA

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A substantial number of patients diagnosed with idiopathic hypereosinophilic syndrome (HES) or chronic eosinophilic leukemia (CEL) are positive for the *FIP1L1-PDGFR α* fusion gene which results from a cytogenetically invisible interstitial chromosomal deletion on chromosome 4q12. The pathogenesis is similar to BCR-ABL positive chronic myeloid leukemia (CML) with constitutively activated tyrosine kinase of the fusion protein and excellent response to treatment with imatinib. By now, we have identified 17 *FIP1L1-PDGFR α* positive patients initially diagnosed with HES or CEL ($n=10$), acute myeloid leukemia (AML, $n=3$), systemic mastocytosis with eosinophilia (SME, $n=3$) or T-cell lymphoblastic lymphoma with eosinophilia ($n=1$). Sequence analysis revealed a substantial heterogeneity of the fusion transcripts due to the involvement of several *FIP1L1* exons, variable breakpoints within *PDGFR α* and the insertion of intron-derived sequences of different length and origin in $>50\%$ of cases. For unknown reasons the vast majority of patients were male (16/17 patients, 94%). The median age at diagnosis was 50 years (range, 30-65). Three patients had a history of significant eosinophilia of more than 10 years, further three patients of more than 5 years, respectively. Treatment with imatinib was initiated in 14 patients and a clinical follow-up of at least 3 months was available from 10 patients (median 11.5 months, range 5.5-21). All seven patients with CEL or SME achieved a rapid complete hematologic remission after a median time of 1.0 month (0.2-2.7) and six patients achieved complete molecular remission after a median time of 6.8 months (1-19.8). One patient with acute eosinophilic leukemia was not eligible for conventional chemotherapy and received imatinib as monotherapy. Currently, 16 months after start of imatinib, he is in complete hematologic and molecular remission. Two patients with AML received imatinib after one course of conventional induction chemotherapy. Both patients are in sustained complete hematologic remission, one additionally in complete molecular remission 9 months after initiation of imatinib. We conclude that all patients who present with sustained non-reactive hypereosinophilia should be monitored for the *FIP1L1-PDGFR α* fusion gene by RT-PCR or FISH. There is a significant clinical variability ranging from a complete asymp-

tomatic disease to acute, possibly secondary acute myeloid leukemia similar to blast crisis in CML. Cytogenetic analysis is recommended in *FIP1L1-PDGFR*A negative cases in order to exclude rearrangements of genes coding for tyrosine kinases that are known to be involved in hematologic disorders associated with eosinophilia, e.g. *PDGFRB* on 5q31, *FGFR1* on 8p11 or *JAK2* on 9p24. Patients with rearrangements of *PDGFR*A and *PDGFR*B are candidates for treatment with imatinib even if they present with progressive disease, e.g. secondary AML.

0355

RIMM: A NATIONWIDE PROSPECTIVE REGISTRY OF 1107 PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA

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Myelofibrosis with myeloid metaplasia (MMM) is a rare chronic myeloproliferative disease. Knowledge about natural history, prognosis and therapy is burdened by selection bias and limited size. The Italian Registry of Myelofibrosis (RIMM) is a nationwide network of 702 clinical and pathology units: its aims are to characterize the epidemiologic, clinical, and prognostic issues of myelofibrosis in a population-based prospective cohort. Since June 1999, 1107 patients were referred to RIMM: after clinical and pathologic revision, diagnosis was confirmed in 1042 patients (365 females), providing an estimated incidence rate of 0.34/100.000/year. Median age was 72 years (0.5% <30 years; 8% <50 years; 21% >80 years). Cluster of atypical megakaryocytes in bone marrow biopsies were reported in 699 out of 782 (89%) complete histopathological analyses. Bone marrow cellularity was usually high (median 80%), but in 22% of patients it was <50%. Diffuse bone marrow fibrosis was an originally necessary diagnostic criterion (Italian Consensus Conference on Diagnostic Criteria, 1999), however, 9 cases were a-posteriori classified as prefibrotic MMM according to WHO (2001). Out of 386 patients with a cytogenetic analysis performed on peripheral blood at diagnosis, 66 presented with cytogenetic abnormalities (17%): deletions (36 patients), especially in chromosomes 7, 13 and 20, and trisomies (23 patients), especially in chromosomes 8 and 9, were the most frequent abnormalities. In 817 patients with a complete clinical record, mean hemoglobin was 10.7 g/dL, but it was below 8 g/dL in 16% of patients. Mean WBC count was $13.4 \times 10^9/L$: 7% of patients had values $>30 \times 10^9/L$ and 14% $<4 \times 10^9/L$. Mean platelet count was $351 \times 10^9/L$, but in 6% of patients the count was $<50 \times 10^9/L$ and in 17% $>600 \times 10^9/L$. Prognostic Lille score was low in 51% and high in 14% of patients. Mean spleen size was 6.8 cm from the costal arc (18 cm longitudinal US diameter; $r=0.74$): in 48% and 25% of patients, spleen was minimally enlarged at palpation (<5 cm from costal arc) and at US (10-15 cm), respectively. Overall, 172 (20%) patients reported a previous diagnosis of essential thrombocythemia (ET) or polycythemia vera (PV): they had a significantly lower age and lower (ET) or higher (PV) spleen size. Overall, 209 patients had circulating CD34⁺ cells assessed at diagnosis: only 4% had counts <4/mL, and 17% <15/mL. Forty-four percent of patients were diagnosed at Internal Medicine or Oncology Units: they had an older age (73 vs 68 years; $p<0.001$), lower hemoglobin (10.2 vs 11.0 g/dL; $p<0.001$), lower platelet count (310 vs $390 \times 10^9/L$; $p=0.002$) and higher Lille score ($p<0.001$) than those diagnosed at Hematology Units. After a median follow-up of 30 months, 18% of patients has died: the principal causes of death were cardiovascular, infective and hematologic (major bleedings, blastic transformation). Data from the largest cohort of consecutive MMM patients support a shift to older age of the patient cohort. Clusters of atypical megakaryocytes in bone marrow biopsies and CD34⁺ count in peripheral blood are sensible diagnostic parameters.

0356

A GENOME WIDE SCREENING FOR GENETIC IMBALANCES OF CML BY ARRAY CGH

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Chronic myelogenous leukemia (CML), a clonal myeloproliferative disorder of the hematopoietic stem cell, typically evolves in three distinct clinical stages. The expression of the chimeric BCR/ABL fusion gene resulting from the disease-specific t(9; 22) is necessary for malignant transformation but not sufficient to maintain the disease progression. The appearance of various chromosomal and molecular alternations in the accelerated and terminal phase of CML is well documented without any causal relationship. The presence of the cryptic deletion at the translocation site in the der (9) chromosome detectable by FISH was shown to correlate with a short chronic phase and hence-short survival. The consequences of the der (9) deletions and mechanism of formation remain unclear, as does our understanding of the molecular events behind the disease evolution. Here we present a genome wide screening for genetic imbalances by array CGH of 50 samples from CML patients in chronic, accelerated or blast phase including 10 CML cell lines and controls. There are two samples from 5 CML patients, taken at diagnosis and onset of blast stage. We used arrays at a resolution of 1Mbp, representing some 2,7000 BAC clones (Spectral Genomic Inc.). Twelve patients' samples and all cell lines have a comprehensive molecular cytogenetics profile, including classical CGH and colour karyotyping (Gribble *et al.*, *Cancer Gen & Cytogen.* 1999 and *GenesChrom&Cancer*, 2003). Out of some 2000 loci found to display imbalances in all samples, only aberrations presented by fluorescence ratio in excess of 3 standard deviations were considered significant. Our findings fall into the following groups: (i) Single BAC gains/losses as recently reported in 10% of normal individuals and termed large-scale copy number variations (LVCs) by Lanfrate *et al.*, *Nat Gen*, 2004. Combinations of 6 LCVs were seen in over 90% of the CML patients (mean of 3.2 per patient) forming haplotypes; (ii) Common breakpoints delineating extended (segmental) loss in 8p13 of the region flanked by RP11-91J19 (proximally) and RP11-89M20 (distally) as well as the amplifications affecting the region at 8q24.12 flanked by RP11-94M13 (proximally) and RP11-45B19 (distally). (iii) A common breakpoint at 17p11.2 associated with the presence of iso (17q) marker chromosome. (iv) Recurrent gains of the 6q16.1/qter and 11q22/qter segments. (v) New imbalances affecting the ABL/BCR breakpoint regions at der (9) chromosome. These findings bring new insights into the CML genomic profile and draw attention to the previously unappreciated large scale genomic heterogeneity and its possible association with either disease specific gene rearrangements, susceptibility to diseases or reaction to specific environmental stimuli.

0357

WIDESPREAD OCCURENCE OF THE JAK2 V617F MUTATION IN CHRONIC MYELOPROLIFERATIVE DISORDERS

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We have investigated samples from 679 patients and controls for the JAK2 V617F mutation using a tetra primer ARMS assay. Of the 480 samples with a known or suspected diagnosis of an

MPD, 129 (27%) were positive for the V617F JAK2 mutation and 351 (73%) were negative. Sequence analysis on selected cases (n=51) was fully concordant with the ARMS results. The proportion of positive cases per disease subtype was PV 58/72 (81%), ET 24/59 (41%), IMF 15/35 (43%), idiopathic HES 2/134 (2%), aCML/CML 17/99 (17%), unclassified atypical MPD 13/53 (25%). The mutation was not identified in patients with systemic mastocytosis (n=28), CML (n=18), AML (n=17), secondary erythrocytosis (n=4) or normal controls (n=160). Of the 14 V617F mutation negative PV patients, 13 were male (p=0.005) but no other significant associations between sex and mutation status were identified. Mutation analysis of JAK2 exon 12 in V617F negative cases did not reveal any additional sequence variants. In many cases the intensity of the mutant band was stronger than the wild type band, suggesting that the mutation was homozygous. To investigate this further we developed a Pyrosequencing assay, since this technique provides robust allele ratios. Overall, homozygosity was seen in 55 of the 129 (43%) mutant samples. The frequency of homozygotes relative to heterozygotes was not significantly different from the average of all cases in any of the subgroups apart from ET, in which the proportion of homozygous mutants was significantly lower than average (p=0.009). Homozygous status was closely correlated with chromosome 9p uniparental disomy, as determined by microsatellite analysis to determine 9p marker zygosity and multiplex probe ligation amplification to determine JAK2 copy number. In 53 cases analysed (PV, n=15; ET, n=32; IMF, n=6), the median level of PRV1 expression was significantly higher in V617F positive cases compared to cases without the mutation. We conclude that V617F is widespread in MPDs. Detection of this mutation is likely to have a major impact on the way MPD patients are diagnosed, as well as serving as an obvious target for signal transduction therapy.

Hodgkin's lymphoma

0358

CONVENTIONAL PROGNOSTIC FACTORS IN CLINICAL STAGE IA/IIA HODGKIN'S LYMPHOMA (HL) AFTER TREATMENT WITH ABVD-BASED COMBINED MODALITY THERAPY (CMT)

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ABVD-based CMT has emerged as the standard of care for early stage HL during the last decade. However, risk stratification is still based on conventional prognostic factors, mainly derived from radiotherapy (RT) treated patient populations. We have adopted ABVD-based CMT for the treatment of all patients with early-stage HL, since 1988. *Aims.* To evaluate the role of conventional demographic, clinical, and laboratory prognostic factors in the prognosis of patients with clinical stage IA/IIA HL under current standard therapy. Patients and *Methods.* We analyzed the clinical data of 387 consecutive patients with clinical stage IA/IIA HL, who were scheduled to receive ABVD-based CMT in our Unit between 1988 and 2004. In brief the median age of the patients was 30 years (14-82), 55% were males, 38% and 62% had clinical stage IA and IIA, 28% had ≥ 3 involved sites, 11% pure infradiaphragmatic disease, 30% bulky disease (mediastinal and/or peripheral), 19% extranodal extension (E-disease), 18% had anemia, 35% leukocytes $\geq 10 \times 10^9/L$, 4% severe lymphocytopenia, 21% serum albumin < 4 g/dL, 46% ESR ≥ 30 mm, and 32% elevated LDH levels. The histologic subtype was nodular sclerosis in 65% of the patients, mixed cellularity in 20%, and lymphocyte predominance in 14%. The ABVD and EBVD (E=Epirubicine) regimens were administered in 55% and 45% of the patients respectively. 22 patients (6%)

did not actually receive RT because of refusal or early progression. Involved field RT was administered in 82% and mantle or inverted Y in 7% of the patients. The median RT dose was 2800 cGy. *Results.* At 5 and 10 years failure free survival (FFS) rates were 89 \pm 2% and 87 \pm 2% respectively. Multivariate analysis demonstrated that independent adverse prognostic factors for FFS were: Age ≥ 45 years (p=0.002), extranodal extension (p=0.002), leukocytes $\geq 10 \times 10^9/L$ (p=0.03), bulky disease (p=0.03), and male gender (p=0.03). The percentages of patients with 0, 1, 2 or 3-5 adverse factors were 12%, 42%, 33%, and 13%, respectively. The 10-year FFS rates of patients with 0, 1, 2 or 3-5 adverse factors were 97 \pm 3%, 93 \pm 3%, 83 \pm 5%, and 64 \pm 7% (p<0.0001). The corresponding 15-year overall survival rates were 100%, 96 \pm 2%, 75 \pm 8%, and 80 \pm 8%, respectively (p=0.01), while 15-year HL-specific survival rates were 100%, 98 \pm 1%, 86 \pm 6%, and 80 \pm 8% (p=0.005). *Conclusions.* After ABVD-based CMT, approximately 55% of patients with clinical stage IA/IIA HL-those with 0 or 1 adverse factors- have a very favorable outcome. Patients with 2 adverse factors (33% of the total) have intermediate outcomes, but 13% of patients with 3 or more adverse features have more aggressive disease with a prognosis similar to that of advanced stages.

0359

BEACOPP CHEMOTHERAPY FOR ADVANCED HODGKIN'S DISEASE: LATEST RESULTS FROM THE GERMAN HODGKIN STUDY GROUP TRIALS FOR TREATMENT OPTIMISATION

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Escalated-dose BEACOPP proved more effective than conventional COPP/ABVD or standard-dose BEACOPP in the HD9 trial. In HD12 we tested a variant with more moderate use of escalated BEACOPP to reduce toxicity. *Aims.* We assess long-term survival and second acute leukemias with escalated-dose BEACOPP in HD9 and the mid-term effectiveness of the new variant with/without radiotherapy in HD12. *Methods.* HD9: In 1993-98, 1201 patients aged 16-65, stage IIB or IIIA with risk factors or stage IIIB-IV were randomised to 4x (COPP+ABVD) (4CA), 8x standard-dose BEACOPP (8SB) or 8x escalated BEACOPP (8EB), with radiation of initial bulky and residual disease. HD12: In 1999-2002, 1396 patients aged 16-65 in stage IIB with risk factors or stage III-IV were randomised in a factorial design to 8EB (arms A, B) vs. 4x escalated plus 4x standard BEACOPP (4E4S) (arms C, D), and then randomised to radiation of initial bulky and residual disease (arms A, C) vs. no irradiation (B, D). All BEACOPP cycles were 21 days. An expert panel reviewed results after chemotherapy and advised on radiation independent of arm. *Results.* With 82 months median follow-up in this update to HD9, the estimated freedom from treatment failure (FFTF) and overall survival (OS) rates were: 4CA 67% and 79%, 8SB 75% and 84%, 8EB 85% and 90% (p<0.001 and p=0.004) respectively. 61 second malignancies were documented, with similar rates in each arm. More second acute leukemia/MDS occurred with 8EB (11/466) than 8SB (5/469) or 4CA (1/260). In the 4th interim analysis of HD12 with 30 months median follow-up, the overall complete remission rate was 91% and the early progression rate 2%. 6% of patients have died, including 3% due to acute toxicity. The 30-month FFTF rate was 88% (95% confidence interval: 86-90%) and the 30-month OS rate was 94% (92-95%) for the total cohort. Radiation was given in arms A+C in 60% and in arms B+D in 9% (mostly upon panel recommendation). There were 29 second malignancies; the rate of second acute leukemia/MDS (11/ 1396) at this early analysis was lower than in HD9. Group sequential analysis showed no significant differences in FFTF according to chemotherapy arm or irradiation arm (irradiation according to panel recommenda-

tion was not counted as treatment failure). Summary/conclusions. Escalated-dose BEACOPP increases the risk of second acute leukemia but achieves an OS superior to that of COPP/ABVD or standard-dose BEACOPP. The new BEACOPP variant using 4 escalated and 4 standard cycles appears to be equivalent to 8x escalated BEACOPP, but longer follow-up is needed. Progress of the current trial HD15 assessing alternative reductions of BEACOPP (6x escalated BEACOPP; 8x standard BEACOPP with an accelerated 14-day cycle) will also be reported.

0360

THE IMPACT OF SOCIOECONOMIC STATUS ON THE COMPLETE REMISSION RATE IN PATIENTS WITH HODGKIN'S DISEASE IN BRAZIL

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Socioeconomic status (SES) has been shown to be a determinant of clinical outcome in various types of cancer. The aim of this study was to analyse the impact of the socioeconomic status on the complete remission (CR) rate of patients with Hodgkin's disease (HD). *Methods.* From November 2001 to October 2004, 156 consecutive patients were prospectively followed in five institutions (three public and two private) in Rio de Janeiro. Data regarding disease and treatment features were collected, and patients were classified according to the International Prognostic Score (IPS). Each patient answered a questionnaire with information about their socioeconomic status, including educational level, household income, ownership of household goods (radio, TV, refrigerator, washing machine, VCR/DVD and car), presence of housemaid, and housing features such as electricity, source of drinking water, type of toilet and sewage facilities, type of floor, roof and wall, and number of persons per sleeping room. Most of these features were used to calculate two indexes of socioeconomic status: the 'Criteria for Economic Classification' developed by the National Association of Public Opinion Research, which has been validated in publicity and political polls in Brazil, and the 'Assets Questionnaire' developed by the Health, Nutrition and Population/Poverty Thematic Group of The World Bank. A total of 138 patients have completed primary treatment and are available for this analysis. Patients were divided in two groups according to their socioeconomic status: higher SES (classes A1 to C) and lower SES (classes D and E). IPS score risk was also categorized in low risk (2 or less risk factors) and high risk (more than 2 risk factors). *Results.* According to the 'Criteria for Economic Classification', one patient was classified in social class A1 (1%), six in A2 (4%), 27 in B1 (20%), 28 in B2 (20%), 44 in C (32%), 31 in D (22%) and one in E (1%). The overall CR rate was 78% (107/138 patients). The CR rate was higher in patients with a higher SES than those with a lower SES (82% versus 66%, $p=0.047$). There was no statistically significant correlation between the SES group and the individual variables comprised in the IPS, nor with the histopathologic subtypes. The median time from the beginning of symptoms to diagnosis was 4 months (1-36) in the higher SES group and 6 months (2-30) in the lower SES group ($p=0.26$). A comparison of the CR rates between the higher and lower quintiles of the score generated by the World Bank Assets Questionnaire did not show any difference. The CR rate was higher in patients with a low risk IPS (91% vs. 66%, $p=0.002$). In a multivariate analysis including the IPS and the SES groups, only the IPS remained significantly associated with the CR rate. Summary/conclusion: Patients with a higher socioeconomic status had a higher complete remission rate. It is possible that some of the variables in the IPS reflect SES, but we could not identify them, possibly due to the low sample size.

0361

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IMPROVES SURVIVAL IN HIV-ASSOCIATED HODGKIN'S DISEASE

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Hodgkin's disease (HD) is the most common non-AIDS-defining malignancy in patients (pts) infected with the human immunodeficiency virus (HIV). HIV-associated HD (HIV-HD) has been reported to be of a more aggressive clinical behavior as compared with HD in HIV-uninfected patients. *Aims.* The purpose of the study was to evaluate the evolving characteristics and outcome of HIV-HD with respect to the use of highly active antiretroviral therapy (HAART). This multicenter retrospective cohort study included pts with histologically proven HIV-HD diagnosed from June 1984 to February 2004 at 11 institutions in Germany. Data on pts diagnosed with HIV-HD prior to the introduction of HAART (1984-1996) were compared with those who were diagnosed in the HAART-era (1997-2004). HAART-response was defined as increase in the CD4⁺ lymphocyte count of at least 100/ \times L and/or at least one plasma HIV-RNA viral load below 500 copies/mL. Of 66 pts. with HIV-HD 47 (71%) presented with stage III/IV disease, 47 (71%) had at least one extranodal manifestation, and 38 pts (58%) were found to have an AIDS-defining illness at diagnosis. 59 of 66 patients (89.4%) underwent curative intended chemotherapy (CT), consisting of ABVD alone or in alternating cycles with COPP (n=36), CHOP (n=9), EBOEP alone or in alternating cycles with COPP (n=8), BEACOPP (n=4), and other (n=2). The median number of completed CT-cycles was 4.8 (range 0-13). 1 patient received curative radiotherapy only. CT and concomitant HAART was administered in 33 pts. Of 56 pts evaluable for response, 33 (59%) achieved a complete response (CR) and 14 (25%) a partial response. 9 pts (16%) experienced progressive disease. The CR rate was 67% in pts with concomitant HAART as compared to 44% of pts in the pre-HAART group ($p=0.01$). A CD4 cell count $<200/\times$ L at HD diagnosis was observed in 70% of pts in the pre-HAART era as compared to 50% of pts in the HAART group. After a median follow-up of 13 months the median progression-free survival and overall survival (OS) of the entire group of pts. was 12 and 22 months, respectively. The estimated 3-year survival probability was significantly higher in pts who presented with CDC stage A/B as compared to CDC stage C (58% vs 33%, $p=0.01$). Pts receiving HAART (n=33) had a better 3-year OS as compared to those in the pre-HAART group (72% vs 15%, $p<0.0001$). The 2-year OS probability of HAART-responders was 92% as compared to 12% in pts without a HAART-response ($p<0.0001$). Summary/conclusions: HAART and, in particular, HAART-response significantly improved the CR rate and the 3-year overall survival in pts with HIV-HD.

0362

GLUTATHIONE-S-TRANSFERASE (GST) P1 GENOTYPE AS PROGNOSTIC MARKER IN HODGKIN'S LYMPHOMA

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Glutathione-S-Transferase (GST) P1 is a member of the GST enzyme superfamily which is important for the detoxification of several cytotoxic drugs and their by-products. A single

nucleotide polymorphism results in the substitution of Isoleucine (Ile) to valine (Val) at codon 105 causing a metabolically less active variant of the enzyme. Recently, the GSTP1 105Val genotype has been associated with favorable prognosis following chemotherapy with drugs known to be GSTP1 substrates in a variety of malignancies, such as pediatric acute lymphoblastic leukemia, myeloma, breast and colon cancer. *Aims.* We assessed the impact of the GSTP1 codon 105 genotype on treatment outcome in patients with Hodgkin's lymphoma. *Methods.* The Ile105Val polymorphism in the GSTP1 gene was analyzed using a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) technique. DNA was extracted either from peripheral blood or paraffin-embedded lymph node biopsies from 180 patients with Hodgkin's lymphoma (median age 31 years, range 13-71 years; 78 females and 102 males). 168 Patients were treated with standard chemotherapy regimens: 95 patients received ABVD, 32 pts a modified Stanford V regimen (substituting 6 mg/m² methchloramine with 650 mg/m² cyclophosphamide), 24 pts MOPP (+ABVD), 17 pts BEACOPP. Associations with patient characteristics and treatment outcome were analyzed. *Results.* GSTP1 genotype was assessed in 180 patients with Hodgkin's lymphoma, of which 16 (9%) were homozygous for the 105Val/105Val genotype, 58 (32%) were heterozygous (105Ile/105Val) and 106 (59%) were homozygous for 105Ile/105Ile GSTP1 genotype. The GSTP1 Ile105Val polymorphism was associated in a dose-dependent fashion with an improved failure-free survival in patients with Hodgkin's lymphoma ($p=0.02$). The probability of 5-year survival for patients homozygous for the 105Val/105Val GSTP1 genotype was 100%, for heterozygous patients 74% (95% C.I., 54-81), and 51% (95% C.I., 39-62) for patients homozygous for the 105Ile/105Ile genotype. When the analysis was restricted to 95 patients treated with ABVD chemotherapy, essentially the same differences in failure-free survival were observed. In univariate analysis of established prognostic factors, stage proved to be of prognostic value in our patient group (limited disease in stage I-IIA vs advanced disease in stage IIB-IV, $p=0.01$). The Cox multivariate analysis showed that GSTP1 codon 105 genotype and stage were independent prognostic factors ($p=0.028$, respectively). *Conclusions.* Our study indicates that the GSTP1 genotype predicts clinical outcome in patients with Hodgkin's lymphoma and points to the importance of pharmacogenetics to eventually identify patients with altered metabolism of cytotoxic drugs modifying their response to the treatment.

Chronic lymphoblastic leukemia and related disorders - Clinical

0363

OUTCOME IS IMPROVING IN CHRONIC LYMPHOBLASTIC LEUKEMIA: A POPULATION-BASED STUDY ON 9,756 PATIENTS DIAGNOSED IN SWEDEN 1973-2001

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B-cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia in the Western world with an incidence of 2-4.5 per 100,000 in the general population. Patients with CLL have a widely variable clinical course. After decades of relatively conservative approach the overall clinical management of CLL patients has changed considerably over the last years. Contributing factors are an improved understanding of biologic properties of the disease, new tools to risk-adapt treatment, and recently developed therapeutic agents and strategies includ-

ing supportive care measures. *Aims.* To define the impact of diagnostic and treatment strategies introduced during the last three decades, on outcome by estimating relative survival rates (RSR) in all diagnosed CLL patients in Sweden 1973-2001; and relate changes to age, gender and calendar period. *Methods.* Records on all patients with CLL reported to the Swedish Cancer Register between 1973 and 2001 were linked to the nationwide Cause of Death Register. The study period was divided arbitrarily in four calendar periods: 1973-1979, 1980-1986, 1987-1993, and 1994-2001. Patients were grouped according to age at diagnosis (0-40, 41-50, 51-60, 61-70, 71-80, and 80+) and gender. Survival analyses were performed by computing RSR, defined as the ratio of observed versus expected survival. Excess mortality was modeled using Poisson regression. All statistical estimates were applied by using SAS 8.2 (Cary, NC, USA). *Results.* A total of 9,756 patients (6,000 males and 3,756 females, mean age 70.5 years) were diagnosed with CLL in Sweden between January 1st 1973 and December 31st 2001. The overall five-year RSR estimates were 50%, 61%, 64%, and 72%, respectively, for the four calendar periods. The overall ten-year RSR was 26%, 37%, and 40% for the first three calendar periods; the twenty-year RSR was 13% and 21% for the first two calendar periods (ten-year and twenty-year RSR could not be calculated for the latter calendar periods). The increase in five-year and ten-year RSR was observed in all age categories over the studied calendar periods, while the increase in twenty-year RSR was restricted to patients <70 years. Younger age at onset was associated with an improved survival in all calendar periods. Differences in survival by age at diagnosis and calendar period were highly statistically significant ($p<0.0001$). Females had a better prognosis ($p<0.0001$) compared to males, after adjusting for age and calendar period. The gender effect did not vary over time. *Summary/conclusions.* The results of the present study show an improved prognosis over time in a population-based study including approximately 10,000 CLL patients diagnosed during almost 30 years. Despite potential differences in diagnostic classification and reporting over time, therapeutic developments have clearly contributed to this finding. Of special interest is that ten-year RSR has improved in all age groups over the study period. In addition, in patients <70 years at diagnosis, the twenty-year RSR improved over the first calendar periods. Males, the elderly and patients diagnosed during early calendar periods experienced higher excess mortality.

0364

CLADRIBINE WITH CYCLOPHOSPHAMIDE VS FLUDARABINE WITH CYCLOPHOSPHAMIDE AS FIRST-LINE TREATMENT IN CHRONIC LYMPHOBLASTIC LEUKEMIA: AN EARLY REPORT OF PROSPECTIVE, RANDOMIZED STUDY (PALG CLL3)

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The study comparing activity and toxicity of cladribine and cyclophosphamide (CC) versus fludarabine and cyclophosphamide (FC) in previously untreated progressive or symptomatic chronic lymphocytic leukemia (CLL) has started in January 2004. Eligible patients are assigned to either 2-CdA 0.12 mg/kg/d and cyclophosphamide 250 mg/m²/d for 3 consecutive days or fludarabine 25 mg/m²/d and cyclophosphamide 250 mg/m²/d for 3 consecutive days. The courses are administered at 28-day intervals or longer if myelosuppression develops.

The treatment is stopped after 3 courses in nonresponders. In responding patients it is continued up to 6 cycles. The preliminary results of our study indicate that CC and FC programs used as first line therapy give similar complete (CR) and overall (OR) responses and comparable frequency of drug-induced

thrombocytopenia, neutropenia and infections in both groups. So far, death rates have also been similar in patients treated with CC and FC. Larger number of patients and longer observation are necessary for final conclusions.

Supported by grant from MNIi (2P05B01828)

0365

ADDITION OF RITUXIMAB TO FLUDARABINE IMPROVES CLINICAL OUTCOME IN UNTREATED ZAP-70 NEGATIVE CHRONIC LYMPHOBLASTIC LEUKEMIA

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Clinical trials of monoclonal antibodies in combination with chemotherapy have reported previously unattained response rates in B-CLL. Nevertheless, the analysis of unmutated VH genes, CD38 and/or ZAP-70 protein could explain the discordant outcome independent of treatment observed. Recent data indicate that unmutated VH genes, CD38 and/or ZAP-70 protein overexpression predict a worse outcome. Studies from Cancer and Leukemia Group B have demonstrated that rituximab plus fludarabine (Flu) for symptomatic, untreated CLL allows to achieve higher remission rates, longer progression free (PFS) and overall survival (OS). We performed a phase II study that added rituximab sequentially to Flu for symptomatic, untreated CLL in order to evaluate the clinical outcome. ZAP-70 protein and CD38 antigen were determined on mononuclear cells by flow cytometry. Minimal residual disease (MRD) was assessed by a multiparametric flow cytometric method. Sixty B-CLL patients, median age 59 years, received six monthly courses of Flu (25 mg/sqm for 5 days) and four weekly doses of rituximab (375 mg/sqm) starting on an average of forty days after completion of the Flu therapy. According to modified Rai stages, 5 patients had low-risk disease, 52 patients had intermediate-risk and 3 patients had high-risk. Five of 58 pts, treated with rituximab, experienced mild infusion-related symptoms consisting of fever, chills and rigors. There were 13 opportunistic infections, including 3 herpes zoster, 7 herpes simplex, 2 cases of grade 3 pulmonary toxicity and 1 fulminant B hepatitis. Hematologic toxicity included neutropenia (grade 1 and/or 2 in 12 pts, grade 3 and/or 4 in 29 pts), thrombocytopenia (grade 1 and/or 2 in 4 pts, grade 3 and/or 4 in 3 pts) and anemia (grade 2 in 3 pts). Based on NCI criteria, 47/60 (78.3%) patients achieved a complete remission (CR), 9/60 (15%) a partial remission (PR) and 4/60 (6.7%) no response or progressive disease. Noteworthy, our patients experienced a long PFS from treatment (68% at 3 years). Twenty-eight patients (46.7%) were found to have ZAP-70>20% and 19/60 patients (31.7%) presented CD38>20%. MRD performed on bone marrow by flow cytometry was positive (>5% CD19⁺CD5⁺CD79b⁻ CLL cells) in 15/60 (25%) patients.

Treatment	Patients (n=72)		DR (%)	CR (%)	Transients/pts (n=36)	Remission at 30%	Infections	Dwt
	Flu+Rit	Flu						
CC	82	30	28 (34.1%)	11 (14.7%)	5 (14.7%)	11 (34.7%)	21 (64.7%)	2 (6.3%)
FC	83	32	28 (33.7%)	12 (37.5%)	3 (9.4%)	11 (31.2%)	18 (53.1%)	2 (6.2%)
p-value			0.27	0.1	0.22	0.45	0.09	0.67

With regard to clinical outcome, all cases (n=32) with ZAP-70<20% obtained a CR or PR (p=0.0009) and a higher overall response rate was found in CD38- patients (98% vs 84%, p=0.02). A significantly shorter PFS was observed in ZAP-70+ patients (25% vs 100% at 3 years; p=0.00005), in CD38+ patients (16% vs 91% at 3 years; p=0.0002) and in those with higher MRD (36% vs 76% at 2.5 years; p=0.001). OS was significantly shorter in ZAP-70+ patients (68% vs 100% at 3 years; p=0.006) and in MRD+ patients (72% vs 89% at 3 years,

p=0.002). Therefore, the addition of monoclonal antibodies to chemotherapy, allowed us to obtain improved responses and outcome. Moreover, the stratification of patients by means of ZAP-70 and CD38, might allow us to offer more aggressive and/or experimental approaches to high risk B-CLL subsets.

0366

FLUDARABINE PLUS CYCLOPHOSPHAMIDE (FC) IS SUPERIOR TO FLUDARABINE (F) ALONE IN FIRST LINE THERAPY OF CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL): RESULTS OF A PHASE III STUDY OF THE GERMAN CLL STUDY GROUP (GCLLSG)

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In younger CLL pts. F has become the standard first line therapy. The combination therapy FC may further improve the quality of the response and prolong survival. To evaluate the potency of the FC combination, the GCLLSG initiated a phase III study, the CLL4 protocol, to directly compare the safety and efficacy of F vs. FC as first-line therapy for advanced CLL. *Methods.* Between July 1999 and July 2003 375 pts (mean age 59.0 [range 33-65] years) were randomized to receive either F (n=190) or FC (n=185). This analysis was performed with all information available as of August 2004. 7.7% of the pts were in Binet stage A with severe symptoms requiring therapy, 52.3% in stage B, and 32.0% in stage C. Staging data were not yet available for 8.8% of the pts. There was no significant difference between the two treatment arms regarding Binet stage or other clinical parameters. Pts in the FC arm received F (30 mg/m²/day (d) IV) plus C (250 mg/m²/d IV) for 3 consecutive days, every 28 days for up to 6 cycles (median 5.0 courses). Pts in the F arm received F (25 mg/m²/d IV) for 5 consecutive days, every 28 days for up to 6 cycles (median 5.2 courses). Anti-infective prophylaxis and growth factors were not given routinely. *Results.* 299 pts were evaluable for response. The overall response rate (ORR) was significantly higher in pts treated with FC (141/148; 95.3%) than pts treated with F alone (127/151; 84.1%) (p=0.002). The CR rate was significantly higher with FC therapy as well (20.3% vs. 8.6%) (p=0.004). After a median observation time of 19.3 months (mo) median time to progression was 46.7 mo in the FC arm vs. 21.0 mo in the F arm (p = 0.003). No difference in the overall survival was observed so far. Safety data were available in 160 FC treated and 163 F treated pts. Myelotoxicity was significantly more frequent (91.9% vs. 74.8% of the pts; p<0.001) and more severe in the FC arm (64.4% vs. 37.4% of the pts with common toxicity criteria (CTC) grade 3+4; p<0.001). Especially leukocytopenias were more common (83.8% vs 55.2%; p<0.001) and severe (25.2% vs. 55.0% CTC grade 3+4; p<0.001) in the FC arm. Thrombocytopenia was more frequent in the FC arm as well (36.8% vs. 48.8%; p=0.03). The incidence of severe infections was similar in both arms (8.1% vs. 8.0% p=0.9). There were two treatment-related deaths in the FC arm and three in the F arm, due to autoimmune haemolytic anaemia, neutropenic sepsis and tumour lysis syndrome. *Results.* of a final analysis will be presented in June 2005. *Conclusions.* FC induces a significantly higher ORR and CR rate than F, as well as significantly longer progression free survival. Though leukocytopenia was more frequent and severe with FC, the incidence of infections was similar in both arms. Due to these results, the GCLLSG will recommend FC as the standard first line treatment in younger CLL pts.

0367

FLUCAM—A NEW, 4-WEEKLY COMBINATION OF FLUDARABINE AND ALEM-TUZUMAB FOR PATIENTS WITH RELAPSED CHRONIC LYMPHOBLASTIC LEUKEMIA

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Background. Purine analogs, particularly fludarabine (Fludara®), have had a major impact on the management of chronic lymphocytic leukemia (CLL), achieving overall response rates (ORRs) of 60%–80% as a single agent in treatment-naïve patients. Despite high ORRs relative to other agents, patients continue to have detectable minimal residual disease (MRD) and eventually relapse; even with the most highly active combination regimens, chemotherapy alone cannot cure CLL. Monoclonal antibodies such as alemtuzumab (Campath®), that have been developed against antigens expressed on the surface of CLL cells act synergistically with fludarabine *in vitro* and also appear to have synergistic properties *in vivo*. **Aims.** We therefore evaluated the safety and efficacy of a new, 4-weekly combination regimen consisting of fludarabine and the anti-CD52 monoclonal antibody alemtuzumab (FluCam) for patients with CLL in a phase II study. Objectives of this study were to evaluate the feasibility, ORR, duration of response (DR), and the presence of MRD following treatment with FluCam. **Methods.** The FluCam regimen consisted of fludarabine 30 mg/m²/day IV over 15–30 min (Days 1–3) immediately followed by alemtuzumab 30 mg IV over 2 h (Days 1–3); prior to FluCam, a short period of alemtuzumab dose escalation was implemented, during which the dose of alemtuzumab was gradually increased from 3 mg to 10 mg to 30 mg on consecutive days. The FluCam combination was repeated on Day 29 for up to 6 cycles. MRD was measured by 4-color flow cytometry. **Results.** Of 36 enrolled patients, 34 have been evaluated. The median age of the patients was 61.0 years (range, 38–80), 26/34 (76%) were male, 26/34 (76%) had Binet stage C disease, and the median number of prior treatment regimens was 2 (range, 1–8). The ORR for FluCam was 85%, including 10 (29%) complete responses and 19 (56%) partial responses. One (3%) patient had stable disease, while 4 (12%) others had progression of their disease. MRD-negativity was achieved in the peripheral blood for 15/34 (44%) patients. Reactivation of cytomegalovirus occurred in 2 patients: 1 patient had PCR-confirmed CMV and died due to E. coli sepsis, and another had a subclinical CMV reactivation that was successfully treated with IV ganciclovir. Two patients with refractory disease developed fungal pneumonia. Notably, 7 patients who entered the trial with active autoimmune hemolytic anemia and/or autoimmune thrombocytopenia were successfully treated with FluCam. Moreover, FluCam treatment was successful in 9 other patients with transfusion-dependent thrombocytopenia and/or anemia due to bone marrow infiltration prior to therapy. **Summary/conclusions.** In conclusion, results from the analysis of this new, 4-weekly dosing regimen of FluCam suggest that combination therapy with fludarabine and alemtuzumab is feasible, safe, and effective in treating patients with relapsed and refractory CLL, even in those patients with inherent poor prognostic factors, those who had received multiple prior therapies, or those who were refractory to fludarabine or alemtuzumab monotherapy. Based on these promising results, a prospective, randomized, phase III trial has recently started (CAM314) comparing FluCam to fludarabine alone in patients with relapsed CLL.

Thrombosis and thrombophilia

0368

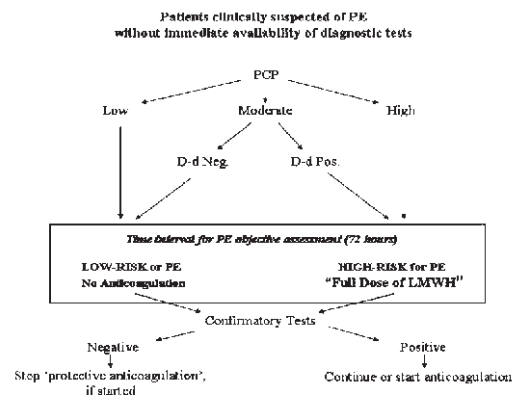
OBJECTIVE ASSESSMENT OF PULMONARY EMBOLISM CAN BE DEFERRED WITHOUT RISK

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Management of patients with suspected Pulmonary Embolism (PE) is problematic if diagnostic imaging is not available. Aim. In this situation, we evaluated whether Pre-test Clinical Probability (PCP) and D-dimer (D-d) assessment allow objective appraisal of PE to be deferred for up to 72 hours. **Methods.** During a period of 4 years, 336 consecutive patients, who could not undergo immediate imaging for PE, were managed according to the below reported algorithm. Briefly, patients with high PCP or a moderate PCP with positive D-d (high risk patients) received a protective full-dose of LMWH; the remaining patients were discharged without anticoagulants. All patients underwent objective tests for PE within 72 hours (figure). **Results.** At the short-term follow-up (72 hours), that was the time allowed for performing objective tests, only a single thromboembolic event (0.3%, upper 95% CI 0.8%) had occurred. None of the patients had major bleeding events. At the timing of diagnostic imaging, venous thromboembolism was confirmed in 22.6% (95% CI 18.2–27) of patients.



Our study demonstrates that this approach allows a safe deferral of diagnostic imaging procedures for PE for up to 72 hours.

0369

LOW PROTEIN Z PLASMA LEVELS AND THE RISK OF VENOUS THROMBOEMBOLISM

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Background. Protein Z (PZ) is a vitamin-K dependent protein that serves as cofactor for PZ-dependent protease inhibitor, inactivating factor Xa. Therefore, deficiency of PZ could lead to a prothrombotic phenotype. Little and inconsistent data are available on the relationship between PZ and venous thromboembolism (VTE). **Aims.** We carried out a case-control study on 443 patients with deep vein thrombosis of the lower limbs and/or pulmonary embolism who discontinued oral anticoagulant ther-

apy and 444 controls, similar for age and sex. *Methods.* PZ was measured with an enzyme immuno-assay (Diagnostica Stago, France). A thrombophilia screening including DNA testing for factor V Leiden and G20210A prothrombin mutation and measurements of plasma antithrombin, protein C, protein S, and homocysteine (fasting and post-methionine load) was performed. The use of oral contraceptives at the time of VTE for patients and blood sampling for controls was recorded. *Results.* No difference in mean (\pm SD) PZ levels was found between patients (2.08 ± 1.04 μ g/mL) and controls (2.06 ± 1.1 μ g/mL; $p=0.2$), also when divided by sex and quartiles of age. Low levels of PZ (below the 10th percentile of the distribution in controls, i.e., 0.89 μ g/mL) were not associated with an increased risk of VTE (odds ratio 1.16, 95% CI 0.75-1.78). *Results.* did not change after adjustment for age, sex and the presence of thrombophilia. Since factor V Leiden, G20210A prothrombin, antithrombin, protein C and protein S deficiency taken together, hyperhomocysteinemia and the use of oral contraceptives were significantly associated with an increased risk of VTE, we looked at the interaction between these risk factors and low PZ levels, finding additive interaction with thrombophilia (odds ratio 8.0, 95% CI 1.0-60.0) and multiplicative interaction with hyperhomocysteinemia (odds ratio 9.9, 95% CI 1.1-88.2) and oral contraceptive use (odds ratio 11.2, 95% CI 2.3-54.4). *Conclusions.* Low PZ levels per se are not a risk factor for VTE, but increases the risk of VTE in association with thrombophilia, hyperhomocysteinemia and oral contraceptive use.

0370

POST-THROMBOTIC SYNDROME, RECURRENCE AND DEATH 10 YEARS AFTER THE FIRST EPISODE OF VENOUS THROMBOEMBOLISM TREATED WITH WARFARIN FOR 6 WEEKS OR 6 MONTHS

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There are only limited studies on the long-term outcome after venous thromboembolism. *Aims.* We aimed to investigate the long-term sequelae of venous thromboembolism in patients randomised to different duration of secondary prophylaxis. *Methods.* In a multicentre trial comparing secondary prophylaxis with vitamin K antagonists for 6 weeks or 6 months in 897 patients we extended the originally planned 2-year follow-up to 10 years. The patients had annual visits and at the last visit clinical assessment of the post-thrombotic syndrome (PTS) was performed. A radiologist, blinded to treatment allocation, adjudicated recurrent thromboembolism. Causes of death were obtained from the Swedish Death Registry. Observed numbers of death from different causes were compared with expected numbers on national mortality data and the standardised incidence ratios (SIR) were calculated. *Results.* Of the 897 patients randomised, 545 could be evaluated at the 10-year follow-up. The probability of developing severe PTS was 4.8% and any sign of PTS was seen in 55.6% of the evaluated patients. In multivariate analysis, old age and signs of impaired circulation at discharge from the hospital were independent risk factors at baseline for development of PTS after 10 years. Recurrent thromboembolism occurred in 29.1% of the patients with a higher rate among males, older patients, those with permanent triggering risk factor-especially with venous insufficiency at baseline-signs of impaired venous circulation at discharge, proximal deep vein thrombosis or pulmonary embolism. Death occurred in 28.5%, which was a higher mortality than expect-

ed with a SIR of 1.46 (95% CI 1.30-1.60), mainly due to a higher mortality than expected from cancer (SIR 1.83; 95% CI 1.44-2.23) or from myocardial infarction or stroke (SIR 1.28; 95% CI 1.00-1.56). The duration of anticoagulation did not have a statistically significant effect on any of the long-term outcomes. *Conclusions.* The morbidity and mortality during 10 years after the first episode of venous thromboembolism is high and not reduced by extension of secondary prophylaxis from 6 weeks to 6 months. A strategy to reduce recurrence of venous thromboembolism as well as mortality from arterial disease is needed.

0371

THE IVS1-401 T-ALLELE OF THE ESTROGEN RECEPTOR (ER)-A IS AN INDEPENDENT PREDICTOR OF LATE FETAL LOSS

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Estrogens are involved in the regulation of placental function and fetal development through their interaction with estrogen receptor α (ER- α). Sequence variants in the gene encoding for ER- α could disturb estrogen-dependent mechanisms in pregnancy maintenance, probably leading to fetal loss. *Aim and Methods.* We determined the IVS1-401C/T polymorphism of the human ER- α , the G1691A mutation of the factor V gene (factor V Leiden), the G20210A mutation of the prothrombin gene, and the C677T polymorphism of the methylenetetrahydrofolate-reductase (MTHFR) gene in 104 women with fetal loss and 277 normal women. Inclusion criteria for the women with fetal loss were either recurrent early fetal loss (three or more consecutive fetal losses at < 12 weeks gestation and no late fetal loss) or at least one late fetal loss (> 12 weeks gestation). Only women with post-embryonic loss after ultrasonic disappearance of fetal pulse from the intrauterine fetal pole were included in the study. Documented first trimester preclinical and blighted ovum abortions as well as fetal losses that were the result of documented fetal malformation or the result of an infectious complication were excluded. The women enrolled with recurrent fetal loss had no previous history of venous or arterial thromboembolic disease, diabetes mellitus, chronic hypertension, thyroid dysfunction, systemic lupus erythematosus, pregnancy-induced hypertension, or preeclampsia. They all had a detailed investigation which was negative for potential causes of fetal demise including fasting glucose, basal FSH, LH and estradiol levels on day 3 of a natural cycle, TSH and prolactin levels and antinuclear factor. In addition, transvaginal scanning was performed in all patients included to verify ovarian morphology. Women with three or more first trimester or one or more second or third trimester pregnancy losses underwent a hypersalpingography and/or hysteroscopy to confirm uterine cavity normalcy, and both partners were also investigated for chromosomal aberrations. The 277 normal women had at least one previous pregnancy and no previous fetal loss or late pregnancy complications, and no history of previous arterial or venous thromboembolism. *Results.* In a subgroup analysis of women with recurrent early fetal loss ($n=34$), the prevalence of the genetic markers did not differ significantly between women with early fetal loss and normal women. In contrast, in the subgroup analysis of women with at least one late miscarriage ($n=70$), the prevalences of the ER- α IVS1-401 T-allele (TT vs. CC, odds ratio 2.85, $p=0.018$, TT+CT vs. CC, odds ratio 2.28, $p=0.043$) and of heterozygous factor V Leiden (odds ratio 3.2, $p=0.002$) were significantly higher among women with late fetal loss than among normal women. Carriers of both risk determinants have an at least additive increase in risk for late abortions (odds ratio 7.0, $p=0.0004$). The fraction of all late abortions that would be attributable to the genetic variants (population attributable risk) was 13.9 percent for factor V Leiden and 49.2 percent for the ER- α IVS1-401 T-allele. *Conclusions.* Women with the IVS1-401 T-allele of the ER- α and/or factor V Leiden are at increased risk of late fetal loss.

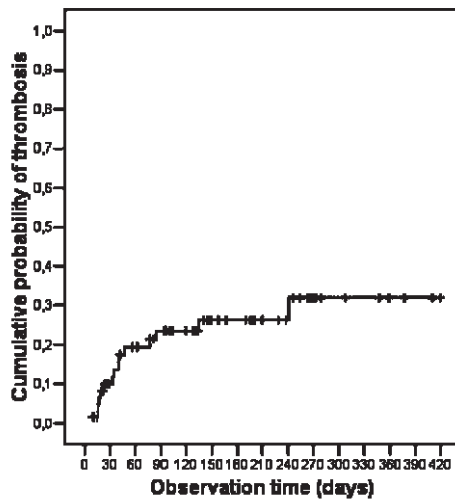
0372

HIGH INCIDENCE OF SYMPTOMATIC VENOUS THROMBOEMBOLISM IN PATIENTS WITH GLIOBLASTOMA

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Patients with malignancy have an increased risk of venous thromboembolism (VTE). The aim of the present study was to investigate the occurrence of symptomatic VTE in patients with high grade glioma after surgery and during consecutive treatments in the light of advanced thromboprophylaxis in the immediate post-surgical period and new developments in neurosurgical techniques. Patients with newly diagnosed and histologically confirmed glioma (WHO grade III/IV) were enrolled from 10/2003 to 12/2004 and followed prospectively after surgery. Study endpoints were symptomatic or fatal VTE. Observation of the patients' clinical course ended on 12-12-2004, and patients who had not developed VTE were censored at their last follow-up visit or at death.



Sixty-three patients (36 f/27 m; median age: 58yrs.; interquartile range 48 - 66 yrs.) with newly diagnosed glioma (WHO grade III/IV) were enrolled. Histological diagnoses were glioblastoma multiforme in 51 patients, anaplastic astrocytoma in 6, oligodendroglioma III in 3, ependymoma III and anaplastic glioma III and anaplastic oligoastrocytoma in 1, respectively. 56 patients were treated with radiochemotherapy, 6 with radiotherapy only and 1 with chemotherapy only. All patients had received prophylactic low-molecular-weight heparin for the first 10 postoperative days.

During a median observation period of 104 days, 15 patients (24%; median age=63yrs.) developed VTE including 5 deep vein thrombosis (DVT) of the leg, 1 DVT of the upper extremity, 6 (2 fatal) pulmonary embolisms (PE) and 3 PE and DVT. The median time interval between surgery and VTE was 35 days. The cumulative probability of VTE was 24% after 3 months and 32% after 1 year. 13 (87%) of the 15 events occurred during the first 3 months of observation. *Conclusions.* Patients with high grade glioma still have a very high incidence of symptomatic or fatal VTE within the first 3 months after surgery. Interventional trials to study efficacy and safety of prolonged anticoagulation are urgently needed.

Infectious diseases

0373

EARLY HOSPITAL DISCHARGE WITH ORAL ANTIMICROBIAL THERAPY IN LOW RISK PATIENTS WITH FEBRILE NEUTROPENIA FOLLOWING CHEMOTHERAPY FOR HAEMATOLOGICAL MALIGNANCIES-AN INTERIM ANALYSISH. Cherif¹, E. Johansson¹, M. Kalin¹, M. Björkholm²¹Karolinska Institute, STOCKHOLM, Sweden; ²Karolinska University Hospital, STOCKHOLM, Sweden

Early intravenous administration of antimicrobial agents is still the standard choice for the empirical management of fever in neutropenic patients with hematological malignancies. After defervescence, the continued inpatient treatment with intravenous antibiotics may be unnecessary for patients who are at low risk for development of complications. *Aims.* In this prospective study the capacity of the Multinational Association of Supportive Care of Cancer (MASCC) risk-index score to identify febrile neutropenic patients at presentation who are at low risk of medical complications is evaluated. In addition, the safety of early hospital discharge with oral antibiotics in low risk patients is determined. Between November 2003 and February 2005, we prospectively identified 164 adult patients (median age 58 years, range 20-93) with haematological malignancies presenting with 240 episodes of febrile neutropenia after chemotherapy. The MASCC risk-index score, which is based on seven independent factors, was calculated in all episodes at fever onset. Patients with a score of ≥ 21 were regarded as low risk and patients with a score of < 21 as high risk. All patients were hospitalized on the first day of fever, treated with broad-spectrum intravenous antibiotics and monitored for medical complications. Twenty-four hours after fever defervescence, discharge from hospital was considered in low risk patients. If oral antibiotic therapy was feasible and medical stability of the patient was ensured, patients continued with oral broad-spectrum antibiotics for 5 days and were followed as outpatients. Final evaluation was done one month after discharge. *Results.* Ninety-two of the 240 episodes (38%) were classified as low risk. Serious medical complications (hypotension, respiratory/renal failure, intensive-care admission, confusion, cardiac failure, bleeding requiring transfusion, cardiac arrhythmia, fungal infection, and/or an allergic reaction) were recorded during 17 (18%) of these episodes indicating a positive predictive value of 82%. A total of 38 low risk patients/episodes were excluded from oral therapy due to multi-resistant bacterial infection (n=14), suspected/proven fungal infection (n=8), gastrointestinal disorder (n=4), deteriorating general condition (n=4), or other disorders prohibiting oral antibiotic administration (n=8). Thus, 54 low risk patients/episodes were discharged with oral antibiotics 24 hours after defervescence. At final evaluation of this group two patients (3%) were registered as failure due to readmission with fever relapse (both caused by fungal infections). No complications were recorded in remaining patients/episodes (success rate 97%). Overall no low risk patient died from infectious complications. *Conclusions.* The MASCC risk-index score is a reliable tool for the identification of low risk patients at presentation with febrile neutropenia. Low risk patients can safely be discharged from hospital with oral antimicrobial therapy 24 hours after fever defervescence.

0374

DISSEMINATED INTRAVASCULAR COAGULATION (DIC) IN SEPSIS: ISTH SCORE FOR OVERT DIC PREDICTS FATALITY AND ORGAN DYSFUNCTIONS. Zeerleder¹, C. Voves², W.A. Wuillemin²¹Central Hematology Laboratory, BERN, Switzerland; ²Division of Hematology, LUCERNE, Switzerland

Background. Although disseminated intravascular coagulation (DIC) is a frequent complication of sepsis, diagnosis is difficult since no International standard exists. In 2001, the International

al Society for Thrombosis and Haemostasis (ISTH) published a score system to evaluate DIC. *Methods.* 32 patients suffering from severe sepsis and 8 patient with septic shock were evaluated following the DIC score (DICs) of the ISTH. Points were given according to thrombocytes and fibrinogen levels, prolongation of prothrombin time as well as fibrin related markers (FRM). Fibrin monomer (FM) and Ddimer (DD) had been chosen as FRM, respectively. Coagulation activation, fibrinolysis parameters as well as inflammatory mediators had been measured in these patients. Moreover, organ dysfunction scores (MODS) had been calculated. *Results.* Total DICs was calculated for each patient with both FRM, FM and DD, respectively. Using FM as well as DD as FRM, DICs for non-survivors (n=13) as well as for septic shock patients were higher (p<0.04) as compared to survivors and patients with severe sepsis, respectively. By the ISTH definition, patients with a DICs ≥5 suffered from overt, whereas patients with a DICs <5 had non-overt DIC. By using FM as FRM, 28 patients suffered from non-overt, whereas 12 had a overt DIC. For DD, 30 had overt as compared to 10 with non-overt DIC. For both FRM, higher thrombin-antithrombin complexes and plasminogen activator inhibitor type-1 levels had been found (p<0.005) in patients with overt as compared to non-overt DIC, whereas no difference in plasmin- α 2-antiplasmin was found. In the DICs calculated by using FM as FRM, lower levels of FVII and FV (p<0.015) were measured in patients with overt as compared to those with non-overt DIC, whereas no difference was found for FII and FX, respectively. Using the DICs with DD as FRM, only lower FVII levels in the overt as compared to the non-overt group was found (p=0.009). Patients with overt DIC had a sig. higher risk to die (Odds Ratio (OR) 5 for both scores) and to develop septic shock (OR>4). Using FM as FRM sig. worse organ function (calculated by 3 different MODS scores) was found in patients with overt as compared to non-overt DIC. *Summary/Conclusions.* The ISTH DICs using FM as well as DD as FRM increases with advanced coagulation activation and predicts fatality as well as disease severity. MODS correlates well with DICs using FM as FRM. FVII level is a sensitive marker for coagulation activation in sepsis.

0375

EPIDEMIOLOGY OF FUNGAL INFECTIONS IN HEMATOLOGICAL MALIGNANCIES IN ITALY: SEIFEM-2004 STUDY (SORVEGLIANZA EPIDEMIOLOGICA INFEZIONI FUNGINE NELLE EMOPATIE MALIGNHE)

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Background/Aims. To evaluate the incidence and the outcome of fungal infections in patients affected by haematological malignancies and admitted in Italian centres. *Methods.* A retrospective study, conducted over 1999-2003, in patients with hematological malignancies (HM), admitted in 18 hematology divisions in tertiary cares or university hospitals, who developed fungal infections. *Results.* Our population included 11,802 patients: 3,012 with AML (25.5%), 1,173 with ALL (9.9%), 596 with CML (5%), 1,104 with CLL (9.4%), 1,616 with MM

(13.7%), 3,457 with NHL (29.3%), 844 with HL (7.2%). Patients who underwent autologous or allogenic HSCT were included in a specific different analysis. A proven or probable fungal infection occurred in 538 patients, with an incidence of 4.6%; in particular we registered 346 episodes sustained by moulds (incidence 2.9%) and 193 by yeasts (incidence 1.6%). The incidence rate depends upon underlying malignancy (12.3% in AML, 6.5% in LLA, 2.7% in CML, 0.6% in CLL, 0.5% in MM, 1.6% in NHL, 0.9% in HL). Among moulds, the detected etiological agents were Aspergillus spp (310 episodes, incidence 2.8%), Mucorales spp (14 episodes, 0.1%), Fusarium spp (15 episodes, 0.1%), and other rare fungi (7 episodes, 0.1%). Among yeasts we registered only septicemia sustained by Candida spp (175 patients, incidence 1.6%). Other yeast infections were caused by Cryptococcus spp (8 pts, incidence 0.1%), Trichosporon spp (7 pts, 0.1%) and other rare agents (2 pts). As for aspergillosis, the identification of the specific subtype of agent was possible only in the 108 cases (35%); A.fumigatus was identified in cases (15%), A. flavus in (12%), A. terreus in (5%), A.niger in (2%). It is worth noting that the number of infections caused by A.flavus increased from 1999 (5 pts, 8.8% of the total cases of aspergillosis registered during the year) to 2003 (14 pts, 18.4%); relative risk was about 2.10 (IC95% 0.8-5.49; p-value 0.117). Conversely all other subtypes showed a stable incidence. The lethality rate registered in the population was about 39%, with differences between aspergillosis (42%) and candidemia (33%). In particular the lethality due to aspergillosis ranged from 40% in 1999 to 45% in 2003 without significant variation (RR 1.11; IC95% 0.74-1.66; p-value 0.613), as well as the lethality in patients affected by candidemia not significantly increased from 30% in 1999 to 37.5% in 2003 (RR 1.25; IC95% 0.67-2.32; p-value 0.478). *Summary/conclusions.* Our study confirms the general trend already described for hematological patients: infections due to moulds continue to be more frequent than those caused by yeast. Among all fungi, Aspergillus spp remains the main etiologic agent. AML represents the most frequently involved category. The mortality rate is actually about 40%, with a remarkable decrease when compared to past years.

0376

LOW DOSE LIPOSOMAL AMPHOTERICIN B (L-AMB) AS PROPHYLAXIS OF INVASIVE FUNGAL INFECTIONS IN PATIENTS (PTS) WITH PHASED NEUTROPENIA: PRELIMINARY RESULTS OF A RANDOMIZED PROLONG III TRIAL

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Background. This trial was designed to compare the incidence of invasive fungal infections (IFI) in L-AmB prophylaxis to no systemic prophylaxis. *Aims.* In order to evaluate the indication for early trial stopping (significant difference in mortality) we performed a preliminary analysis. *Methods.* 140 pts with hematological malignancies (HM) and expected neutropenia (neutrophils <500/ μ l) >10 days (D) following intensive chemotherapy or autologous hematopoietic stem cell transplantation (HSCT) were randomized to either Arm 1) 50 mg L-AmB i.v. every second D started 1-2 D before neutropenia or Arm 2) No systemic antifungal prophylaxis. Treatment continued until neutrophil recovery, breakthrough IFI, intolerable toxicity or dead. Assuming 82 eligible pts in each arm the study had 0,80 power to detect 50% difference in the incidence of proven, probable or possible IFI (primary endpoint). All p-values reported are two-sided. *Results.* Pt Characteristics: Eligible pts Arm 1: 40; Arm 2: 45. Reasons for exclusion were: Neutropenia<10 D (50), stop of treatment due to skin rash (2), baseline infection (2) and patients decision (1). Pretreatment characteristics were equally balanced for age (mean 55.4 years), underlying disease (59 AML, 16 ALL, 10 NHL), duration of neutropenia (mean 18.4 D) and treatment (primary 61, secondary 16, transplant 8). Primary endpoint: Incidence of IFI was 2 of 40 pts (5%) in Arm 1 and 22 of 45 pts (49%) in Arm 2 (p < 0.001, RR = 10.0, CI 2.5-40.0). Key secondary endpoints: Fever of unknown origin (FUO) occurred in 16 pts (40%) vs. 30 pts (67%) (p = 0.017, RR = 1.67, CI 1.08-

2.57), systemic antifungals were used in 10 pts (25%) vs. 29 pts (65%) ($p < 0.001$, RR = 2.58, CI 1.45-4.60) and death occurred in 1 pt (2.5%) vs. 6 pts (12%) ($p = 0.11$, RR = 5.3, CI 0.67-42.4). Toxicity: No grade 3 or 4 toxicity occurred. Laboratory values, including creatinine and liver enzymes, were not different between the treatment groups. *Conclusions.* The highly significant lower incidence of IFI in pts treated with low dose L-AmB prophylaxis supports its use in prolonged neutropenia. Due to not significant differences in mortality study-recruitment was continued.

0377

THE EUROPEAN NETWORK ON THE EPIDEMIOLOGY, PATHOPHYSIOLOGY AND TREATMENT OF SEVERE CHRONIC NEUTROPENIA-A SUMMARY REPORT

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Developing from the Severe Chronic Neutropenia International Registry (SCNIR) founded in 1994, a European Network on Severe Chronic Neutropenia (SCN) was established in 2001. It was supported by an EU commission grant for 3 years. SCN is a rare haematological disorder occurring isolated or in context with other diseases. It is characterized by permanent or recurring neutrophil counts of less than 500/ μ L peripheral blood for a period of more than 6 months. The aim of the network is to increase the knowledge on SCN and to strengthen and expand the cooperation within Europe and to neighbouring countries. The number of countries involved in this project increased from 13 (already involved in the activities of the SCNIR) to currently 22. As a central core facility of the European network an ongoing internet-accessible database was established that provides the opportunity of both remote data entry and statistical data analyses. The patient information collected includes data on the clinical course, treatment, specific clinical events e.g. pregnancy and osteopenia/osteoporosis, and late sequelae of SCN patients, the most alarming being transformation to MDS/leukaemia in approximately 10% of congenital patients. As of August 31st 2004, 375 patients were included in the database with 303 of them being adopted from the database of the SCNIR. The diagnostic categories include 211 patients with congenital neutropenia, sub diagnosed as severe congenital (156), Shwachman-Diamond syndrome (18), glycogen storage disease (17), Barth syndrome (4), and other congenitals (16), 47 cyclic and 114 idiopathic neutropenia patients, the latter including 37 autoimmune neutropenias and others (3). Mean observation time was roughly 4 years for congenital (range 21 years) and cyclic (range 13 years), and approximately 2.5 years for idiopathic neutropenia patients (range 8.5 years). Treatment with Granulocyte-Colony Stimulating Factor (G-CSF) was administered to a total of 300 patients (79.2%) at a median dose of 5 μ g/kg/d for severe congenital (range 0.17 to 180), 2 μ g/kg/d for cyclic (range 0.1 to 18.5) and 1.4 μ g/kg/d for idiopathic patients (range 0.13 to 9.5). The median duration of treatment was roughly 7 years for cyclic and congenital patients, and 4.5 years for the group of idiopathic patients. In addition to the collection and analysis of epidemiological and clinical data, the database provides a basis for the correlation of molecular and genetic findings such as mutations in the gene for neutrophil elastase or acquired mutations of the G-CSF receptor gene with clinical findings, the most important being the transformation to MDS/leukaemia. Dissemination of relevant information is provided by a continuously updated comprehensive multi lingual web page (www.severe-chronic-neutropenia.org) and annual meetings of all partners involved in the European network with international experts from the SCNIR allowing direct personal exchange of specific information. Thus, the European Network on SCN has proven to be a valuable tool for the connection of basic research to clinical practice. It is intended to continue the intensive European cooperation and data collection even after the conclusion of the grant period and to further expand the network to other European and neighbouring countries.

Cytogenetics and molecular diagnostics in hematological malignancies

0378

ASSESSMENT OF PML-RAR REAL-TIME QUANTITATIVE PCR FOR DEFINITION OF MOLECULAR RELAPSE IN APL PATIENTS (RESULTS OF THE APL STUDY GROUP)

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Background. Although patients Acute Promyelocytic Leukaemia (APL or AML M3) and the classical t(15;17) translocation can now benefit from efficient treatments, some 10 to 20% of patients still relapse at 5 years. *Aims.* In order to improve the clinical outcome of APL patients, the early detection of disease reactivation should allow treatment at molecular relapse rather than at complete haematological relapse. To this end accurately defining molecular relapse is compulsory. Recent studies suggest that RQ-PCR methods applied to the detection of minimal residual disease (MRD) could provide such criteria. *Methods.* and Patients: Our laboratory has established a RQ-PCR method (Cassinat et al., 2001) using Taqman technology. Standard curves are established and the PML-RARa copy number is normalized according to the expression of the house-keeping gene PBGD. A total of 260 APL patients (APL study Group) have been followed from 2000 to 2004 with a total of 970 samples analyzed. Median follow up for all these patients was 24 mths (range: 1-83 mths) with a median number of samples per patient of 6 (range: 1-28). During this period, 38 patients presented at least one positive result after a median negative period of 13 mths and 13 of them relapsed (median follow-up of 17 mths, range from 3 to 43mths).

	Group A = 10 copies n=17	Group B 10 - 100 copies n=13	Group C = 100 copies n=8
Median follow-up from diagnosis (months)	32	21	29
Negative status median follow-up (months)	12	8.5	21
Mean number of negative samples before positive RQ-PCR (range)	2.7 (range 1 to 8)	2.3 (range 1 to 4)	2.3 (range 1 to 3)
Relapses	11 / 0 / 6	11 / 0 / 3	7 / 0 / 1
% of Relapse	0 %	38 %	100 %
Median delay to relapse (months)	0	8	2

To evaluate the value of this re-positivity, we tried to determine whether a predictive threshold of PML-RAR copy as assessed by RQ-PCR method could allow detecting patients with molecular relapse. *Results.* At the time of the first positive result patients were in hematological CR. Patients were grouped according to the normalized PML-RARa copy number and the risk of relapse was studied in each group. No relapse (n= 0/17; 0%) was noted in patients with less than 10 copies (group A). 38% relapses (n= 5/13) in patients with 10 to 102 copies (group B) and 100% relapses (n=8/8) in patients with more than 102 normalized copies (group C). Negative RQ-PCR was reached again in all patients that did not relapse. In groups B and C, the median interval to hematological relapse was 3.5 months (range:

1 to 9 months). Interestingly, interval to hematological relapse appeared to be related to the number of PML-RAR copies at first positivity. For 3 positive patients of group B, 3 consecutive samples were analysed until relapse: copy numbers increased exponentially but with a different slope from one patient to another. *Conclusions.* From these results we conclude that RQ-PCR is a powerful tool to define the molecular relapse in APL patients as it is able to accurately identify two subgroups of patients with either a 100% or no risk of relapse with median follow up of 24 mths. These patients with true molecular relapse should receive salvage therapy without waiting for the haematological relapse.

0379**THE DETECTION OF COPY NUMBER CHANGES IN AML USING SNP GENOTYPE**

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Background. Chromosome abnormalities, both structural and numerical are well recognised in acute myeloid leukaemia (AML) yet approximately 40% display a normal karyotype at diagnosis using routine chromosome analysis. Fluorescence in situ hybridisation (FISH) at diagnosis is used as an adjunct to G-banded analysis to improve the abnormality pick up rate since cytogenetics represents one of the most important independent prognostic indicators in AML. FISH, however, provides limited information only on the probes selected for investigation. The development of techniques such as array-based analysis of single nucleotide polymorphisms (SNPs) allows the rapid determination of genome-wide allelic information at high density and we have recently demonstrated the use of SNP analysis to identify large regions of homozygosity representing somatically acquired loss of heterozygosity (LOH), due to partial uniparental disomy (UPD) (Raghavan et al, Cancer Res 2005;65(2):375-8). *Aims.* We sought to determine whether chromosomal imbalance could be determined using the SNP assay (Affymetrix 10K array). *Methods.* Sixty-four presentation AML samples showing typical cytogenetic profiles were screened; normal karyotype [n=40], t(8;21) [n=5], t(15;17) [n=4], inv16 [n=3], 11q23 [n=2], -7 [n=3], +8 [n=2] and other abnormalities [n=7]. The mean signal values were calculated for each chromosomal arm and compared with the mean signal values in the remission bone marrows of the same patient. *Results.* The following numerical abnormalities, already identified by cytogenetic analysis; +3, +8, +10, +11, +13, +19, +22, +22 were readily detected in the array data from 6 AMLs. In another sample showing additional chromosomal material attached to either arm of a deleted chromosome 7 by chromosome analysis, SNP analysis pinpointed its chromosomal origin and FISH confirmed that the material was derived from distal 13q. Cytogenetically visible deletions of 5q, 7q, and monosomy 7 were also demonstrated by LOH and could be discriminated from the acquired UPD events since the deletions were associated with reduced hybridisation signal values of SNPs in those regions (diagnostic samples relative to remission samples) unlike the UPDs where there was no net change in value. *Conclusions.* SNP arrays offer a valuable new approach to the analysis of AML permitting the simultaneous analysis of acquired UPD and genomic imbalance thereby providing an alternative methodology to array comparative genomic hybridisation. The use of higher resolution SNP arrays should further refine the chromosomal imbalance map and improve the genetic characterisation of AML.

0380**ACQUIRED UNIPARENTAL DISOMY FOR CHROMOSOME 13 IS COMMON AND ASSOCIATED WITH FLT3-ITD MUTATIONS IN AML**

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Mutations of FLT3, on chromosome 13, are common in acute myeloid leukaemia (AML). They usually involve an internal tandem duplication (itd) of between 3 and 400 bp in exons 14 or 15, and have an adverse effect on prognosis. FLT3-itd mutations were identified in 27/158 (17%) patients referred with possible diagnoses of AML at presentation or potential relapse. 3/27 of the FLT3-itd patients had a FLT3-itd to wild type (wt) ratio greater than 1, implying more copies of the FLT3-itd gene than of the wt gene. These patients had normal karyotypes. Interphase FISH showed no cryptic loss or gain of chromosome 13. DNA microsatellite (STR) analysis of informative markers on chromosome 13 showed the ratio between the two alleles, for each patient, to be consistent with acquired uniparental disomy (AUPD) of chromosome 13. The STR ratios correlated well with the FLT3-itd:wt ratios. AUPD represents a novel mechanism of cryptic chromosome abnormality, allowing a doubling of an oncogenic event, without the deleterious effects of genomic imbalance due to chromosomal gain or loss. AUPD may occur frequently in AML, probably also in other neoplasia, and may be of prognostic significance. Screening for AUPD may become part of the routine diagnostic testing.

0381**AUTOMATED SCREENING FOR GENOMIC IMBALANCES IN MULTIPLE MYELOMA USING MICROARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION (M-CGH)**

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Background. Chromosomal abnormalities are detectable in virtually all patients with multiple myeloma (MM) by the use of molecular cytogenetic techniques (e.g. fluorescence in situ hybridization, FISH; comparative genomic hybridization, CGH). Genomic rearrangements play a crucial role in the pathogenesis of clonal plasma cell disorders and can deliver important prognostic information. Microarray-based comparative genomic hybridization (mCGH) is an innovative technique that enables a genome-wide, high-resolution tumor cell screening for chromosomal imbalances in a single experiment. The aim of the present study was the detailed characterization of genomic gains and losses in MM-in particular the delineation of critical regions-by the use of mCGH. *Methods.* So far, bone marrow specimens from 55 patients with Salmon & Durie stage II or III MM were analyzed. To achieve a plasma cell proportion greater than 50% before hybridization, CD 138-enrichment by immunomagnetic separation was performed whenever necessary. mCGH chips consisting of approximately 6.400 DNA clones, among them ~3.200 clones covering the whole human genome in 1 Mb increments, the remaining forming contigs from specific genomic regions or containing tumor-relevant genes were applied. *Results.* 52 out of 55 patients (94.5%) were evaluable by mCGH. Gains and losses of chromosomal material were found in 51 out of 52 patients (98%). In one case, mCGH did not exhibit imbalances but +9q34 and +11q25 was diagnosed by FISH for yet unknown reasons. Genomic losses most frequently involved chromosomes 13 (65%), 1p (39%), 16 (31%), 6 (27%), and 12 (21%), while gains commonly affected chromosomes 1q, 9, 11, 15 (46%), and 19 (35%). High-level amplifications were identified on chromosome 16 in two cases (overall three amplicons involving chromosome bands 16p11-p13, 16q12, and 16q21, respectively) as well as on chromosomes 8 (three amplified segments, one of them containing c-myc at

8q24) and 20q (2 amplifications involving bands 20q13.1 and 20q13.3) in one case each. Data on chromosome 13 deletion was consistent with monosomy 13 in 28 out of 34 cases (82.3%). For the remaining 6 cases with a partial 13q loss, no commonly deleted region could be delineated. In contrast, single critical regions were identified on other chromosomes, e.g. chromosome 14 (+14q23.2-q24.3). A small genomic region defined by only four closely adjacent 11q DNA clones was gained in 23 out of 24 (96%) cases with chromosome 11 extra copies. **Conclusions.** mCGH allows the detection of genomic imbalances including high-level amplifications in almost all patients with MM and enables a precise delineation of critical regions in this disease. Evaluation of mCGH raw data is ongoing and confirmative FISH analyses are currently under way.

Supported by grants from the Deutsche José Carreras Leukämie-Stiftung (DJCLS-R04/04) and the Wilhelm Sander-Stiftung (No. 2002.098.1) to P.L.

0382

A NEW RECURRENT INVERSION, INV (7) (P15Q34), LEADS TO TRANSCRIPTIONAL ACTIVATION OF HOXA10 AND HOXA11 IN A SUBSET OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIAS

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T-cell acute lymphoblastic leukemia (T-ALL) represents 10-15% of childhood and 25% of adult ALLs and are associated with an intermediate prognosis within the total group of ALLs. Chromosomal translocations with breakpoints in T-cell receptor (TCR) genes are recurrent in T-cell malignancies. These translocations involve the TCR- α gene (14q11), the TCR β gene (7q34) and to a lesser extent the TCR- γ gene (7p14) and juxtapose T-cell oncogenes next to TCR regulatory sequences leading to deregulated expression of those oncogenes. The finding of a cryptic t(7;11)(q34;q24) in a child with T-ALL, as the result of a Multi-color Fluorescence In Situ Hybridization (M-FISH) study on T-ALLs with complex karyotypes, prompted us to screen for cryptic rearrangements of the TCR- α locus (7q34) in a large cohort of patients (94) with T-ALL. Disruption of the TCR α locus was assessed by dual color FISH with TCR, flanking probes (RP11-1220K2: centromeric to TCR β and RP11-556113: telomeric to TCR- α) and involvement of the TCR α gene was further confirmed using TCR α covering clones (RP11-785K24: centromeric on TCR- α and RP11-701D14: telomeric on TCR β). Chromosomal rearrangement of the TCR α locus was found in 19 of 94 T-ALL cases. In 6 of these 19 cases, the distal probe for TCR α was translocated to recurrent TCR α partner genes (TAL1, RBTN2, RBTN1, TAN1 and HOXA11) as confirmed by FISH with the appropriate probes. In 8 of the 19 cases showing TCR α rearrangement, involvement of known partner genes was excluded. Interestingly, in five other cases with split signals for the TCR α flanking probes, the telomeric BAC clone for TCR α moved to the distal end of the short arm of chromosome 7, thus revealing the presence of a pericentric inversion with an unknown partner gene. Since the HOXA gene cluster is known to be involved in normal human T-cell development, this locus was considered as candidate partner gene for rearrangement with the TCR α locus. Therefore, dual color hybridization was performed on the inv (7) positive cases using probes flanking the HOXA gene cluster located at 7p15 (RP1-167F23 telomeric and RP5-110315 centromeric clones). In all five inv (7) positive cases, a split for the HOXA flanking probes was observed, with the telomeric clone being inverted to distal 7q. Subsequent expression analysis using real time quantitative RT-PCR for all HOXA genes revealed high expression levels for HOXA10 and HOXA11 in all inv (7) positive cases compared to inv (7) nega-

tive cases. Overexpression of these genes is most probably due to juxtaposition near strong enhancers embedded within the TCR- α locus. This study strongly supports the role of deregulated HOXA gene expression in T-cell oncogenesis in a subset of T-ALLs and further supports the presumed role of HOXA10 and HOXA11 in normal thymocyte development.

Chronic myeloid leukemia

0383

ARSENIC TRIOXIDE (TRISENOX) IN COMBINATION WITH IMATINIB MESYLATE IN PATIENTS WITH IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE: RESULTS OF A PHASE I/II STUDY

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Background. Imatinib mesylate has demonstrated remarkable single-agent activity in patients with chronic myeloid leukemia (CML) in the chronic phase. However, a defined subset of patients has less than major cytogenetic response, predictive for increased risk of disease progression. Resistance to single agent imatinib has become increasingly better characterized and is most often the result of point mutations in the BCR-ABL kinase domain or overexpression of the protein. Given that the BCR-ABL kinase remains central to disease pathogenesis even with resistance, rational approaches to treat relapsed disease include agents that target the oncoprotein. These include alternate ABL inhibitors that maintain activity against kinase domain mutant BCR-ABL proteins, as well as agents that reduce the expression of BCR-ABL, such as arsenic trioxide. Arsenic trioxide was found to have single-agent activity in preclinical studies of CML, and has demonstrated single agent activity in patient samples taken during imatinib-resistant blast crisis and CML cell lines expressing the highly imatinib resistant BCR-ABL kinase domain mutant, T315I. Combinations of imatinib and arsenic trioxide have additive effects in CML cell lines expressing wild type BCR-ABL. Recent work from our laboratory has demonstrated additive to synergistic activity of arsenic trioxide combined with imatinib against several imatinib resistant cell lines. Phase I clinical studies in patients with hematologic malignancies, including CML, have demonstrated safety as well as clinical activity. Based on these observations, we completed a phase I/II trial combining imatinib with arsenic trioxide in patients with imatinib-resistant or relapsed chronic phase CML. **Aims.** To study the safety, tolerability, and efficacy of combination therapy with imatinib mesylate and arsenic trioxide in patients with chronic myeloid leukemia (CML) in chronic phase. **Methods.** 16 patients with chronic phase CML who either lost a complete hematologic response or major cytogenetic response or failed to achieve a major cytogenetic response after imatinib therapy were treated on a phase I/II study combining imatinib mesylate with arsenic trioxide. In the phase I portion, imatinib was administered at a dose of 400 mg/day with allowance for escalation to 800 mg/day after 12 weeks of therapy (n=6) while in the phase II portion patients continued their pre-study imatinib dose, up to 800 mg/day, throughout the study. Arsenic trioxide was administered by IV infusion daily for 5 days at a dose of 0.25 mg/kg then twice weekly at a dose of 0.25 mg/kg for all patients. **Results.** Dose limiting toxicity was not encountered in the phase I portion. Overall toxicity was acceptable but greater than with imatinib alone; during study therapy in both phase I and II, grade 4 neutropenia and grade 3 myelosuppression, dyspnea, diarrhea, anorexia, and hematemesis were encountered. Three of 16 patients had responses by protocol criteria: complete hematologic response (n=1) and major cytogenetic response (n=2). **Summary/Conclusions.** Therapy with imatinib

combined with arsenic trioxide at the doses described in patients with imatinib-resistant CML is feasible and responses to therapy, including major cytogenetic responses, were observed. Further exploration is warranted in larger phase II studies.

0384

FREQUENCY, DISTRIBUTION AND PROGNOSTIC VALUE OF ABL MUTATIONS IN DIFFERENT SUBSETS OF IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIA PATIENTS: AN ITALIAN MULTICENTER STUDY BY THE GIMEMA-CML WORKING PARTY

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Background. ABL kinase domain mutations are associated with imatinib (IM) resistance in chronic myeloid leukemia (CML) patients (pts). **Aims.** of this study in a large series of IM-resistant CML pts were: a) to assess and compare the incidence and distribution of ABL mutations in subsets of pts differing for phase of disease and for type/degree of IM-resistance; b) to evaluate the clinical/prognostic relevance of ABL mutations. **Methods.** Using D-HPLC and sequencing, we screened for ABL mutations 165 IM-resistant CML pts. At the time IM was started at 400-600 mg/d, 143 pts (87%) were in chronic phase (CP) (27 previously untreated, 116 post-IFN failure), 4 pts (2%) were in accelerated phase (AP), and 18 pts (11%) were in blast crisis (BC). At present, clinical data are available for 119 pts. Median age at IM start was 48 years (range, 17-70). Median delay between diagnosis and IM start was 40 months (range, 0-160). Median duration of IM was 22 months (range, 9-55). **Results.** Evaluable pts were 134/165 (81%). At the time of analysis, 91/134 (70%) pts were in CP (14 previously untreated, 77 post-IFN failure), 14 (10%) pts were in AP and 29 (22%) pts were in BC. Sixty-six pts had primary resistance to IM; 68 had acquired resistance. Sixty-one mutations were identified in 56/134 (42%) pts. In 5 pts (4 BC; 1 CP post-IFN) two mutations simultaneously occurred. Mutations mapped to 14 codons, the most frequent ones being E255K/V (12 pts), Y253F/H (8 pts), F359V/I (6 pts), M244V (6 pts), G250E (6 pts), M351T (6 pts). Three novel amino acid substitutions (F311I; E355D; F359I) and a novel mutated codon (P296H) were detected; biochemical/structural characterization will be presented. Mutations were detected in 23/91 (26%) CP pts (2/14 (14%) previously untreated, 21/77 (27%) post-IFN), 8/14 (57%) AP patients and 25/29 (83%) BC pts (CP vs. AP, $p=0.02$; AP vs. BC, $p=0.03$; CP vs. BC, $p<0.0001$). Mutations were associated in 14/66 (22%) pts with primary resistance (2/2 hematologic and 12/64 cytogenetic) and in 42/68 (62%) pts with acquired resistance (5/14 pts who lost CCgR, 6/11 pts who lost HR, 31/43 pts who progressed to AP/BC) (primary vs. acquired, $p<0.0001$). Ten out of 25 pts with P-loop mutations had already progressed to AP/BC at the time of mutation detection; 9 additional pts subsequently progressed within 2 to 12 months. Three out of 4 pts with the T315I mutation had already progressed to AP/BC; one progressed after 3 months. In contrast, only four of the 27 remaining pts with mutations had progressed or subsequently progressed ($p<0.0001$). Detailed correlation analysis of mutation status according to clinical features/outcome will be reported for the whole series of patients. **Conclusions.** We conclude that: a) there is a significantly higher probability of mutations according to disease phase (BC>AP>CP); b) there is a significantly higher probability of mutations in pts with acquired resistance vs. pts with primary resistance; c) P-loop and T315I mutations are significantly associated with disease progression and seem to confer a worse outcome.

0385

AUTOGRAFTING IN CHRONIC MYELOID LEUKAEMIA: A META-ANALYSIS OF SIX RANDOMIZED TRIALS

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Interferon- α (IFN) was the first agent to produce sustained reduction in the numbers of Philadelphia positive cells in patients with chronic myeloid leukemia (CML) in chronic phase; it has been demonstrated to prolong survival in comparison with conventional cytotoxic drugs. Because it was possible that an autologous stem cell transplant might further prolong survival for CML patients treated with IFN, a series of prospective randomized trials were initiated in various countries in the 1990s. With the advent of imatinib these trials closed prematurely. To collate and report the results of these randomized trials and thus to ascertain whether there was any clinical benefit derived from autografting. Searches were undertaken to identify all relevant trials. Data for each patient were collected centrally, checked and analysed by standard methods of meta-analysis. **Results.** for each trial were confirmed with the respective trialists.

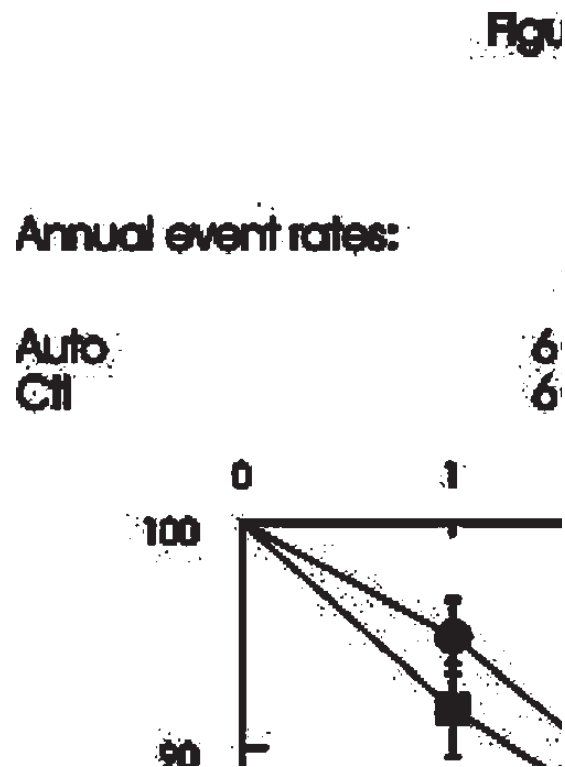


Figure Autograft v Control Survival

Six trials were identified in which patients were randomly allocated to receive an autograft or to continue treatment in the control arm. Data were received from all six trials. The control arms included IFN, usually with a target dose of 5MU/m²/day; in some trials patients also received cytosine arabinoside (araC). For patients allocated to the autograft arm, mini-ICE (idarubicin, araC and etoposide), high dose hydroxyurea, IdAC (idarubicin and araC) or araC alone, were used for stem cell mobilisation.

All trials used busulphan or busulphan plus melphalan as cytoreduction before autografting. 416 patients were included; 38% were low risk, 35% intermediate and 27% high risk by Sokal criteria. 216 patients were randomized to autograft and 200 to control. 71% of those in the autograft arm received an autograft in chronic phase, and 7% of the control arm. The median follow-up was 3.5 yrs. There was no evidence of a difference in incidence of haematological, or of cytogenetic, responses within the first year after randomization. Nor was there evidence of a difference in survival between the two arms (odds ratio = 1.06, 95% CI 0.69–1.62); at 4 years the estimated survival was 78.7% in the control arm and 77.3% in the autograft arm. When imatinib became available, approximately half the patients in each arm received this agent starting at a median time of just over 2 years from randomization. Analysis of survival censoring patients at date of starting imatinib did not alter the results. There was no differential effect of treatment in any trial or in any subgroup defined by age, gender or Sokal risk group. **Conclusions.** Interpretation of this analysis is limited by the relatively small number of patients and premature termination of individual trials. With these reservations there was no evidence that autografting patients in chronic phase confers survival benefit.

Submitted on behalf of the CML Autograft Trialists Collaboration.

0386

AMN107, A NOVEL AMINOPYRIMIDINE INHIBITOR OF BCR-ABL, HAS SIGNIFICANT ACTIVITY IN IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIA (CML)

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AMN107, a novel aminopyrimidine ATP-competitive inhibitor of Bcr-Abl, is 10 to 50 fold more potent than imatinib against Bcr-Abl expressing cell lines, including most imatinib-resistant Bcr-Abl mutants. The increased potency and broader activity of AMN107, relative to imatinib, may result in clinical benefit for patients (pts) with CML. In a Phase I/II study of AMN, 78 of 98 total pts (other 20 had lymphoid disease) had imatinib-resistant CML in different phases. Median age was 61 years (range 15-83). CML phase was: chronic (CP) 12 pt, accelerated (AP) 47 pts; myeloid blastic (BP) 19 pts. AMN daily doses were (mg/day): 50 - 6 pts, 100 - 5 pts, 200 - 7 pts, 400 - 8 pts, 600 - 3 pts, 800 - 14 pts, 1200 - 9 pts, 400 mg BID-17 pts, and 600 mg BID-10 pts. Patients have been treated for 2 to 267 days. In the overall cohort, no MTD dose level is defined. AMN107 is well tolerated up to 1200 mg daily and 400 mg BID with rare skin (1 pt G3 rash) and hepatic toxicities (3 pts G3 ALT, 2 pts G3 AST). Transient elevations (grade 3) in indirect bilirubin in the absence of hemolysis is observed in 15% of patients at total daily doses > 600 mg. Possibly related Grade 3 or 4 hematologic AE's are observed in 13% of patients (11% thrombocytopenia) usually in responding patients. Transient Grade 4 bone marrow aplasia at 800 mg (pt recovered and restarted at 400 mg QD). Other possibly related toxicities included grade 2 pancreatitis (1 pt), grade 3 fatigue (1 pt), and subdural hematoma (1 lymphoid pt), and subarachnoid hemorrhage (1 lymphoid pt). In AP, 28/35 pts (80%) responded: 16 CHR, 5 marrow CR (MR), and 7 return to chronic phase (RTC). 12/28 pts (43%) had so far cytogenetic (CG) responses: complete 8, minor (Ph 35-90%) 4. In myeloid BP, 8/18 pts (44%) responded: 3 CHR, 5 RTC; and 3/8 (37.5%) had a CG response. In CP, 3/4 patients responded (75%) 2 CHR and 1 MR and 1/4 had a CGPR. Mutational data available in 38 pts showed 10 mutations in 17 pts: G250E (4); M351T (4); E355G (2); E459Q, Y253F, F317L, F359V, T315I, M244V, E255K (1 each). 14/17 pts with mutations responded to AMN. Pharmacokinetic (PK) studies showed steady-states

reached by day 8. Both Cmax and AUC were dose proportional from 50 to 400 mg with a median Tmax of 3 hours but were not increased with 800-1200 mg/daily. Using a 400 mg BID dose, daily exposure was 50% greater than in the 800 mg qd regimen. Preliminary data indicate that 600 mg BID may not be associated with a further increase in exposure over 400 mg BID. The apparent half-life was 16 hours. AMN107 has significant activity in advanced imatinib-resistant CML.

0387

LONG-TERM SURVEILLANCE OF CML PATIENTS ON IMATINIB THERAPY. FOLLOW-UP OF GERMAN PATIENTS TREATED WITHIN THE IRIS TRIAL

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High rates of complete cytogenetic response (CCR), the availability of sensitive methods to detect and quantify residual disease, and options for treatment optimization are the basis for molecular monitoring of patients (pts) with chronic myeloid leukemia (CML) treated with imatinib. We sought to determine BCR-ABL transcript levels in 132 CML pts recruited into the IRIS study in Germany over a period of 52 months. Pts were randomized to receive imatinib (n=69) or interferon α +Ara-C (IFN, n=63). Due to intolerance or lack of response 43 pts crossed over from IFN to imatinib, one patient from imatinib to IFN. Response to therapy was sequentially monitored by conventional cytogenetics from bone marrow metaphases (n=1080) and quantitative RT-PCR (Q-PCR) in 1543 blood samples using the LightCycler technology. In case of low level (<10 transcripts/2ul cDNA) or neg Q-PCR, nested PCR was performed using 5ul cDNA. Total ABL transcripts were quantified as internal controls. In pts on 1st-line imatinib median ratios BCR-ABL/ABL gradually decreased: 4.8% at mo 3, 0.88% at mo 6, 0.22% at mo 12, 0.17% at mo 18, 0.058% at mo 24, 0.066% at mo 30, 0.023% at mo 36 and 0.027% at mo 42. After crossover to imatinib similar levels were achieved: 11.7% at mo 3, 1.6% at mo 6, 0.34% at mo 12, 0.077% at mo 18, 0.11% at mo 24, 0.041% at mo 30, 0.054% at mo 36 and 0.016% at mo 42. Ratios BCR-ABL/ABL <0.12% representing a 3-log reduction from baseline have been reached in 52 pts (75%) on 1st-line and in 22 pts (51%) on 2nd-line imatinib therapy. After a median follow-up of 45 mo (1-52) 41/69 pts (59%) on 1st-line imatinib were still Q-PCR pos, 19 pts (28%) were Q-PCR neg but nested PCR pos, and in 4 pts (5.8%) BCR-ABL became undetectable by Q- and nested PCR. After a median of 28 mo (3-50) on 2nd-line imatinib 25/43 pts (58%) were Q-PCR pos, 13 pts (30%) were Q-PCR neg and nested PCR pos, and in 5 pts (12%) BCR-ABL was undetectable by Q- and nested PCR. In one patient, BCR-ABL reappeared after two treatment free intervals of 4 weeks. Four pts (6.5%) on 1st-line and 3 pts (7.0%) on 2nd-line imatinib therapy experienced cytogenetic relapse after a median time of 9 mo (4-21). Best molecular response in pts with subsequent relapse was significantly higher compared to pts with continuous remission (1st-line: 0.35% vs 0.010%, p=0.0022; 2nd-line: 0.45% vs 0.022%, p=0.031). In none of the relapsing pts BCR-ABL mutations were detected. We conclude that (i) treatment with imatinib in newly diagnosed CML pts is associated with a rapid and steady decrease of BCR-ABL transcript levels, (ii) a short trial of IFN does not jeopardize molecular response to subsequent imatinib therapy, (iii) an increasing minority of pts achieve complete molecular remission, (iv) the use of 5ul cDNA in a nested PCR increases sensitivity of residual BCR-ABL detection, and (v) pts with undetectable BCR-ABL may harbor leukemic cells with the potential to cause relapse.

Molecular abnormalities in MDS/AML

0388

PROGNOSTIC GENE-EXPRESSION SIGNATURES IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS WITH NORMAL KARYOTYPE

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Background. Acute myeloid leukemia (AML) encompasses a large number of morphologically similar but molecularly distinct variants. Recurrent cytogenetic aberrations have been shown to constitute markers of diagnostic and prognostic value. However, despite recent successes in detecting novel molecular markers like FLT3 (fms-related tyrosine kinase 3) mutation, treatment stratification is still difficult, especially for the 40-45% of patients with intermediate-risk, normal karyotype disease. To better characterize AML at the molecular level, and to address the need for improved risk stratification, we recently profiled gene expression in a large series of adult AML patients (Bullinger et al., *N Engl J Med* 350:1605, 2004). By unsupervised analysis we identified new prognostically-relevant AML subgroups, and using a supervised learning algorithm we constructed a gene-expression based outcome predictor, which accurately predicted overall survival across all patients, including for the subset of AML cases normal karyotype. **Aims.** Having demonstrated the presence at diagnosis of normal karyotype signatures correlating with clinical outcome, we have now sought to refine a prognostic signature specific for normal karyotype disease. **Methods.** Towards this goal, we have now profiled 119 samples of adult AML patients with normal karyotype using 42k cDNA microarrays from the Stanford Functional Genomics Facility. **Results.** By semi-supervised analysis using the supervised principal component method (Bair et al., *PLoS Biology* 2:511, 2004), we built a cross-validated gene-expression based outcome predictor in a randomly partitioned training set (n=60 samples). This outcome signature, comprising only 16 genes, significantly predicted outcome class for normal karyotype samples in the independent test set (n=59 samples; p=0.001). In multivariate analysis, the 16-gene signature was a strong [odds ratio=0.35 (0.13 to 0.91); p=0.01] factor in predicting overall survival, independent of known prognostic factors including FLT3 mutations and preceding malignancy. **Conclusions.** Our findings support the utility of expression profiling for improved risk stratification and clinical management of the clinically important subclass of AML patients with normal karyotype disease.

0389

EV11, A GENE FREQUENTLY INVOLVED IN MYELODYSPLASTIC SYNDROME CAUSES ERYTHROID DYSPLASIA IN A CONDITIONAL TRANSGENIC MOUSE MODEL

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Background. Patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) harboring chromosome 3q26 translocations frequently show overexpression of the nuclear zinc-finger protein EVI1. Moreover, aberrant expression of this proto-oncogene is observed in 10% of AML and 30% of MDS patients without abnormalities in 3q26, as a result of currently unknown mechanisms. Clinical data show that AML/MDS with high EVI1 expression responds poor to anti-leukemic therapy. It is generally accepted that AML is caused by a combination of genetic alterations, suggesting that other genetic defects cooperate with EVI1 in transformation. This is exemplified by the fact that MDS with 3q26 aberrations frequently evolve into AML. **Aims.** Development of a transgenic EVI1 mouse model with conditional expression that allows us

to study: (a) hematopoietic lineage specific effects of ectopic EVI1 expression and (b) the role of EVI1 in MDS and AML. Since MDS is characterized by a severe anemia, we applied the novel EVI1 mouse model to investigate whether erythroid lineage specific EVI1 overexpression directly affects erythropoiesis. **Methods.** We generated a construct in which EVI1 transcription is blocked by the presence of a loxP-flanked transcriptional stop sequence located 5' of the transgene. In vitro experiments with a CMV-loxP-stop-loxP-EGFP/EVI1 expression construct showed recombination between the loxP sites, excision of the transcriptional stop sequence and nuclear expression of EVI1 exclusively in the presence of Cre recombinase. For the *in vivo* experiments, transgenic lines with conditional EVI1 expression were established using either a Vav1-loxP-stop-loxP-EGFP/EVI1 or a Vav1-loxP-stop-loxP-FLAG/EVI1 expression construct. The Vav1 promoter and regulatory sequences in these constructs drive expression of a transgene specifically in the hematopoietic lineages (Adams et al., 1999, *Oncogene* 18, 5268-77). The EVI1 transgenic lines were crossed with the erythroid lineage specific pEV-Cre line (Gutierrez et al., 2004, *Development* 131, 3183-93) to study the immediate effect of EVI1 on erythroid development in day 12.5 fetal livers. **Results.** We observed deletion of the stop sequence and induction of EVI1 mRNA and protein expression exclusively in Vav1-loxP-stop-loxP-EVI1 / pEV-Cre double transgenic fetal livers. Fetal livers from double transgenic animals were small, pale and contained decreased cell numbers as compared to livers from single transgenic or wild type littermates. Colony assays demonstrated that double transgenic fetal livers contained less CFU-E erythroid progenitors. Furthermore, a partial block in terminal erythroid differentiation was observed, that was accompanied by a significant increase in the number of apoptotic and dysplastic fetal liver erythroid cells. **Summary/Conclusions.** Our data convincingly demonstrate that EVI1 interferes with the erythroid differentiation program and that overexpression of this gene may be a key event causing an erythroid defect in MDS patients carrying 3q26 abnormalities. We have established a conditional EVI1 transgenic mouse model that in combination with other inducible or lineage specific Cre-lines, e.g. Mx1-Cre can be applied to study the involvement of EVI1 in MDS and AML.

0390

MOLECULAR BASIS OF TRANSCRIPTIONAL SILENCING IMPOSED BY PML-RAR

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Background. Many human cancers are characterized by alterations in the balance of DNA methylation. In cancer cells, a large part of the genome undergoes dramatic hypomethylation, which is often linked to genome instability, concomitantly with regional gain of methylated sequences at sites usually unmethylated (CpG islands). The major outcome of promoter hypermethylation appears to be long-term silencing of the associated gene. Silencing of tumor suppressors due to such a mechanism can provide a growth advantage to cancer cells. The ability of the PML-RARA fusion protein to block hematopoietic differentiation and to induce acute promyelocytic leukemia is based on aberrant gene repression. Mechanistically, PML-RARA inactivates its target genes by recruiting histone deacetylase (HDAC) and DNA methyltransferase activities to the promoters. Aim Understanding the contribution of epigenetic events in early steps of leukemogenesis imposed by PML-RARA. **Methods & Results.** We show that MBD1 is required for silencing the PML-RARA target promoter, RAR β 2. Following PML-RARA-induced promoter hypermethylation, MBD1 is recruited to and remains associated with the silenced RAR β 2 promoter. Mutations in the MBD and TRD domains of MBD1 restore RAR β 2 transcriptional activity. We provide evidence that HDAC3 is a common interactor for both PML-RARA and MBD1. Chromatin immunoprecipitation analysis revealed that MBD1 association to PML-RARA target genes is not confined to the promoter region but instead is spread over the locus. Retroviral expression of dominant negative mutants of MBD1 in hematopoietic pre-

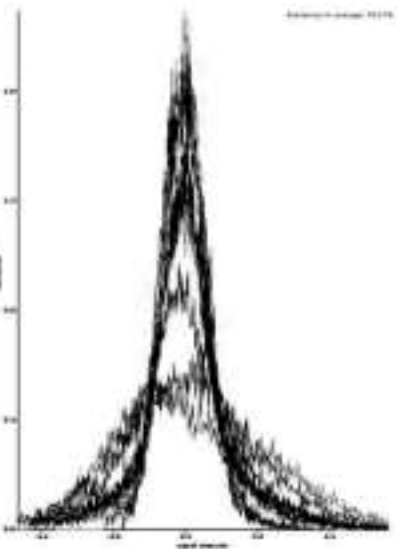
cursors compromised the ability of PML-RAR α to block their differentiation and thus restored cell differentiation. *Summary/Conclusions.* Together these results identify MBD1 as a critical mediator of PML-RAR α -induced gene silencing subsequent to promoter hypermethylation. Further characterization of the PML-RAR α -co-repressor complex, which establishes and allows spreading of the silenced state, will provide insight into crosstalk among the different epigenetic layers as well as into the molecular pathology of leukemia.

0391**EPIGENOMICS OF MYELOPOIESIS AND ACUTE PROMYELOCYTIC LEUKEMIA**

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Background. The etiology of cancer is often characterized by a simultaneous deregulation in the expression of a wide range of genes. Successful therapeutic interference into such pathologies thus requires the identification of the gene regulatory programs affected. Acute promyelocytic leukemia (APL) serves in this respect as an important model disease. *Aims.* By blending different novel technologies we attempt the characterization of the primary set of deregulated genes during APL - the signature gene program. We thereby try to link genome sequence information, and regulatory predictions by means of bioinformatics and statistics to whole-genome experimental data. *Methods.* We have developed the ace - annotation of complex/combinatorial enhancers algorithmic platform that allows us to generate heuristic genome-wide probability predictions of leukemia relevant genes, and cross-correlate these predictions with micro-array and sysChIP data. Furthermore, about eighty individual micro-array experiments were recorded, simultaneously monitoring the expression of ~30.000 human genes from different myeloid leukemia cell lines treated with retinoic acid and GCSF.



Multiple-subtraction, pass-filtering, kinetic, and principal component analysis was performed using our algorithmic platform ace in order to identify those genes that correspond to the signature of APL. Finally, using heuristic probability-based predictions, we have predicted regulatory sites for retinoic acid receptors in general and PML-RAR α in particular on a genome-wide basis. These predictions are used to in situ synthesize up to 400.000 oligonucleotides in form of ultra-high density arrays. These arrays are hybridized with fluorescence labeled Chromatin Immunoprecipitated DNA from APL cell lines using anti-RAR and anti-PML antibodies. These experiments should reveal

active binding sites for both transcription factors, and through correlation analysis with the micro-array transcriptome data single-out the primary regulatory circuit. *Results.* We will discuss here the ace platform and the successful identification of a gene signature for APL through the micro-array transcriptome studies. Furthermore, we will outline the progress on the validation of the signature through quantitative RT-PCR on patient samples, as well as the sysChIP experiments.

0392**DECITABINE: WHERE IS THE TARGET?**

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Background. The demethylating effect of 5-aza-2'-deoxycytidine (decitabine, DAC) has been well characterized. *Aims.* The molecular events downstream of methylation inhibition are less well known. *Methods. and Results.* Here, decitabine was shown to induce apoptosis in acute myeloid leukemia (AML) cells (p53 mutant and wildtype), but not in epithelial or normal peripheral blood mononuclear cells (PBMCs). Apoptosis was characterized by activation of the mitochondrial, but not the receptor death pathway as demonstrated by release of cytochrome c and loss of mitochondrial membrane potential. Activation of caspase-3 but not -6 and -8 was detectable using Western blot analysis and measurement of caspase enzymatic activity. Decitabine treatment resulted in the induction of the cell cycle inhibitor p21, which correlated with the arrest of AML cell lines in the G1 phase. Induction of p21 expression was independent of the methylation status of its promoter, but was mediated via decitabine-induced reexpression of the tumor suppressor p73, an upstream regulator of p21. The p73 promoter was found to be hypermethylated in AML cell lines and in primary AML cells but not in epithelial cells that were resistant to decitabine. *Conclusions.* In conclusion, decitabine-induced specific killing of leukemic myeloid cells via caspase activation might be dependent on the ability to revert p73 promoter hypermethylation and therefore to reexpress p73

Tyrosine kinase inhibition in ALL**0393****CHEMOTHERAPY-PHASED INTERMITTENT IMATINIB FOR ADULT PH/BCR-ABL+ ACUTE LYMPHOBLASTIC LEUKAEMIA**

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Background/Aims. Ph/BCR-ABL+ ALL has a very poor outlook due to early development of chemoresistance. Imatinib (IM) is therapeutically effective blocking the BCR-ABL-related tyrosine kinase, and is being evaluated in trials with/without associated chemotherapy. We studied an intermittent schedule (that could minimize the selection of resistant clones) in which IM was given with concurrent chemotherapy in induction and before/during each consolidation cycle as potential chemosensitizing agent. *Methods.* IM 600 mg/d po was given for 7 days (from d 15, to allow for the detection of Ph/BCR-ABL) with IVAP induc-

tion (IDR/VCR/ASP/PDN); and in consolidation starting 3 days before each cycle (VCR/IDR/CYCLO/DEX x4, HD-MTX/ARAC x2, IVP x1). All patients were eligible to an allogeneic SCT or, alternatively, to further high-dose therapy (HD-VP/6MP/MEL x2; HD-MTX/ARAC x2; IM as above; autograft support) followed by maintenance with IM and 6MP/MTX (alternating every 14 dd for 2 yy). The results obtained in the first 19 patients are compared with those from 35 cases treated without IM (IM+ vs. IM-). **Results.** Patient characteristics: male gender 11/19 vs. 19/35; age 39 (23-66) vs. 50 (19-65) years; blast count 16 (0.2-267) vs. 15 (0.2-242) $\times 10^9/L$. Outcome to induction therapy: CR 17 (89%) vs. 29 (83%); NR 0 vs. 6 (17%) ($p=0.05$); ED 2 (10%) vs. 0 ($p=0.05$). Two early deaths in IM+ group were caused by cerebral thrombosis and haemorrhagic alveolitis, respectively. Other toxicities were (induction only): neutropenia <0.5 (median days) 16 vs. 13 ($p=0.03$); thrombocytopenia <20 (median days) 8 vs. 9; febrile episodes (no.) 76% vs. 31% ($p=0.002$); documented infections 70% vs. 31% ($p=0.008$); hepatic/gastrointestinal (CTC grade >2) 65% vs. 49%; thromboembolic 2 vs. 0. The allogeneic SCT rate was 65% vs. 47% ($p=0.03$). The relapse rate was 12% vs. 59% ($p=0.002$); 4 patients died in remission in each group, mainly from SCT-related toxicity. Median overall survival and DFS were 15 vs. 14 mos. and 13 vs. 10 mos. respectively ($p=NS$). **Conclusions.** Chemotherapy-phased intermittent IM is highly active in adult Ph/BCR+ ALL, reducing the early refractory rate (0%) and allowing more patients to reach the SCT phase (65%). As yet, this has not improved survival, owing to treatment-related deaths (6 in IM+ group) rather than relapse. In induction, myelotoxicity was increased by IM (despite G-CSF), and severe thromboembolic toxicity was registered. This requires anthracycline (IDR) dose reduction, intense antimicrobial prophylaxis and withdrawal of ASP. The role of short IM pulses (7 days) can only be established by studying the residual molecular disease in remission patients.

0394

TREATMENT OF PHILADELPHIA CHROMOSOME (PH⁺)-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) WITH CONCURRENT CHEMOTHERAPY AND IMATINIB MESYLATE

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Background. and aims. Imatinib mesylate as a single agent has modest activity in refractory/relapsed Ph-positive ALL. Use of concurrent chemotherapy and imatinib mesylate in newly diagnosed Ph-positive ALL was explored. Patients and methods. Thirty patients with *de novo* Ph-positive ALL were included in a prospective trial. Induction chemotherapy consisted of imatinib mesylate (400 mg/d, p.o.) and VCR (1.5mg/m²/wk), DNR (60 mg/m²/wk) and PDN (60mg/m²/d) for 4 weeks if adequate bone marrow response ($<5\%$ blast cells) at day +14 was observed. If not, mitoxantrone (12 mg/m², d15, 16, 17) and HD-ARAC (1,000mg/m²/12h d 18, 19) were administered. Consolidation chemotherapy (C1) included imatinib mesylate and MP, HD-MTX (1.5 g/m²), VM-26 and ARA-C. Patients with a HLA-identical family or MUD donor were submitted to SCT. If no donor was found a second consolidation cycle (C2) with imatinib mesylate, VCR, DNR, DXM and CPM was administered. Patients without MUD donor after 6 months of active search were submitted to autologous SCT. After SCT imatinib mesylate was administered from the sustained hematological recovery to the eventual relapse or at least up to 1 year of continuous molecular remission. Sequential MRD studies were performed by cytofluorometry and quantitative RT-PCR. **Results.** Up to October 2004, 30 patients (18 males, mean [SD] age 45[12] years, range 17-62, p190bcr-abl in 80% of the cases) were

included. Two patients died in induction and 1 was refractory, the remaining 27 (90%) attained CR. Slow bone marrow response, defined as the persistence of $>10\%$ bone marrow blasts at day +14 of induction, was observed in 9 of them. Ten patients still are in consolidation treatments, 1 abandoned the protocol because of induction toxicity, 3 relapsed before and 1 after allo-SCT. SCT has been performed in 13 (43%) patients (4 auto, 5 related allo (2 of them with a RIC regimen), 3 MUD and 1 UCSCT. Two deaths were transplant-related events. With a median follow-up of 5 (1-18) months, 7 patients died (2 induction toxicity, 1 consolidation toxicity, 2 TRM, 2 relapse), 2 are alive in second CR and 21 remain in first CR. 1-year OS probability was 55% (95%CI 28-84) and 1-year DFS was 41% (95%CI 17-66). The CR rate of a previous protocol (PETHEMA ALL93AR) not including imatinib was 70%, 1-year OS was 53% and 1-year DFS 31%. Three log reduction in BCR-ABL/GUS was observed in 13/28 (46%) patients at the end of induction therapy and there was a further 1 log median reduction of BCR-ABL transcripts at the end of C1. No additional reduction of transcripts was observed between C1 and SCT. Molecular and cytofluorometric MRD data presented an adequate correlation. **Conclusions.** Use of concurrent chemotherapy and imatinib mesylate in newly diagnosed Ph-positive ALL patients is feasible and the preliminary data are promising in terms of high rate of clinical and molecular CR. SCT is feasible in a high proportion of patients in CR after consolidation. A longer follow-up of the current cohort is needed to evaluate the impact of this approach on DFS and OS.

0395

ACTIVITY OF AMN107, A NOVEL AMINOPYRIMIDINE INHIBITOR OF BCR-ABL, IN IMATINIB-RESISTANT BCR-ABL POSITIVE LYMPHOID MALIGNANCIES

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AMN107, a novel aminopyrimidine ATP-competitive inhibitor of Bcr-Abl, is 10- to 50-fold more potent than imatinib in inhibiting cellular proliferation and autophosphorylation of Bcr-Abl expressing cell lines, including two p190-Bcr-Abl ALL cell lines. It is also effective against most cell lines expressing imatinib-resistant Bcr-Abl mutants. In an ongoing phase I study, AMN107 was given orally, once or twice daily dosing schedule. Twenty of 98 total pts entered had lymphoid disease: lymphoid blast phase CML (CML LBP) 8 pts; Ph+ acute lymphocytic leukemia (Ph+ ALL) 12 patients. Median age was 45 years (range 28-80). AMN doses were: QD dose cohorts (mg/day); 50 (1 pt), 100 (2 pts), 200 (3 pts), 400 (2 pts), 600 (3 pts), 800 (5 pts), 1200 (1 pt); BID dose cohort; 400 mg BID (3 pts). Pts have been treated for 15 to 140+ days. In the overall cohort (98 pts), no MTD dose level is defined. AMN107 is well tolerated up to 1200 mg daily and 400 mg BID with rare skin (1 pt G3 rash) and hepatic toxicities (3 pts G3 ALT, 2 pts G3 AST). Transient elevations (grade 3) of indirect bilirubin in the absence of hemolysis were observed in 15% patients at total daily doses >600 mg. Possibly related Grade 3 or 4 hematologic AE's were observed in 13% of patients (11% thrombocytopenia) often in responding patients. Transient grade 4 bone marrow aplasia was observed at 800 mg (pt recovered to grade 2 after 4 days and restarted at 400 mg QD). Other possibly related toxicities included grade 2 pancreatitis (1 pt), grade 3 fatigue (1 pt), and subdural hematoma (1 lymphoid pt), and subarachnoid hemorrhage (1 lymphoid pt) Clinical activity has been observed in 7/20 (35%) patients with lymphoid disease: Ph + ALL (PR 1 pt, hematological improvement 2 pts) LBP (CHR 2 pts, marrow response/no evidence of

leukemia 2 pts) 1 pt with LBP had a cytogenetic CR. One pt with Ph+ ALL MRD (PCR positive) has maintained a CR for 5 months. Mutational data are available for 2 of the responding patients: Y253H (hematologic improvement), and E355G (partial response). Pharmacokinetic (PK) studies showed steady-states reached by day 8. Both C_{max} and AUC were dose proportional from 50 to 400 mg with a median T_{max} of 3 hours but were not increased with 800-1200 mg/daily. Using a 400 mg BID dose, daily exposure was 50% greater than in the 800 mg qd regimen. Preliminary data indicate that 600 mg BID may yield no further increase in exposure over 400 mg BID. The apparent half-life was 16 hours. AMN107 has significant activity in patients treated with imatinib-resistant CML LBP or Ph+ ALL.

0396

IMATINIB CONCURRENT WITH INDUCTION CHEMOTHERAPY IS SUPERIOR TO ALTERNATING CHEMOTHERAPY AND IMATINIB AS FRONT-LINE THERAPY FOR NEWLY DIAGNOSED PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Chemotherapy induces remissions in 60-80% of adult patients with Ph+ALL but is associated with relapse rates up to 90% unless followed by allogeneic stem cell transplantation (SCT). Even after SCT, relapse and transplant related mortality limit the prognosis. Imatinib during first-line therapy may improve treatment outcome and reduce the development of imatinib-resistance, but the best schedules for using Imatinib during induction have not been established. **Aims.** and study design: In a prospective, multicenter GMALL study, we compared the safety and efficacy of two schedules of imatinib, either alternating with (cohort 1) or given simultaneously with (cohort 2) induction and consolidation chemotherapy. Patients enrolled in cohort 1 needed to have achieved a CR after preceding induction phase I, whereas in cohort 2 patients were eligible irrespective of their initial response to induction. In cohort 2, imatinib was given parallel to weeks 4-6 of induction and continued throughout and for up to 8 weeks after the first consolidation cycle. Primary study objectives were safety and MRD response by quantitative RT-PCR. **Results.** Cohort 1 [n=47, median age 46y (21-65)] received imatinib at 400mg (n=35) or 600mg (n=12) for a median of 28 days. 26 patients again received imatinib after the first consolidation cycle. No patient relapsed during post-induction imatinib, 3 (6.4%) patients relapsed prior to SCT, there was one death in CR after consolidation chemotherapy due to septicemia. Imatinib-related toxicity was limited to WHO grades I and II. In cohort 2 [n=45, median age 41 yrs (19-63)], 56% of patients had achieved a CR after induction phase I prior to imatinib. After completion of induction chemotherapy parallel with imatinib, 97% of patients (38/39 evaluable) were in CR, one patient(3%) failed to respond and died during induction. Median bcr/abl transcripts prior to and after induction II showed no significant decrease (0.9 log) in cohort 1 in contrast to a 1.4 log reduction (p=0.02) in cohort 2. The impact of parallel administration of imatinib and induction on bcr/abl transcript levels was more pronounced prior to consolidation I: compared with pre-induction II levels, there was a 1.5 log reduction in cohort 1 versus a 4.9 log reduction in cohort 2. Bcr/abl transcripts became undetectable by RT-PCR prior to the first consolidation cycle in 19% and 52% in cohorts 1 and 2, respectively. Cytopenias and/or infectious complications entailing imatinib dose reductions and interruptions occurred in 74% of pts. The most frequent grade III/IV non-hematologic toxicity was hepatic and occurred in 42% of evaluable patients. There were 3 (6.7%) treatment related deaths in CR after induction phase II (n=1) and consolidation (n=2), three pts. relapsed prior to SCT (6.7%). **Summary.** Imatinib is highly effective first-line

treatment for adult Ph+ALL when given concurrently with and subsequent to induction and consolidation chemotherapy. This strategy is more effective than alternating chemotherapy and imatinib cycles, although hematologic and non-hematologic toxicity is considerable. The impact on overall treatment outcome remains to be determined, particularly in light of subsequent allogeneic SCT in a large majority of patients.

0397

HIGH SINGLE DRUG ACTIVITY OF COMPOUND GW506U78 IN RELAPSED T-LYMPHOBLASTIC LEUKEMIA (T-ALL) AND T-LYMPHOBLASTIC LYMPHOMA (T-LBL) OFFERS OPTION FOR CURE WITH STEM CELL TRANSPLANTATION

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In T-ALL/LBL outcome has improved substantially in the past decades. At relapse, however, the disease is highly refractory and rapidly progressive. In the GMALL study 05/93 in 'early' relapse during therapy the CR rate with HD regimens was 29% and the survival 8%. The major problem was achievement of CR to offer the option of SCT. Therefore the T-cell specific purine analogue compound GW506U78 (NSC 686673, Nelarabine) was evaluated. The compound was provided by the National Cancer Institute and administered as single drug (1.5 g/m² day 1,3,5) in 50 adult patients. The median age was 31 (19-81) years. 46 (92%) had T-ALL and 4 T-LBL. 43 patients presented BM and 7 only extramedullary involvement. All patients had heavily pretreated, refractory disease. 34 (68%) were included in 1st 'early', 7 (14%) in 2nd relapse, 7 (14%) in relapse after SCT (3 sibling, 3 MUD, 1 auto). 2 (4%) had never obtained CR. 32/34 patients in 1st relapse were refractory to >= one salvage therapy (FLAG-IDA 9, Cladribine/VP16/HDAC 17, other HDAC/HDMTX based 6). 25/49 evaluable patients (51%) achieved CR, 6 PR (12%) and 17 (35%) were refractory. In 2 of them disease transformed to AML. 3/7 patients with relapse after SCT achieved CR. 19/25 CR patients (76%) were transferred to SCT (4 sibling, 14 MUD, 1 auto). Median time to SCT was 41 (20-83) days. 6 patients had no SCT due to age (N=3) or previous SCT (N=3). 7/25 CR patients are in continuous CR at median 13 (1-36) months. 4 patients died in CR (1 sepsis/liver failure, 3 transplant related). 14 patients relapsed, 10 after SCT. Time to relapse was median 5 (1-8) months. 2 patients developed AML in relapse after SCT. 10 patients were included in the programme a second time, 8 in relapse after SCT. 4 CRs, 1 subjective improvement and 5 failures were observed. At present overall 15 patients (30%) are alive at median 5 (0.3-43) months. Moderate bone marrow suppression and elevated liver enzymes were the most frequent toxicities. Neurotoxicity was encountered in only two patients (reversible psychosyndrome with agitation and somnolence). We conclude that the compound has a impressive single drug activity in highly resistant relapsed T-ALL and is well tolerated even in heavily pretreated patients. A treatment attempt should be made at any stage of relapsed T-ALL/LBL. Exploration in earlier stages e.g. molecular relapse, in front-line therapy and in combination is warranted. Since a durable remission can not be expected with chemotherapy a high proportion of CR patients was transferred to SCT. Importantly no extraordinary mortality or morbidity was observed after SCT. Long-term relapse free survival was achieved in some patients. The major problem were relapses. Potential solutions are reduction of tumor load before SCT by consolidation cycles with the compound, early detection of relapse by MRD analysis and interventions e.g. reduction of GvHD prophylaxis, donor lymphocyte infusions or repeated cycles with the compound. Partly supported by Deutsche Krebshilfe (M84/92Ho1), NCI/CTEP and GlaxoSmithKline

Multiple myeloma

0398

A PROSPECTIVE RANDOMIZED TRIAL OF ORAL MELPHALAN, PREDNISONE, THALIDOMIDE (MPT) VS ORAL MELPHALAN, PREDNISONE (MP): AN INTERIM ANALYSIS

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Background. In newly diagnosed multiple myeloma (MM) patients, the combination melphalan, prednisone and thalidomide induces a fast tumor response with a high complete remission rate. In a prospective randomized trial, we compare the efficacy and toxicity of oral MPT and MP. **Aims.** The end points of the study were: response, EFS, OS and toxicity. **Methods.** An interim analysis was conducted after the first 250 newly diagnosed myeloma patients, median age 72, range 56-85, entered the study, between January 2002 and December 2004. At present, 177 patients were evaluated for toxicity and response on an intent to treat basis. The MPT regimen included 6 monthly courses of oral melphalan 4 mg/sqm and prednisone 40 mg/sqm for 7 days every month plus thalidomide 100 mg/day continuously until any sign of progressive disease or relapse. The dose of thalidomide was reduced to 50% when grade II toxicity occurred, and suspended for any grade III. On December 2003, the protocol was amended and enoxaparin prophylaxis was added. The MP regimen was as MPT without thalidomide. **Results.** According to the EBMT/IBMTR criteria, the response rate among patients who received MPT was: 22.2% immunofixation negative CR (CR), 5.5% immunofixation positive near CR (nCR), 49.4% partial remission (PR) (M-protein reduction 50-99%), 14.5% stable disease (SD) (M-protein reduction 0-49%) and 8.4% progressive disease (PD). The response rate after MP was 4.2% CR, 1.2% nCR, 41.3% PR, 25.3% SD and 28% PD. Response was followed by significant improvement of performance status, skeletal pain, anemia and transfusion requirement. After a median follow up of 18 months, 38 patients relapsed: 11 (29%) after MPT and 27 (71%) after MP. The median EFS was 25.2 months after MPT and 13.7 after MP (P < 0.001). The median OS has not been reached. Treatment-related mortality was 5% after MPT (1 septicemia, 1 pulmonary thrombo-embolism, 1 renal failure and 1 heart failure), and 5% after MP (1 myocardial infarction, 1 heart failure, 1 septicemia and 1 disease progression). The major adverse events of MPT vs MP were: deep-vein thrombosis (19% vs 4%), grade III-IV infections (10% vs 1%), grade I-II neurotoxicity (32% vs 11%), grade III-IV hematologic toxicity (18% vs 25%). Thalidomide discontinuation was recorded in 33.8% of patients (8 thromboembolic events, 4 neurotoxicities, 4 constipations, 2 infections, 3 miscellaneous); dose-reduction in 24.2% of patients (8 neurotoxicities, 3 constipations, 4 miscellaneous). **CONCLUSIONS** MPT significantly improves response rate and EFS in elderly myeloma patients with a median age of 72 years. An update of these data will be presented.

0399

THALIDOMIDE-DEXAMETHASONE VERSUS MELPHALAN-PREDNISOLONE AS FIRST LINE TREATMENT IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA: AN INTERIM ANALYSIS

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Background. Thalidomide-Dexamethasone (TD) is active in patients with relapsing/refractory multiple myeloma (MM). Recent phase II and III studies revealed an even higher response rate in previously untreated patients. **Aims.** In the present trial we compare TD with standard Melphalan-Prednisone (MP) in previously untreated elderly patients with multiple myeloma. **Methods.** The trial is designed to include 350 patients with MM, 151 patients have been enrolled so far (median age: 72 years, stage I: 7 (5%), stage II: 47 (31%), stage III: 97 (64%). Patients are randomized to Thalidomide 200mg/day and Dexamethasone 40mg, days 1-4 and 15-18 (on odd cycles) and days 1-4 (on even cycles) or Melphalan 2.5 mg/kg day 1-4 and Prednisone 2 mg/kg days 1-4, q 4-6 weeks. Thalidomide should be dosed up to 400mg/day, if feasible. Patients achieving response or stabilization are randomized to maintenance treatment either with Thalidomide (maximal dose 200mg/day)-Interferon α -2b (3Mega U, TIW) or Interferon α -2b (3Mega U/TIW). All patients are scheduled for monthly Zometa (4mg) during the entire period. Response is defined according Blade's criteria, statistical results are given by intend to treat analysis. **Results.** 125 patients are evaluable for response as yet. Best response to TD was: CR 6 (10%), NCR 7 (12%), VGPR 9 (15%), PR 9 (15%), MR 10 (16%) yielding an ORR of 67%. Four patients had SD (7%) and 16 PD or failure (26%). The respective results in patients on MP were: CR 2 (3%), NCR 4 (6%), VGPR 5 (8%), PR 13 (20%), MR 7 (11%), ORR 48% (p<0.05). Analysis per protocol revealed an ORR of 89% in the TD and 66% in the MP group (p<0.02). Time to response and time to best response was significantly shorter in the TD (8, 11 weeks, respectively) compared to the MP group (10, 39 weeks, respectively; p<0.01, p<0.0047, respectively). Due to the interim nature of the analysis, survival data will be presented only for both groups combined at the meeting. Grade III-IV thrombocytopenia was more frequent and leukopenia was statistically significant more common in patients on MP (4 (7%) vs. 1 (2%); ns. and (8 (13%) vs. 1 (2%); p<0.05, respectively). Patients on TD had more grade II-III neuropathy 15 (25%), psychological toxicity 12 (20%) and, skin toxicity 7 (12%) compared to those on MP (5 (8%), 5 (8%), 3 (3%), respectively). Thromboembolic complications were seen in 5 (8%) patients on TD and in 2 (3%) on MP. **Conclusions.** Time to response and time to best response was significantly shorter in patients on TD. Both, the intend to treatment and per protocol analysis showed a higher response rate in TD treated patients (67% vs. 48%, p<0.05 and 89% vs. 66%, p<0.02, respectively). Leukopenia was more frequent in patients on MP and neuropathy more common in TD treated patients. There was a tendency for increased incidence of thromboembolic complications, psychological disturbances and skin toxicity in patients on TD. In relation to the high median age (72 years) of patients studied, TD was well tolerated.

0400

BORTEZOMIB IS MORE EFFECTIVE THAN HIGH-DOSE DEXAMETHASONE AT FIRST RELAPSE AND PROVIDES BETTER OUTCOMES WHEN USED EARLY RATHER THAN AS LATER SALVAGE THERAPY IN RELAPSED MULTIPLE MYELOMA

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APEX, an international phase 3 study and the largest randomized trial in relapsed multiple myeloma, compared bortezomib (VELCADE™) with high-dose dexamethasone. At the final analysis, bortezomib achieved a significant benefit over dexamethasone, providing a longer median time to progression (TTP), higher response rates, and improved median overall survival. **Aims.** This prospective subgroup analysis of APEX was performed to determine the potential benefit of starting bortezomib at first relapse by analyzing outcomes with bortezomib versus dexamethasone among patients who had received only 1 versus > 1 prior line of therapy. **Methods.** Patients who had received 1–3 prior treatments and were not refractory to dexamethasone were randomized to either bortezomib 1.3 mg/m² IV bolus on days 1, 4, 8, and 11 for eight 3-week cycles followed by 1.3 mg/m² IV bolus on days 1, 8, 15, and 22 for three 5-week cycles (n = 333), or dexamethasone 40 mg po on days 1–4, 9–12, and 17–20 for four 5-week cycles followed by treatment on days 1–4 for five 4-week cycles (n = 336). The European Blood and Marrow Transplantation criteria were used to evaluate response. Randomization was stratified at entry by number of prior therapies (1 versus > 1). Among the 251 patients who had received only 1 prior therapy (table), those receiving second-line bortezomib had a significantly longer median TTP and a higher response rate (complete response [CR] + partial response [PR]) versus those receiving second-line dexamethasone.

	Bortezomib	Dex	P
One prior line, n	132	119	
- Median TTP, mo	7.3	5.6	0.0021
- CR/PR, % (n/N)	45 (57/128)	26 (29/110)	0.0035
- CR/PR by prior treatment			
- Prior transplantation, % (n/N)	48 (40/83)	29 (19/66)	0.0039
- Prior thalidomide, % (n/N)	29 (22/74)	12 (3/25)	0.0937
- No prior thalidomide, % (n/N)	48 (50/104)	31 (28/85)	0.0105
- Prior vincristine, % (n/N)	45 (42/93)	26 (18/71)	0.0055
- Prior arabinoside, % (n/N)	47 (45/95)	26 (19/74)	0.0025
- Prior alkylating agent, % (n/N)	45 (50/110)	29 (26/81)	0.0064
> 1 prior line, n	200	217	
- Median TTP, mo	4.9	2.9	< 0.0001
- CR/PR, % (n/N)	34 (64/187)	13 (27/202)	< 0.0001

After a median follow-up of 8.3 months for all patients, second-line bortezomib provided significantly improved overall survival (hazard ratio 0.42, $p=0.0130$) versus second-line dexamethasone. Comparing patients who received bortezomib as second-line versus as later salvage therapy, the median TTP appeared longer and the response rates higher in patients who received bortezomib earlier. Response rates were significantly higher with bortezomib than with dexamethasone, regardless of type of prior therapy, except among patients who received

prior thalidomide. The response rates to both dexamethasone and bortezomib appeared lower in the limited number of patients who received prior thalidomide; although the advantage for bortezomib persisted, the difference did not reach significance. Among patients who had received > 1 prior line of therapy, the median TTP, response rate, and overall survival (hazard ratio 0.63, $p=0.0231$) were higher with bortezomib compared with dexamethasone. **Conclusions.** Bortezomib provided higher response rates, longer overall median survival, and improved TTP compared with dexamethasone at first relapse and beyond. Response to bortezomib was superior to that to dexamethasone regardless of the type of prior therapy; the benefit of bortezomib persisted in patients who received thalidomide, although it did not reach significance. TTP and response rates appeared more favorable when bortezomib was administered earlier at first relapse compared with its use as later salvage therapy. These data support the use of bortezomib for patients as second-line therapy rather than at later relapse.

0401

REDUCED INTENSITY CONDITIONING ALLOGENEIC TRANSPLANTATION IN HIGH RISK MULTIPLE MYELOMA: LACK OF PLATEAU IN EVENT FREE AT THE LONG TERM FOLLOW UP?

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Although allogeneic transplantation is the only curative approach for patients diagnosed with multiple myeloma (MM), the high transplant related mortality (TRM) associated to this procedure has stimulated the investigation of reduced intensity conditioning regimens (RIC). Nevertheless, long term results at the long term using these RIC are still lacking and, although preliminary data has confirmed that TRM has been decreased among patients undergoing RIC the efficacy of this procedure is still under investigation. To evaluate the results of RIC at the long term among 70 MM patients undergoing RIC. All but 6 patients received a previous autologous stem cell transplantation (ASCT). Conditioning regimen consisted of fludarabine and melphalan in 67 patients; two received busulphan and one 200 cGy TBI instead of melphalan. Among the 64 patients who received a previous ASCT, 33 had either relapsed (n=29) or did not respond (n=4) to ASCT. Seventeen patients were included in a sequential auto-miniallo approach. **Results.** Median age was 54 years (30-66); only 6 (8.7%) patients were in 1st or 2nd CR at transplant, 36 (51.4%) were in PR while 19 (27.4%) were in progression at the time of transplant. At day +100, 89%, 83% and 100% of patients reached complete chimaerism in granulocytes, lymphocytes and bone marrow, respectively. At last follow up 16 (25%) patients are in CR, 15 (24%) are in PR, 17 (26%) have progressive disease and 16 (25%) have died. Projected overall survival at 5 years is 47% and EFS is 29% (patients in CR + PR) and 16% (patients in CR). Acute GVHD adversely affected OS in multivariate analysis [HR=3.38 (95% CI=1.3-8.77), $p=0.01$] while cGVHD favorably influenced on OS [HR=7 (95% CI=2.03-24), $p=0.002$]. Among characteristics at transplant only disease status at transplant (categorized as response / no response) significantly affected OS [HR = 2.63 (95% CI = 1.11-6.27), $p=0.02$]. Regarding EFS only patients who developed cGVHD were event free at 5 years although, even within this subset of patients, relapses may occur when immunosuppressive treatment is started. While TRM was low (24% for the whole series of patients and 12% for patients included in the

sequential trial), results at the long term regarding EFS were hampered by a high incidence of relapse with 66% relapses which occurred even >3 years after transplant. *Summary.* According to our results among high risk MM patients, allo-RIC has allowed to decrease TRM after allogeneic transplantation. In addition, our study confirms GVM effect as the most important factor for disease control after allo-RIC leading to disease response in a significant proportion of poor prognostic patients. Nevertheless, in spite of this GVM effect, most patients have disease recurrence which may occur specially in the absence of GVHD or when GVHD therapy is started. Thus, in patients requiring rescue therapy, allo-RIC should only be considered as a platform for additional therapeutic strategies after transplantation in order to take advantage of the GVM effect

0402

EVALUATING ORAL LENALIDOMIDE (REVLIMID-) AND DEXAMETHASONE VERSUS PLACEBO AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Two International, Randomized, Double-Blind Phase III Studies *Background.* High-dose dexamethasone (Dex) remains a standard therapy for relapsed or refractory multiple myeloma (MM). Lenalidomide is a novel, orally administered, immunomodulatory drug (ImiD) that has single-agent activity against MM and additive effects when combined with Dex. *Methods.* Two randomized, multicenter, double-blind, placebo-controlled studies (MM-009 in North America (N=354); MM-010 in Europe and Australia (N=351)) are fully enrolled. Patients with relapsed or refractory MM were randomized to receive oral lenalidomide (25 mg daily for 3 weeks every 4 weeks) plus Dex (40 mg on Days 1-4, 9-12, 17-20 every 4 weeks for 4 months, then 40 mg on Days 1-4 every cycle thereafter) or placebo plus Dex. A pre-planned interim analysis of the primary endpoint (Time to Progression, TTP), response, and safety data was performed by an Independent Data Monitoring Committee (IDMC). *Results.* The treatment arms in both studies were well balanced. Over 50% of patients in both studies had been treated with high-dose chemotherapy and stem cell transplantation and had failed at least 2 prior conventional chemotherapy regimens. The median TTP for lenalidomide/Dex-treated patients was not reached in either study (exceeds 60 weeks and 47 weeks in MM-009 and MM-010, respectively). In contrast, the median TTP for patients in the Dex alone group was 19.9 weeks in MM-009 and 20.4 weeks in MM-010. The difference was highly significant (p<0.00001). The overall response rate was significantly greater in patients treated with lenalidomide/dexamethasone compared to dexamethasone alone in both MM-009 (51.3% vs. 22.9%; p<0.0001) and MM-010 (47.6% vs. 18.4%; p<0.001). Grade 3 or 4 neutropenia adverse events were reported more frequently in patients given combination therapy than in patients treated with Dex alone (MM-009, 24.1% vs 3.5%; MM-010, 16.5% vs 1.2%), however grade 3 or 4 infection adverse events were reported with similar frequencies between treatment groups in both studies. Otherwise, the safety profile of lenalidomide/dexamethasone was similar to that of Dex alone. *Conclusions.* Treatment of patients with relapsed/refractory MM with combination lenalidomide and Dex induced a marked improvement in TTP and response rate compared to therapy with Dex alone. The differences in TTP surpassed the pre-specified O'Brien-Fleming boundary for superior efficacy (p<0.0015) in both studies and the IDMC recommended the data be released to all study participants.

Immunotherapy

0403

RETROVIRAL TRANSFER OF T CELL RECEPTORS SPECIFIC FOR LEUKEMIA-ASSOCIATED H-Y ANTIGENS GENERATES POTENTIAL GVL-EFFECTOR T CELLS

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Background. The superior Graft-versus-Leukemia (GVL) effect of the female-to-male stem cell transplantation (SCT) is partially independent from the concomitant Graft-versus-Host reactivity. However, the antigenic basis of this selective GVL response remains unknown, since no H-Y antigens with the hematopoietic-restricted expression have been identified. *Aims.* In this study the nature of H-Y epitopes that may mediate GVL reactions was investigated and possible causes of their differential recognition were studied. Furthermore, we explored the feasibility of immunotherapeutic targeting of these epitopes using the TCR-transfer approach. *Methods.* We isolated two female T cell clones, YKIII.8 and YKII.39, that specifically recognized normal and malignant male B and T cell lymphoblasts, but not fibroblasts, resting lymphocytes or monocytes. The respective antigens were identified via either cDNA expression library screening or screening of known H-Y antigens. The antigenic epitopes of these proteins were found by testing for T cell recognition of products of truncated H-Y genes and their candidate peptides. Next, TCR α - and beta-chains of T cell clones were cloned into separate pMX retroviral vectors and the specificity of TCR-transduced donor T cells was studied. *Results.* YKII.8 recognized the HLA-B*5201-restricted TIRYPDPVI epitope of the RPS4Y protein, while YKII.39 recognized the HLA-B*5201-restricted MQQMRHKEV epitope of the UTY protein. Western blot analysis demonstrated the overexpression of RPS4 proteins in normal and malignant lymphoblasts. However, for UTY, Real Time RT-PCR failed to reveal differential expression of UTY mRNA in lymphoblasts, indicating that posttranscriptional regulation of UTY protein expression and/or atypical processing of this epitope in lymphoblasts are likely causes of its differential recognition. Subsequently, we evaluated the feasibility of transfer of the RPS4Y- and UTY-specific T cell receptors (TCR-RPS4Y and TCR-UTY, respectively) to naive T cells to specifically eliminate RPS4Y- and UTY-expressing lymphoblasts. TCR-RPS4Y/TCR-UTY-transduced T cells efficiently lysed B*5201-positive EBV-LCL (100%/92%), PHA blasts (72%/72%), and B*5201-transduced Raji (71%/56%), YT (21%/53%) and RPMI 8226 (28%/47%) cell lines, in the absence of significant recognition of B*5201-transduced fibroblasts. In addition, the amount of IFN- γ produced by TCR-UTY-transduced T cells was significantly higher (5455 \pm 1286 pg/mL) than that of the original clone (721 \pm 134 pg/mL) (p=0.003). Similarly, IFN- γ production by the original RPS4Y-specific clone and TCR-RPS4Y-transduced T cells was 391 \pm 35 vs. 1498 \pm 431 pg/mL (p=0.01). TCR-transduced T cells also displayed a significant increase in stimulation-induced production of IL-2 and TNF- α in comparison to T cell clones. Moreover, they produced less IL-10 and, unlike the original clones, demonstrated great proliferative potential. This is the first demonstration of the possibility to preserve the specific pattern of a T cell response to a differentially recognized antigen after TCR-transfer and to augment the amplitude of this response concomitantly. *Conclusions.* Our findings suggest that CTL specific to some epitopes of ubiquitously expressed H-Y antigens may specifically target lymphoblasts, contributing to the selective GVL effect of female-to-male SCT. Furthermore, our experiments support the feasibility of the selective targeting of male B and T cell lymphoblasts by female donor T cells transduced with UTY- and RPS4Y-specific TCRs.

0404

MEMBRANE-BOUND CD95L EXPRESSED ON HUMAN ANTIGEN-PRESENTING CELLS MEDIATES SELECTIVE DEPLETION OF ANTIGEN-SPECIFIC T CELLS WITHOUT LOSS OF VIRAL AND THIRD-PARTY T CELL RESPONSES

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Background. Allogeneic bone marrow transplantation (BMT) is a common curative treatment for hematological diseases and severe autoimmune defects. However, graft-versus-host disease (GVHD) is the primary limitation of BMT since it presents the major course of mortality after transplantation. Mediators of GVHD are donor-derived host-specific T cells transduced together with the hematopoietic stem cells. Complete elimination of T cells in the transplant reduces the incidence of GVHD but increases the risk of opportunistic infections, graft failure and disease relapse. **Aims.** Selective depletion of alloreactive T cells would prevent GVHD induction but preserve early immune function due to the non-depleted transferred T cells. Therefore, we analyzed whether antigen-presenting cells (APC) expressing death inducing CD95 ligand could be used in a counterattack model to delete antigen-specific activated T cells by CD95/CD95L interaction. **Methods.** We transfected the HLA-A1 expressing lymphoblastoid cell line C1R.A1 with membrane-bound CD95L (m-CD95L), which was stably expressed on the cell surface due to a mutation in the metalloproteinase cleavage site. HLA-A1 negative T cells were weekly stimulated with m-CD95L expressing C1R.A1 cells (C1R.A1.CD95L) or with the mock transfectant (C1R.A1.puro). After different rounds of stimulation modulation of the alloimmune response and antigen-specific deletion of HLA-A1 restricted T cells by m-CD95L expressing APC was compared to the immune response induced by the mock transfectant. **Results.** While T cells stimulated in the presence of the mock transfectant strongly increased in numbers and developed into CD8+ T cells the presence of m-CD95L expressing APC inhibited T cell expansion and T cell cultures consisted predominantly of CD4+ T cells. Inhibition of T cell proliferation in the presence of m-CD95L was not due to impaired cytokine secretion. After 3 rounds of stimulation by m-CD95L expressing APC, HLA-A1 restricted proliferative T cell response was abolished in the CD4+ and CD8+ T cell population. Non-depleted T cells, however, maintained reactivity to third party antigens, autologous EBV transformed cells and respond to tetanus and tuberculosis antigens. During the whole stimulation process no HLA-A1 restricted cytotoxicity was detected in cultures stimulated in the presence of m-CD95L while T cells activated by C1R.A1.puro developed into HLA-A1-restricted CTL. However, expression of perforin and granzymeB and cytotoxic lysis of unrelated targets and autologous EBV cell lines indicated that T cells spared from deletion preserved their cytotoxic potential. **Summary/conclusion:** In conclusion, the present data provide strong evidence that an antigen-specific immune response can be abrogated by m-CD95L expressing APC. This approach might present an attractive strategy to selectively deplete host-reactive T cells from donor-derived BM in order to avoid GVHD induction but retain T cell immunity to environmental antigens.

0405

TELOMERASE - A TUMOR ANTIGEN IN CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL) INDUCES SPONTANEOUSLY AUTOLOGOUS CYTOTOXIC T LYMPHOCYTES EFFICIENT IN ERADICATING CLL CELLS

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Background. It is well known that most tumor cells display a high expression of human telomerase reverse transcriptase (hTERT), the catalytic domain of the functional telomerase com-

plex, which most normal human adult cells do not express, or express only transiently. Considering that high telomerase activity has been reported also in B-CLL, we performed this study to assess whether hTERT could be a potential target for cancer immunotherapy in this disease. **Aim.** To evaluate the presence of spontaneously occurring telomerase-specific T cells in B-CLL patients with telomerase-positive leukemic cells and to investigate whether these T cells could be expanded by means of DC pulsed with hTERT peptide. **Methods.** Peripheral blood mononuclear cells (PBMC) from 25 B-CLL patients were tested for telomerase expression by RT-PCR. Monocyte-derived dendritic cells (DC) were generated from PBMC of 5 telomerase-positive and 3 telomerase-negative B-CLL patients. DC were pulsed with a 16 amino acid long peptide from hTERT and with a 17 amino acid long Ras peptide as control. To assess telomerase-specific T cells, we performed IFN-g production (ELISPOT), ³H thymidine incorporation, cytotoxicity and MHC blocking assays. **Results.** hTERT expression was detected by PCR in 19 out of 25 patients. Among the 19 telomerase-positive pts, 5 were selected for hTERT-specific CTL expansion. In all 5 pts DC pulsed with hTERT peptide could generate CTL against the autologous leukemic cells upon two rounds of restimulation with a specific lysis of 53.8±10% (mean±SEM). The corresponding figures for Ras stimulated T cells were 14.4±3% lysis (p<0.001). In one experiment, MHC class I and MHC class II blocking assay showed that the cytolytic activity was MHC I-dependent, but not MHC II-dependent. In 3 telomerase-negative pts telomerase specific CTLs against the autologous leukemic B cells could not be expanded. Telomerase-expanded T cells induced 3.5±1.8% lysis of tumor B cells and the control Ras peptide 2.1±1.2% lysis. Difference in lytic capacity between telomerase-positive and telomerase-negative pts was statistically significant (p=0.02). In 3 out of 5 telomerase-positive pts a much higher proliferative response against hTERT (10234±2718 cpm) than against Ras (4249±828 cpm) was noted in fresh purified T cells (p=0.04). No restimulation was done. MHC class I and MHC class II blocking revealed that the proliferative response was both MHC I and MHC II-dependent. No INF-g was detected in fresh isolated T cells stimulated once with the peptide. **Conclusions.** Our data show that B-CLL pts with telomerase-positive leukemic cells have spontaneously occurring telomerase specific T cells able to lyse the leukemic cells. This indicates that telomerase might be a valid target structure for vaccine development in B-CLL.

0406

IN-VIVO THIRD PARTY ACTIVATION OF DONOR T CELLS IMPAIRS THE ISOLATION OF CLL-REACTIVE T CELLS FOR ADOPTIVE TRANSFER

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By CD40 crosslinking in the presence of IL-4 chronic lymphocytic leukemia (CLL) cells can be modified into good antigen presenting cells (APC). However, attempts to induce primary leukemia-reactive T cell responses from HLA-matched donors by repeated *in vitro* stimulation using these leukemic-APC as stimulator cells revealed that the likelihood of generating a good immune response against CLL is highly unpredictable. Since the length of the *in-vitro* culture period has been shown to be inversely correlated with the potential of the cells to survive and expand *in-vivo*, we developed a method facilitating early activation, detection and rapid isolation of leukemia-reactive T cells based on their secretion of interferon- γ (IFN γ) using the IFN γ cytokine capture assay (Miltenyi Biotech). In order to maximally enrich for leukemia-reactive T cells and to synchronize the production of IFN γ , donor T cells were first stimulated with the leukemic APC with addition of low dose IL-2 (10 IU/mL) after 7 days of culture, resulting in re-entry of the majority of the T cells into a quiescent state after 14 days of culture. Then, the cells were specifically restimulated with the leukemic APC resulting in synchronized production of IFN γ by the leukemia-reactive T cells allowing efficient isolation of these

T cells. Although we were able to isolate T cell populations containing a high frequency (15-50%) of leukemia-reactive T cells from primary immune responses against CML and ALL blasts, the induction of responses against CLL-APC was less predictable. In individual cases despite minor histocompatibility antigen disparities between donor and patient no specific anti-leukemia immune response could be detected. It appeared that prior to exposure to the leukemic-APC *in vivo* activated T cells were observed in the responder T cell population of these donors containing a high frequency of virus-specific T cells as well as inhibiting regulatory T cells defined as CD4+/CD25+ or CD4+/CD152+. We hypothesized that these regulatory T cells might actively inhibit the induction of an anti-leukemic T cell response, whereas recently activated virus-specific T cells might further hamper the enrichment of leukemia-reactive T cells by their spontaneous IFN γ production. Therefore, we investigated whether removal of the *in-vivo* activated T cells from the responder material prior to the initial activation with the CLL-APC could enhance the efficacy to isolate CLL-reactive T cells. In a representative case, no cytotoxic activity against primary CLL or CLL-APC could be isolated from unmodified responder material by isolation of the IFN γ secreting cells (only 1/288 clones was cytotoxic), whereas the IFN γ producing T cells isolated from the response induced after depletion of the *in-vivo* activated T cells were capable of exerting massive cytotoxicity against both the primary CLL (55%) and the CLL-APC (70%). Single cell cloning of this response revealed that 35 of the 129 T cell clones (>25%) were capable of minor histocompatibility antigen specific recognition. From these results we conclude that the likelihood of generating a primary anti-leukemic immune response is largely influenced by the number of *in-vivo* activated T cells in the responder cell population.

0407

MESENCHYMAL STEM CELLS INHIBIT THE GENERATION AND FUNCTION OF DENDRITIC CELLS

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Background. Mesenchymal stem cells (MSCs) are considered to be multipotential nonhematopoietic progenitor cells that have been studied extensively regarding their effects on T lymphocytes, but their effect on the initiators of the immune response, the dendritic cells (DCs), are relatively unknown. Aim. We therefore investigated the effects of mesenchymal stem cells

on the differentiation of monocytes into DCs with respect to phenotype and functional activities. **Methods.** Monocytes (CD1a-CD14+) were obtained from PB and cultured with IL-4 and GM-CSF to induce differentiation into CD14-CD1a+ immature DCs in the presence or absence of MSCs. MSCs were generated from fetal lung tissue as reported previously (Exp. Hematol. 2002; 30: 870-878). The phenotype (CD1a, CD14, CD80, CD86, CD83, HLA-DR, CD209) of the cells was analyzed by flow cytometry; cytokine production (IL-12) was examined by enzyme-linked immunosorbent assay (ELISA) and T cell stimulatory capacity was determined by a mixed lymphocyte reaction (MLR). **Results.** The presence of MSCs during differentiation completely prevented the generation of immature DCs (CD1a+CD14-), even at a ratio of MSCs:monocytes of 1:1,000. Supernatants (50% v/v) from MSCs partially suppressed the generation of DCs. In addition, MSCs in the upper wells of a transwell culture system inhibited the differentiation of monocytes in the lower wells, indicating that the suppressive effect was partially mediated by soluble factor (s). Kinetic experiments showed that a complete suppression of DC development was only obtained when MSCs were added at day 0. Addition of MSCs at a later time point resulted in partial suppression of differentiation. Upon removal of MSCs cultured in a transwell after 48h, differentiation of monocytes towards DCs was restored, indicating that the suppressive effect of MSCs was reversible. DCs generated in the presence of MSCs were unresponsive to signals inducing maturation (CD40 ligand, lipopolysaccharide, TNF- α , IL-1), as demonstrated by the absence of CD83, CD80, CD86 and HLA-DR upregulation and the decreased production of the inflammatory cytokine IL-12 (from 672 pg/mL to 332 pg/mL). In addition, the T cell stimulatory capacity of mature DCs generated in the presence of MSCs was strongly reduced. MSCs also affected the maturation induced by CD40L of already differentiated immature DCs (CD1a+CD14-) and, inhibited the upregulation of CD80, CD86, CD83 and HLA-DR, and suppressed the secretion of IL-12. **Summary/conclusions.** Taken together, these results show that MSCs, next to the anti-proliferative effect on T cells, inhibit the generation, maturation and function of DCs. Although the soluble factors mediating the suppression are presently unknown, our data indicate that MSCs are able to modulate immune responses at multiple levels. Studies are ongoing to further study the biological significance of these observations in a mouse allograft rejection model.

PRESIDENTIAL SYMPOSIUM

Six best abstracts

0408

NUCLEOPHOSMIN MUTATIONS IN CHILDHOOD ACUTE MYELOGENOUS LEUKEMIA WITH NORMAL KARYOTYPE

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Background. Childhood acute myelogenous leukemia (AML) is a clinically and molecularly heterogeneous disease. The identification of recurrent chromosomal abnormalities allows to define different prognostic subgroups. Unfortunately, this is not yet feasible in a large proportion of cases (20-25%) in which no chromosomal abnormalities are visible by conventional karyotyping. Nucleophosmin (NPM1), a nucleus-cytoplasm shuttling protein involved in rearrangements in leukemia and lymphomas, was found mutated at its C-terminal region in 35% of primary adult non-M3 AML, causing an aberrant cytoplasmic expression, mainly associated with normal karyotype (60% of cases). Since NPM1 is a multifunctional protein involved in the regulation of the Arf-p53 pathway, it is likely that the mutation and/or ectopic location of the protein may play a leukemogenic role. **Aims.** We investigated the prevalence of NPM1 mutations in a large group of childhood AML patients and correlated this finding with the major biological and clinical features. **Methods.** We successfully evaluated the prevalence of NPM1 exon 12 gene mutation in 107/111 (96.4%) non-M3 childhood AML patients consecutively enrolled in the ongoing protocol of the Associazione Italiana di Ematologia e Oncologia Pediatrica. Standard cytogenetics, RT-PCR for major translocations, FISH for MLL gene, and FLT3 mutational analyses were available for 96/107 patients (89.7%). **Results.** NPM1 mutations were found in 7/107 patients (6.5%). All mutations consisted in either the insertion of 4 nucleotides, or in alternative the deletion of 5 nucleotide and the insertion on the same position of 9 new nucleotides. Six sequence variants were observed among our cases. All mutations caused a frame shift in the region encoding the C-terminal of the NPM protein, resulting in the replacement of the last seven amino acids (WQWRKSL) with 11 different residues. As for the adult cases, NPM mutant proteins showed mutations in at least one of two cryptic tryptophan residues, and shared the same last five amino acid residues (VSLRK). The mutations were heterozygous and were related only to the leukemic clone, since they were not present in a BM specimen tested at the time of complete remission. NPM1 mutated patients showed different FAB subtypes and were CD34 negative. All the NPM1 mutated patients carried a normal karyotype (7/26 cases, 26.9%). AML children aged more than 10 years showed a higher NPM1 mutation rate (5/45, 11.1%). Moreover, 5 out of 14 childhood AML patients older than 10 years and with normal karyotype were NPM1 mutated (35.7%). **Conclusions.** we demonstrated that the NPM1 gene mutations are frequent in childhood AML patients with normal karyotype and older age, although the prevalence is lower respect to adult AML. The NPM1 mutated children and adults also share morphological, phenotypical, and clinical features, such as wide morphological spectrum, lack of CD34 expression, and a good response to induction chemotherapy. Our findings confirm in the childhood AML setting the importance of deregulated NPM1 in tumorigenesis, and may have diagnostic and clinical relevance. NPM1 mutations may represents a new target to monitor minimal residual disease in AML, and a potential candidate for alternative and targeted treatments.

0409

A UNIQUE CLONAL ACTIVATING MUTATION IN JH2 DOMAIN OF JAK2 IS PRESENT IN MYELOPROLIFERATIVE DISORDERS OUTSIDE CHRONIC MYELOID LEUKEMIA

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Chronic myeloproliferative disorders (MPD) are clonal hematopoietic stem cell malignancies. In contrast to chronic myeloid leukemia (CML), the pathogenesis of polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF) is poorly understood. To understand the molecular bases of these disorders, we focused our studies on the mechanisms involved in spontaneous erythroid differentiation in PV. We showed that a JAK2 chemical inhibitor (AG490) hampered spontaneous erythroid terminal differentiation, and confirmed these results by using an anti-JAK2 siRNA. We then sequenced all the JAK2 coding sequence of 3 PV patients and 2 controls. In 2/3 PV patients we found a mutation in exon 12 leading to a substitution of valine to phenylalanine at position 617 (V617F). This mutation is located in the JH2 regulatory pseudo-kinase domain of JAK2, which is involved in the auto-inhibition of its tyrosine kinase activity. In a second time, the V617F mutation was found in 40/45 PV patients tested. Beside, the mutation was not found in controls nor in samples from 35 patients with secondary erythrocytosis. By transient transfection, we have shown that this mutation leads to consecutive JAK2 tyrosine auto-phosphorylation inducing STAT5 signaling pathway activation in the absence of EPO. Interestingly, the wild-type (wt) JAK2 act as a dominant-negative for the mutated V617F JAK2. Retroviruses coding for wt or mutated V617F JAK2 cDNAs were introduced in growth factor dependent cell lines. The dose response curve to EPO showed that the mutated JAK2 induced an EPO hypersensitivity compared to WT JAK2. In addition, a part of the cells became cytokine-independent. The V617F mutation was acquired because it was found in myeloid lineages (granulocytes-platelets-erythroid cells differentiated from CD34+ cells) but not in T cells. Interestingly, only the mutated nucleotide was detected in bone marrow cells from around 30% of PV patients. In the other 70% PV patients the normal and the mutated allele were detected. Two hypotheses could account for this observation, either a chimerism between normal clones and a homozygously mutated malignant clone, or a monoclonal hematopoiesis but with a monoallelic-mutated clone. As PV, ET and IMF are closely related disorders, we looked for the presence of the mutation in ET and IMF patients. The mutation was found in about 30% of ET and IMF patients. Thus we have identified a unique, clonal, acquired, JAK2 mutation in PV patients, which induces a constitutive JAK2 signaling. As it was also found in a large proportion of ET and IMF, this mutation may be the major molecular event of these diseases, having the same significance as bcr-abl in CML. Further experiments are now required to understand how a unique mutation could give rise to phenotypically different MPD. Nevertheless, the presence of the V617F mutation can be a diagnosis test of MPD and opens the possibility for new targeted therapies.

0410

A GAIN OF FUNCTION MUTATION IN JAK2 IS FREQUENTLY FOUND IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

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Background. Polycythemia vera (PV), essential thrombo-

cythemia (ET) and idiopathic myelofibrosis (IMF) are three myeloproliferative disorders (MPD) characterized by clonal hematopoiesis arising from a multipotent progenitor. Loss of heterozygosity of chromosome 9p (9pLOH) was described as a recurrent clonal aberration in MPD implicating that this genomic region may harbor a mutation causing clonal expansion of myeloid cells. *Aims.* The goal of this study was to find a mutation involved in the expansion of hematopoietic cells in MPD patients with 9pLOH. *Methods.* We performed microsatellite mapping of the 9pLOH region and DNA sequencing in 244 patients with MPD (128 PV, 93 ET and 23 IMF). *Results.* Microsatellite mapping defined a common 9pLOH region that included the gene for Janus kinase 2 (JAK2). Sequencing in patients with 9pLOH revealed a homozygous G>T transversion resulting in a valine to phenylalanine substitution at position 617 (V617F) of the Jak2 protein. All MPD patients with 9pLOH (51/51; 100%) carried the V617F mutation. Patients without 9pLOH were either heterozygous for V617F (66/193; 34%) or carried the wild type allele (127/193; 66%). V617F was most frequent in patients with PV (83/128; 65%), followed by IMF (13/23; 57%) and ET (21/93; 23%). V617F was found in hematopoietic cells only and represents a somatic mutation. Our data imply that 9pLOH is caused by mitotic recombination and is responsible for the transition from heterozygous to homozygous V617F. Genetic evidence *in vivo* and studies on proliferation and phosphorylation *in vitro* indicate that the V617F mutation confers a proliferative and survival advantage. Patients carrying the V617F mutation had significantly longer disease duration, more complications (fibrosis, hemorrhage and thrombosis) and were more frequently treated with cytoreductive therapy than patients with the wild type Jak2. *Conclusions.* A large proportion of patients with MPD carry a dominant gain-of-function mutation of JAK2, which may provide a basis for a new molecular classification of MPD. The invariant nature of the V617F mutation makes the mutant Jak2 an attractive candidate for targeted drug therapy in patients with MPD.

0411

HOMOCYSTEINE LOWERING BY B VITAMINS AND THE SECONDARY PREVENTION OF DEEP-VEIN THROMBOSIS AND PULMONARY EMBOLISM. A RANDOMISED, PLACEBO-CONTROLLED, DOUBLE BLIND TRIAL

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Hyperhomocysteinemia is a risk factor for venous thrombosis and arterial vascular disease. Supplementation with B-vitamins (folic acid, vitamin B12 and vitamin B6) reduces homocysteine concentrations by 25-30%. Until now, no clinical trial has been carried out to test whether homocysteine lowering by B-vitamins affects the risk of venous thrombosis. The VITRO (Vitamins and Thrombosis) study investigated the effect of daily supplementation with folic acid (5 mg), pyridoxine (50 mg) and cyanocobalamin (0.4 mg) or placebo in the secondary prevention of deep-vein thrombosis (DVT) and/ or PE. Patients with a first DVT or PE who were registered for out-patient treatment at an anticoagulation clinic were asked to participate. Patients between 20 to 80 years old with a first objectively confirmed proximal DVT or PE in the absence of risk factors - divided into a hyperhomocysteinemic (HH) and normohomocysteinemic (NH) group - were entered into the study. End-points were recurrent DVT or PE. 701 patients were enrolled: 360 belonged to the HH group and 341 to the NH group. 353 (177 HH and 176 NH) were randomised to treatment with multivitamins, and 348 (183 HH and 165 NH) to placebo. Multivitamins reduced homocysteine values by 42% in the HH group and by 30% in the NH group. The number of recurrent events of venous thrombosis was 43 out of 348 in the vitamin group

(54/1000yr) and 50 out of 353 in the placebo group (64/1000yr). The relative risk associated with vitamin treatment was 0.84 (95%CI 0.56-1.26): 1.14 (95%CI 0.65-1.98) in the HH group and 0.58 (95%CI 0.31-1.07) in the NH group. The difference in the HH and NH group is contrary to what was expected. The risk of recurrence associated with a 5-umol/L increase in homocysteine level at baseline in the placebo group was 1.12 (95%CI 1.05 to 1.20) and in the vitamin group 1.16 (95%CI 1.03 to 1.31) confirming that homocysteine is a risk factor for recurrent DVT/PE. The results of our study are compatible with the absence of an effect of homocysteine lowering by B-vitamin supplementation on the risk of recurrent venous thrombosis. However, the point estimate of effect does not exclude a small effect, close to what could be expected from etiologic studies on first-time venous thrombosis. The question is whether such a small effect, if indeed confirmed in larger trials, is clinically relevant. The difference in the annual incidence of recurrence was only 1%. This implies a number needed to treat of 100 patients during 1 year to prevent one case of recurrent venous thrombosis. Although multivitamin supplementation seems to be safe - though negative effect of vitamins at these pharmacological doses cannot be excluded - and is not expensive, this number needed to treat indicates that vitamin supplementation at most can play a minor role in the prevention of recurrent venous thrombosis. At this point of knowledge we do not advise vitamin treatment for DVT/PE however.

0412

BONE MARROW ANGIOGENESIS IS INCREASED IN THE PRE-LEUKEMIC INHERITED MARROW FAILURE DISORDER, SHWACHMAN-DIAMOND SYNDROME (SDS)

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Background. Angiogenesis, as measured by microvessel density (MVD), is increased in marrows from patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Angiogenesis has not been studied in the inherited or acquired bone marrow failure syndromes. SDS is an inherited bone marrow failure syndrome that is characterized by a high propensity for MDS/AML. The gene associated with SDS, SBDS, has been identified, but its function is still unknown. We have previously shown that multiple myelodysplastic features characterize SDS marrows prior to overt malignant myeloid transformation. Importantly, we have found that similar to the stroma in adult MDS, SDS marrow stroma does not support normal hematopoiesis established from CD34+ cells. *Aims.* To test whether SDS marrows harbor increased angiogenesis prior to the development of malignant transformation and to evaluate the individual contribution of the bone marrow haematopoietic and stromal cell compartments to the establishment of a pro-angiogenic environment. *Methods.* Eight SDS and six normocellular age-control bone marrow biopsy specimens were stained with anti-Factor VIII antigen antibody to highlight endothelial cells. The average MVD count of three areas with the highest vessel density was measured. To screen for candidate genes that might stimulate blood vessel formation in SDS, we analyzed gene expression in marrow cells from 5 patients and 5 age-matched controls by cDNA microarray. Bone marrow haematopoietic cell expression of VEGF and VEGFR-2 was measured by western blot and flow cytometry. SDS marrow stromal cell expression of VEGF was measured by semi-quantitative RT-PCR. Stromal secretion of the angiogenic factors VEGF, bFGF, EGF, and IL-6 was measured by ELISA. *Results.* MVD counts were statistically significantly higher in SDS compared to controls. Gene expression analysis showed that several known pro-angiogenic genes were overexpressed, most notably TAL1. No well-known angiogenesis inhibitors were down regulated. These results were confirmed by real-time PCR using specific primers for the TAL1 gene and showed statistically significant higher expression of the gene in the SDS mar-

row samples compared to controls. Expression of VEGF and VEGFR-2 in haematopoietic cells and secretion of the measured pro-angiogenic cytokines by stromal cells were comparable between patients and controls. **Summary.** We conclude that SDS marrows are characterized by increased angiogenesis and TAL1 expression prior to the development of malignant transformation. The increase in marrow angiogenesis might promote the growth of malignant clones and potentially leads to the establishment and progression of malignant transformation in this syndrome. TAL1 is an important pro-angiogenic factor and dysregulation of its expression is seen commonly in T-cell leukemia, but also in subtypes of AML. In SDS, perturbation of TAL1 expression might render the marrow microenvironment pre-leukemic by accelerating angiogenesis.

0413

BORTEZOMIB IS SIGNIFICANTLY MORE EFFECTIVE THAN HIGH-DOSE DEXAMETHASONE IN HIGH-RISK AND ELDERLY PATIENTS WITH RELAPSED MYELOMA: AN APEX SUBGROUP ANALYSIS

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Background. Advanced age, increased κ 2-microglobulin, and prior treatment failure are adverse prognostic factors in multiple myeloma. In the final analysis of a large randomized international multicenter phase 3 trial (APEX), bortezomib (VELCADE®) was significantly more effective than high-dose dexamethasone among patients with relapsed myeloma. Improved time to progression and overall survival, and higher response rates were observed with bortezomib. In this APEX subgroup analysis, the safety and efficacy of bortezomib were compared with that of high-dose dexamethasone in elderly (≥ 65 y) and other high-risk patients. **Methods.** 669 patients were randomized to receive bortezomib 1.3 mg/m² by intravenous bolus on days 1, 4, 8, and 11 for up to eight 3-wk cycles, followed by treatment on days 1, 8, 15, and 22 for up to three 5-wk cycles (n = 333), or dexamethasone 40 mg po on days 1–4, 9–12, and 17–20 for up to four 5-wk cycles, followed by treatment on days 1–4 for up to five 4-wk cycles (n = 336). Randomization was stratified at entry by 3 factors: serum κ 2-microglobulin level (< 2.5 vs > 2.5 mg/L), therapeutic history (1 vs > 1 prior line of treatment), and response to last treatment regimen (refracto-

ry vs not). Criteria of the European Group for Blood and Marrow Transplantation were used to evaluate response (complete response [CR]+ partial response [PR]). Patients at high-risk were defined as those with age ≥ 65 y (bortezomib n = 124; dexamethasone n = 119), > 1 prior line of therapy (n = 200 and 217, respectively), κ 2-microglobulin > 2.5 mg/L (n = 244 and 257, respectively), or refractory to prior treatment (n = 212 and 219, respectively). **Results.** Elderly patients and those with other high-risk factors who received bortezomib had a significantly improved median time to progression and significantly higher response rates than patients receiving dexamethasone (table). Slightly increased rates of grade 3/4 adverse events were reported with bortezomib compared with dexamethasone among patients overall (75% vs 60%), among those age ≥ 65 (75% vs 64%), and among those receiving > 1 prior line (75% vs 64%). In bortezomib-treated patients, the incidence of gastrointestinal symptoms (89% vs 90%), nervous system disorders (74% vs 71%), peripheral neuropathy \geq grade 3 (11% vs 11%), infections (64% vs 65%), and hematologic disorders (53% vs 58%) was similar regardless of age (< 65 vs ≥ 65 y, respectively). The rates of serious adverse events (SAE) were balanced between groups treated with bortezomib or dexamethasone.

Risk Factor		Bortezomib	Dex	P
Age ≥ 65 y	RR, %	40	18	0.0004
	TTP, d	168	132	0.0019
> 1 prior therapy	RR, %	34	13	< 0.0001
	TTP, d	148	87	< 0.0001
β_2 -M > 2.5 mg/L	RR, %	39	18	< 0.0001
	TTP, d	170	106	< 0.0001
Refractory to prior treatment	RR, %	35	13	< 0.0001
	TTP, d	168	85	< 0.0001

β_2 -M = β_2 -microglobulin; Dex = dexamethasone; RR = response rate; TTP = median time to progression.

Overall, SAE were reported in 44% bortezomib vs 43% dexamethasone; for patients age ≥ 65 , SAE were reported in 46% vs 47%, respectively, and for patients receiving > 1 prior line of therapy, SAE were reported in 47% vs 43%, respectively. **Conclusions.** Bortezomib was significantly more effective than high-dose dexamethasone in patients with relapsed myeloma and adverse prognostic factors, such as age ≥ 65 y, > 1 prior therapy, refractory to prior treatment, and increased serum κ 2-microglobulin levels. Bortezomib should be considered an appropriate therapeutic option in such patients.

POSTER SESSION II

Acute lymphoblastic leukemia

0414

ACUTE CHILDHOOD LEUKAEMIAS AND EXPOSURE TO ELECTRO MAGNETIC FIELDS GENERATED BY VERY HIGH VOLTAGE POWER LINES

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Many investigators have studied the effects of Electro Magnetic Fields (EMF) generated by power lines, as a risk factor in the pathogenesis of acute leukaemias in children, but there is no such study on very high voltage overhead lines. Children living (in slums) industrializing major cities in developing countries, sometimes live very close to very high voltage (i.e.; 132000 - 230000 volts) power lines, by a negligence on housing safety standards. In this study we have analyzed 60 consecutive patients with acute leukaemias, and 59 matched controls living in a provincial capital city in North-Western Iran. The cases consisted of 58 patients (pts) with ALL, and 2 with AML, 35 males (58%) and 25 females (42%), 58 alive, and 2 died, with a mean age of 12.3 years. After a written consent, a detailed questionnaire was filled in, by the help of mothers as the main interviewees. A visit to the present (and for the cases, the previous) residential areas of the study groups was arranged. The locations of the power lines were detected in each area, if present, and their distances from the houses under study were detected. The expected intensity of the EMF generated (microTeslas, μ T) was calculated having the mean intensity of the electrical current ($I = 1000$ amperes, $HZ = 50$) by means of the relevant equations. The distances ≥ 2000 meters regarded potentially safe ($= 0 \mu$ T). Fourteen pts (23.5%) in the case group were living near the power lines in a distance equal to or less than 500 meters (range 67-500) experiencing a constant mean EMF intensity of 0.6 microteslas (range 1.7-0.2). In the control group the figure was only 2 persons (3.5%). On statistical analysis, residency to very high voltage power lines in distances ≤ 500 meters significantly increased the likelihood of acute leukaemias in children. (95% CI = 1.87-40, Odds ratio = 8.67, P value = 0.001).

0415

RAPID AND RELIABLE DETERMINATION OF THE IGH-VDJ REARRANGEMENTS SPECIFIC FOR B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN

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[Background] Monitoring of minimal residual disease (MRD) in the samples from bone marrow (BM) as well as peripheral blood (PB) is widely accepted to provide strong prognostic information in childhood ALL. The complementary-determining region 3 (CDR 3) sequence derived from VDJ recombination in the immunoglobulin heavy chain (IgH) gene is unique to each rearrangement generating the diversity of B cell repertoire and therefore used as molecular probes to identify clonal B-cell expansion. For MRD detection, preparation of clone-specific oligonucleotides is required from sequencing the VDJ regions in each of B-ALL patients. [Aims] To develop rapid and reliable determination of the leukemic clone-specific VDJ rearrangements, we analyzed the size of IgH-CDR3 segments by spectratyping with modification, which could identify the clone-specific CDR3 sizes and VH gene usage in a simple PCR-based technology. [Methods] Genomic DNAs of diagnostic/relapse BM samples from 11 patients with B-ALL were PCR amplified

using a series of 6 VH family FR1 consensus primers and a JH consensus primer (1st PCR). The second PCR was performed using an aliquot of each of 1st PCR products with a FAM-labeled VH FR3 consensus primer and a HEX-labeled JH 2nd consensus (nested) primer. PCR cycles were adjusted to reduce non-specific amplification. The PCR products were applied to an automated DNA sequencer for separation of the different CDR3 lengths in each VDJ combination. Nucleotide sizes and areas of the observed CDR3 peaks were determined using GeneScan (Applied Biosystems) software. TA cloning and sequencing of the PCR products were performed to confirm the CDR3 sizes and VHDHJH gene usage. [Results] IgH-CDR3 size spectratyping of the samples from normal BM/PB showed Gaussian distribution of the peaks representing a diverse population of cells. The samples from leukemic patients demonstrated skewing pattern of the peaks representing the loss of diversity and indicating clonal expansion. Using a set of different fluorophore-labeled forward and reverse primers, we were able to exclude the non-specific noise and identify the specific peaks with dual fluorescence. Six of 11 patients showed a single clonal expansion and 5 patients indicated the presence of oligoclonal leukemic clones (Table). In 16 clones from 11 patients, the frequency of the utilization of the specific VH segments, identified with the VH primers in the 1st PCR step, was consistent with that in the previous reports. To evaluate the sensitivity of CDR3 size spectratyping for detection of leukemic clone-specific peaks, we tested the leukemic DNA samples in 1 to 103-fold dilutions with normal PB DNA. The leukemic clone-specific peaks were successfully detected in the sample of 103-fold dilution. [Conclusions] The IgH-CDR3 size spectratyping with dual fluorophore-labeled primers could rapidly identify the clone-specific IgH-VDJ rearrangements characterized with the CDR3 size and VH family usage. Detectable levels of the clone-specific rearranged products were defined as 10-3. The spectratyping of IgH-CDR3 could be useful and reliable for monitoring of MRD in B-ALL. E-mail: saikawa@ped.m.kanazawa-u.ac.jp Picture 1: http://www.parthen-impact.com/pco/6_05EHA/public/admin/sgml/1156_fig1.jpg Caption 1: Patient profiles and CDR3 spectratyping

0416

PROPHYLACTIC TIME-SEQUENCED ADMINISTRATION OF G-CSF IS ASSOCIATED WITH IMPROVED OUTCOME IN ADULT T-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA

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In a previous randomized 4-96 study by the Polish Adult Leukemia Group (PALG) we demonstrated that prophylactic time-sequenced administration of G-CSF (lenograstim) was associated with improved survival of adults with newly diagnosed acute lymphoblastic leukemia (ALL). A subgroup analysis revealed that the effect was restricted to T-lineage ALL. Since the number of subjects was small we currently decided to extend the analysis including patients treated later with the same protocol although the use of G-CSF was optional. Induction therapy consisted of epirubicin+vincristin on days 1, 8, 15, 22, prednisone (PDN), and L-asparaginase; consolidation treatment included twice high dose cytarabine and cyclophosphamide (HD AraC/Ctx), twice methotrexat+etoposide (Mtx/Vep), CNS irradiation and intrathecal Mtx+AraC+PDN. During induction patients received G-CSF 150 μ g/m² sc. on days 2-6, 9-13, 16-20, 23-until the neutrophil recovery $> 1.0 \times 10^9/L$, starting 36 hours after Epi/Vcr, finishing 48 hours before the next dose; in consolidation-following each

HDaraC/Ctx course on days 5-16. High risk patients having a donor were performed alloHCT in first complete remission (CR), whereas those without a donor were given autologous transplant. Among 47 T-lineage ALL patients (age 25 (16-54) years), 14 received G-CSF prophylactically and 33 were not given G-CSF or only in case of severe neutropenic infection. CR rate for the above subgroups equaled 100% vs. 82%, respectively ($p=0.09$). At 7 years the probability of OS and LFS was 69% vs. 14% ($p=0.006$) and 68% vs. 18% ($p=0.007$). G-CSF administration was associated with reduced risk of relapse (32% vs. 78%, $p=0.02$). In a multivariate analysis including other recognized risk factors, the prophylactic use of G-CSF remained an independent factor associated with improved OS (RR 0.2 (0.06-0.72), $p=0.007$), LFS (RR 0.2 (0.04-0.87)), $p=0.02$ and reduced relapse incidence (RR 0.22 (0.05-0.99), $p=0.03$). The effect remained significant when censoring observations at the time of HCT. Similar findings were not observed in patients with B-lineage ALL ($n=176$). We conclude that the prophylactic time-sequenced use of G-CSF during induction and consolidation improves outcome of adult T-lineage ALL mainly by reducing relapse incidence. This may be a consequence of more efficient disease eradication resulting from better adherence to the chemotherapy protocol.

0417

FLUDARABINE, CYTARABIN, AND MITOXANTRONE (FLAM) FOR TREATMENT OF RELAPSED AND REFRACTORY ADULT ACUTE LYMPHOBLASTIC LEUKEMIA. A PHASE II STUDY BY THE POLISH ADULT LEUKEMIA GROUP (PALG)

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Outcome of adult ALL patients who fail to achieve CR or who relapse soon after initial response is poor. The goal of this phase II study by the Polish Adult Leukemia Group (PALG) was to evaluate in this group of patients safety and efficacy of a new FLAM regimen consisting of fludarabine 2x30 mg/m² iv on d. 1, 2, 8, 9 + AraC 8x100 mg/m² iv on d. 1, 2, 8, 9 + mitoxantrone 10 mg/m² on d. 3, 10. Fifty patients were included with primary ($n=11$) or secondary ($n=3$) refractoriness, early (before 6 months) 1st relapse ($n=21$), 1st relapse after HSCT regardless CR duration ($n=10$), and 2nd or subsequent relapse ($n=5$). Median age was 30 (18-60) years, gender: m/f-54%/46%. 32% of pts were bcr/abl-positive. 50% of patients achieved CR after FLAM therapy. CR rate was significantly higher in a subgroup of patients aged <30 vs. ≥30 years (67% vs. 33%, $p=0.02$) and was particularly high for patients with primary refractoriness (69%). Among those who failed to respond, 8 pts died in aplasia (bacterial sepsis-13, cerebral bleeding -1, cardiotoxicity -1) and 17 pts had leukemia regrowth after initial cytoreduction. Five out of 8 patients who died of severe adverse events were over 45 years old. At 3 years, the probability of disease-free survival for patients who achieved CR equaled 16%. Five patients were performed alloHCT. All patients but one experienced grade IV neutropenia and thrombocytopenia. Median time to ANC >0.5 G/L and PLT >50 G/L recovery was 26 (0-36) d. and 29 (13-43) d., respectively. Non-hematological grade III-IV toxicity was as follows: infections -62%, vomiting -18%, bleeding -6%, cardiotoxicity -6%, hepatotoxicity -3%, and constipation -3%. Patients required 5 (0-12) RBC and 5 (0-16) PLT transfusions. 69% of pts received G-CSF for 11 (3-25) d. Median duration of iv. antibiotics was 27 (8-41) d. Patients stayed in hospital for a median of 32 (12-46) d. Our results indicate that FLAM regimen has a potent anti-leukemic activity and let achieve CR in half of poor-prognosis resistant and relapsed adult ALL. This therapy may serve for cytoreduction before referring patients to alloHCT. Because of pronounced toxicity including prolonged cytopenia, FLAM should be considered with caution for patients aged >45 years.

0418

CENTRAL NERVOUS SYSTEM INVOLVEMENT IN ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA: RETROSPECTIVE ANALYSIS FROM NORTHERN ITALY LEUKAEMIA GROUP (NILG) ON 687 TOTAL PATIENTS (1979-2004)

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Background/Aims. The exact implications of central nervous system (CNS) involvement in adult ALL are not entirely clear due to the disease heterogeneity and the variable design of treatment protocols. We analyzed, in a large series of patients with ALL, the relationships between CNS disease, ALL subtype, prophylactic intervention and outcome, aiming to highlight the critical points concerning this specific treatment issue. **Methods.** The database of NILG trials conducted between 1979-2004 was analyzed. Clinico-diagnostic features and outcome of patients with CNS involvement at presentation or later CNS progression were assessed and correlated with prophylactic regimens including single or triple intrathecal therapy (ST: MTX or Ara-C; TT: MTX/Ara-C/PDN), cranial irradiation (RT), and systemic high-dose (HD) CNS-active therapy (Ara-C 2 g/m² dosing, MTX 1.5 g/m² dosing). **Results.** Six-hundred eighty-seven patients were treated on NILG ALL trials. Of them, 26 (4.5%) of 578 evaluable had proven CNS involvement at presentation: 4/22 (18%) in B-ALL, 10/124 (8%) in T-ALL, and 12/422 (3%) in B-precursor ALL ($p=0.009$). Twenty one CNS +ve patients entered CR (81%) and 11 subsequently relapsed (9 marrow, 1 CNS, 1 combined). Long-term survival did not differ between CNS +ve and CNS -ve cases: median 16 vs. 20 mos.; 30% at 5 years in either group. With regard to remission patients ($n=553/687$), there were 20 isolated CNS relapses after a median of 7.8 mos., and 10 combined (CNS + marrow/other) relapses after a median of 7.7 mos. Thus CNS relapse, of any type, occurred earlier than isolated marrow relapse. The probability of CNS recurrence (isolated+combined) correlated with ALL subset: 4+1/20 (20%+5%) in B-ALL, 9+5/115 (8%+10%) in T-ALL, and 6+4/376 (1.6%+1.4%) in B-precursor ALL ($p=0.000$). Notably, the risk of CNS progression was comparable among different prophylaxis regimens (ST/RT: 10/192, 5.2%; TT/RT: 3/63, 4.7%; TT/RT/HD: 13/275, 4.7%), with the only exception of higher recurrence rates in a small group not given RT (ST/HD-ara-C: 3/23, 13%). As regards survival, outcome was universally poor, with no survivor beyond 1 year after combined CNS relapse and a single cured patient at 14.5 years after isolated CNS relapse (18/19 remaining patients dying within 2 years). **Conclusions.** CNS involvement is a sizeable problem in adult ALL, and more so in the relapse setting, where it appears to sustain an early systemic spread of ALL that ultimately leads to treatment failure. Despite intensified prophylaxis with TT, RT and HD-Ara-C/MTX, T-ALL is still a challenging condition, whereas results were improved in B-ALL using specific treatment protocols. Higher-dose Ara-C (3 g/m²), MTX (2.5-5 g/m²), and intrathecal liposome-entrapped Ara-C (DepoCyte) should be evaluated, particularly when omission of RT prophylaxis is planned.

0419**CD20+ ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL): DISTINCTIVE DIAGNOSTIC PROFILE BUT NOT A UNIQUE CLINICAL ENTITY**

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Background/Aims. In ALL immunophenotype plays a crucial diagnostic role and is prognostically informative. CD20 is a maturation B-lineage marker with variable expression in B-precursor ALL and uncertain prognostic significance. Because CD20 can be therapeutically targeted by Rituximab and has been preliminarily associated with poorer clinical outcome, we elected to analyze the clinical characteristics and treatment response of CD20+ ALL in NILG trial 09/00. The study aimed to compare major diagnostic features and treatment outcome in CD20+ and CD20- cases, respectively. **Methods.** All patients entered on trial since the year 2000 were evaluable (FAB L3/B-ALL excluded). In this study, homogeneous multidrug induction-consolidation (8 cycles) is followed by MRD-adapted therapy (maintenance; high-dose, autograft-supported cycles; allogeneic SCT). Positive CD20 expression on marrow blast cells was set at 20% or higher. **Results.** Out of 193 evaluable cases, CD20 was expressed in 61/155 (39%) with B-precursor ALL and 2/38 (5%) with T-ALL. The comparative analysis was restricted to the former group and showed the following (CD20+ vs. CD20-; P value only when <0.05): median age 41 vs. 37 years; male gender 56% vs. 48%; FAB L2 71% vs. 64.5%; median blast count(x10e9/L) 4 vs. 6; pro-B phenotype 3.3% vs. 27% (p=0.000); pre-B CD10+ phenotype 83% vs. 53% (p=0.000); CD34+ 70% vs. 84% (p=0.04); CD13+/33+ 20%/21% vs. 29%/30%; Ph/BCR-ABL+ 41% vs. 24% (p=0.03); t(4;11)/MLL-AF4+ 0% vs. 11% (p=0.04). In summary, two different ALL subsets were significantly associated with CD20 expression: Ph/BCR-ABL+ ALL (n=46, 50% CD20+) and standard risk CD10+ ALL with blast count <10 (n=49, 51% CD20+), whereas the lowest CD20 expression was observed in cases with higher blast count and/or immature CD10+/CD34+ phenotype (n=58, 15.5% CD20+; p=0.000). As regards treatment, similar CR rates (88% vs. 82%), early negative MRD rates (42% vs. 40%) and survival/DFS rates were observed. The relapse incidence was tentatively higher in CD20+ (45% vs. 29%, p=0.06), possibly reflecting the higher prevalence of Ph/BCR-ABL. **Conclusions.** CD20+ ALL displays the features of B-precursor ALL at intermediate maturation stage (CD10+) and is often associated with Ph/BCR-ABL or, alternatively, with lower blast cell count as in standard risk ALL. To date, we could not confirm a significantly worse prognosis associated with CD20 antigen expression. These observations suggest that many high-risk patients with CD20+ Ph/BCR-ABL+ ALL are potential candidates to targeted therapy with rituximab plus imatinib and/or other synergistic combinations (e.g. rituximab /cyclophosphamide/fludarabine).

0420**CENTRAL NERVOUS SYSTEM (CNS) RELAPSE IN ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): FREQUENCY AND PROGNOSIS IN 467 PATIENTS WITHOUT CRANIAL IRRADIATION FOR CNS PROPHYLAXIS**

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Background. Recurrence of ALL in CNS in adults is considered a poor prognostic feature but few studies have analyzed this issue. The objective of this study was to analyze the frequency, prognosis and predictive factors for CNS involvement and recurrence of adult patients treated with 4 PETHEMA protocols not including cranial irradiation for CNS prophylaxis. **Methods.** From June 1989 to December 2003, 467 adult patients (≥15 years-old) diagnosed with ALL were treated with one of the four consecutive protocols of PETHEMA group: ALL-89 (standard and high risk, n=108), ALL-93 (high risk, n=222), ALL-96 (standard risk, n=84), and ALL-97 (Burkitt's leukemia, n=53). CNS prophylaxis consisted of intrathecal injection of methotrexate (12 mg), cytarabine (30 mg) and hydrocortisone (20 mg), 12 courses over the first year, together with high-dose systemic methotrexate and cytarabine during the early intensification phase. Cranial or craniospinal irradiation was not used in any case. **Results.** The median (SD) age was 33 (16) years and 272 (58%) were males. ALL type according to the FAB classification was: L1 28%, L2 61%, L3 11%. Immunological subtypes were: early-pre-B 15%, pre-B 5%, common 45%, mature B 11% and T 24%. CNS involvement at diagnosis was observed in 18 (3,9%) patients. Predictive factors for CNS involvement at diagnosis were: L3/mature B ALL (p=0.007) and mediastinal mass (p=0.008). Overall, complete remission (CR) was achieved in 381 (81%) of the patients. Of them, 159 (34%) relapsed: 137 (29%) in bone marrow (BM) and 22 (4.7%) in CNS (14 isolated and 8 combined CNS and BM). Medians (range) of CR duration prior to CNS recurrence were 1.16 yr (0.25-3.45) for isolated CNS relapse and 0.6 yr (0.22-4.47) for combined CNS and BM relapse (p= n.s.). No differences in patients' and ALL characteristics were observed when patients with BM and CNS relapses (isolated and combined) were compared. Specifically, no correlation was found between initial CNS involvement and CNS relapse. Treatment of CNS relapses consisted of systemic and IT therapy in the 22 cases (cranial irradiation was added to one). CR was attained in 7 (32%) out of 22 patients with CNS relapse. Stem cell transplantation was performed in 4 patients. Three patients had a second CNS recurrence. The medians of overall survival (OS) after recurrence were 0.7 yr for isolated CNS relapse, 0.13 yr for combined relapse and 0.41 yr for BM relapse (p=0.11). **Conclusions.** The frequency of CNS relapse in adult ALL patients receiving IT and systemic therapy for CNS prophylaxis is similar to that observed in protocols including cranial irradiation. No predictive factors for CNS recurrence were identified. Adult patients with CNS recurrence have a poor prognosis, although in this study it was not different from that observed in BM relapses. Supported in part by grant P-EF/04 from Jose Carreras International Leukemia Foundation

0421**ACUTE LYMPHOBLASTIC LEUKAEMIA CELLS CARRYING THE T (12;21) TRANSLOCATION ARE HIGHLY SENSITIVE TO ALEMTUZUMAB MEDIATED CELL LYSIS**

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Background. Alemtuzumab is an anti-CD52 humanised IgG1 antibody used for the treatment of B-CLL and for non-myeloablative conditioning in allogeneic bone marrow transplantation. CD52 is expressed on mature T and B lymphocytes, monocytes, monocyte derived dendritic cells and on most leukaemia and lymphoma cells derived from these haematopoietic cell populations. **Aims.** 1) To determine the levels of expression of CD52 in acute lymphoblastic leukaemia (ALL) cell lines and freshly isolated samples of different subtypes. 2) To investigate the capacity of alemtuzumab to lyse ALL cells through complement activation. **Methods.** CD52 expression was analysed by standard immunophenotyping and FACS analysis. Complement dependent cytotoxicity (CDC) was measured *in vitro* using human serum as source of complement. Cell death was determined with the alamar blue vital dye as well as by FACS analysis. **Results.** CD52 was expressed in 4 out of 9 ALL cell lines studied, with 62-100% of the cells staining positively in these. However only two of these lines, the pre B-ALL REH (t(12;21), TEL-AML1) and the B-ALL Silti (t(8;22), MYC-IGL) expressed CD52 at relatively high density (MFI 351 and 274, respectively). Analysis of alemtuzumab induced CDC of the four cell lines showed that only the REH cell line was lysed efficiently, with 50% lysis in presence of 10 microgr/mL alemtuzumab. The other CD52+ cell lines all showed either weak or no lysis (0-8%). Lysis was complement dependent. We then analysed CD52 expression in a panel of 56 freshly isolated adult or pediatric ALL samples. CD52 was expressed at varying levels on 84% of cases. CD52 expression did not correlate with leukemia subtype, except that all t(4;11) pro-B ALL cases were CD52 negative (4/4 cases). Furthermore all t(12;21) ALL cases were CD52 positive and expression levels in this subgroup was on average slightly higher than in all other subgroups (MFI 684 compared to 400). CDC was then analysed: interestingly 8/8 cases of t(12;21) cases showed very high CDC (mean 96% lysis with 10 microgr/mL alemtuzumab, range 85-100%) compared to a mean of 14% in the other 20 CD52+ cases bearing other or no translocations (0-50%). In t(12;21) cases, efficient CDC was obtained with as low as 1 microgr/mL alemtuzumab. Measurement of deposition of the complement components C3 and C9 indicated that more efficient initiation of the complement cascade took place in t(12;21) cells compared to non t(12;21) cases. Furthermore the CD55 and CD59 complement inhibitors were expressed at similar levels in all leukemia subtypes and were not responsible for poor lysis in resistant ALL cells. Although t(12;21) cells express relatively high levels of the CD52 antigen, this factor alone does not fully explain the particularly high susceptibility of these cells to alemtuzumab mediated CDC compared with other leukaemia subtypes. **Conclusions.** pre B-ALL cell lines and freshly isolated samples bearing the t(12;21) translocation express CD52 and are particularly sensitive to alemtuzumab and complement mediated lysis *in vitro*. We thank Schering SpA, AIRC, AIL-Paolo Belli section, Bergamo and MIUR (Firb project to JG) for support.

0422**GEMTUZUMAB OZOGAMICIN (MYLOTARG) HAS THERAPEUTIC ACTIVITY AGAINST CD33+ ACUTE LYMPHOBLASTIC LEUKAEMIAS IN VITRO AND IN VIVO**

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Background. Gemtuzumab Ozogamicin (GO) is a humanised anti-CD33 antibody conjugated to the cytotoxic drug calicheamicin. It has been approved for the treatment of relapsed acute myeloid leukemia (AML) and is particularly effective in acute promyelocytic leukemia (APL). 15-25% of acute lymphoblastic leukaemias (ALL) are also CD33+. **Aims.** To investigate the cytotoxic activity of GO on CD33+ ALL cells *in vitro* and *in vivo*. **Methods.** The anti-proliferative activity of GO was measured *in vitro* at different concentrations using thymidine uptake assays and cytotoxic activity using the vital dye alamar blue. The cells isolated from patient ALL-2 were passaged *in vivo* by intraperitoneal injections of 50x10⁶ cells in SCID mice. After 4 weeks, passaged ALL-2 cells could be recovered from the ascites fluid. These remained CD33+ like the original leukaemic clone. These cells were then used to set up an *in vivo* model of CD33+ ALL by i.v. injection of the passaged cells in SCID mice. **Results.** *In vitro*, 10 ng/mL GO induced 45-95% inhibition of thymidine uptake and 30-70% cell death in four freshly isolated and in the *in vivo* passaged CD33+ ALL-2 cells. Furthermore an *in vivo* model of CD33+ ALL was established. 5x10⁶ passaged ALL-2 cells inoculated in the tail vein of SCID mice expanded in haematopoietic organs reaching at 5 weeks a mean of 70%, 61%, and 69% in bone marrow, spleen and liver, respectively. To test the therapeutic activity of GO *in vivo*, 50 or 100 µg immunotoxin were inoculated i.p. on days 7, 11 and 15 following tumour cell inoculation. GO treatment dramatically inhibited expansion of ALL-2 cells in all tested organs and increased survival of tumour injected animals by 28-41 days relatively to controls. All animals however eventually succumbed to the leukaemia. Cells recovered from GO treated animals were CD33+ suggesting that treatment had not selected for a CD33 negative variant. In order to try and increase the therapeutic activity of GO *in vivo*, ALL-2 injected animals are being treated with 50 µg GO in combination with 150 µg cyclosporin A. The results will be presented. **Conclusions.** These data demonstrate that GO is active both *in vitro* and *in vivo* against CD33+ ALL cells. We thank AIRC, AIL- sezione paolo Belli- Bergamo and MIUR (Firb project to JG) for their support

0423**COMPASSIONATE USE OF INTRATHECAL DEPOT CYTARABINE (DEPOCYTE®) AS TREATMENT OF CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT IN ACUTE LEUKEMIA (AL) IN SPAIN: REPORT OF 5 CASES**

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Background. and aim. Depot formulation of cytarabine (DepoCyt®) has proven to be useful in clinical trials as treatment of neoplastic meningitis and lymphomatous meningitis. Its value in the treatment of CNS involvement in AL has been demonstrated in individual cases, but few series have been reported. **Methods.** Retrospective review of all cases in which IT depot cytarabine was employed as compassionate therapy for CNS involvement of AL in Spain. In each patient, the following parameters were evaluated: Type of AL, previous CNS prophylaxis, number of IT doses of depot cytarabine administered, response and side effects. **Results.** From March 2004 to January 2005 five cases (4 females) were recorded (ALL, 3, AML, 1, and

lymphoid blast crisis of CML, 1). The median (range) age was 24 (5-50) yr. Two patients (ALL and AML) had CNS involvement at diagnosis. In one (AML) IT depot cytarabine was administered after failure of triple intrathecal therapy (TIT), whereas the remaining (ALL) received IT depot cytarabine as adjuvant therapy after TIT and cranial radiotherapy. The remaining 3 (2 ALL, 1 blast crisis of CML) had CNS relapse. The two cases of ALL received CNS prophylaxis with TIT and TIT plus radiotherapy, respectively, whereas the patient with blast crisis of CML did not receive any prophylaxis. IT depot cytarabine was administered as treatment of a second CNS relapse in the two ALL patients and as adjuvant to TIT plus radiotherapy in the patient with blast crisis of CML. In the 3 evaluable cases (AML and 2 cases with ALL in second CNS relapse) treated with IT depot cytarabine as the only drug, clearance of blasts in cerebrospinal fluid (CSF) was observed with sustained response in two (at 9 and 11 months). The remaining patient presented neurologic progression at 3 months. The median number of IT depot cytarabine administrations was 4 (1-10) and the total number of the doses administered was 27. Concurrent dexamethasone therapy was administered with each dose of IT depot cytarabine. Side effects included headache (3 patients), dizziness (2) and vomiting (1). **Conclusions.** Depot formulation of cytarabine was effective in achievement CNS remission in AL patients with CNS involvement or relapse. The administration of depot formulation of cytarabine was well tolerated. This justifies the development of a clinical trial to evaluate the efficacy and safety of IT depot cytarabine in meningeal involvement of AL.

0424

TREATMENT OUTCOME OF ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA ON ALL-BFM-90M AND ALL-MB-91 PEDIATRIC PROTOCOLS

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Background. till now in city hematological departments of Moscow modern technologies for treatment of adolescents and young adults with an acute lymphoblastic leukemia (ALL) have not been introduced. According to own analysis the 6-years event-free survival (6y-EFS) of young adults with ALL treated in Moscow city hospitals for the period 1993 - 1996 was the lowest and has made 20.7%. In attempt to improve the treatment outcome of adolescents and young adults with ALL us have used pediatric protocols ALL-BFM-90m and ALL-MB-91. **Aims.** to carry out the analysis of treatment outcome of adolescents and young adults with ALL on ALL-BFM-90m and ALL-MB-91 protocols. **Patients and treatment:** during 1997 - 2003 in this study 54 patients in the age of from 15 till 29 years have been enrolled. With the purpose of the comparative analysis of results of treatment it has been allocated two age groups: 15-19 years (m-19, f-11, median 16.8 year)-30/55.6% and 20-29 years (m-17, f-7, median 21.6 year)-24/44.4% patients. The analysis of the most significant initial prognostic attributes has been executed with the help of Cox regression model. **Results.** The complete remission (CR) has been achieved at 86.7% of patients in the age of 15-19 years and 87.5% - 20-29 years (p=0.9). Age differences concerning an early lethality it has not been registered: 6.7 and 4.2% (p=0.9). Overall event free survival (EFS) estimates were at 6 year 63.3% in group 15-19 years and 54.2% -20-29 years (p=0.564). 6-year cumulative rates of relapse were 19.4%. The cumulative rates of relapse was not different according to patient ages: 4/13.3% for patients of 15-19 years and 6/25.0% for patients 20-29 years (p=0.5). Relapse free survival (RFS) at 6 year also was enough high: 15-19 years-84.6% and 20-29 years-71.4% (p=0.4). In the single-step Cox regression model it has been revealed that on therapy under reports ALL-BFM-90m and ALL-MB-91 prognostic values have: hyperleukocytosis $\geq 50.000/\mu\text{L}$ (p=0.002) and BFM-RF > 0.8 (p<0.001) and CNS involving (p=0.003) and risk group (p<0.001) and the poor answer to steroids at 8 day of therapy (p=0.008) and M3 (> 25% blasts) bone marrow status at 15 day (p=0.001) and M2/M3 (>

5% blasts) at 33/36 day of treatment (p < 0.001). Age (p=0.4) and a sex of the patient (p=0.5) and T or B immunological variant of ALL (p=0.3) and augmentation of a mediastinum (p=0.1) do not carry in itself independent prognostic value. **Summary.** results of this study can serve as a substantiation of rationality of introduction risk - adapted of protocols ALL-BFM-90m and ALL-MB-91 for treatment of adolescents and young adults with ALL. The basic prognostic factors earlier revealed in research ALL-BFM-90m and ALL-MB-91 at children keep the value concerning adolescents and young adults with ALL treated on the same protocols.

0425

OVEREXPRESSION OF CD123: A USEFUL MARKER IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Overexpression of interleukin-3 receptor (IL-3R) α chain (CD123) provides proliferative advantage to neoplastic myeloid blasts and represents a marker of transformed phenotype in acute myeloid leukemia. However, only few studies have investigated the expression of CD123 in normal lymphoid precursor cells and/or in acute lymphoblastic leukemia (ALL). **Aims.** The aims of our study were (1) to examine the expression of CD123 in normal B-cells at different maturational stages and compare it to patterns of expression in ALL cells at diagnosis, and (2) to investigate if patterns of CD123 expression correlate with any genetic alterations in ALL. In addition, (3) use of CD123 as a marker of minimal residual disease was evaluated. **Methods.** 98 diagnostic samples from 95 children (<18 years old) consecutively diagnosed at Karolinska University Hospital, Stockholm, and subsequently enrolled in NOPHO ALL-2000 pediatric protocol were examined. 89 patients were diagnosed with *de novo* ALL and 6 had relapse of previously diagnosed leukemia. The diagnostic specimens included bone marrow aspirate (n=94) and peripheral blood (n=4) samples. Control bone marrow samples were obtained from children with non-malignant conditions. Expression of CD123 was assessed using phycoerythrin (PE)-conjugated anti-CD123 antibody (Becton Dickinson) on CD19-gated cells (in precursor B ALL) or CD7-gated cells (in precursor T ALL) by four-color flow cytometry. The mean fluorescence intensity (MFI) of leukemic population was compared with MFI of three maturation stages of B cells as defined by BIOMED-1 concerted action report. **Results.** Non-malignant B-cells showed dim expression of CD123 at mid-stage (CD10+, CD20+/-) and mature-stage (CD10-, CD20+). Early-stage B-cell precursors (CD10+, CD20-) showed no expression of CD123. B-cell precursor ALL (85 cases) showed bright expression of CD123 in 29 cases (34%), dim CD123 expression in 49 cases (59%) and no expression in 6 cases (7%). Interestingly, 15 of 16 ALL cases (94%) with hyperdiploid karyotype (≥ 52 chromosomes) showed bright CD123 expression. All 7 cases with t(12;21) translocation showed dim CD123 expression. 9 of 10 cases (90%) of precursor-T ALL showed no expression of CD123. The sole T ALL patient with dim expression of CD123 had 11q23 rearrangement and later relapsed as undifferentiated leukemia. The aberrant CD123 expression persisted in follow-up samples positive for MRD. **Conclusions.** Our study suggests that overexpression of CD123 is an aberrant phenotype present in a subset of precursor B ALL. This phenotype correlates with hyperdiploid genotype, a well-defined category with prognostic significance in pediatric precursor B ALL. Expression of CD123 may be used as a marker of residual disease as early B-cell precursors lack expression of this antigen. Majority of T ALL cases do not express CD123.

0426**FLAG-IDA IN THE TREATMENT OF RELAPSED/REFRACTORY ADULT ACUTE LYMPHOBLASTIC LEUKEMIA**G. Specchia¹, D. Pastore¹, P. Carluccio¹, A. Liso², A. Mestice¹, R. Rizzi¹, L. Ciuffreda¹, G. Pietrantonio¹, S. Sibilla¹, V. Liso¹¹University of Bari, BARI, Italy; ²University of Foggia, FOGGIA, Italy

Relapsed or refractory adult acute lymphoblastic leukemias (ALL) have poor prognosis. The strategy for treating these patients is by re-induction chemotherapy followed by allogeneic stem cell transplantation, provided that the toxicity of the salvage regimen is acceptable. We evaluated the efficacy and the toxicity profiles of the combination of fludarabine, high-doses of cytosine arabinoside (AraC), idarubicin and granulocyte colony-stimulating factor (G-CSF) in refractory/relapsed ALL patients. Between January 2001 and February 2004, 23 adult refractory/relapsed ALL patients were treated with FLAG-IDA (fludarabine 30 mg/m², AraC 2 gr/m² for 5 days, idarubicin 10 mg/m² for 3 days and G-CSF 5 µg/Kg from day +6 until neutrophil recovery). There were 14 men and 9 women with a median age of 32 years (range 16-62). Cytogenetic studies were made in all cases: 8 cases had t(9;22), 10 cases had a normal karyotype and no mitoses were obtained in 5 cases. Median WBC and blasts cells in the bone marrow (at the start of FLAG-IDA) were 7.4 x10⁹/L (range 3.7-114) and 62% (range 20-88%), respectively. Five patients were refractory to first line chemotherapy at a median of 40 days (range 28-49) from the start of front-line chemotherapy; the first line therapy consisted in an induction regimen with vincristine, daunorubicin, L-asparaginase and prednisone according to the GIMEMA protocols. Eighteen patients were in first relapse; the median duration of first complete remission (CR) was 6 months (range 3-16), being shorter than 12 months in 15 cases and longer in the other 3 cases. Nine patients achieved CR following salvage therapy (39.1%), whereas twelve patients (56.5%) were refractory and one patient died during the aplastic phase due to a bacterial infection (*P. aeruginosa*). The CR rate was 0/5 in primary refractory and 9/18 (50%) in relapsed patients; the CR rate was 7/15 (46.6%) and 2/3 (66.6%) in early and late relapses, respectively. During the neutropenic phase 5/23 episodes of documented sepsis (2 due to *Staphylococcus aureus*, 1 *Pseudomonas aeruginosa* and 1 *Escherichia coli*), 1 fungemia due to *Trichosporon capitatum* together with 13 cases of fever of unknown origin (FUO) were observed. The non-hematological toxicity was acceptable; the most common side effects were mucositis (18/23 or 78.2%), increased serum bilirubin (6/23 or 26%). All 9 patients who achieved CR received one consolidation course with 2nd FLAG-IDA and 7 patients underwent allogeneic stem cell transplantation (4 from a matched donor, 1 from a mismatched donor and 2 from an unrelated donor) while 2 did not reach this stage due to early relapse; no transplant-related mortality was observed. The median OS for all 23 patients is 4.5 months (range 2-38); for the 9 responder patients the DFS and the OS were 6 (range 3-38) and 9 (7-38) months, respectively; for the 7 patients who received allogeneic stem cell transplantation the DFS was 10 (range 7-38) months. We suggest that FLAG-IDA is an acceptable salvage regimen in adult relapsed/refractory ALL patients, which can render a transplant procedure feasible in patients who achieve complete remission.

0427**IRON OVERLOAD IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) OFF-THERAPY**

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Background. Secondary hemosiderosis related to multiple transfusions received during intensive chemotherapy is a possible sequela in long-term adult survivors of acute leukemia (15-

20%). Up today one pediatric study has found, by liver biopsy, long-term iron overload in at least 14% of children after therapy for ALL. **Aims.** To quantificate liver iron concentration (LIC) in children with ALL off-therapy and its correlation with other parameters. **Methods.** In our study LIC was measured not invasively by SQUID biomagnetic susceptometry in a consecutive series of children with ALL at first complete remission and off-therapy. Iron parameters were assessed by standard methods. The patients were screened for gene mutations of hereditary hemochromatosis (C282Y, H63D, S65C). Statistical analysis was performed by descriptive statistics, linear correlation and T-test for independent samples, by Statistica v.6, Stat-Soft Inc. **Results.** We studied 67 consecutive patients treated according to the AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica)'95 ALL protocol (4 low, 58 intermediate, 5 high risk). Mean age at diagnosis was 6.3 + 4.5 years (range 0.9-18). Males/females ratio was 37/30. During therapy, patients received a mean of 10 + 6 (range 2-36) packed red cells units, equivalent to 1558 mg of iron + 1073 (range 272-5758). The mean LIC value was 1.5 + 1.1 mg/gdw (range 0.3-5.3); 11 (16%) patients showed normal LIC value (< 0.7 mg/gdw). Five (7%) patients resulted homozygous or compound heterozygous for the HFE mutations, 10 (15%) were heterozygous. No patient had signs of HBV or HCV infections, nor iron-related complications as cardiopathy or endocrinopathies. The amount of transfused iron correlated (p < 0,001) with LIC; the relationship of LIC and ferritin was poor, even if the highest serum ferritin values were found in patients with the highest LIC. Two patients with severe iron overload (LIC 5,3 and 4,6 mg/gdw respectively) normalized the iron stores after a well-tolerated phlebotomies period. Both had other risk factors: one of them was \square thalassemia heterozygous and H63D homozygous, the other one was H63D heterozygous. **Conclusions.** Because the increasing number of long-term survivors of childhood ALL, issues regarding the late effects of therapy as iron overload are becoming more and more important. The evaluation of iron status and the presence of other factors as thalassemia and HFE mutations should be investigated in ALL patients. Phlebotomy may easily normalize iron stores and prevent secondary complications.

0428**INCREASED ANGIOGENESIS IN THE BONE MARROW OF PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA**M. Bakrac¹, V. Cemerikic², J. Bila², M. Colovic², I.E. Elezovic², M.P. Petrovic³, D.T. Tomin³, D. Boskovic³¹Institute of Haematology, BELGRADE, Serbia; ²Institute of Haematology, BELGRADE, Serbia; ³Institute of Haematology, BELGRADE, Serbia

It has been shown that solid tumors progress in concerne with an induction of tumor angiogenesis. Emerging data suggest an involvement of angiogenesis in the pathophysiology of hematologic malignancies as well. To investigate the role of angiogenesis in acute lymphoblastic leukemia (ALL), bone marrow biopsies from 30 adults with newly diagnosed untreated ALL were evaluated, also from 24 pts in the first remission, and from 16 pts in the first relapse of disease. We evaluated expression of VEGF (vascular endothelial growth factor) and microvessel density (MVD) by using immunohistochemical staining on slides from paraffin embedded bone marrow biopsies. Assessment of MVD was characterized immunohistochemically by anti-CD34 antibody with the use of a labeled streptavidin-biotin peroxidase method. Control specimens were obtained from pts without hematological malignancy. We countered the number of vessels per 400x high/power microscopy field (HPF) in the area of most dense vascularisation. All samples were further analyzed for the expression pattern of vascular endothelial growth factor (VEGF) in the bone marrow lymphoblasts. The intensity of staining for VEGF was graded as weak (0-30% lymphoblasts positive), moderate (31-60% lymphoblasts positive), or strong (>60% lymphoblasts positive). Microvessel counts in

untreated ALL pts (17.10/x400 field) were significantly higher than those in normal controls (7.11/x400 field) and in the bone marrow remission (BMR) ALL pts (9.12/x400 field) ($p < 0.001$) but slightly lower than at relapsed pts (19.21/x400) ($p > 0.05$). The VEGF was significantly higher in newly diagnosed ALL pts (strong positivity) than of the control group ($p < 0.05$). There was a positive correlation between VEGF expression and MVD. A higher rate of VEGF expression was confirmed at diagnosis in contrast to low level at remission. Furthermore, in relapsed pts strong activity of angiogenesis by VEGF and MVD expression was detected. Our data suggest that increased angiogenesis confirmed by immunohistochemical expression of VEGF and MVD may play an important role in the pathophysiology of ALL with prognostical implications. The expression of VEGF raises the possibility of using angiogenesis inhibitors as a novel therapeutic strategy in adult ALL.

0429**GENETIC PROFILE OF ACUTE LYMPHOBLASTIC LEUKEMIA IN A PATIENT WITH THE NOONAN SYNDROME**

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Background. Germline mutations in PTPN11, the gene encoding SHP-2, occur in the Noonan syndrome (NS), a disorder characterized by short stature, dysmorphic face, congenital heart disease and skeletal abnormalities. Children with NS are also predisposed to a spectrum of hematological diseases. Somatic mutations in PTPN11, the most frequent molecular lesion in Juvenile Myelomonocytic Leukaemia, are also found in MDS, AML, and pediatric ALL (1-2). Aim To investigate the genetic profile of ALL arising in NS using cytogenetics and molecular analysis. **Methods.** A 17-year-old male with NS was referred to the Hematology Unit, Perugia General Hospital because of thrombocytopenia (PLT 19000/mm³) and anemia (Hb 12.5 gr/dL). Blood film showed 90% of lymphoid blasts (WBC $4.46 \times 10^9/L$). ALL with common B phenotype was diagnosed on bone marrow biopsy. The bone marrow karyotype was: 46 XY [8]/ 41-45/XY [6]/ 53 XXY,+4,+17,+18,+20,+21,+21 [1]. Interphase FISH (I-FISH) was performed with probes for the aliphoid centromeric sequences of chromosomes 8, 13/21, 15, 17, and 18. BCR/ABL1, MLL/AF4, ETV6/AML1, and E2A-PBX1 were studied by I-FISH or RT-PCR. Mutation analysis of PTPN11, AML1, K-RAS, N-RAS, FLT3-ITD and FLT3835 was performed as described elsewhere (2-5). dHPLC analysis and sequencing for amplimers with abnormal denaturing profiles was conducted as reported (1). **Results.** I-FISH found hyperdiploid clones with centromeric probes for chromosome 13/21 (25% and 50% of nuclei with five and six signals, respectively), chromosome 17 (60% and 10% of nuclei with three and four signals, respectively), and chromosome 18 (40% of nuclei with three signals). Probes for centromeres 8 and 15 gave two hybridization signals in 100% and 92% of nuclei, respectively. Rearrangements of BCR/ABL1, MLL/AF4, ETV6/AML1, and E2A/PBX1 were not detected. The germline mutation of PTPN11 G (179)C; Gly (60) Ala in exon 3, was found in bone marrow cells and skin fibroblasts. Screening was negative for AML1/RUNX, FLT3, K-RAS and N-RAS mutations. **Summary/Conclusions.** In paediatric leukaemia chromosome translocations that arise during foetal haemopoiesis probably constitute a first-hit mutation that, in itself, is insufficient for the development of clinical leukaemia. The germline PTPN11 mutation may act as the first-hit which, when associated with other genetic lesions, induces ALL. In this case of NS cytogenetics and molecular analysis of well-known major rearrangements and mutations did not identify the second hit, which was related to hyperdiploidy without structural changes like atypical subgroup of ALL with favourable prognosis.

Supported by: Fondazione Cassa di Risparmio, Perugia, Italy
Acknowledgements: DNA probes were kindly provided by Dr. M Roc-

chi, University of Bari, Italy and TIGEM, Ospedale San Raffaele, Milan, Italy

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0430**IS IT SAFE TO RESUME L-ASPARAGINASE AFTER CENTRAL NERVOUS SYSTEM THROMBOSIS IN CHILDREN?**

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Background. L-Asparaginase (ASP) is an important component of combination therapy for treatment of pediatric acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma. A significantly worse 5-year event-free-survival has been documented in patients who receive less than the planned dose of ASP. Thrombosis is a major complication of ASP, caused by changes in plasma proteins involved in coagulation and fibrinolysis, mainly fibrinogen and antithrombin III (AT III). **Aims.** To evaluate the feasibility and safety of reintroduction of ASP in patients recovered from a central nervous system (CNS) thrombotic event, occurred under ASP therapy. **Methods.** The clinical, laboratory and neuroradiologic data of four children with CNS thrombosis during ASP therapy were retrospectively evaluated. **Results.** There were 2 boys and 2 girls, aged between 4 and 12 years. All had *de novo* disease - three children had ALL (precursor B in 2, T cell in 1) and were receiving intensification therapy according to DFCI 91-01 protocol (high risk); one child had a T-lymphoblastic lymphoma, treated in Average Risk 2 (AR2) arm of EORTC ALL 58981 protocol. Two children developed CNS thrombosis under pegaspargase (2500 IU/m²), respectively after the second and third intramuscular dose. The third ALL patient thrombosis occurred after the 4th dose of native E. Coli ASP (25000 IU/m²). The other patient was in the late intensification of AR2 arm, receiving pegaspargase (1000 IU/m²). All ALL patients had had prophylactic cranial irradiation, completed between 1 and 14 days before the thrombosis. No patient had evidence of infection; two children had central venous line. Two patients presented with seizures, the other 2 with apathy, headache and prostration. Longitudinal superior sinus thrombosis was diagnosed by angio-MRI in all. Coagulation studies were abnormal, with low levels of fibrinogen and antithrombin III (ATIII). Therapy included correction of coagulation factors and low-molecular-weight heparin (LMWH). All patients had a full recovery, clinical and by MRI, with re-perfusion of the thrombosed veins. After recovery, all patients restarted therapy with ASP, under LMWH prophylaxis. Before each dose of ASP, evaluation and correction, as needed, of coagulation factors was performed, including administration of ATIII concentrate. All but one have already completed full doses of ASP as recommended by protocol. The other one is still on treatment, without complications. **Conclusions.** It was possible and safe to resume and complete ASP therapy in these 4 patients, after resolution of the CNS event. The evaluation and correction strategy for coagulation studies has now been applied to all patients who need ASP therapy, and since May 2004 no more thrombosis were diagnosed in our Department.

0431**BCR-ABL MUTATIONS IN PATIENTS WITH PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ALL) RECEIVING IMATINIB-BASED THERAPY**

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Resistance to the Abl kinase inhibitor imatinib has become a

critical issue for patients in advanced phases of chronic myelogenous leukemia (CML) and Ph+ALL. Imatinib-resistance frequently develops as a consequence of point mutations within the Bcr-Abl kinase domain (KD) that prevent imatinib from binding. In CML patients, more than two dozen mutations have been identified, located primarily in one of three domains referred to as the P-loop, the catalytic domain and the activation loop. These various mutations differ in the degree of imatinib resistance they confer. In advanced Ph+ALL, mutations have likewise been associated with clinical resistance, but the pattern and relative abundance of Bcr-Abl resistance mutations emerging in the presence of imatinib alone or in combination with chemotherapy have not been established. **Aims.** To determine the frequency and distribution of mutations of the Abl KD in patients with relapsed/refractory or newly diagnosed Ph+ALL or lymphoid blast crisis of CML, and their occurrence in relation to imatinib therapy given as single agent versus in combination with chemotherapy. **Methods.** and patients: In this study, we retrospectively investigated the bcr-abl mutational status at the time of relapse in 64 pts. who were enrolled a) in the initial phase II trials of imatinib monotherapy for advanced Ph+ALL/LyBC [n=45], b) in a subsequent GMALL study of elderly pts. with *de novo* Ph+ALL receiving imatinib in combination with extended chemotherapy [n=12] or c) in a GMALL study of younger pts. with *de novo* Ph+ALL receiving imatinib-based induction therapy [n=7]. Bone marrow and/or PB samples collected at the time of relapse were analyzed by direct sequencing of the abl KD. **Results.** Mutations were detected in 39 patients overall (61%). In the phase II trials of imatinib monotherapy, 29/45 patients (64%) developed a mutation; the frequency of mutations in elderly patients with *de novo* Ph+ALL receiving parallel imatinib and chemotherapy was 83% (10/12). The overall frequencies of these mutations were: Y253F/H (n=8; 20%), Q252H (n=4; 10%), E255K (n=13; 33%), G250E (n=4; 10%), M248V (n=2; 5%), in the P-loop region and T315I (n=8; 20%) - a mutation in a position in direct contact with imatinib. Only 1 mutation (2.5%) in the catalytic domain (F359V) and 1 mutation (2.5%) in the activation loop (H396R) were observed. In 10 patients (16%) more than one mutation was detected. Comparison of patients with p190bcr/abl and p210bcr/abl revealed no significant difference in either the frequency (69% vs. 78%) or profile of mutations. **Summary.** In Ph+ALL, clinical resistance to imatinib is associated with a high frequency of abl KD mutations, with conspicuous predominance of mutations located in the P-loop, most of which confer high level resistance to imatinib. In addition, 20% of patients were found to harbor the T315I mutation which confers absolute resistance to imatinib as well as to other ABL TK inhibitors in clinical development. The mutation profile is comparable in patients with advanced leukemia treated with imatinib monotherapy and in patients with newly diagnosed Ph+ALL receiving imatinib in combination with chemotherapy.

Acute myeloid leukemia Clinical trials II

0432

ALLO-GENIC BONE MARROW TRANSPLANTATION SHOULD BE CONSIDERED IN ACUTE MYELOID LEUKEMIA PATIENTS WITH MRD LEVELS >0.1% AT THE END OF TREATMENT

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Assessment of minimal residual disease (MRD) by multiparameter flow cytometry (FCM) in acute myeloid leukemia (AML) has a documented prognostic value. However, clinically significant time-points and MRD levels have not as yet been

established for various groups of patients and in different treatment programs. The aim of the study was to evaluate the prognostic significance of sequential MRD detection in children and adults treated for AML. **Methods.** MRD levels were evaluated in 108 patients (87 adults >18 yrs old, 21 children) with AML achieving morphological complete remission at Karolinska Hospital in Stockholm between July 1994 and June 2001. The diagnostic flow cytometry panel included membrane CD2, CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD45, CD56, CD65, CD117, HLA-DR, and intracellular CD3, CD79a, MPO and TdT. Based on results at diagnosis phenotypic aberrancies were defined and used in follow-up samples in three color antibody combinations as custom-built probes. CD34 or CD117 counts and MRD levels were determined after induction treatment(1), during consolidation (2) and after the end of chemotherapy treatment or before allogeneic SCT (3).

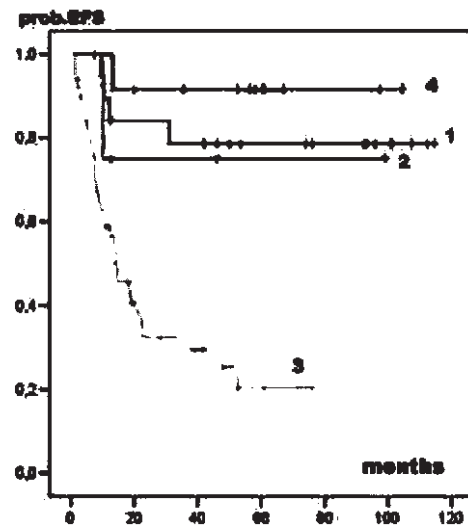


Figure 1. Kaplan-Meier curves for EFS in four groups of AML patients: (1) MRD <0.1%, no transplantation, (2) MRD <0.1%, allo-transplantation, (3) MRD >0.1%, no transplantation, (4) MRD >0.1%, allo-transplantation, log-rank test, $p < 0.0001$

The Cox proportional hazard model was used in survival analysis. Levels of cells with immature phenotype (CD34, and in patients with CD34 negative AML-CD117) significantly correlated with MRD levels at time-points (1) [$p < 0.0001$, $R = 0.505$] and (3) [$p = 0.03$, $R = 0.274$]. In univariate analysis event-free survival (EFS) was determined by detectable MRD >0.1% at time points (1) and (3) [$p = 0.004$] and [$p = 0.006$], respectively] and also by CD34/CD117 levels at the same time-points [$p = 0.034$] and [$p < 0.0001$], respectively]. However, in multivariate analysis only measurements of CD34/CD117 and MRD at time-point(3) were found to be the independent prognostic factors for EFS. Age was the only independent prognostic factor for overall survival (OS). Furthermore, analysis within age groups <18, 18-60 and >60 years old, showed that MRD levels above 0.1% were significant only for the group of the oldest patients [$p = 0.028$ and $p = 0.018$ at time-points (1) and (3), respectively], but not for children and younger adults. This may be influenced by the fact that MRD at the end of treatment(3) was significant for EFS in patients who were not allo-transplanted ($p = 0.019$), but not significant for allo-transplanted patients (Figure 1). However, in all adult patients (>18 years old) both MRD (3) and allo-transplantation were independently significant for EFS. **Conclusions.** Our results suggest that the presence of MRD at the level >0.1% at the end of chemotherapy may be a strong indication for allogeneic SCT. Also, simple measurements of CD34+ or CD117+ cell levels reveal prognostic information and correlate well with MRD levels.

0433**CLINICAL TRIAL OF VALPROIC ACID AND ALL-TRANS RETINOIC ACID IN PATIENTS WITH POOR-RISK ACUTE MYELOID LEUKEMIA (AML)**G. Bug¹, M. Ritter², B. Wassmann³, C. Schoch³, T. Heinzel⁴, K. Schwarz¹, A. Romanski¹, O.H. Kraemer¹, M. Kampfmann¹, D. Hoelzer¹, A. Neubauer², M. Ruthardt¹, O.G. Ottmann¹¹Hospital of J.W. Goethe-University, FRANKFURT, Germany;²Hosp. of Philipps-University, MARBURG, Germany; ³University Hosp. Grosshadern, MUNICH, Germany; ⁴Georg-Speyer-Haus, FRANKFURT, Germany

The histone deacetylase (HDAC) inhibitor valproic acid (VPA) induces *in vitro* differentiation of primary AML blasts, an effect enhanced by all-trans retinoic acid (ATRA). Clinical responses to VPA were recently observed in patients with myelodysplastic syndrome (MDS). *Aims.* Herein, we describe the results of a clinical trial with VPA plus ATRA in 26 patients with poor-risk AML. *Methods.* VPA (5–10 mg/kg starting dose) and ATRA (45 mg/m²) were administered orally. Low-dose AraC or hydroxyurea (HU) were permitted to control leukocytosis. Biological activity of VPA was confirmed by serial analysis of HDAC2 protein levels in peripheral blood (PB) mononuclear cells. *Results.* 19 of 26 patients completed at least 4 weeks of VPA/ATRA treatment, 7 patients were withdrawn prematurely because of rapidly progressive disease (n=3) or unacceptable neurological and cardiovascular toxicity (n=4). Additional cytoreductive treatment was required in 58% of the patients enrolled. Median treatment duration was 3 months. No patient achieved a complete remission. Two patients with secondary AML arising from a myeloproliferative disorder (MPD) achieved a partial remission and clearance of PB blasts, respectively. Strikingly, these responses were accompanied by profound granulocyte and erythrocytosis in both patients, necessitating additional HU. Patient #18 had an AML arising from a Philadelphia Chromosome-negative myeloproliferative disorder of 2.5 years duration and was enrolled after failing one cycle of standard induction therapy. Within four weeks of initiating VPA/ATRA treatment, significant neutrophilia and erythrocytosis developed, with 80 erythroblasts and 10.5% reticulocytes in the differential blood count. The hemoglobin increased from 12.7 to 17.5 g/dL after 4 weeks on VPA/ATRA, at which time a phlebotomy was performed. During this time period, circulating blast cells gradually decreased, with complete clearance from the peripheral blood by week 7, paralleled by a reduction of bone marrow CD34+ blasts from 23.5% to 14% by week 6. Patient #6 presented with an AML arising from a mixed myeloproliferative disorder/myelodysplastic syndrome with an isolated i(17)(q10). Approximately 10% residual blast cells were detected in this patient's BM after one cycle of induction chemotherapy with AraC and daunorubicin. By week 4 of VPA/ATRA administration a profound leukocytosis with a granulocytic left shift was observed, concurrently with a reduction of BM blasts below the level of detection by cytology. Cytogenetic monitoring of the efficacy of therapy were based on detection of i(17)(q10) positive BM cells. At the time of hematologic response the i(17)(q10) was detected in 20% of the CD34+ stem and progenitor cells, but in none of the mature granulocytes. *Conclusions.* We show that combination treatment with VPA/ATRA is clinically feasible on an outpatient basis and was generally well tolerated, despite the development of considerable neurological and cardiovascular toxicity in a minority of patients. Overall, antileukemic efficacy was marginal and restricted to patients with AML evolving from an antecedent myeloproliferative disorder. Additional cytoreductive treatment was required in more than half of the patients, including the 2 responders. However, cytogenetic analysis of isolated CD34+ cells and granulocytes in one responding patient did not reveal terminal differentiation of leukemic blasts.

0434**IMATINIB MESYLATE IN THE TREATMENT OF NEWLY DIAGNOSED OR REFRACTORY/RESISTANT C-KIT POSITIVE ACUTE MYELOID LEUKEMIA. RESULTS OF AN ITALIAN MULTICENTRIC PHASE II STUDY**P.P. Piccaluga¹, G. Martinelli¹, M. Malagola², M. Rondoni¹, M. Arpinati¹, P.L. Tazzari¹, M. Amabile¹, E. Ottaviani¹, R. Fanin³, D. Cilloni⁴, G. Saglio⁴, F. Lauria⁵, G. Visani⁶, D. Russo², M. Bacarani¹¹University of Bologna, BOLOGNA, Italy; ²University of Brescia, BRESCIA, Italy; ³University of Udine, UDINE, Italy; ⁴University of Torino, TORINO, Italy; ⁵University of Siena, SIENA, Italy; ⁶Department of Hematology, PESARO, Italy

Background. Imatinib mesylate specifically inhibits ABL, c-KIT and PDGFRs *in vivo*, and induces clinical responses in tumor expressing those kinases, such as chronic myelogenous leukemia, Ph+ acute lymphoid leukemia, gastro-intestinal stromal tumors, and hyper eosinophilic syndromes. *Aims.* We designed a phase II clinical trial in order to evaluate the clinical efficacy and the toxicity profile of Imatinib in c-KIT positive acute myeloid leukemia (AML). *Methods.* From February 2003 to November 2003 we treated with imatinib 36 c-kit positive AML patients who were not amenable to conventional chemotherapy. Fifty-one percent of the patients were refractory/relapsed and 49% were previously untreated. All the patients had c-kit positive leukemic cells (median 55%, range 16-96%), with a high mean fluorescence index. One patient aberrantly expressed PDGF-receptor beta (PDGFRb) on blastic cells (46%). No patient was found to carry the bcr/abl rearrangement. The median imatinib dose was 600 mg/day (range 200-700), for a median of 31 days (range 2-311+). *Results.* No patient achieved a complete or a partial remission. In two patients the disease remained stable as defined by no peripheral blood or bone marrow modification. They are now continuing imatinib after 270 and 311 days. The patient with PDGFRb aberrant expression had a platelet increase which lasted for one year with transfusion independence. In this meanwhile, blast cells became PDGFRb negative. Six patients died while on therapy with imatinib (2 with multi organ failure, 2 of disease progression, and 2 of cerebral stroke). Fifteen patients died during the follow-up, mainly due to disease progression. Non hematological toxicity was mild and not different from the one found in chronic myeloid leukemia imatinib trials. Intriguingly, by flow cytometry we found that c-kit de-phosphorylation after imatinib administration was not related with the clinical response.

Conclusions. We conclude that imatinib alone is not effective in c-kit positive AML. Further studies are warranted in the subset of patients with PDGFRb expression because positive blast cells may be more sensitive to imatinib therapy.

0435**□ TOCOPHEROL SUCCINATE INDUCES MOLECULAR REMISSION AND PROLONGS SURVIVAL IN AN ACUTE PROMYELOCYTIC LEUKEMIA TRANSGENIC MOUSE MODEL**R.S. Abreu e Lima¹, P.S. Scheucher¹, B.A.A. Santana¹, M.C.T. Pintao¹, A.S.G. Silva¹, A.B. Garcia², F.P. Saggiaro¹, R.P. Falcao¹, E.M. Rego¹¹Medical School of Ribeirao Preto, RIBEIRAO PRETO, Brazil; ²Medical School of Ribeirao Preto, RIBEIRAO PRETO, Brazil

□ tocopherol succinate (VES) is a vitamin E analog with antiproliferative activity against several cancer cell types. VES has strong redox activity and has been shown to inhibit protein kinase C (PKC) and target Akt and JNK pathways, and thus activating the intrinsic cell death mediators of caspase-9 and -3. Likewise, As2O₃ [an agent that has successfully been employed in the treatment of patients with acute promyelocytic leukemia (APL)] was shown to induce apoptosis through activation of APAF-1, caspases-3 and -9 and cleavage of Bid. Unfortunately, As2O₃ treatment is unavailable to most APL patients in Latin

America. *Aims.* a) to determine if VES is effective against t(15;17)/APL; b) to study its mechanism of action and c) to characterize if there is synergism between VES and As2O3. *Methods.* We used a syngenic transplantation model of APL: blasts from hCG-PML/RAR α transgenic mice (TM) were IV injected in irradiated non-transgenic littermates. Massive infiltration of bone marrow (BM), spleen and liver was detected by 21st Day. Recipient mice were treated with: VES (50UI/g/d) (n=15), As2O3 (2.5 μ g/g/d) (n=15), VES + As2O3 (n= 15) at the same doses, or vehicle (Control; n=15) starting from Day 4 for 21 days. Molecular remission was determined by RT-PCR for PML/RAR α . Another group of twelve mice was treated with VES or DMSO for 6 days, and the percentage of apoptotic CD117+ leukemic cells in spleen and liver was determined by flow cytometry. Differentiation was evaluated morphologically on Leishman stained BM cytospin preparations after 72h of treatment with VES. Furthermore, we compared the gene expression profile of BM cells obtained from leukemic mice treated with VES or DMSO (n=6, per group) using nylon microarrays representing 598 genes. *Results.* The mean survival time in the Control group was of only 25,6 days (95% C.I. = 20,9-30,3), whereas in the VES it was 160,40 (95% CI = 134,2-185,6); in the As2O3 of 162,1 (95% CI = 137,97 -186,3) and in the VES+ As2O3 of 163 (95% CI = 139,93-186,1). Compared to controls, all treatments significantly prolonged survival. Molecular remission was attained in 86,5% of mice in the VES arm, 80% in the As2O3, and 86,5% in VES + As2O3. No significant organ toxicity was found by histopathological analyses. The mean percentage of CD117+ apoptotic cells in VES treated mice was significantly higher (41,17 \pm 5 versus 62,11 \pm 4%, in spleen p<0,03; and 94,65 \pm 1% versus 12,94 \pm 11,95%, in liver, p<0,01). No significant difference in the number of mature granulocytic cells was detected. Data mining from our microarray results revealed the higher expression of Mitogen Activated Protein Kinase 3, Protein Kinase C delta and BCL2 antagonist Killer-1 genes in VES treated samples. In conclusion, our results demonstrate that VES induces prolonged remissions and that it activates of signaling pathways leading to apoptosis. Moreover, VES in combination with As2O3 was well tolerated and effective. Therefore, our experimental data suggest that VES may be an alternative to As2O3 for APL treatment.

0436

MITOXANTHONE AND CYTARABINE IN UNTREATED ELDERLY ACUTE MYELOID LEUKEMIA (AML): A RETROSPECTIVE SINGLE CENTRE ANALYSIS

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Background. The value and exact type of intensive chemotherapy of unselected elderly AML patients in terms of overall survival (OS) and quality of life remains controversial. The lack of large randomized trials comparing intensive to low-dose treatment or to just support, as well as the selection bias observed in smaller studies in patients >60 yrs contribute significantly to the dilemma. Despite improvements in supportive measures during chemotherapy, elderly AML patients continue to exhibit fewer remissions, higher toxicity, more relapses and worse survival. *Aim & Methods.* The present analysis evaluates retrospectively the outcome of 31 homogeneously-treated AML patients >60yrs during a period of 33 months (2001 to 2004). The protocol schedule included an initial course of mitoxantrone+cytarabine 3+5 (12mg/m²/d and 100mg/m² q12h respectively) followed by a second abbreviated course of mitoxantrone+ cytarabine 2+5, followed by a final course of idarubicin+cytarabine+thioguanine 2+7+7 (10mg/m²/d, 100mg/m² q12h and 100mg/m²/d respectively). G-CSF was added to all

courses. Our patient population consisted of 16 cases of *de novo* AML and 15 of secondary AML (MDS 14, NHL 1) classified according to FAB as follows: M0 (4), M1 (3), M2 (14), M4 (3), M5 (2), M6 (4), hybrid-leukemia (1). Their median age was 70yrs (range 63-80) and M/F ratio was 21/10. Cytogenetic analysis was performed in 26/31 cases: 3/26 failed to produce metaphases, 17/26 revealed standard-risk abnormalities (fourteen normal karyotype, three trisomy 8) and 6/26 were poor-risk by MRC criteria. Leukocytosis > 50X10⁹/L was noted in 5/31 cases and leukopenia <5X10⁹/L in 14/31 patients. *Results.* After a median observation period of 9 months (range 1-33) 15/31 (48.4%) patients entered CR (9 *de novo*, 6 secondary) post-course 2 and their median OS is 12 months (range 2-24). An additional 5/31 (16%) cases returned to a myelodysplastic phase without excess of blasts achieving PR. 2/31 (6.4%) patients deceased during induction. The remaining 11/31 (35.4%) proved primary-resistant and deceased after a median of 2.5 months (range 1-8). Two resistant cases survive 7 and 8 months, one in CR attained off-protocol and the other with on-going disease. Within the group of remitters, 3/15 deceased from complications (MI, fungal infection, sepsis) before the next chemotherapy course. The remaining 8/15 remitters relapsed after a median of 8.5 months; six of them deceased and two are alive after salvage treatment. Finally 4/15 cases remain disease-free (two interrupted after the second course). Median OS for the entire cohort is estimated to date at 7.5 months. *Conclusions.* Treatment with mitoxantrone+cytarabine is well tolerated and reasonably effective in elderly AML patients fit enough to undergo chemotherapy, regardless of karyotype and antecedent dysplasia. With 64.4% overall responses (CR+PR) and a low induction death rate, this schedule deserves further evaluation in larger AML populations. Moreover, our data validate the improvement in survival of those patients achieving CR as reported by other studies.

0437

DOSE REDUCTION IN NEW CHEMOTHERAPY REGIMENS -EFFECT OF GEMTUZUMAB OZOGAMICIN ALONE AND IN COMBINATION WITH LOW DOSE ARA-C AND ETOPOSIDE ON ACUTE MYELOID LEUKAEMIA BLASTS IN VITRO

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Background. First remission in AML is achievable using conventional chemotherapy, however 70% of patients subsequently relapse, thus requiring the development of novel therapies. Gemtuzumab Ozogamicin (GO or Mylotarg) comprises anti-CD33 antibody conjugated to the antibiotic calicheamicin. On binding to CD33, GO is internalized, calicheamicin is released and binds to DNA. *Aims.* The aim of current work is to investigate the effect of GO alone and in combination with low dose Ara-C and Etoposide *in vitro* with the intention to develop effective treatment strategies which would allow dose reduction of cytotoxic drugs. In a number of cases we have measured the level of CD33 and Pgp expression and Pgp function to assess correlation with GO induced cytotoxicity. *Methods.* Presentation blasts from AML patients were isolated from bone marrow (BM) or peripheral blood (PB) and cultured in 96 well plates at 1x10⁶ cells/mL in McCoy's 5A medium containing 15% FCS, GM-CSF (100ng/mL), SCF (10ng/mL) and IL-3 (10ng/mL). In 32 experiments GO alone was added to cultures at 0.1-1000ng/mL. In 13 experiments Ara-C and Etoposide alone were added at 0.1-100ng/mL. GO (10 and 100ng/mL) was then added in combination with the Ara-C or Etoposide (0.1-100ng/mL). Plates were incubated at 37°C in 5% CO₂, 5% O₂, 90% N₂. Apoptosis assays were carried out between 18-96h incubation using the annexin-V PE apoptosis detection kit. Cell proliferation was evaluated on day 5 of incubation using the thymidine uptake assay. CD33 and Pgp (using MRK-16 ab) expression was quantified by flow cytometry. Pgp function was assayed using the Multi Drug Quant assay kit. *Results.* Exposure of AML blasts to GO resulted in a dose-dependent inhibition of proliferation in 30 out of 32 patients. An increase in apoptosis in response to GO was however only observed in 4 patients out of 32. Patients with higher levels of CD33 were significantly more sensitive to

GO at 1µg/mL than patients with lower levels (p=0.005). No correlation between sensitivity to GO and PgP expression/function was observed although further data is being accrued. Apoptosis was detected in 2 out of 13 patients in response to GO, Ara-C or Etoposide alone with combinations of GO and Ara-C or Etoposide giving up to a 38% increase in apoptosis levels in these patients. However combinations of GO with Ara-C or Etoposide resulted in a dose-dependent inhibition of proliferation in all 13 patients, inhibition of proliferation was up to 46% greater with drug combinations than with GO alone. **Conclusions.** In future the additive effect of GO and Ara-C/Etoposide may be exploited *in vivo* to provide an effective lower dose chemotherapy regimen which may be particularly useful in older patients.

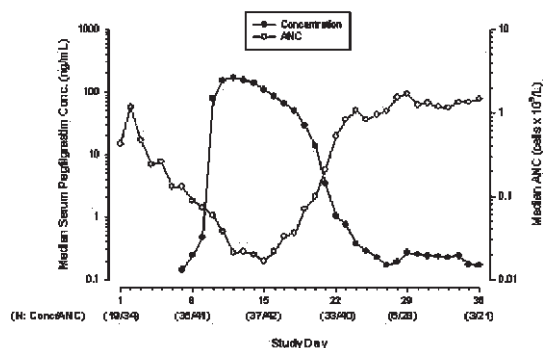
0438

A SINGLE DOSE OF PEGFILGRASTIM SUCCESSFULLY SUPPORTS RECOVERY FROM PROLONGED NEUTROPENIA FOLLOWING INDUCTION CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA

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Background. Pegfilgrastim, produced by covalently attaching a 20-kd polyethylene glycol to the N-terminus of filgrastim, has been shown to support chemotherapies having varying degrees of myelosuppressive potential. Pegfilgrastim is predominantly cleared via a neutrophil-mediated clearance mechanism, in which serum levels are maintained during periods of neutropenia and clearance is increased as the absolute neutrophil count(ANC) recovers. Acute myeloid leukemia (AML) is typically associated with an extended duration of neutropenia due to the nature of the disease and the administration of induction chemotherapy. **Aims.** The pharmacokinetics of pegfilgrastim and ANC profiles after standard induction chemotherapy for treatment of AML were evaluated. **Methods.** A multicenter, double-blind, randomized, phase 2 study comparing pegfilgrastim with filgrastim among patients with *de novo* AML was conducted. Patients were randomized (1:1) to receive either pegfilgrastim 6 mg (single dose) or filgrastim 5 mcg/kg/day (until ANC > 10.0×10⁹/L for one day or >1.0×10⁹/L for 3 consecutive days) 24 hours after standard induction and consolidation chemotherapy. Pharmacokinetic and ANC samples were collected daily from day 1 of induction chemotherapy until ANC recovery. Serum samples were assayed for pegfilgrastim or filgrastim concentration using a validated ELISA.



Results. Eighty-three patients received study medication (42 pegfilgrastim, 41 filgrastim), and all had severe neutropenia (ANC < 0.5×10⁹/L) during induction chemotherapy. Median

time to ANC recovery (first of 2 consecutive days with ANC > 0.5×10⁹/L) was 22.0 days for both groups (95% CL for difference: -1.9, 1.9), with nearly superimposable ANC profiles up to study day 21. For the pegfilgrastim cohort, median pegfilgrastim serum concentrations peaked 12 days after the start of chemotherapy (at 167 ng/mL) and fell below 2 ng/mL (a therapeutic threshold derived from pharmacokinetic/pharmacodynamic modeling) 22 days after the start of chemotherapy, at which time the median ANC had recovered from the nadir (Figure 1). There is a positive correlation (Spearman rank correlation = 0.485, p = 0.004) between the time to ANC recovery (ANC > 0.5×10⁹/L) and the time to pegfilgrastim concentration falling below 2 ng/mL. **Summary/conclusions:** Pharmacokinetic data support the use of a single dose of pegfilgrastim to facilitate ANC recovery after induction therapy for AML.

0439

INTENSIVE CHEMOTHERAPY IN ADULT ACUTE MYELOID LEUKEMIA (AML): OUTCOME OF THE FINNISH AML-92 STUDY

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Background. The optimal treatment of acute myeloid leukemia (AML) in adults is still a challenge despite more than three decades intensive investigation. Aim of the present study was to investigate the effect of idarubicin-based induction regimen and of a cyclical postremission chemotherapy in adult AML. **Methods.** Altogether 327 consecutive patients with *de novo* AML aged 16-65 years (median 48 years) were recruited into the study during 1992-2001. Acute promyelocytic leukemia was excluded. Idarubicin was combined in the first induction regimen with conventional dose cytarabine and thioguanine, and in the second regimen with high dose cytarabine. After remission achievement three intensive consolidation cycles each containing high or intermediate dose cytarabine were given. End points were remission achievement, cumulative incidence of relapse (CIR) and death (CID), and overall survival (OS). Allogeneic transplantation was offered in the first remission to patients with a sibling donor (except to those with favorable karyotype) and in the second remission to patients with an unrelated donor. Transplanted patients were censored at the time of transplantation. **Results.** Of the patients, 237 were less than 56 years, 90 were 56 years or more, 10% had favorable*, 67% intermediate**, and 23% unfavorable*** karyotype at diagnosis. Complete remission was achieved with the induction treatment in 82% (n=195) of the patients less than 56 years and in 81% (n=73) of the patients 56 years or more. Remission rate was 85%, 68% and 56% in the patients with favorable, intermediate and unfavorable karyotype, respectively. CIR at 1 year was 16% in the favorable, 31% in the intermediate and 62% in the unfavorable group. CID in first remission at 1 year was 3% for the patients less than 56 years and 15% for the patients 56 years or more. The median OS was 63 months (95% CI 32-94 months). Projected 5 year survival was 59% for the patients less than 56 years, 39% for the patients 56 years or more, and 82%, 47% and 25% for the patients with favorable, intermediate and unfavorable karyotype, respectively. **Conclusions.** Idarubicin-containing regimen is very effective in remission induction in patients up to 65 years of age. Postremission therapy including three intensive cycles with high and intermediate dose cytarabine is feasible with acceptable toxicity in adult AML. Cytogenetics predicts remission achievement, CIR and OS, while age is associated with CID and OS. * t(8;21) +/- -Y, inv16/t(16;16). ** Normal karyotype, all other abnormalities not belonging to either favorable or unfavorable group including unknown karyotypes of five patients. *** -5/5q-, -7/7q-, 3q, 11q, 17p-, 20q, 21q, t(6;9), t(9;22), complex karyotypes with at least 3 abnormalities.

0440**COMBINATION OF FLUDARABINE AND CYTARABINE IS EFFECTIVE AS INDUCTION TREATMENT OF POOR PROGNOSIS MDS AND AML**M. Tassara¹, F. Ciceri¹, R. Milani², J. Peccatori¹, F. Lunghi¹, A. Pescarollo³, L. Camba¹, P. Servida¹, V. Calbi¹, M. Bregni¹, M. Bernardi¹¹San Raffaele Scientific Institute, MILAN, Italy; ²Istituto Nazionale Tumori, MILAN, Italy

Background. response rate of advanced MDS, sAML and refractory/relapsed AML to conventional chemotherapy is poor, namely about 35-50%, compared to 75-80% of *de novo* AML; usually, standard dose cytarabine plus an anthracycline (daunorubicin/idarubicin/mitoxantrone), with or without etoposide, is the treatment of choice as these are the most active drugs on leukemic blasts. Complete remission (CR) should be pursued as the only option of cure for these patients (pts) is to receive an allogeneic stem cell transplant(SCT) with a minimal residual disease in their bone marrow; consolidation of CR with high dose chemotherapy and autologous stem cell (ASCT) rescue is an option for patients without a HLA suitable stem cell donor. Fludarabine has no direct activity on MDS/AML but improve cytotoxicity of cytarabine increasing intracellular concentration in leukemic blasts of its toxic metabolite, ara-P. Fludarabine and cytarabine containing regimens (FLA) have shown promising results in CR induction of poor prognosis MDS/AML. **Aims.** to retrospectively evaluate the efficacy of FLA regimens in poor prognosis MDS/AML pts. **Methods.** period 5/1997-2/2005, 78 pts, median age 53 (18-72), 25 > 60; diagnosis was MDS for 31 pts (IPSS INT2/HIGH=15, INT1=4, NV=2), sAML for 27, *de novo* AML for 2, refractory/relapsed AML for 18, therapy related MDS/AML for 18. Cytogenetics (74 pts): normal=32, high risk=26, other=12, good risk=4. As induction therapy, 70 pts received a FLAG-IDA regimen, 7 FLAG, 1 FLAG + liposomal DAUNO. Forty-one patients underwent further CT, (35 for CR consolidation, 6 for reinduction), 29 of them received a FLA regimen. **Results.** overall response rate (RR) to induction was 76.9%, CR 66.6% (1 pt. after second FLAG-IDA), PR 10.2%, NR 17.9%. RR for *de novo* and refractory/relapsed disease was 80.7% (CR 72.4%) and 66.6% (CR 52.3%) respectively (p=ns). There was no difference in RR and CR comparing different subgroups of pts. according to diagnosis (MDS, AML and T related MDS/AML), age and cytogenetics. TRM was 3.8% (3 pts), in all cases due to sepsis. Forty-one patients (54%) underwent sequential SCT after a median of 2 cycles (range 1-4): 10 ASCT, 31 allogeneic SCT. At transplant 26 pts (63%) were in CR, 3 in PR (7%), 11 (27%) with overt disease, 1 not evaluable (hypoplastic marrow). After a median follow-up of 37 months (range 5-89) 26 patients (33.3%) are alive, 20 (25.6%) in CR; 49 patients (65.4%) are died, 9 in CR, 3 were lost to follow-up (1 in CR). **Conclusions.** our results suggest that a FLA containing regimen should be of choice for first line treatment of poor prognosis MDS and AML. We obtained optimal tumor debulking (CR+PR) in the majority of patients, with tolerable toxicity; one third of them received a SCT as consolidation of the CR. Elderly pts., at least up to 70, could be safely treated too. A phase III study is warranted to clarify if addition of an anthracycline to the FLA combination actually improves the initial response.

0441**FLUDARABINE, CYTARABINE, IDARUBICIN AND ETOPOSIDE (FLAIE) COMPARED TO FLUDARABINE, CYTARABINE, G-CSF AND IDARUBICIN (FLAG-IDA) AS INDUCTION TREATMENT FOR DE NOVO AML PATIENTS (<60 YEARS)**M. Clavio, I. Pierri, M. Balocco, G.L. Michelis, R. Varaldo, M. Miglino, A. Albarello, N. Abdal, G. Catania, A. Garrone, S. Aquino, A. Bellora, R. Cerri, F. Ballerini, L. Canepa, M. Gobbi
Clinica Ematologica, GENOVA, Italy

Background. The addition of etoposide to a regimen including synergistic drugs, such as intermediate dosage Ara-C, idarubicin and fludarabine might reduce treatment failure. The relatively short duration of chemotherapy might, on the other hand, reduce toxicity and allow prompt and safe administration of high dose therapy (HDT). Patients and methods. The induction regimen (FLAIE) included fludarabine (30 mg/sqm), followed four hours later by a 2-hour infusion of Ara-C (2 g/sqm) and etoposide on days 1-5, and by a 30 minute infusion of idarubicin (10 mg /sqm) on days 1,3,5. High dose therapy with stem cell rescue was planned for all patients in first CR after high dose Ara-C and idarubicin consolidation course and in good clinical conditions. Patients' features and results were compared with an historical group of patients who received in the same center fludarabine, Ara-C, G-CSF and idarubicin (FLAG-Ida) followed by consolidation chemotherapy and HDT. **Results.** Patients treated with FLAIE and FLAG-Ida have been 23 and 43, respectively. Median ages were 53 and 50. Almost all patients had *de novo* AML. Karyotype belonged to the unfavorable prognosis group in 2 (9%) and 2 (5%) patients, respectively. An intermediate prognosis karyotype was detected in 21 (91%) and 39 (91%) patients receiving FLAIE and FLAG-Ida regimens, respectively. Days to PMN > 0.5 x 10⁹ / l have been 17 (10-33) and 17 (10-28); days to Plt > 50 x 10⁹ / l have been 18 (13-43) and 17 (12 - 38), respectively. Median number of erythrocyte transfusions were 5 (2-10) and 7 (2 - 18). Median numbers of platelet transfusions have been 5 (2-18) and 5 (1 - 17). No episodes of cardiovascular toxicity have been recorded in both groups. Sepsis have been reported in 6 (26%) and in 7 (16%) patients treated with FLAIE and FLAG-Ida, respectively, and pneumonia in 4 (9%) patients receiving FLAG-Ida. Deaths in induction have been 4 (17%) and 1 (2%), complete responses (CR) have been achieved in 16 (70%) and 35 (82%) patients, respectively. Allogeneic BMT has been performed in 5 (22%) and in 6 (14%) patients. Autologous PBSC transplants have been done in 1 (4%) and 17 (39%) patients receiving FLAIE and FLAG-Ida induction regimens, respectively. Relapses have been recorded in 3 (13%) and 18 (42%) patients. Median duration of survival is 9 (1-24) months and 20 (1-83) months. Median duration of DFS is 10 (2-23) and 17 (3-66) months, respectively. **Conclusions.** The low number of enrolled patients and the short follow up in the FLAIE group, and the historical nature of the comparison do not allow to draw any definitive conclusion on the efficacy and tolerability of the two induction regimens. Anyway, compared to our previous experience with FLAG-Ida, the addition of etoposide does not seem to increase the antileukemic efficacy in patients with intermediate risk AML and might reduce tolerability (as indicated by deaths in induction and incidence of infections).

0442**AUTOLOGOUS BONE MARROW TRANSPLANT FOR ACUTE MYELOID LEUKAEMIA: A SINGLE CENTRE EXPERIENCE**

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Background. The role of autologous transplantation as consolidation therapy in adults with acute myeloid leukaemia (AML) remains poorly established. Several large studies have shown no significant improvement in overall survival and have raised concerns regarding the late effects of conditioning therapy. We present our single centre experience of long-term follow up of patients with AML who received an autologous bone mar-

row transplant(BMT) between September 1981 and November 1993. **Aims.** To evaluate our centre's cumulative long-term experience of autologous BMT in AML in terms of overall survival, disease free survival, relapse rate and the late effects of conditioning therapy. **Method.** This was a retrospective case note audit. All eligible patients were identified from local BMT records. **Results.** 80 patients with a diagnosis of AML received an autologous BMT during the stated time period. There were 70 evaluable patients (follow up data were not available or incomplete for 10 patients). The median age at BMT was 34 years. 5 patients were transplanted in relapse, 5 were in second complete remission (CR) and the remainder were in first CR. The majority of patients (54) received conditioning with Cyclophosphamide and total body irradiation (TBI), 10 patients received Melphalan and TBI and 6 received Busulphan and Cyclophosphamide. Cytogenetics studies from time of diagnosis were available in only 30 patients. Of these 8 were good risk (t(15;17), t(8;21), inv (16)) and 5 were poor risk (complex, monosomy 7, 11q23 abnormalities). Overall survival at a median of 17years was 30% with a disease free survival of 25.7%. Of the 70 evaluable patients 26 died of relapsed disease (+/- sepsis). Transplant related mortality was 17.1% (7.0% infection, 2.9% veno-occlusive disease, 2.9% intracerebral bleeding, 2.9% respiratory failure/pneumonitis, 1.4% multiorgan failure). In terms of late effects of conditioning therapy, evaluable patients were assessed for the development of iron overload, secondary malignancy including myelodysplastic syndrome, chronic lung disease, hepatitis C infection, cataracts and endocrinopathy. Only 1 patient(1.4%) developed a secondary malignancy (colonic carcinoma). 4 patients (5.7%) had acquired hepatitis C, presumed to be transfusion-transmitted. 13 patients (18.6%) had clinically significant iron overload with abnormal liver function tests. 15 patients (21.4%) had endocrinopathy. Most female patients experienced premature ovarian failure and 5 patients (7.1%) had hypothyroidism. 2 patients developed chronic lung disease (pulmonary fibrosis) and 1 patient developed chronic renal failure. **Conclusions.** Our local experience is broadly comparable with other contemporary published series with long-term outcomes similar to those achieved with chemotherapy alone in analogous patient groups. Although the incidence of secondary malignancy and myelodysplasia appears lower than in other series we demonstrate that the cumulative incidence of the late effects of transplant are clearly significant, with transfusional iron overload and endocrinopathy being particularly common.

0443

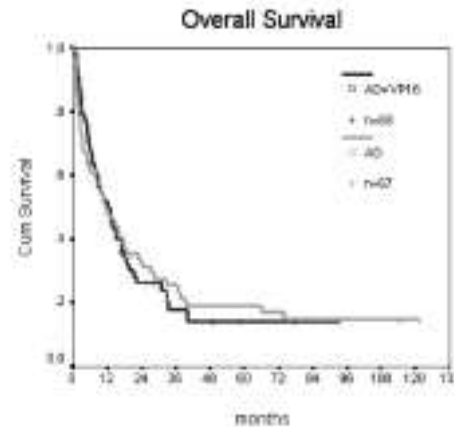
SINGLE INSTITUTION EXPERIENCE DURING INDUCTION FOR NAÏVE ACUTE MYELOID LEUKEMIA IN ADULTS: VP16 IT DOES NOT MATTER

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VP16 had been proposed to increase complete response (CR) rate during induction for naïve acute myeloid leukemia (AML) in adults suitable to receive high dose chemotherapy. We report our experience with Ara-C+ Daunorubicine (AraC-D) based regimen with and without VP16 in two historical cohorts: comparing CR rate, overall survival (OS) and disease free survival (DFS). Between January-1995 to December-2004, 387 AML patients were attended in our institution, median age 53 years old (range 13-91). One hundred fifty-two patients were not eligible for high dose chemotherapy, 27 patients underwent allogeneic stem cell transplantation (SCT) after CR was achieved, 40 patients were excluded because previous treatment received in other institutions, 30 patients were diagnosed AML-M3 and 3 received pediatrics AML regimens. Sixty-seven patients were treated with Ara-C 100 mg/m² continuous infusion (c.i.) over 24 hours for 7 days + Daunorubicine 60 mg/m² c.i. over 3 hours for 3 days (group A: 7*3). Sixty-eight patients were treated with

7*3 plus VP16, 100 mg/m² c.i. over 3 hours for 3 days (group B: 7*3+3). Consolidation was followed after CR was achieved with 5*2 and 5*2+2 regimen for group A and B respectively; at less 2 intensification was given with AraC 1.5 g/m² c.i. over 3 hours bid for 4 days plus Daunorubicine 60 mg/m² or VP16, 100 mg/m² c.i., over 3 hours for 2 days. Median age for group A and B were 50 years (range 16-78) and 44 years (range 16-74)



For group A and B: secondary AML cases were 10 and 16 respectively; bifenotypic/M0 cases were 3 and 9 respectively; AML FAB M4-5 cases were 23 and 13 respectively. Cytogenetic risk was determinate in 34 (51%) and 43 (63%) cases respectively. High risk by cytogenetic were 2 and 10 cases for group A and B respectively. For group A and B: CR was achieved in 50% and 53% respectively (not significance). Median OS was 11.7 months SD 3.3 (CI 95% 5,3-18,1) and 12,9 months SD 2.4 (CI 95% 8,2-17,6) for group A and B respectively, with no significance (p=0,98). Median DFS was 11,1 months SD 3.1 (CI 95% 5,0-17,2) and 9,2 months SD 2,2 (CI 95% 4,8-13,5) for group A and B respectively, with no significance (p=0,54). Our experience indicated that adding VP16 to AraC-D treatment does not improved CR rate, OS and DFS in our patient population. The low CR observed was because unselected cohorts treated and patients with CR whom underwent to allogeneic SCT were excluded to this analysis. Finally, new drugs and regimen including Idarubicine, Mitoxantrone, Fludarabine and Clofarabine are suitable to be used for increasing response rate for adult AML during first induction.

0444

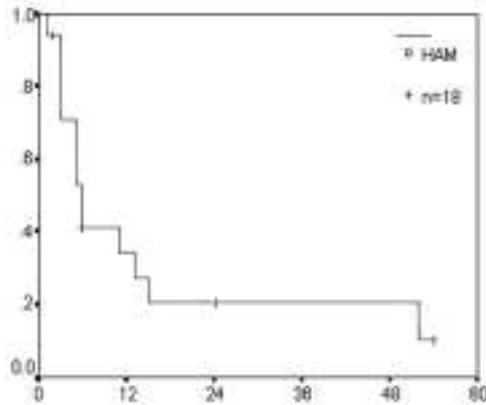
HIGH DOSE ARA-C PLUS MITOXANTRONE FOR REFRACTORY - RELAPSED ACUTE MYELOID LEUKEMIA IN ADULTS

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High doses Ara-C plus mitoxantrone (HAM) had been proposed for refractory-relapse acute myeloid leukemia (AML) in adults suitable to receive high dose chemotherapy for re-induction. We report our experience with HAM in our institution regarding complete response (CR) rate, hematological recovery treatment, overall survival (OS) and disease free survival (DFS). Between January-1995 to May-2004, 365 AML patients were attended in our institution, median age 52 years old (range 13-91). Thirty-eight(10,4%) refractory or relapsed adults with AML received AraC 1.5 g/m² c.i. over 3 hours bid for 4 days plus

Mitoxantrone 12 mg/m² c.i., over 3 hours for 2 days. Median age was 38 years (range 16-59) Secondary AML cases were 5, AML with fibrosis was one case and M0 cases were 3; AML FAB M4-5 cases were 7. Median treatment number was 3 (range 1-7). Cytogenetic risk was determinate in 29 cases (76%), cytogenetics risk was: high (n=8), intermediate (n=16) and low (n=5). CR was achieved in 17 (45%) cases with 4 (10%) cases with treatment related mortality, not response in the remaining 17 cases (45%). Median OS was 6 months SD 1,7 (CI 95% 2,6-9,3). Median DFS for the 17 cases whom achieved CR was 6 months SD 1,2 (CI 95% 3,6-8,3). Median time for neutrophils recovery ≥ 500 mm³ and platelets recovery $\geq 50,000$ mm³ were 15 days (range 0-30) and 12 days (0-31) respectively.



Forty-six infections were reported mainly in central venous lines (n=11), skin (n=10) and lung (n=6). Our experience indicated that HAM treatment for refractory-relapse AML in adults had acceptable toxicity but does not for OS and DFS in our patient population. The low OS observed was because unselected patients treated and only 2 patients whom achieved CR underwent to sibling allogeneic SCT, none un-related or mismatch SCT were performed. Finally, we argue that new drugs and regimen including Idarubicine, Fludarabine and Clofarabine following with allogeneic STC are suitable to be used for in this high risk group of patients.

0445

ACUTE MYELOID LEUKAEMIA IN SOUTHERN SWITZERLAND: A POPULATION BASED ANALYSIS

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Background / Aims. To evaluate the outcome for all adult patients from the Italian-speaking part of Switzerland with *de novo* acute myeloid leukaemia (AML) from January 1983 to December 2003 and to identify prognostic factors for the time to progression (TTP) and overall survival (OS). Patients and **Methods.** Data of all adult patients known to the Oncology Institute of Southern Switzerland (IOSI) was collected retrospectively and then completed with the tumor registry data. Univariate and multivariate analysis for TTP and OS were performed. **Results.** The incidence of AML in the adult population in Southern Switzerland is 2.6 / 100'000 per year for the examined period. Complete clinical and pathological data and follow-up information were available for 128 patients treated at the IOSI in the examined period. The median age was 67 years (range 18-94). The median follow-up time was 97 months with

a median overall survival of 6 months, a median TTP of 3 months. 35 patients with a median age of 80 years (26-94) were given best supportive care and / or palliative chemotherapy. The median survival in this subset was 2 months. Of the 93 patients treated with myelosuppressive chemotherapy with curative intent 48 were older than 60 years. The CR-rate was 74% for patients younger than 60 years in comparison with 29% for those older than 60 years (p<0.0001). The overall survival rates at 2-years were 48% and 12% respectively (p<0.0005). The relapse rate after initial CR was 58% and only 8 patients reached a 2nd CR. 52% of the patients treated with curative intent were included in a clinical trial. Their median age is significantly lower compared to the patients not included in a trial; 57 vs. 66 years (p<0.0001). Patients on a trial had a significant better prognosis with a median survival of 12 vs. 6 months. High dose Ara-C was given in 25 of 93 patients with curative intent and showed a survival benefit compared to standard Ara-C doses (p<0.0005). The outcome of the patients treated after 1993 is significantly better (p=0.026) compared to the previously treated cohort. At multivariate analysis without cytogenetic data (available only for 51 cases) only age (p=0.005), PS > 1 (p=0.001) and treatment given before/ after 1994 (p=0.044) were found to be an independent prognostic factors for both OS and TTP. **Conclusions.** The majority of patients with AML are older than 60 years and their outcome is still disappointing. For younger patients the prognosis is superior if they receive high-dose Ara-C as part of the first line treatment and if they are treated in the setting of a clinical trial.

0446

THERAPEUTICAL RESULTS IN AML PATIENTS OLDER THAN 60 YEARS. COMPARATIVE STUDY BETWEEN CURATIVE AND PALLIATIVE TREATMENT

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Background. It is well established that obtaining complete remission in patients with acute myeloid leukemia (AML) improves survival rates. The introduction of new therapies contributes to obtain complete remission in 50% of AML patients which are older than 60 years. **Aims.** We made a retrospective analysis which includes all patients older than 60 years, without any other selection, treated in the Hematology Clinic of the County Clinical Hospital of Timisoara in the last 10 years (1995-2004). We compared the medium survival duration of the patients which received curative treatment with the medium survival duration of those that received palliative treatment. **Methods.** We analyzed 96 patients (50 males and 46 females) with ages between 60 and 94 years which were diagnosed with AML (excepting AML3). We administrated intensive induction therapy in 39 patients (40,62%) with medium age of 65 years, palliative therapy in 41 patients (42,71%) with medium age of 70 years and symptomatic treatment in 16 patients (16,66%) with medium age of 75 years. We determined the medium survival duration in the analysed groups. **Results.** Medium survival duration in the curative therapy study group was 4,3 months (0,03-40 months). Complete remission after first induction was obtained in 25% of the patients. No one received allogeneic stem cell transplantation, but 2 patients are still in complete remission. Patients which received palliative treatment had a medium survival duration of 2,5 months (0,03-6,9), and those who received symptomatic treatment lived between 0,02 and 2 months. **Conclusions.** Patients older than 60 years and a good prognostic score are indicated for curative therapy which can contribute to increase medium survival duration. The prognosis of those AML patients remains unfavourable.

Allogeneic HSCT - Mechanisms

0447

THE IMPACT OF MONITORING EPSTEIN BARR VIRUS PCR IN PAEDIATRIC BONE MARROW TRANSPLANT PATIENTS: CAN IT SUCCESSFULLY PREDICT OUTCOME AND GUIDE INTERVENTION

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Epstein Barr virus (EBV) associated lymphoproliferative disease (LPD) is a complication of haemopoietic stem cell transplantation (HSCT). In certain groups (unrelated and mismatched donor transplants, T-cell depletion) the risk may be as high as 25% with significant morbidity and mortality. Strategies to predict the impending development of this disorder and allow early intervention have therefore assumed importance. We routinely screen the peripheral blood of all recipients of allogeneic HSCT to detect EBV DNA by quantitative polymerase chain reaction (PCR) technology and report here how this correlates with clinical disease and management. Data on 28 successive patients who underwent HSCT at our institution were reviewed. The median age at time of transplant was 6.5 years. 17 patients received an unrelated donor transplant and 1 a haplo-identical transplant. The remainder (n=9) received a matched family donor transplant. 23 patients received either Alemtuzumab (n=19) or ATG (n=4). 13 patients had leukaemia, 5 had a mucopolysaccharide syndrome, 4 a congenital immune deficiency and 6 patients a non-malignant haematological condition. EBV reactivation occurred in 68% of patients however only 7% developed clinical LPD. Patients with high level reactivation (n=9), defined as >log4.5 copies/mL had more frequent episodes of reactivation and all patients who progressed to overt LPD were found in this group. In addition all had received either Alemtuzumab or ATG as part of the preparative regimen. In contrast, none of those patients with low level reactivation (n=10) or persistently negative results (n=9) showed any signs of clinical disease. Anti-CD20 monoclonal antibody (Rituximab) therapy was instigated in both cases of proven LPD and 3 cases of high level reactivation with successful outcomes. Response to treatment was associated with a prompt decline in viral copy number. Our results indicate that EBV reactivation is a common occurrence in the paediatric allogeneic transplant setting and that only a proportion of patients will progress to LPD. Monitoring may help predict those at greatest risk and guide intervention.

0448

COMBINATION OF CYCLOSPORINE A, METHOTREXATE AND DACLIZUMAB FOR THE PROPHYLAXIS OF ACUTE GRAFT-VERSUS-HOST DISEASE IN PEDIATRIC PATIENTS WITH SEVERE APLASTIC ANEMIA: A SINGLE CENTER EXPERIENCE

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Combination of cyclosporine A and methotrexate was found to be effective for prevention of acute GVHD in SAA patients, but the incidence of this potentially lethal complication is still remains very high. Availability of novel monoclonal antibodies had led to new approaches in the reduction of GVHD occurrence and severity with the help of combinational therapy, acting on different pathways of the aGVHD cascade. Daclizumab is a 144-Da humanized monoclonal antibody that specifically binds to the α subunit of the human high affinity interleukin-2 receptor, expressed on the surface of the activated lymphocytes, and achieves immunosuppression by competitive antagonism of IL-2-induced T cell proliferation. In this study, daclizumab was added to the CsA/methotrexate based acute GVHD prophylaxis for pediatric SAA patients, underwent SCT from HLA

matched related donors, with the aim to reduce the probability of acute GVHD and graft rejection. 38 pediatric patients affected with acquired SAA received 43 allogeneic stem cell transplantation in Russian Institute for Pediatric Hematology, Moscow. The majority of patients (n=21) received the conditioning regimen composed of cyclophosphamide (200 mg/kg) and ATG (90 mg/kg). Ten patients received the conditioning with ATG (100 mg/kg), fludarabine (100 mg/kg) and cyclophosphamide (100 mg/kg). Nine patients received busulfan (8 mg/kg) and cyclophosphamide (200 mg/kg). 3 patients were regrafted without any conditioning. All 43 transplantations were performed with unmanipulated bone marrow (n=24) or PBSC (n=19). GVHD prophylaxis consists of CsA (3 mg/kg day i.v.) and methotrexate (Mtx) (10 mg/m² i.v. on days +1, +4, +7). 16 patients received daclizumab as the addition to the standard CsA/Mtx prophylaxis of the GVHD. Daclizumab was given at a dose 1 mg/kg on days +1, +4 and +14. In the cases of fever persistence with the absence of infection and in spite of broad-spectrum antibiotics the additional doses of daclizumab was administered on days +8 and +28. The median number of days required to achieve neutrophils engraftment was 19 days in both group. The platelet recovery to more than 20x10⁹/L required a median of 24 days (range 11-56) in the group of patients, received daclizumab and 28 days (range 16-83) in the group, received standard GVHD prophylaxis. All but one patient engrafted in the group of patients, received daclizumab. Four patients failed to show engraftment in the group of standard GVHD prophylaxis. All the patients with graft failure received second transplantation from the same donor. Two of them successfully engrafted, one showed autologous reconstitution after the second transplantation. Ten of the 38 pediatric patients with SAA expired (26%); 3 in the group received daclizumab and 7 in the group received standard GVHD prophylaxis. The median follow-up of survived patients was 964 (205-1448) and 1287 (150-3650) days respectively after SCT. The incidence of acute GVHD was similar (63% vs 50%, p=0.78) in both groups. The addition of daclizumab did not affect the overall survival (81%±9 vs 73%±9, p=0.42) and incidence of chronic GVHD. This study is failed to show positive role of daclizumab in the prevention of aGVHD in SAA.

0449

QUANTITATIVE ASSESSMENT OF HEMATOPOIETIC CHIMERISM AFTER ALLOGENEIC STEM CELL TRANSPLANTATION VIA SNP REAL-TIME PCR

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Distinguishing between host- and donor-hematopoiesis (chimerism) is critical to observe the engraftment of donor stem cells after allogeneic stem cell transplantation (SCT). Single nucleotide polymorphisms (SNPs) represent a huge source of human genetic diversity and enable the distinction of any individual from each other. Specific SNPs for host and donor DNAs are selected from a panel of 30 SNP-loci and used furthermore as markers for quantification via TaqMan probes and LightCycler technology. For assessment of the percental proportion of recipient cells the values of SNP PCR setups in dependence of the GAPDH value as DNA-standard were compared to the SNP values of the patients prior to transplantation. Peripheral blood samples from 7 patients were examined by VNTR- and SNP-analysis in parallel. The detection limit of host-cells was 5% for VNTR PCR and 0,0005% for quantitative SNP real-time PCR. Dynamic changes in the proportion of recipient cells were observed via SNP-analysis even in samples with complete donor genotypes in VNTR analysis. An increase of recipient cells shortly prior to relapse was detected whereas only donor genotypes were detected in VNTR analysis until clinical observed relapse. Until now 27 SCT recipients and their HLA-identical donors were screened for specific SNP-markers. For all pairs at least one SNP-locus specific for donor and host DNA, respectively, was identified and used in the follow up of the patients for

quantitative assessment of hematopoietic chimerism. Sixteen patients received standard condition regimens (8 AML, 2 Ph+ ALL, 2 Ph+ CML, 1 ALL, 1 CLL, 1 MDS, 1 SAA) and 11 patients reduced intensity regimens (4 solid tumors, 3 AML, 2 NHL, 1 MM, 1 Ph+ ALL). The proportion of recipient cells decreased in all patients after Tx and reached negativity (less than 0,0005% host-cells) in some patients. However, in most patients host-cells persisted at low levels (0,001 to 1%) over time. In seven patients an increase of recipient cells was observed prior to relapse (2 to 25 months after Tx). From 2 Ph+ ALL patients results from bcr/abl quantification and SNP real-time PCR after Tx were available. Both markers had a parallel course during follow up, whereby bcr/abl had a sensitivity of one tumor-cell in 3×10^{-5} leukocytes and is a specific marker for tumor cells whereas SNPs are specific for both malign as well as benign host-cells. In conclusion, the SNP real-time PCR is a fast and reliable method for quantitative assessment of hematopoietic chimerism after allogeneic SCT. The sensitivity is four logs higher compared to VNTR PCR and it offers the possibility to observe chimerism changes at a very low detection limit.

0450

IMMUNE RECONSTITUTION FOLLOWING NON-MYELOABLATIVE TRANSPLANTATION USING IN VIVO T CELL DEPLETION WITH ALEMTUZUMAB IS SIMILAR TO T CELL REPLETE MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Delayed immune recovery after Allogeneic stem cell transplantation (Allo-SCT) is associated with poor outcome, with a low total lymphocyte count (TLC) at 1 month post transplant being predictive for relapse in AML and ALL. Lower TLC at 6 months is predictive of treatment failure (relapse, death or graft failure) following T cell depleted Allo-SCT. Alemtuzumab, used as *in vivo* T cell depletion, has a long plasma half-life resulting in prolonged lymphopenia. The higher rate of infective complications (e.g. CMV and adenovirus) reported in T deplete NST has therefore been attributed to a persistent lymphopenia lasting well beyond 6 months with associated poor outcome. **Aims.** To analyse TLC recovery in 50 sequential adult patients with haematological disorders post non-myeloablative transplantation (NST) or myeloablative transplantation (MST) at a single centre between March 2000 and July 2003. **Methods.** Patients receiving ATG, T replete NST or T deplete MST using alemtuzumab were excluded. TLCs were collected retrospectively at day 0 and then every 30 days until day +270. Sequential TLCs were included if within +/- 7 days of each time point and 78% (307) were available for analysis. Median follow up was 443 days (range 18–1542). Five variables were tested for their impact on TLC recovery: acute and chronic GVHD, post transplant immunosuppression with steroids, number of lines of therapy prior to transplant and use of *in vivo* alemtuzumab. Time to recovery of TLC to $>1.0 \times 10^9/L$ was then analysed using Cox proportional hazard modelling, censoring at death if before TLC recovery. **Results.** 27 patients received a NST (leukemia n=16, lymphoma n=8, myelodysplasia n=1, myeloma n=1, paroxysmal nocturnal hemoglobinuria n=1) using fludarabine, melphalan and *in vivo* alemtuzumab and 23 patients received a MST (leukemia n=22, myelodysplasia n=1). 63% undergoing NST had >1 line of therapy versus 26% undergoing MST. We demonstrate a quicker recovery of median TLC in alemtuzumab treated patients compared with previous reports with a sustained TLC $>1 \times 10^9/L$ by 6 months. Patients receiving 100mg alemtuzumab did not have slower TLC recovery than those receiving lower doses. There was no significant difference in TLC recovery between alemtuzumab treated NST and T cell replete MST. Post transplant immunosuppression with steroids, acute GVHD and chronic GVHD did not affect TLC recovery which was only significantly delayed by greater pre-transplant therapy (1 versus 2 or more lines of therapy) (p=0.03). **Conclusions.** NST aims to minimise procedure related toxicity, allow-

ing treatment of higher risk patients due to age or amount of prior therapy. Heavy pretreatment may have been a confounding factor when prolonged lymphopenia has been identified following alemtuzumab treated NST. The impact of prior therapy on immune reconstitution is important, especially with the increasing use of autografts and purine analogues. Although functional impairment of lymphocytes may persist after TLC recovery, the effect of alemtuzumab on reconstitution seems less severe than previously thought since TLC recovery following T cell deplete NST using alemtuzumab is similar to T replete MST.

0451

PURIFIED T DEPLETED PERIPHERAL BLOOD AND BONE MARROW CD34 TRANSPLANTATION FROM HAPLOIDENTICAL MOTHER TO CHILD WITH THALASSEMIA

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Approximately 60% of thalassemic patients can not apply to 'gene therapy today' which the insertion of one allogenic HLA identical stem cell into the empty bone marrow as the vector of the normal gene for beta globin chain synthesis. We studied the use of the haploidentical mother as the donor of hematopoietic stem cells assuming that the immuno-tolerance established during the pregnancy will help to bypass the HLA disparity and allow the hemopoietic allogeneic reconstitution in the thalassemic recipient of the transplant. We have employed a new preparative regimen for the transplant in ten thalassemic children aged 3 to 8 years (median age 5 years) using T cell depleted peripheral blood stem cell (PBSCTs) plus bone marrow (BM) stem cells. All patients received hydroxyurea (OHU) 60 mg/kg and azathioprine 3 mg/kg from day -59 until day-11, fludarabine (FLU) 30 mg/m² from day -17 to day -11, busulphan (BU) 14 mg/kg starting on day -10, and cyclophosphamide (CY) 200 mg/kg, Thiotepa 10 mg/kg and ATG Sangstat 2.5 mg/kg, followed by a CD34 + t cell depleted (CliniMacs system), granulocyte colony stimulating factor (G-CSF) mobilized PBSC from their HLA haploidentical mother. The purity of CD34+ cells after MACS sorting was 98-99%, the average number of transplanted CD34+ cells was $15,4 \times 10^6/kg$ and the average number of infused T lymphocytes from BM was $1,8 \times 10^5/Kg$. The patients received cyclosporin after transplant for graft versus host disease (GVHD) prophylaxis. Four patients rejected the transplant and are alive with thalassemia: one patient received a different dose of CD3 without cyclosporine after transplant, two patients received a lower dose of CD34+, in the fourth patient the donor has been the haploidentical father instead than the mother. One of the ten patients, after the failure of the transplant from the mother, received a second transplant using purified CD34+ cells from the father, using the same preparative regimen and achieved a complete hematopoietic reconstitution. Seven patients are alive disease free with a median follow up of 19 months (range 7-30). None of the seven patients showed AGVHR. This preliminary study suggest that the transplantation of megadose of haploidentical CD34+ cell from the mother is a realistic therapeutic option for those thalassemic patients without genotypically or phenotypically HLA identical donor.

0452**ALLOGENEIC TRANSPLANTATION OF STEADY-STATE BONE MARROW VS G-CSF-PRIMED BONE MARROW FOR ADULT AML PATIENTS**

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Background & Aims. The relative effects of different types of allogeneic hematopoietic stem cell transplantation on the engraftment rate, incidence of acute and chronic graft-versus-host disease (GVHD), and relapse rate together with other transplant-related complications (TRC) or transplant-related mortality (TRM) have been specifically investigated and comparatively analyzed in 216 adult AML patients according to the cytogenetic-risk groups. **Methods.** We electively performed graft engineering. In all, 101 patients received G-CSF primed unmanipulated bone marrow (uBM) and the other 115 patients received steady BM cells (sBM). The patients in these two groups were stratified non-randomly according to donor and recipient ages (≥ 40 or < 40), status of leukemia (advanced or not), presence of preceding treatment-related sequelae, weight discrepancy between donor and recipient i.e. if the difference was over 10kg, and presence of major organ dysfunction. The median follow-up duration for all patients was 48 months (range, 1–98). Sex, age, and subtype of disease were adjusted between two groups. **Results.** As for each group, the median CD34+ cells infused were $3.6 \times 10^6/\text{kg}$ (range, 2.1–13.7) (uBM group), $2.7 \times 10^6/\text{kg}$ (range, 1.7–12.2) (sBM), respectively. The median days for recovery of absolute neutrophil count to 500/ul were 18 (range, 11–27) and 17 (range, 9–29), and platelet count to 20,000/ul without transfusion were 18 (range, 12–27) and 17 (range, 11–24) in order. When we compared the incidences of acute and chronic GvHD, interestingly, we did not find any differences between groups. The novel finding of a long-term disease-free survival benefit, remarkably in case of developing either acute or chronic GvHD, especially in uBM group may be of great interest for the treatment of high-risk and even intermediate-risk AML patients. Of note, when the patients developed acute GvHD in uBM, most of them showed progressive type of chronic GvHD. There was no difference in the context of TRC and TRM between uBM and sBM groups. **Conclusions.** Thus, these findings suggest a possibility that we can not only overcome various pre-transplant co-morbid conditions of AML patients using uBM but also enhance graft-versus-leukemia effect without increasing severe GvHD, TRC, and TRM.

0453**RECOGNITION OF DANGERS SIGNALS BY EOSINOPHILIC GRANULOCYTES DERIVED FROM ALLOGENEIC PERIPHERAL BLOOD STEM CELL RECIPIENTS WITH AND WITHOUT GRAFT-VERSUS-HOST DISEASE**C. Wennerås¹, A.-L. Stenfeldt², L. Svensson², P. Wernstedt¹, M. Hardling¹, E. Telemo², M. Brune¹¹Sahlgrenska University Hospital, GÖTEBORG, Sweden; ²Göteborg University, GÖTEBORG, Sweden

Background. Increased numbers of eosinophilic granulocytes in blood appears to precede the development of acute graft-versus-host disease (GVHD) in patients after allogeneic stem cell transplantation (allo-SCT). Eosinophils also infiltrate tissues afflicted by GVHD, in particular the gastrointestinal tract. Our hypothesis is that eosinophils act as sentinels in the tissues they normally inhabit, alerting the host to the presence of danger. Tissue damage and the translocation of the resident bacterial flora across leaky mucosal membranes are examples of putative danger signals. **Aims.** To compare eosinophils from allo-SCT patients with and without GVHD, respectively, with regard to their capacity to recognize and become activated by danger signals, e.g. necrotic epithelial cells, bacteria and formylated peptides. The reactivity of control eosinophils from healthy, non-transplanted individuals to danger signals was also assessed. The long term goal of the project is to understand the role

played by the eosinophil in GVHD. **Methods.** Eosinophils were isolated from heparinized blood of stem cell recipients at two time points, either 2 or 6 months following transplantation, or at the onset of GVHD. The cells were co-incubated with the above listed danger signals and the activation patterns displayed by the eosinophils were evaluated. Specifically, the capacity of eosinophils to migrate *in vitro* in a chemotaxis microwell system, to release the granule proteins eosinophil cationic protein (ECP) (Pharmacia's EIA UniCAP) and eosinophil peroxidase (EPO) (enzymatic activity) and to generate reactive oxygen species (chemiluminescence) was determined. **Results.** Eosinophils from patients with GVHD had a greater propensity to migrate *in vitro* toward formylated peptides and necrotic intestinal epithelial cells compared to cells from transplant recipients without GVHD. A similar migratory rate toward the anaerobic bacterium *Clostridium perfringens* was seen among transplanted patients independent of GVHD status. Patients with GVHD had a greater eosinophilic release of EPO *in vitro* in response to necrotic epithelial cells compared to those without GVHD. The eosinophils derived from stem cell recipients produced more reactive oxygen specimens upon incubation with formylated peptides than did cells from healthy controls. There was a tendency toward a superior secretion of ECP from eosinophils of GVHD patients compared to patients without GVHD, after stimulation with either *C. perfringens* or necrotic epithelial cells. **Summary.** Eosinophils from patients with GVHD displayed both similar and dissimilar activation patterns compared to what was observed in allo-SCT patients without GVHD. Our results suggest that necrotic epithelial cells, the anaerobic bacterium *C. perfringens* and formylated peptides may constitute danger signals to eosinophils derived from patients with GVHD. Hopefully, this line of research may help elucidate the role, beneficial or deleterious, played by the eosinophil in this serious medical condition.

0454**CYTOKINE GENE POLYMORPHISMS AND ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD) IN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (ALLO-HCT)**A. Hadzidimitriou¹, P. Kalogiannidis¹, H. Kartsios¹, I. Sakellari¹, A. Anagnostopoulos², A. Fassas¹¹G. Papanicolaou Hospital, THESSALONIKI, Greece; ²Evangelismos Hospital, ATHENS, Greece

Polymorphisms of cytokine genes and their promoter regions may alter transcription as well as cytokine production. Given that the initial phase of GVHD after Allo-HCT involves cytokine release during conditioning of the recipient, cytokine polymorphisms may modulate immune responses and outcome of hematopoietic cell transplantation. In the present study, we analyzed 19 polymorphic positions in 12 cytokine genes in 28 pairs of donors and recipients of hematopoietic cells. Our patient group included 18 males and 10 females with a median age of 33 years (range, 9–59) who underwent allo-HCT for acute myeloid leukemia (n=10), non-Hodgkin's lymphoma (n=9), acute lymphoblastic leukemia (n=5), chronic myeloid leukemia (n=3) and multiple myeloma (n=1). Twenty-three donors were HLA-matched siblings while 5/28 were HLA-matched unrelated volunteers (VUD). Fourteen donors were men, with 9 male recipients receiving hematopoietic cells from a female donor. Conditioning regimens were those appropriate to disease, remission status, prior treatment and type of donor (sibling vs. VUD); 8 patients underwent allo-HCT with reduced intensity conditioning regimens. The genes and polymorphic positions analyzed were the following: IL-1, promoter -889; IL-1 promoter -511, pos. +3962; IL-1R pos Pst1 1970; IL-1RA pos. Mspa1 11,100; IL-4R pos. +1902; IL-12 promoter -1188; AIFN pos. UTR 5644; TGF- β , exon 1: codons 10 and 25; TNF- α promoter, -308, -238; IL-2 promoter -330, pos. +166; IL-6 promoter -174, pos. +nt 565; IL-10 promoter, -1082, -819, -592. The data obtained were analyzed by ANOVA using Kruskal-Wallis test; multivariable forward stepwise logistic regression analysis (Cox) was performed using SPSS software. Kruskal-Wallis test demon-

strated that both patient and donor genotype G/G at polymorphic site IL6 -174 were statistically related ($P < 0.05$) to acute GVHD (aGVHD) occurrence, in keeping with previous reports (Cavet et al., Blood 2001; 98: 1594/ Cozen et al., Blood 2004; 103: 3216). On multivariate analysis (variables tested: polymorphism analysis data, CMV status, recipient's age, disease type and donor gender), both patient and donor genotype G/G at polymorphic site IL6 -174 retained prognostic significance. In this context, the IL 6 -174 G allele has been associated with higher *in vitro* and *in vivo* IL6 production while, in contrast, IL 6 -174 CC homozygosity is underrepresented in inflammatory conditions (eg, juvenile chronic arthritis). In conclusion, assessment of cytokine genotype may contribute to more accurate prediction of GVHD and assist in appropriate adjustment of GVHD prophylaxis.

0455

VITAMIN D RECEPTOR GENE POLYMORPHISMS MAY BE ASSOCIATED WITH THE OUTCOME OF NON-MYELOABLATIVE STEM CELL TRANSPLANTATION

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Non-myeloablative stem cell transplantation (NMSCT) can enable effective treatment of haemopoietic malignancy in patients who are ineligible for a conventional myeloablative protocol because of significant co-morbidities or advanced age. Despite reduced conditioning complications, including infections and GVHD, are common. DNA markers capable of identifying potential post-transplant complications would help to improve patient management and reduce morbidity and mortality following NMSCT. Among the immunomodulatory factors vitamin D (D3) and its receptor (VDR) are of interest because of their importance in a range of normal and pathological processes. Polymorphisms in VDR gene have been shown to be associated with GVHD and survival in myeloablative stem cell transplantation. The critical importance of graft versus leukaemia effect (GVL) in NMSCT makes D3 and VDR important study targets. *Aims.* To investigate a possible relationship between DNA polymorphisms in VDR gene and clinical outcome of NMSCT. *Materials and Methods.* 30 recipients of NMSCT (median age 51 (29,63)) and their donors (median age 42 (21,66)) were investigated. The patients were treated for AML (10), NHL (5), CLL (4), MM (3), HD (2), CML (2), and Waldenstrom's macroglobulinaemia (1) and myelofibrosis (1). All patients received the same conditioning consisting of fludarabine (25 mg/m²/day x5), melphalan (140mg/m² x1), ATGAM 15 mg/kg/day x10 and GVHD prophylaxis with cyclosporin 3 mg/kg/day and mycophenolate mofetil 15 mg/kg BD. Genotyping of SNPs Fok I (exon 2), Apa I and Bsm I (intron 8) and Taq I (exon 9) that were previously reported to be associated with various pathologies, was performed by restriction endonuclease (RE) analysis of PCR-amplified genomic DNA. 36 unrelated healthy people were also genotyped as controls to assess haplotype distribution. *Results.* No major differences in haplotype distribution between patients, donors and controls were found and genotype frequencies were also similar. Recipient's Apa I-Bsm I-Taq I haplotype was found to be significantly associated with survival ($p=0.0146$). Recipients with haplotype b-a-T (T= no Taq I RE recognition site) appeared to have more favourable outcome to those with B-A-t haplotype (no Bsm I and Apa I RE recognition sites) but the difference was not statistically significant. Taq I polymorphism in donor showed a strong trend for chronic GVHD development ($p=0.052$) following NMSCT. *Conclusions.* This preliminary VDR polymorphism analysis indicates that genotyping recipient and donor VDR may be a useful risk indicator for patients undergoing NMSCT. A larger prospective study of NMSCT is required to verify our findings. The mechanism linking VDR with clinical course of NMSCT is unclear.

0456

SIMULTANEOUS TRANSPLANTATION OF MULTIPLE ALLOGENEIC CORD BLOOD UNITS IN ADULTS WITH HIGH-RISK LEUKEMIA

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Absence of related or unrelated marrow donor creates a significant problem in cases where allogeneic transplantation is the only known effective therapeutic option. Currently, practically for each such patient it is possible to find appropriately-matched cord blood unit (CBU) but the cell number in such a unit is usually insufficient for adult patient. Hence, simultaneous transplantation of more than one unit was suggested in order to achieve necessary minimal number of cells. *Aims.* To explore the feasibility of simultaneous transplantation of multiple allogeneic CBU in adult high risk leukemia lacking marrow donor. *Material and Methods.* Eleven consecutive patients with advanced hematological malignancies and lacking both related and unrelated (BMDW database) donors have been considered. For all of them it was possible to find in BMDW database two or more CBUs matched at least 3/6 HLA antigens. Seven patients did not reach the transplant phase for various reasons, and four patients have been finally transplanted. Two patients (No 1 and No 2-males) had primarily resistant acute myeloblastic leukemia, aged and weighted 21 years/85 kg and 24 years/87 kg respectively. The third patient-male, 22 years old, weighted 98 kg and had acute lymphoblastic leukemia Ph+, BCR/ABL+ in complete hematological remission and the fourth was female, 24 years old, with body weight of 53 kg, with imatinib-resistant chronic myelogenous leukemia, in advanced chronic phase. All patients received myeloablative conditioning chemotherapy: Patients Nr 1, 2 and 4 received busulphan 16 mg/kg, cyclophosphamide 200 mg/kg and thymoglobuline (Sangstat, Fresenius). The patient No 3 underwent 1200 cGy total body irradiation and received cyclophosphamide 60 mg/kg, etoposide 40 mg/kg and thymoglobuline. The characteristics of CBU see Table.

Patient No	Number of CBU	Number of mismatches in each CBU (out of 6)	Total NC number (x 10 ⁷ /kg)	Total CD34 number (x 10 ⁵ /kg)	ABO group (Recipient / Donors)
1	2	1, 2	2.0	0.58	O+ / A-, B+
2	3	2, 3, 2	3.38	0.95	A+ / A+, O+, O+
3	3	3, 2, 2	3.36	2.3	O+ / AB+, O+, O+
4	2	2, 2	3.7	0.6	A+ / O+, A+

Patient No 1 became reconstituted with progeny of one of the two transplanted units but died day +103 of brain aspergillosis. Patient No 2: died before reaching the engraftment on day 42 due to Gram negative sepsis. Patient No 3 is fully reconstituted with progeny of one of the three transplanted units, but at day +100 he still had 5% of BCR/ABL positive cells in FISH being in complete hematological remission. Patient No 4 as of day +60 is neutrophil recovered but with still platelet transfusion dependency. Chimerism studies on day +50 have revealed mixed chimerism. *Conclusions.* These data confirm the notion on the validity of this approach to patients who require transplant but lack marrow donor. They also represent the first report of simultaneous transplantation of three cord blood units (patients 2 and 3) into one recipient.

0457**PREEMPTIVE DONOR LYMPHOCYTE INFUSIONS IN ACUTE LEUKEMIAS RELAPSING AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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Donor lymphocyte infusions (DLI) are now an established procedure to treat malignant relapse after allogeneic hematopoietic stem cell transplantation (HSCT), particularly in chronic myelogenous leukemia. However, their role is less clear in acute leukemias, due to the potentially rapid increase in leukemic relapse burden. For acute lymphoblastic leukemia (ALL), DLI are estimated to be of no value at all. Here we report our single center ten years experience with DLI as preemptive therapy of acute leukemias relapsing after allogeneic HSCT. We performed 33 DLI courses for relapsed *de novo* acute myeloid leukemia (AML; 15/33), secondary acute myeloid leukemia evolved from myelodysplastic syndromes (sAML/MDS; 10/33), or ALL (8/33); two thirds of which had been high risk leukemias. Transplantations were from sibling (15/33) or unrelated donors (18/33), after reduced intensity (8/33), standard (17/33) or intensified conditioning (8/33). Reasons for DLI were molecular relapse or progressive mixed chimerism (9/33), but most often frank relapse (24/33). As adjunctive to DLI, chemo-, immuno- or molecular targeted therapies were administered in 13/33, of whom 7 achieved hematological remission before DLI. 1-4 (median: 2) incremental DLI doses were given, with cumulative doses of 1×10^6 - 1.9×10^8 (median: 1.8×10^7) CD3⁺ cells/kg. Overall response rate was 55% (18/33) and complete remission (CR; complete hematological remission plus return of stable complete donor chimerism) rate was 52% (17/33). 16 of 18 responders have since remained in continuous CR with a median follow-up of 24+ (1+77+) months. After DLI, acute and chronic GvHD occurred in 15/33 and 16/25, respectively; two patients (6%) died from GvHD or GvHD-related infection. Overall survival is 52% and disease-free survival 42%. Conditioning regimen, remission duration after transplant, hematologic remission before DLI, acute GvHD post DLI, and response to DLI had a significant influence on overall survival. Subgroup analysis showed that the best results were obtained in *de novo* AML with 73% CR, 67% overall survival, 60% disease-free survival, and almost half the subgroup already being long-term survivors. Interestingly, even in sAML/MDS and ALL, both response rates and overall survival reached 40% and 38%, respectively. *Conclusions.* In our experience, DLI are effective in the majority of patients with acute leukemias relapsing after allogeneic HSCT.

0458**ALLOGENEIC TRANSPLANTATION OF STEADY-STATE BONE MARROW VS G-CSF-PRIMED BONE MARROW FOR ADULT AML PATIENTS**H.J. Kim¹, K.S. Eom², Y.J. Kim², C.K. Min², D.W. Kim², J.W. Lee², W.S. Min¹, C.C. Kim¹¹*St Mary's Hospital, Catholic Univ Korea, SEOUL, South-Korea;* ²*St Mary's Hospital, Catholic Univ Korea, SEOUL, South-Korea*

Background & Aims. The relative effects of different types of allogeneic hematopoietic stem cell transplantation on the engraftment rate, incidence of acute and chronic graft-versus-host disease (GvHD), and relapse rate together with other transplant-related complications (TRC) or transplant-related mortality (TRM) have been specifically investigated and comparatively analyzed in 216 adult AML patients according to the cytogenetic-risk groups. *Methods.* We electively performed graft engineering. In all, 101 patients received G-CSF primed unmanipulated bone marrow (uBM) and the other 115 patients received steady BM cells (sBM). The patients in these two groups were stratified non-randomly according to donor and recipient ages

(≥ 40 or < 40), status of leukemia (advanced or not), presence of preceding treatment-related sequelae, weight discrepancy between donor and recipient i.e. if the difference was over 10kg, and presence of major organ dysfunction. The median follow-up duration for all patients was 48 months (range, 1-98). Sex, age, and subtype of disease were adjusted between two groups. *Results.* As for each group, the median CD34⁺ cells infused were 3.6×10^6 /kg (range, 2.1-13.7) (uBM group), 2.7×10^6 /kg (range, 1.7-12.2) (sBM), respectively. The median days for recovery of absolute neutrophil count to 500/ μ l were 18 (range, 11-27) and 17 (range, 9-29), and platelet count to 20,000/ μ l without transfusion were 18 (range, 12-27) and 17 (range, 11-24) in order. When we compared the incidences of acute and chronic GvHD, interestingly, we did not find any differences between groups. The novel finding of a long-term disease-free survival benefit, remarkably in case of developing either acute or chronic GvHD, especially in uBM group may be of great interest for the treatment of high-risk and even intermediate-risk AML patients. Of note, when the patients developed acute GvHD in uBM, most of them showed progressive type of chronic GvHD. However, there was a big difference in the context of TRC (21% vs 45%, $p=0.007$), and a marginal difference of TRM (6% vs 10%, $p=0.09$), between uBM and sBM groups. The 3-yr estimated event-free-survival rate for these two groups were 65% vs 58%, respectively. *Conclusions.* Thus, these findings suggest a possibility that we can not only overcome various pre-transplant co-morbid conditions of AML patients using uBM but also enhance graft-versus-leukemia effect without increasing severe GvHD, TRC, and TRM.

0459**STEM CELL TRANSPLANTATION (SCT) BEYOND FIRST REMISSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)-A POTENT GVL EFFECT OF CHRONIC BUT NOT ACUTE GVHD**H. Hallböök¹, H. Hägglund², D. Stockelberg³, P.G. Nilsson⁴, K. Karlsson⁴, M. Björkholm², M. Linderholm⁴, A. Wahlin⁴, O. Linder⁴, B. Smedmyr¹¹*Uppsala University Hospital, UPPSALA, Sweden;* ²*Karolinska University Hospital, HUDDINGE, Sweden;* ³*Sahlgrenska University Hospital, GÖTEBORG, Sweden;* ⁴*University Hospital, LUND, Sweden*

On behalf of the Swedish Adult ALL Group. *Background.* and aim: In Sweden, adult patients with ALL and acute undifferentiated leukemia (AUL) have been treated according to national protocols since 1986. SCT has been recommended in first remission for patients with high-risk leukemia (WBC $>30 \times 10^9$ /L, CNS leukemia, late remission, t(9;22), t(4;11) or AUL) and for patients with standard-risk leukemia only after relapse. We retrospectively analysed outcomes for patients who underwent SCT beyond first remission. *Methods.* Patients from all regions of Sweden, diagnosed with ALL 1986-2000, who underwent SCT beyond first remission were included. None of the patients had received a SCT in first remission. Patients were identified through the registers of two previous national studies, each centre's EBMT register and the transplantation records of each centre. Clinical data were retrieved from the same sources. GVHD was evaluated in patients who underwent an allogeneic SCT, and chronic GVHD was assessed in patients who survived at least 100 days after SCT. *Results.* During the period, 63 patients (median age 34 years, range 17-62) underwent SCT beyond first remission (51 patients in second or third remission, 12 in relapse or with resistant disease). Conditioning regimen was reported for 59 patients; the majority (51 patients) received a regimen including TBI. One patient received a reduced intensity conditioning. The overall 5-year disease free survival (DFS) was 14% (CI 5-23%); 18% (CI 6-29%) in patients 17-40 years and 5% (CI 0-15%) in patients 41-62 years, respectively (ns). No significant difference in 5-year DFS was observed between autologous SCT 6% (CI 0-18%), related donor 18% (CI 4-32%) and unrelated donor SCT 16% (CI 0-32%). In patients with less than 24

months from diagnosis to SCT, the 5-year DFS was 7% (CI 0-15%) compared to 27% (CI 8-45%) in patients with >24 months from diagnosis to SCT ($p=0.03$). Evaluation of acute GVHD was reported for the 47 patients who underwent allogeneic SCT. The 5-year DFS was 25% (CI 1-50%) for patients without acute GVHD versus 14% (CI 3-26%) for patients with acute GVHD (ns). Patients who developed chronic GVHD had a significantly better 5-year DFS (42%; CI 15-68%) than patients free from chronic GVHD (12%; CI 0-29%) ($p=0.005$). *Conclusions.* The results indicate that late relapse and the presence of a chronic GVHD predict a better DFS in patients undergoing salvage SCT for adult ALL.

0460

STEM CELL TRANSPLANTATION IN CONGENITAL NEUTROPENIAS: RECOMMENDATION FOR PATIENTS REFRACTORY TO G-CSF TREATMENT AND SECONDARY MDS OR LEUKEMIAS

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Severe congenital neutropenia (CN) characterized by an early stage maturation arrest of myelopoiesis leading to bacterial infections from early infancy has originally been described as Kostmann syndrome with an autosomal recessive inheritance. However, recent pathogenetic investigations have demonstrated that this clinical phenotype includes also autosomal dominant and sporadic cases. The underlying genetic defect is still unknown. Long-term application of recombinant human granulocyte-colony stimulating factor (G-CSF; filgrastim, lenograstim) is first choice treatment, whereas hematopoietic stem cell transplantation (HSCT) is still the only available option for patients refractory to G-CSF treatment (5-10%) or secondary leukemic transformation (12,4%). In the European database HSCT information on 10 patients transplanted for reasons other than malignancy (refractory to G-CSF, $n=5$; neutropenia in the pre-G-CSF era, $n=1$; development of pancytopenia, $n=1$; G-CSF receptor mutations, $n=3$). 6 of 10 patients in the non-malignant group received a graft from an HLA identical sibling. All of these patients are currently alive. However, some patients appear to develop unusual and severe toxicity leading to a significant decrease of their quality of life. Three of 4 patients receiving a transplant from matched unrelated or alternative donors died in the early post transplant phase (1.9 - 3.7 months) due to multi-organ failure or severe GVHD. In general, BU-CY based regimens were tolerated well in non-malignant HSCT. 22 patients were transplanted for secondary MDS or leukemia, compared to 4 patients receiving chemotherapy alone. Outcome of patients treated with chemotherapy alone was fatal in all patients so far. However, outcome of HSCT before 2000 was poor due to HSCT related infections, e. g. aspergillus infections and severe GVHD. Relapse of leukemia was not an issue, since patients died early from transplant related morbidity. In this patient group the majority of patients received a graft from matched unrelated or even haploidentical donors. Data from the international database up to the end of the year 2000 on all patients receiving HSCT after secondary malignant transformation demonstrate a survival rate of 31%. As compared to the non-malignant group the relation between matched unrelated/mismatched donors and HLA identical donors is exactly the opposite. Early transplantation within 3 months after diagnosis of MDS or leukemia and less toxic primary chemotherapy improved health status at HSCT and thus survival of transplant to 60% in the European patient cohort up to date. We therefore recommend the following procedure for CN patients with malignant transformation: HSCT is the only curative treatment; HSCT should be performed as early as possible, at least within 3 months after diagnosis of leukemia; prevention of infection before HSCT is the main objective and therefore intensive AML-type chemotherapy should -in general- not be given prior to HSCT; however, to keep blast counts low, low dose chemotherapy may be required.

0461

PATTERNS OF ENGRAFTMENT OF T-CELLS, GRANULOCYTES, B-CELLS, NK CELLS AND DENDRITIC CELLS (BDCA1 AND BDCA4) FOLLOWING RIC ALLO HSCT

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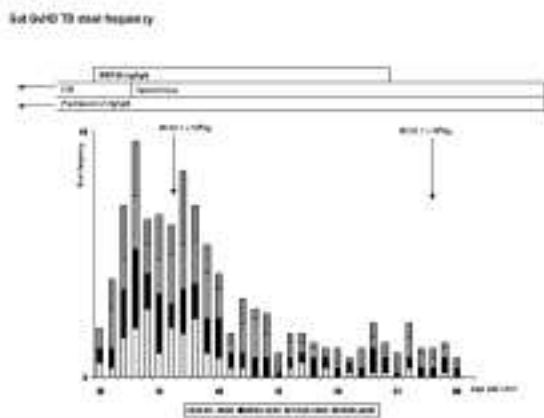
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Background. Patients undergoing RIC Allo-HSCT using alemtuzumab (Campath-1H) rely on the induction of profound recipient immunosuppression in order to permit donor engraftment. The presence of host dendritic cells (DC) after transplantation has been associated with graft versus host disease (GVHD). Alemtuzumab is a humanized IgG1 monoclonal anti-CD52 antibody directed against lymphoid and monocyte cells. In addition it has been shown to deplete host DC hence reducing GVHD. *Aims.* Chimerism investigations were carried out on a cohort of 17 patients with a variety of haematological malignancies (MPD/MDS 7, AML 6, NHL 2, ALL 2), to evaluate correlation between lineage specific sub-sets, engraftment and GVHD. *Methods.* Post transplant peripheral blood was fractionated into T-cells (CD3+), granulocytes (CD15+), B-cells (CD19+), NK cells (CD56+) and DC subsets BDCA1 (myeloid) and BDCA4 (plasmacytoid). Genetic fingerprinting using a multiplex PCR (PowerPlex-16, Promega) yielded fluorescent products separated by PAGE (ABI 377 with genescan software). There were 11 male/6 female recipients. Median age of patients was 51.4 years (35-67). 8 received stem cells from HLA compatible siblings, and 9 from Volunteer Unrelated Donors (6 fully matched, 3 1-antigen mismatched). Stem cell source was 11/17 (64.7%) PBSCH and 6/17 (35.3%) BMH. All patients were in complete remission or good partial remission at time of HSCT. *Results.* The ratio of donor to recipient was calculated as a percentage and four patterns of engraftment were identified. Pattern 1 - Full engraftment of all lineages at day 56: 41% (5-MUD, 2-Sib) sustained to day 100, Pattern 2 - Full engraftment of all lineages at day 56, followed by loss of BDCA1 and BDCA4 in parallel with T-cells and NK cells, but with full granulocyte and B-cell engraftment at day 100: 29% (3-MUD, 2-Sib); Pattern 3 - Initial incomplete BDCA1, BDCA4, T-cell and NK cell, full granulocyte and B-cell at day 56, followed by full engraftment of all lineages at day 100: 18% (2-MUD, 1-Sib); Pattern 4 - Initial incomplete BDCA1, BDCA4, T-cell and NK cell, and full granulocyte and B-cell at day 56, followed by loss of engraftment of all lineages at day 100: 12% (2-Sib). 87% of patients illustrated early engraftment of granulocytes (at day 56) independent of all other lineages. No patients developed acute GVHD (Grade II-IV). Chronic GVHD developed in 9/17 (%) patients, of whom 2/9 had mixed DC chimerism and 7/9 had donor DC chimerism at day 56 post-HSCT. *Conclusions.* In our cohort, T-cell engraftment was highly analogous to that of the DC suggesting a close interaction between these cell subsets during the immune response to BMT. In no case did loss of DC donor chimerism precede that of the T-cells. In addition, there was no correlation between donor-recipient DC chimerism and incidence or timing of onset of GVHD. Further studies involving a larger group of patients is required to confirm these findings and allow the various patterns to be used clinically to predict the outcome of RIC Allo-BMT.

0462

USE OF UNRELATED DONOR THIRD PARTY MESENCHYMAL STEM CELLS IN THE TREATMENT OF ACUTE STAGE IV GRAFT-VERSUS-HOST DISEASE FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATIONL.M. Ball, A.C. Lankester, H. Roelofs, R.M. Egeler, W.E. Fibbe
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Treatment resistant acute graft-versus-host disease (aGvHD) following allogeneic stem cell transplantation is associated with high mortality. Bone marrow derived adult mesenchymal stem cells (MSC's) are immunosuppressive and inhibit proliferation of allo-reactive T-cells. Haplo-identical related MSC's have been used to successfully treat aGVHD.¹ To date, third party unrelated donor MSC's have not been used in the clinical setting of GvHD. A 13 year-old boy with myelodysplastic syndrome (RAEB) underwent HLA identical related sibling bone marrow transplantation. He received myeloablative conditioning plus ATG, a total of 0.5 x 10⁸/kg MNC's and cyclosporine 2 mg/kg/day from day -1 for GvHD prophylaxis. At day +14 he developed an engraftment syndrome responsive to methylprednisolone 2mg/kg/day, tapered to 1 mg/kg/day by day +22. He then developed biopsy proven cutaneous GvHD and diarrhea for which steroids were increased. In addition, mycophenolate mofetil (90 mg/kg/d) was added and tacrolimus substituted for cyclosporine. Despite these measures his aGvHD progressed (skin and GI tract stage IV). On day +30, 1.1 x 10⁶/kg MSC's, from an entirely unrelated donor were administered. MSC's from this donor had been prepared but not used for other clinical use. Permission was obtained from local ethical committee and also the donor for use in this patient. MSC's were expanded under GMP conditions in a nationally accredited laboratory, following a standardized procedure. Morphology and immunophenotype (HLA-I, CD 73, CD 90, CD 105 positive; CD 34, CD 45, HLA-II, CD 80, CD 31 negative) identified the cell population. MSC's were divided into five aliquots of 45 x 10⁶ cells, frozen in DMSO and stored at -70C, to be thawed immediately before clinical use. The MSC's were infused over 5 minutes in a total volume of 55 ml without side effects. Clinically the skin GvHD responded dramatically with evident clinical improvement within the first week. A skin biopsy at day +58 showed no evident GvHD. The frequency and volume of the stool decreased significantly (see Figure 1), although normal defecation did not resume.



Rectal biopsy at day +45 showed evidence of aGvHD pathologically grade 2-3. At day +58 an endoscopic biopsy of the duodenum showed evidence of villi regeneration with a pathological grade 0-1 confirmed in the duodenal and sigmoid biopsies. A further 1.1 x 10⁶/kg MSC's from the same donor were administered, whilst awaiting the outcome of biopsies. Unfortunately, at day +61 he developed a paralytic ileus and (despite pre-emptive

broad-spectrum antibiotics) a *Klebsiella pneumoniae* sepsis. After transfer to intensive care, he died one week later due to multi-organ failure and ARDS. Limited post mortem biopsies showed no evident GvHD. This case demonstrates that MSC's are effective for the treatment of steroid refractory GvHD. We were able to administer frozen unrelated third party MSC's without evident toxicities. Unfortunately, despite pathologically documented response death from overwhelming infection occurred. The use of unrelated stored third party MSC's is possible and allows for a more rapid treatment of GvHD, which could potentially reduce the need for additional immune-suppression.

1. Le Blanc K et al. *Lancet* 2004, 1439-1441.

0463

DOES ABO BLOOD GROUP MISMATCH AFFECT OUTCOME IN REDUCED INTENSITY ALLOGENIC STEM CELL TRANSPLANTS?S.G. Watt¹, E. Tholouli¹, J. Collins², J. Cavet², G.S. Lucas¹, J. Burthem¹, E. Liakopoulou², M. Dennis², J.A. Liu Yin¹¹Manchester Royal Infirmary, Manchester, UK²Christie Hospital, Manchester, UK

ABO blood group mismatch does not significantly affect the overall survival of patients undergoing allogeneic myeloablative transplantation. However, there is evidence of slower engraftment, increased red cell transfusion requirements and an increased incidence of red cell aplasia. There is only limited data concerning reduced intensity stem cell transplant (RISCT) with ABO mismatch and it has been suggested that there may be an increased transplant related mortality. This is a retrospective study of the data from two bone marrow transplant (BMT) units in Manchester, U.K. We analyzed data from 123 patients undergoing RISCT with regards to ABO mismatch and outcome. There were 52 mismatched transplants, including 24 major mismatches, 22 minor mismatches and 6 bidirectional mismatches. All patients were transplanted for haematological malignancies and had various RISCT conditioning regimens. There were no significant differences between groups for age, sex or CMV status. Median age was 53 years (range 29-66 years). There were 52 matched unrelated donor transplants (MUD) and 71 sibling transplants. 50% of the MUDs were ABO mismatched and 42.3% of sibling allografts were mismatched. There was death before engraftment or failure to engraft in 9.0% of major mismatched, 8.3% of minor mismatched and 4.2% of matched RISCT. This was not statistically significant. The 100 day mortality shows no statistically significant difference in survival but a trend towards better outcome in the ABO matched compared with ABO mismatched groups (17.3% v 8.5%, p=0.168). Overall survival of all patients at a median follow up of 36 months (range 3-89 months) was 52.1% in ABO matched, 45.5% in minor mismatched and 30% in major/bidirectional mismatched transplants. This is almost statistically significant (p=0.051). However, the only group that had a worse overall survival which was statistically significant were the major/bidirectional ABO mismatched MUD transplants when compared with the ABO matched MUD transplants (16.7% v 53.6%, p=0.016). Disease free survival (DFS) of all patients at a median follow up of 36 months was 40.9% in ABO matched, 36.3% in minor and 26.6% in major/bidirectional mismatched RISCT. There is a trend towards improved DFS in ABO matched transplants, but this is not statistically significant. As in overall survival, the MUD transplants with major/bidirectional mismatch showed a worse DFS when compared to ABO matched MUD transplants (11.1% v 39.3%, p=0.049). There was no significant difference in transfusion requirements or neutrophil engraftment between groups. Our study shows a trend towards a worse outcome in those patients who underwent a major or bidirectional ABO mismatched transplant. This difference reaches statistical significance in MUD transplantation. Bidirectional ABO mismatched RISCT have done particularly badly in this study and this is consistent with other publications. Minor ABO mismatch in RISCT appears to make little difference to outcome in either MUD or sibling allografts.

Anemia

0464

NOVEL HEMATOLOGIC MARKERS FOR PREDICTING RESPONSE TO *R-HUEPO* IN ANEMIC PATIENTS WITH MULTIPLE MYELOMA AND LYMPHOMA

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Treatment with r-huEPO improves anemia in 60-75% of patients with multiple myeloma (MM) and lymphoma, but is ineffective in about 1/3 of them. The commonly used models often fail to adequately predict response, probably because they do not evaluate both iron status and erythropoiesis. It is well known that disturbances in iron homeostasis (eg functional iron deficiency) participate in the pathogenesis of anemia of chronic disease and hence, anemia of MM and lymphoma. The purpose of this study was to investigate the possibility that a combination of markers of iron status and erythropoiesis could offer a new model, useful in predicting response to, and optimizing therapy with r-huEPO. Patients and *Methods*. Forty (40) newly diagnosed anemic patients (Hb \leq 10.5g/dL) with MM and lymphoma aged 29-83 (median 71 years) were studied. All patients received r-huEPO at a dose of 30000IU/wk for six weeks. In those who responded (Hb increase by \geq 2g/dL after six wks) r-huEPO was continued as needed. Non-responders received iron sucrose 200mg IV/wk for the next 4 wks. Hematologic indices and biochemical markers were measured before treatment (baseline) and on week 1, 2 and 6. Hematologic indices included hemoglobin, MCV, MCH, percentage of hypochromic erythrocytes (HYPO%), percentage of reticulocytes (retics%) absolute reticulocyte count (retics-ab), Immature Reticulocyte Fraction (IRF), High Fluorescence Reticulocytes (HFR), reticulocyte hemoglobin (retics-Hb), reticulocyte hematocrit (retics-Hct), reticulocyte Hemoglobin Content (CHr), reticulocyte Mean Volume (MCVr). All were measured in an ADVIA 120 analyzer. Biochemical markers included endogenous erythropoietin, serum ferritin, transferrin saturation (SAT%), determined with standard techniques, and soluble transferrin receptors (sTfR), measured with the Dade-Behring assay. *Results*. Twenty-six (26) patients responded (65%) to r-huEPO and 14 did not. In non-responders, 10 out of 12 patients who concomitantly received IV iron responded. Univariate analysis revealed statistical significance for response to r-huEPO of baseline HYPO%, retics% wk 2, retics-ab wk 2, retics-Hct wk 2 and retics-Hb wk 2. Statistically significant changes% between baseline and wk 2 were found in retics%, retics-Hb, retics-Hct, retics-ab, and sTfR. Binary logistic regression analysis confirmed baseline HYPO% and a change between baseline and week 2 in retics-Hct as independent predictive markers of response to r-huEPO ($p=0.05$ and 0.008 respectively). A ROC curve revealed that by using a cut-off value of $<5\%$ for HYPO at baseline and of $\geq 50\%$ increment in retics-Hct on wk 2, the sensitivity was 100% and 92% and the specificity was 50% and 85% respectively. The positive and negative predictive value (PPV and NPV) for baseline HYPO% $<5\%$ was 79% and 100% respectively. The PPV and NPV of a model combining baseline HYPO% $<5\%$ and an increment in retics-Hct $\geq 50\%$ on week 2 was 92% and 85% respectively. (i) A combination of baseline HYPO% $<5\%$ and an increment in retics-Hct $\geq 50\%$ on week 2, can be used as a predictive model of response to r-huEPO. 2) HYPO $\geq 5\%$ can recognize patients who will not respond to r-huEPO (NPV=100%), and may benefit from IV iron co-administration.

0465

A PHASE I SINGLE BLIND CLINICAL TRIAL OF A NEW OXYGEN TRANSPORT AGENT (MP4), HUMAN HEMOGLOBIN MODIFIED WITH MALEIMIDE-ACTIVATED POLYETHYLENE GLYCOL

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MP4 (HemospanTM), a hemoglobin-based oxygen carrier, has been designed to deliver O₂ to hypoxic tissues without vasoconstriction. A Phase I clinical trial of MP4 was undertaken to evaluate whether MP4 elicits the clinical side effects associated with previous hemoglobin-based solutions. *3. Methods*. Twelve volunteers were studied. One cohort (n=4) received 50 mg/kg of MP4, a second (n=4) received 100 mg/kg of MP4, and the third (n=4) received Lactated Ringer's solution. Single blind infusions were given at 5 mL/min. Vital signs and symptoms, hematologic, serum chemistry, renal and electrocardiographic measurements were monitored for 15 days after dosing. *4. Results*. Five mild adverse events occurred in the controls and 2 each in the 50 mg/kg and 100 mg/kg MP4 groups. None was severe or judged related to MP4 administration by the principal investigator. There were no clinically significant alterations in blood pressure or heart rate, and there were no gastrointestinal symptoms, abdominal or flank pain, loss of appetite or clinically significant alterations of liver or pancreatic enzymes. In one subject (100 mg/kg of MP4), amylase and lipase were slightly above the upper limit of normal at 4 hours after dosing, but without associated symptoms or signs. Pharmacokinetic analysis of plasma hemoglobin, yields an estimated half-life (T_{1/2}) of 43 hours in the 100 mg/kg MP4 subjects. *5. Summary/conclusions*: MP4 appears to have a favorable safety profile. Subjects in both study groups survived and did no less well than those in the control group.

0466

THE INFLUENCE OF MUTATIONS IN RIBOSOMAL PROTEIN S19 ON ITS STABILITY

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Diamond-Blackfan anemia (DBA) is a rare congenital pure red cell aplasia characterized by a normochromic macrocytic anemia, reticulocytopenia, and normocellular bone marrow with a selective deficiency of erythroid precursors. About 40% of DBA patients have associated physical anomalies and/or growth retardation. In 20-25% of DBA patients mutations in the ribosomal protein S19 (RPS19) gene have been found, but the role of RPS19 in DBA pathogenesis remains elusive. RPS19 is part of a small ribosomal subunit, and it is believed to play an important role in the initiation of translation. *Aims*. The aims of our work were to study the influence of mutations in RPS19 on its stability. Wild type (WT) RPS19 cDNA as well as mutated variants of the protein described previously in Czech DBA patients (Cmejla, BCMD, 2000) were cloned into a plasmid behind the Myc tag (Point mutations: Arg56Gln; Arg62Gln, fusion protein 159 aa. Frameshift mutations: Ins8bp[nt386], fusion protein 202 aa; Del11bp[nt196-206], fusion protein 162 aa). Plasmids were used to stably transfect the K562 cell line. After the G418 selection the cells were harvested and used as a source for RNA isolation and Western blotting (WB). Real-time RT-PCR was performed according to a standard procedure using specific primers to distinguish endogenous and Myc-tagged RPS19. Anti-Myc antibody was used for WB; intracellular c-Myc protein was utilized as an internal load control. *Results*. Real-time RT-PCR results revealed that all variants of Myc-

RPS19 were transcribed at least 10 times more than the endogenous RPS19. Using specific anti-Myc antibody on WB we showed that 1) point mutations affecting conserved amino acids (Arg56Gln; Arg62Gln) did not impair the stability of the RPS19 protein. 2) Frameshift mutations (Ins8, Del11) led to a degradation of mutated RPS19 protein to a nearly undetectable level in comparison with Myc-WT RPS19. **SUMMARY/CONCLUSIONS:** Several types of mutations in RPS19 have been described including missense and nonsense point mutations, insertions and deletions, and splice site mutations, but how the particular mutation influences the RPS19 protein (its production, stability and function) is poorly understood. Mutations leading to a formation of a premature stop codon or its elimination from RPS19 mRNA have been studied so far. Both types of mutations cause the instability of the mutated RPS19 mRNA that is rapidly degraded by nonsense mediated decay or nonstop decay mechanisms (Gazda, BJH, 2004; Chatr-Aryamontri, Hum-Mutat, 2004). Our experiments for the first time considered the stability of point-mutated and frameshift-mutated RPS19 proteins with stable mRNA. In case of mutations affecting highly conserved amino acids Arg56Gln and Arg62Gln, the protein stability was not influenced, implying that mutated RPS19 proteins could compete with WT variants blocking their normal function. On the other hand, RPS19 proteins affected by frameshift mutations that change the RPS19 amino acid sequence significantly (Ins8, Del11) were hardly detectable on WB indicating they were rapidly degraded. In theory, both types of mutations could therefore lead to RPS19 insufficiency by two different mechanisms. Using Myc-tagged mutated RPS19 variants could thus be a valuable tool for further studies on the RPS19 function in DBA pathogenesis.

0467

ERYTHROPOIETIC PROTOPORPHYRIA: GENOTYPE, PHENOTYPE AND FLUOROCYTES COUNT RELATIONSHIP

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Background. Erythropoietic protoporphyria (EPP, MIM 177000) is an autosomal dominant disease with incomplete penetrance, due to reduced activity of ferrochelatase (FECH; EC 4.99.1.1), an enzyme located in the inner mitochondrial membrane that catalyzes the chelation of ferrous iron into protoporphyrin IX, the final step in the heme biosynthetic pathway. Clinical manifestations have a childhood onset and include skin photosensitivity, mild anaemia and, in 5-10% of the cases, progressive hepatic failure. Diagnosis of EPP can be supported by the presence of fluorescent erythrocytes (fluorocytes) in peripheral blood smears and increased protoporphyrin levels in erythrocytes, plasma and faeces. The human ferrochelatase gene (FECH) spans 45kb with a total of 11 exons and maps to chromosome 18q21.3. The FECH gene encodes for a precursor of 423 amino acid residues, the first 54 of which are the putative mitochondrial leader sequence. A single promoter directs both housekeeping and erythroid expression, but two polyadenylation sites produce two mRNAs of different length. So far molecular analysis of FECH gene has allowed the identification of more than 90 different mutations responsible for EPP, showing a high genetic heterogeneity. Phenotypic expression of EPP required coinheritance of a mutated FECH allele and a wild-type low expressed allele. At present, bases of low allele expression are disputed. Studies conducted to identify the causes of this effect suggest two different hypothesis. The first one assumes that an entire haplotype (251G, Ivs1-23T and Ivs2microsatAn9) is able to reduce allele expression, whereas the second indicates the Ivs3-48C polymorphism as the only cause. **Aim and Patients:** In this study we conducted fluorocytes count and haplotype analysis of the most important FECH gene polymorphisms, in order to establish relationship between genotype, phenotype and fluorocytes analysis. We investigated twenty unrelated Italian EPP patients, thirty relatives and fifty normal subjects. **Methods.** We applied flow cytometry screening

test to detect fluorescent red cells in peripheral blood and direct sequencing of three PCR fragments containing -251 A>G, Ivs1-23C>T and Ivs3-48T>C polymorphisms respectively. **Results.** Flow cytometry analysis reveals that ranges for the fluorocytes percentage are 0-0.1% for controls subjects, 0.1-1% for asymptomatic carriers of mutation and 42+/-20% for patients. Sequencing analysis of the FECH gene revealed the presence of -251G, IVS1-23T and IVS3-48C polymorphisms in all patients and their absence in all asymptomatic carriers. Restriction Fragment Length Polymorphism analysis and segregation studies demonstrated that all polymorphisms were in trans to mutation causal for EPP. **Summary.** This study proved that fluorocytes detection is useful to identify asymptomatic and biochemically negative EPP carriers. Indeed, all the subjects with fluorocytes count >0.1% showed a mutation in the coding region of FECH gene. In our results, constant cosegregation of G/T/C haplotype with phenotypic expression of disease suggests a third hypothesis: this entire haplotype can be involved in low expressed allele mechanism. Further studies are in progress to clarify the molecular mechanisms regulating FECH gene expression.

0468

A NOVEL MUTATION D239G IN THE NADH BINDING DOMAIN OF CYTOCHROME B5 REDUCTASE CHANGES ENZYMIC COFACTOR SPECIFICITY IN TYPE I METHAEMOGLOBINAEMIA

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Background. Recessive congenital methaemoglobinaemia (RCM) is an autosomal recessive disorder, which manifests clinically in two forms. In type I individuals are cyanotic but in type II the cyanosis is accompanied by severe neurological dysfunction. Both forms of RCM exhibit a deficiency of the enzyme NADH-cytochrome b5 reductase (cytb5r, EC 1.6.2.2). Cytb5r exists either as a membrane bound protein, which is involved in fatty acid metabolism, or a soluble form present in erythrocytes and catalyses the reduction of the super reduced non-functional met form of haemoglobin. Cytb5r is composed of two cofactor binding lobes, FAD and NADH, linked together by a hinge region. Previously, we described two novel mutations, E255- and G291D, located in the NADH-binding lobe, present in the cyanotic brothers first reported by Gibson in 1948. **Aims.** To identify any molecular defects in the DIA1 gene, which encodes the cytb5r enzyme, in Irish type 1 RCM patients and to relate the effect of any mutations detected to the function of the cytb5r enzyme. **Methods.** Individuals from 4 families with type I RCM were examined for DIA1 gene mutations by PCR-direct sequencing. Haplotype analysis was performed by PCR-direct sequencing using 11 SNPs located within 7-kb of the DIA1 gene. The D239G mutation was expressed in a heterologous expression system and spectroscopic, thermodynamic and thermostability studies were performed. **Results.** were compared to those obtained for the previously identified E255- and G291D NADH binding lobe mutations. **Results.** Sequencing the DIA1 gene in a group of Irish type I RCM patients detected a further novel NADH-binding lobe mutation, D239G, in two unrelated families, one of which belonged to the Irish Travelling Community. Separation of this community occurred during the 1845-1848 Irish famine but they remain genetically identical to the general Irish population. Haplotype analysis indicated that the D239G mutation was associated with the same haplotype in both families. The enzyme kinetic properties and thermostability of the D239G variant were investigated and this variant was found to possess normal protein stability with

only a minor reduction in enzyme activity to 94%. In contrast, both E255 and G291D variants exhibited reduced thermostability and catalytic activity, 38% and 58% of wild type activity respectively. In addition, the D239G mutation exhibited reduced affinity towards the NADH substrate and was more efficient at utilizing the NADPH cofactor. **Conclusions.** The loss of aspartic acid at amino acid 239 of cytb5r impacted on enzyme function by reducing its ability to use the normal NADH substrate thus indicating this amino acid plays the key role in substrate selectivity. The reduced function exhibited by the cytb5r D239G variant is concordant with the type I RCM phenotype manifested by the patients and this mutation may have originated in a common ancestor before the Irish Travelling Community become genetically isolated.

0469

HEMATIDE™, A PEPTIDIC ERYTHROPOIESIS STIMULATING AGENT THAT CORRECTS ANEMIA INDUCED BY PARTIAL NEPHRECTOMY AND ERYTHROPOIETIN-SPECIFIC ANTIBODIES IN RATS

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Hematide is a pegylated, synthetic peptidic erythropoiesis stimulating agent (ESA) that is in clinical development for the treatment of anemia associated with chronic kidney disease and cancer. The amino acid sequence of Hematide is unrelated to native erythropoietin (EPO) or recombinant erythropoietin (rHuEPO). **Methods & Results.** Single intravenous administration of Hematide has been demonstrated to produce a dose-dependent increase of both reticulocytes and hemoglobin (Hgb) of long duration in normocytic rats. In this report, this effect has been confirmed in two separate animal models of anemia. A single intravenous dose of 1 mg/kg was found to correct the anemia exhibited in rats following 5/6 nephrectomy with Hgb increased 6.0g /dL above baseline. Importantly, it was also observed that the drug's clearance from the plasma was nearly doubled in the nephrectomized rats compared to normocytic rats. The development of EPO-specific antibodies following treatment with rHuEPO has been observed in approximately 250 patients and causes pure red cell aplasia (PRCA) which requires the definitive withdrawal of rHuEPO. In this substudy, rats were randomly divided into six groups with 5-6 rats per group as indicated in the table below.

Groups	Route	Regimen	5 week break, then animals regrouped	Test Article	Route	Regimen
Vehicle	SC	TIW x 4		Vehicle	SC	TIW x 4
rHuEPO	SC	TIW x 4		rHuEPO (no antibodies)	SC	TIW x 4
rHuEPO	SC	TIW x 4		rHuEPO (EPO antibodies)	SC	TIW x 4
rHuEPO	SC	TIW x 4		Hematide (EPO antibodies)	SC	QW x 4
Hematide	SC	QW x 4		Hematide	SC	QW x 4
Hematide	IV	QW x 4		Hematide	IV	QW x 4
Hematide	IV	QW x 4		Hematide	IV	QW x 4

Hematide (or vehicle) was administered every week (QW) while rHuEPO was administered three times per week (TIW) at pharmacologically active doses. After 9 weeks, rHuEPO treated-animals were re-assigned based on the presence of EPO-antibodies; the antibody-positive animals were divided into groups to further receive either rHuEPO or Hematide; antibody-negative animals received further rHuEPO treatment. Hematide stimulated erythropoiesis when administered by either IV or

SC routes. Tachyphylaxis and Hematide-antibodies were not observed after repeated administration. In contrast, rHuEPO administration caused the production of rHuEPO-antibodies in 11/17 animals. The appearance of antibodies correlated with significantly decreased Hgb levels. Hematide treatment of a group of animals with EPO-antibodies and low Hgb levels, corrected the antibody-induced anemia. **Conclusions.** Hematide is a potent ESA which corrects anemia induced by nephrectomy and EPO-specific antibodies in rats and may also be potentially used to correct the anemia induced by rHuEPO (PRCA) in humans.

0470

HEMATIDE™, A SYNTHETIC PEPTIDE-BASED ERYTHROPOIESIS STIMULATING AGENT (ESA), DEMONSTRATES DOSE DEPENDENT ACTIVITY IN A PHASE 1 SINGLE DOSE, DOSE ESCALATING STUDY IN NORMAL HEALTHY VOLUNTEERS (NHV)

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Hematide is a synthetic, PEGylated, peptidic ESA that binds to and activates the erythropoietin (EPO) receptor. Although its amino acid sequence is unrelated to that of EPO, Hematide demonstrates *in vitro* activity comparable to EPO and due to sustained plasma persistence is dosed less frequently than EPO in animal studies. A randomized double-blind placebo controlled dose escalation study was conducted to assess safety, pharmacokinetics and pharmacodynamics (PD) of Hematide administered as a single IV infusion to male NHV. Up to 7 cohorts, each with 7 subjects (5 on Hematide and 2 on placebo), were planned with doses escalated according to protocol-specified stopping rules. **Results.** Three successive cohorts were enrolled at increasing doses until the protocol-specified stopping rule for a 1 g/dL increase in hemoglobin (Hgb) was met with Cohort 3. To confirm the results in Cohort 3, a fourth cohort was enrolled at the same dose and hence 28 volunteers completed the study. Hematide appeared to be safe and well tolerated at all doses tested, with a safety profile similar to placebo and no pattern across cohorts. Reticulocyte and Hgb increases were dose proportional with all p values (F-test) <0.0001. See table below. Reticulocytes counts peaked at 7-9 days and returned within baseline at 10-14 days post injection. The mean difference in changes in Hgb from baseline remained > 0.5 g/dL between the high dose group and the placebo group until day 42 after injection.

Group	Mean Peak Reticulocytes (17%)(SD)	Mean CRP _{max} ¹ g/dL (SD)	Mean CRP _{day 2} ² g/dL (SD)
Placebo (n=6)	8%(7)	0.38 (0.26)	0.36 (0.36)
Low Dose (n=6)	14% (23)	0.38 (0.28)	0.36 (0.46)
Median Dose (n=6)	18% (44)	0.07 (0.27)	0.54 (0.34)
High Dose (n=6)	22% (40)	0.61 (0.49)	1.30 (0.36)

¹: mean changes from baseline in Hgb; ²: average of maximum changes from baseline in Hgb

Changes in other PD parameters (increased red blood cell counts and hematocrit, transient decrease in ferritin and in content of reticulocyte hemoglobin, transient increase in transferrin soluble receptor protein, and transient decrease in erythropoietin) were consistent with stimulation of erythropoiesis. A single IV injection of Hematide administered to NHV was well tolerated and demonstrated potent and sustained dose dependent erythropoietic activity. These data are being used to plan Phase 2 studies of Hematide in patients with anemia related to cancer and renal failure. The sustained increase in Hgb of at least once month following a single dose of Hematide is compatible with a reduced frequency of administration compared to that of currently available ESAs.

0471

RESULTS OF A RANDOMISED, DOUBLE-BLIND, ACTIVE-CONTROLLED TRIAL OF DARBEPOETIN ALFA ADMINISTERED ONCE EVERY 3 WEEKS FOR THE TREATMENT OF ANAEMIA IN PATIENTS RECEIVING MULTICYCLE CHEMOTHERAPY

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Background. Chemotherapy-induced anaemia (CIA) results in a significant risk of red blood cell transfusions and is often associated with debilitating fatigue. Darbepoetin alfa (Aranesp®; DA), an erythropoietic protein with a half-life of over 70 hours in cancer patients, is licensed in Europe for the treatment of CIA using either every-3-week (Q3W) or weekly (QW) administration. The ability to administer DA on a Q3W schedule, compatible with most chemotherapy regimens, is of special interest to healthcare providers and their patients. **Aims.** To evaluate the comparability (non-inferiority) of a fixed starting dose of 500mcg Q3W DA with 2.25mcg/kg QW DA with respect to efficacy and safety. **Methods.** This was a randomised, double-blind, double-dummy, active-controlled phase 3 study in 160 centres across Europe. Eligible subjects were ≥18 years of age, anaemic (haemoglobin [Hb]<11g/dL), and diagnosed with a non-myeloid malignancy with ≥12 weeks of planned chemotherapy. Patients were randomized 1:1 to either 500mcg Q3W DA or 2.25mcg/kg QW DA for up to 15 weeks. Randomization was stratified by tumour type, screening Hb (<10 or ≥10g/dL), and region. The primary endpoint was incidence of transfusions from week 5 to end of treatment phase. For this endpoint, a two-sided 95% confidence interval (CI) was calculated for the difference between groups. A pre-specified non-inferiority margin was based on the treatment effect observed in previous randomised, placebo-controlled trials of DA 2.25mcg/kg (Vansteenkiste 2002; Hedenus 2003). Hb target range was pre-specified based on the recommendations from the evidence-based practice guidelines (EBPG) in anaemia (EORTC, NCCN, and ASH/ASCO). The primary analysis was based on the set of patients who received ≥1 dose of study medication and who were enrolled in the study until at least day 29. **Results.** Seven hundred and five patients were randomised; 672 were analysed for the primary endpoint. Demographic characteristics were similar between the two treatment groups. Mean age was 59.0 (range 20 to 86) years, and 55% were female. The mean weight of 68.5 (SD 13.8) kilograms suggests that 500mcg is the appropriate starting dose to deliver 6.75mcg/kg Q3W DA. Adjusting for stratification factors, the incidence of transfusions (95% CI) was 19% (15 to 24) and 28% (23 to 33) for the Q3W and QW groups, respectively (difference -6.7% [-13.2 to -0.2]). The difference between treatments without adjustment was 6.8% (-13.7 to 0.1) in favour of the Q3W arm. Hb profiles over time were identical between groups with over 70% of patients in both groups achieving the EBPG recommended target range (11-13g/dL). In the Q3W arm, the median number of doses was five (compared with 14 doses in the QW arm), with an average dose requirement equivalent to 125.2mcg/week. Overall, DA was well tolerated and no differences in toxicities or thrombotic events were observed between the groups. **Conclusions.** The Q3W regimen was comparable to the QW regimen since the upper limit of the CI of the transfusion incidence fell substantially below the pre-determined non-inferiority margin. These results demonstrate effective anaemia management with less frequent(Q3W) dosing of DA.

0472

PHARMACOKINETIC DATA FROM A PEDIATRIC CLINICAL TRIAL SUPPORTING ONCE-DAILY ORAL ADMINISTRATION OF ICL670 IN PATIENTS WITH TRANSFUSION-DEPENDENT β -THALASSEMIA

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Background. ICL670 (deferasirox) is a once-daily, oral iron chelator in Phase III clinical development that has demonstrated good efficacy and tolerability in adult and pediatric patients with transfusional iron overload. **Aims.** To determine the pharmacokinetics (PK) of ICL670 in pediatric patients with transfusion-dependent β -thalassemia major. **Methods.** In this open-label, multicenter, 48-week Phase II trial, 40 pediatric patients (2 to <17 years) with β -thalassemia major were recruited and stratified by age at baseline: adolescents aged 12 to <17 years (n=20) and children aged 2 to <12 years (n=20). As this was the first pediatric study of ICL670, a conservative daily dose of 10 mg/kg was administered irrespective of baseline liver iron content. In addition, treatment of adolescents commenced prior to treatment of younger children. PK profiles were obtained pre-dose and at specified intervals for 24 hours after the very first dose given and similarly after daily administration for 2 and 4 weeks. Plasma concentrations of ICL670 and its iron complex Fe-[ICL670]₂ were determined by a liquid chromatography-mass spectrometry/mass spectrometry method. Standard non-compartmental PK parameters (AUC, C_{max}, t_{max}, t_{1/2}) were derived from the plasma concentrations of ICL670 and Fe-[ICL670]₂. **Results.** The mean ICL670 plasma concentration increased rapidly in both groups following the first dose administered (median t_{max} of 2 hours; range 1–8 hours). Following multiple ICL670 dosing in both groups, the median t_{max,ss} for weeks 2 and 4 were 2 and 4 hours, respectively (range 1–9 hours). C_{max}, AUC₀₋₂₄ and t_{1/2} of single and multiple doses of ICL670 were comparable in children and adolescents (Table 1).

Table 1. Pharmacokinetic evaluation of ICL670 after single and multiple 10 mg/kg/day doses in pediatric patients

	Group	C _{max} (μ mol/L)	AUC ₀₋₂₄ (h \cdot μ mol/L)	t _{1/2} (hours)
Single-dose (day 1); mean ¹ \pm SD	Adolescents (n=10)	27.5 \pm 11.0	282.8 \pm 82.8	11.5 \pm 6.5
	Children (n=8)	28.8 \pm 10.2	281.1 \pm 94.3	8.8 \pm 2.0
Multiple-dose (week 4); mean ¹ \pm SD	Adolescents (n=10)	46.6 \pm 11.7	619.2 \pm 236.1	13.4 \pm 8.6
	Children (n=9)	40.8 \pm 13.6	536.2 \pm 231.6	10.8 \pm 3.4

¹Anti-heparin solution

Trough ICL670 plasma concentrations showed no accumulation and were stable following daily dosing over 48 weeks; values were comparable to those observed in adult patients. In comparison to day 1, AUC₀₋₂₄ was 60% higher at week 2 (90% CI for the ratio: 1.38, 1.85) and 100% higher at week 4 (90% CI for the ratio: 1.72, 2.31). AUC₀₋₂₄ was 9% higher in adolescents than children (90% CI for the ratio: 0.66, 1.80). However, this may be due to small patient numbers and high interpatient variability. **Conclusions.** The PK characteristics of ICL670 after single and multiple dosing were similar in children and adolescents and suitable for once-daily oral administration in pediatric patients.

0473

MOLECULAR CHARACTERISATION OF FOUR PORTUGUESE PATIENTS WITH PYRIMIDINE 5'-NUCLEOTIDASE DEFICIENT HAEMOLYTIC ANAEMIA

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Background. Pyrimidine 5'-nucleotidase (P5'N) deficiency causes chronic nonspherocytic haemolytic anaemia, characterised by marked basophilic stippling within the erythrocyte, due to the accumulation of pyrimidine nucleotides and ribosomal structures during reticulocyte maturation. Two cytosolic isoforms are known: P5'N-I specific to substrates UMP and CMP, and P5'N-II with preference to deoxypyrimidine nucleotide monophosphates. The anaemia has an autosomal recessive pattern of inheritance, with patients having P5'N-I isoenzyme activity severely reduced. P5'N-I gene (NT5C3) has been reported to have 11 exons (1-2-R-3 to 10), producing 3 mRNAs forms by alternative splicing: one, ubiquitously expressed, lacking the nucleotide sequence corresponding to exons 2 and R, encoding for a polypeptide with 286 aminoacids (p36), and two other mRNAs almost specifically expressed in reticulocytes - P5'N-R, with all the exons, encoding for 285 aminoacids and P5'N-I, lacking exon R, encoding for 297 aminoacids. **Aims.** To establish the molecular basis of P5'N deficient haemolytic anaemia in Portuguese patients. **Patients:** Four unrelated Portuguese patients with chronic haemolytic anaemia due to P5'N deficiency were studied. The diagnosis was based on the clinical history, haematological and biochemical data, marked basophilic stippling, increased reticulocytes count and decreased purine/pyrimidine nucleotides ratio. Age of diagnosis ranged from 3 to 74 years. To exclude other causes for chronic haemolysis, the samples were screened for the most common glycolytic enzyme deficiencies. Reduced P5'N-I isoenzyme activity in red blood cell haemolysates was verified by electrophoretic analysis. **Methods.** After informed consent, genomic DNA and mRNA were extracted from peripheral blood samples. The entire coding sequence and adjacent regions of P5'N-I gene were amplified by PCR, submitted to SSCP analysis and sequenced. RT-PCR studies, followed by DNA sequencing, were performed in one patient. **Results.** Two patients are homozygous for P5'N-I gene missense mutations: the transversion 502G>C in exon 8, predicting the aminoacid substitution 168Gly>Arg, and the transition 773T>C in exon 9, predicting the aminoacid change 258Ile>Thr. The remainder two patients are homozygous for a P5'N-I allele with an Alu element insertion in exon 9, between nucleotides 743-744. Comparative analysis of Alu elements in reference database Repbase showed that P5'N-I Alu elements shared 98.2% identity with the AluYa5 and AluYa1 subfamilies. RT-PCR analysis in one of these patients with the Alu insertion showed that the P5'N-I mRNA transcripts lacked the nucleotide sequence corresponding to exon 9, having the normal exons 8 and 10 sequences. **Summary/conclusions:** In four unrelated Portuguese patients with P5'N deficient anaemia we identified three new P5'N-I gene mutations. The missense mutation 502G>C predicts the nonconservative aminoacid substitution 168Gly>Arg in a conserved region of the polypeptide chain. A second missense mutation, 773T>C, is responsible for the aminoacid change 258Ile>Thr in a region believed to be near the substrate binding site. The third mutation is the insertion of an Alu element in exon 9 leading to the skipping of nucleotide sequence corresponding to exon 9 in the mRNA transcript. All patients have their mutations in the homozygous state, in concordance with the genetic profile for the disease in other populations.

0474

ERYTHROPOIETIN, HYDROXYUREA AND HUMAN STEM CELL FACTOR EFFECTS, ALONE AND IN COMBINATIONS, ON ERYTHROID DIFFERENTIATION AND HEMOGLOBIN PRODUCTIONA. Likousi¹, G. Paterakis², E. Gousetis³, M. Theodosaki³, M. Papadakis⁴, S. Bournazos¹, C. Veneti⁵, B. Tsagari⁵, M. Katelani⁶, N. Stathakis⁷, P. Kollia¹

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Complete understanding of fetal hemoglobin (HbF) production in adult erythroblasts is critical for the treatment of sickle cell anemia and α -thalassemia. Pharmacologic induction of HbF has favorably influenced the course of disease in patients with thalassemic syndromes. In this context, several studies have investigated molecular mechanisms that regulate expression of the α -globin gene, as well as pharmacological agents that stimulate HbF production. In this study, we evaluated the effects of augmented erythropoietin (EPO) doses combined with hydroxyurea (HU) and/or stem cell factor (SCF) on fetal hemoglobin modulation in adult erythroid cells. CD34+ cells from normal donors cultured in serum-free StemSpan medium were exposed to baseline/augmented EPO (3/10 u/mL) +/- SCF (100ng/mL). Different HU concentrations (100/300 μ M) were added to erythroid cells on days 6 or 12. Immunophenotypic analysis was performed by using flow cytometry with monoclonal antibodies directed against glycophorin A (GPA), CD71, CD117 and F-cells. The homogeneity of erythroid cultures was evidenced by high expression of CD71 and GPA (100% and 50%, respectively) on day 7 of the culture. The percentage of cells expressing CD117 at significant levels increased during the first week in culture; thereafter, CD117 levels declined by day 12 and further by HU addition in a dose-dependent fashion. In the absence of SCF, cells eventually became CD117-negative. On day 6, 1-2% F-cells were detected; HU addition led to a 3-4-fold increase; the increase in F-cell numbers was proportional to EPO dose. In the absence of SCF, F-cell numbers by day 12 were decreased. Quantification of α -, β - and γ -mRNA were performed by real-time PCR. Addition of HU (100/300 μ M) for 1-3 days caused a significant time- and dose-dependent increase in α - and β -mRNA and a slightly higher increase in γ -mRNA. α -globin mRNA levels remained stable in SCF-naïve cultures. Hbs and globin chains were separated and measured by ion exchange and reverse-phase HPLC, respectively. On days 14 and 17, fetal and adult globin chains determined for cells grown in Epo+SCF were significantly higher than in cells grown in Epo alone (2-3 fold); EPO effects were dose-dependent. The addition of HU resulted in an even higher elevation of all globin chains. In the absence of SCF, α - and β -globin chain levels increased, while γ -chains became barely detectable. These results indicate that: (i) Epo has a significant synergistic effect with HU; (ii) the presence of SCF is essential both for erythroid cell growth and fetal hemoglobin production. Further understanding of the mechanisms implicated in modulation of HbF production by SCF may assist in the development of new treatments for hemoglobinopathies.

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THE ONCE-DAILY ORAL IRON CHELATOR ICL670 IS WELL TOLERATED AND EFFECTIVE IN TREATING TRANSFUSIONAL IRON OVERLOAD IN DIAMOND-BLACKFAN ANEMIA PATIENTSG. Tchernia¹, E. Vichinsky², M. Jeng³, H. Cario⁴, J. Porter⁵, N. Olivieri⁶, A. Cohen⁷, J.M. Ford⁸, B. Rabault⁸, C. Ressayre-Djaffer⁸, D. Alberti⁸¹Hopital Kremlin Bicetre, LE KREMLIN BICETRE, France; ²Children's Hospital & Research, OAKLAND, USA; ³Stanford Hospital, STANFORD, USA; ⁴Universitaetsklinikum Ulm, ULM, Germany; ⁵University College Hospital, LONDON, UK; ⁶Toronto General Hospital, TORONTO, Canada; ⁷Children's Hosp. Philadelphia, PHILADELPHIA, USA; ⁸Novartis Pharma AG, BASEL, Switzerland

Background. There is currently no curative therapy for Diamond-Blackfan anemia (DBA) other than bone marrow transplantation, so regular transfusion from infancy is required for survival in steroid-resistant patients. The resultant iron overload has a significant impact on patient morbidity and mortality, unless effectively managed with iron chelation therapy. Deferoxamine (Desferal®, DFO), a standard iron chelation therapy, effectively treats iron overload. However, DFO's subcutaneous, slow infusion, five to seven times weekly is a demanding regimen that impacts patient compliance. ICL670 (deferisirox) is an investigational once-daily, oral iron chelator with demonstrated efficacy and acceptable tolerability in patients with transfusional iron overload. **Aims.** To evaluate the efficacy and tolerability of ICL670 in a cohort of transfusion dependent DBA patients, a sub-population of the C1CL670A0108 trial. **Methods.** This open-label, multicenter, 52-week, Phase II trial enrolled 30 patients with DBA from seven countries. Daily ICL670 doses were assigned based on a patient's liver iron content(LIC) at baseline. The iron chelation efficacy of ICL670 was evaluated based on changes in LIC (baseline versus end of study) and serum ferritin measured monthly. **Results.** Depending on baseline LIC, patients (median age 15 years; range 3–42 years) received either ICL670 5, 10, 20 or 30 mg/kg/day (n=1, 3, 8 and 18, respectively). Median duration of exposure to ICL670 was 52.4 weeks (range 23.7–58.1 weeks). At baseline, the mean LIC measured by biopsy or SQUID was 18.8±10.7 mg Fe/g dw, while mean serum ferritin was 3244.9±2438.9 µg/L. After 52 weeks of ICL670 treatment, there were changes for both mean LIC and serum ferritin at all doses: 5 mg/kg/day (n= 1; 3.8 mg Fe/g dw; 2473.0 µg/L); 10 mg/kg/day (n= 3; 2.8±3.1 mg Fe/g dw; 1083.7±391.9 µg/L); 20 mg/kg/day (n= 8; -2.3±3.3 mg Fe/g dw; -363.7±1001.5 µg/L); and 30 mg/kg/day (n = 18; -2.6±7.9 mg Fe/g dw; -366.1±1412.5 µg/L). A median of 13.5 blood transfusions (25–75% percentiles; 13–16) were administered to patients during the study, and the median amount of blood given was 0.38 mL RBC/kg/day (25–75% percentiles; 0.33–0.43). The mean rate of iron excretion (0.43±0.22 mg/kg/day) was slightly greater than iron intake (0.41±0.12 mg/kg/day). A total of 29 patients (96.7%) completed the study; one patient died, suspected to be due to underlying disease. All patients reported at least one adverse event(AE) during the study. All drug-related AEs were mild (14/22; 63.6%) or moderate (8/22; 36.4%), the most common being gastrointestinal AEs such as diarrhea, nausea and abdominal pain. Dose adjustments, primarily reductions, were needed in nine patients, due in 8 patients to AEs or laboratory test abnormalities. Mild serum creatinine increases >33% baseline were seen in 10 patients at 20 and 30 mg/kg, but never >1.5 x ULN. There were no incidences of drug-induced neutropenia or arthralgia. **Conclusions.** Once-daily oral ICL670 is well tolerated and appears to be effective in treating transfusional iron overload in Diamond-Blackfan anemia patients.

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DETERMINATION OF P-GLYCOPROTEIN PROFILE IN CML PATIENTS UNDERGOING IMATINIB MESYLATE THERAPYI. Svoboda-Beusan¹, D. Pulanic², R. Ajdukovic³, A. Sabioncello¹, J. Bozikov⁴, P. Majdak¹, J. Bulum², S. Rabatic¹, B. Labar²¹Institute of Immunology, Zagreb, Croatia; ²University Hospital Rebro, Zagreb, Croatia; ³Clinical Hospital Dubrava, Zagreb, Croatia; ⁴Andrija Stampar SPH, Zagreb, Croatia

Selective inhibition of the BCR-ABL tyrosine kinase by Imatinib mesylate (Glivec) has proven to be a promising frontline therapeutic strategy for patients with chronic myeloid leukemia (CML). Still, while Imatinib does induce a good cytogenetic and hematologic response, the molecular response remains poor with the development of clinical progression in some of the patients. Previously we have evaluated the occurrence of multidrug resistance (MDR) in cells of CML patients treated with Imatinib by monitoring the P-glycoprotein (Pgp) expression and function. Now we wanted to assess the link between the change in Pgp expression/activity and the clinical i.e. hematological, cytogenetic and molecular response to Imatinib. In a long term follow up (4 years in intervals of 3 months during first 2 years, every 6 months later on) of 34 adult CML patients (M/F=19/15; CP/AP=26/8), bone marrow (BM) and peripheral blood (PB) cells were examined using flow cytometry. Pgp phenotype was analyzed by specific antibody and Pgp activity was measured by Rhodamine (Rh123) efflux assay. Imatinib (400 mg/day for CP and 600 mg/day for AP) was started after an inefficient first line (HU, IFN- α and MDR-related) therapy. By now, 25 of 34 patients have been followed for ≥ 9 months. Molecular response at PCR analysis was demonstrated in 5 pts. The pts achieving hematological and cytogenetic response showed stable Pgp activity, well balanced after one year. We created individual Pgp profiles because of great variability in Pgp expression/activity of the BM and PB cells of our patients, especially in the early follow up period. Analyzing the association between the established Pgp profiles and the response to Imatinib, we were able to discern four types of Pgp profiles /response to therapy (Table).

IMPACT OF Pgp STATUS ON THERAPY OUTCOME

Pgp PROFILE	TRENDS OF Pgp ACTIVITY			CLINICAL REMISSION / CHEMORESISTANCE	
	↓	→	↑		
1	1	4	0	MOLECULAR	CURED (N=5)
2	5	7	3	COMPLETE /PARTIAL	SENSITIVE (N=15)
3	0	1	5	MINOR /MINIMAL	STILL SENSITIVE (N=6)
4	0	0	8	CLINICAL RELAPSE	RESISTANT (N=8)

(i) stable Pgp activity/molecular response; (ii) stable Pgp activity/clinical response; (iii) increasing Pgp activity/minor response-development of resistance to Imatinib; (iv) constant increase of Pgp activity/poor response-established resistance to Imatinib. We demonstrated that quantitative determination of Pgp status during Imatinib therapy does show differences in Pgp function, indicating that Pgp efflux could be one of the mechanisms of resistance to Imatinib. The increase of Pgp activity observed in pts refractory to Imatinib or relapsed pts and progression free survival in pts with stable Pgp activity indicate the importance of Pgp screening in pts with CML. From the prognostic point of view, long term follow up of the Pgp status with the determination of Imatinib-Pgp phenotypes may be helpful in the evaluation of the progression/relapse of CML.

Apoptosis and drug resistance II

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SENSITIZATION FOR γ -IRRADIATION-INDUCED APOPTOSIS BY SMAC

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Resistance to current treatment regimens such as radiation therapy remains a major concern in oncology and may be caused by defects in apoptosis programs. Since *Inhibitor of apoptosis proteins* (IAPs), which are expressed at high levels in many tumors, block apoptosis at the core of the apoptotic machinery by inhibiting caspases, therapeutic modulation of IAPs could target a key control point in resistance. Here, we report for the first time that mitochondrial or cytosolic Smac, an inhibitor of IAPs, significantly enhanced γ -irradiation-induced apoptosis and reduced clonogenic survival in neuroblastoma, glioblastoma or pancreatic carcinoma cells. Notably, Smac had no impact on DNA damage/DNA repair, activation of NF- κ B, upregulation of p53 and p21 proteins or cell cycle arrest following γ -irradiation indicating that Smac did not alter the initial damage and/or cellular stress response. Smac significantly enhanced activation of caspase-2, -3, -8 and -9, loss of mitochondrial membrane potential and cytochrome c release upon γ -irradiation. Inhibition of caspases also blocked γ -irradiation-induced mitochondrial perturbations, indicating that Smac facilitated caspase activation, which in turn triggered a mitochondrial amplification loop. Interestingly, mitochondrial perturbations were completely blocked by the broad range caspase inhibitor zVAD.fmk or the relatively selective caspase-2 inhibitor zVDVAD.fmk, whereas caspase-8 or caspase-3 inhibitors only inhibited the increased drop of mitochondrial membrane potential provided by Smac, suggesting that caspase-2 was acting upstream of mitochondria upon γ -irradiation. In conclusion, our findings provide compelling evidence that targeting IAPs, e.g. by Smac agonists, is a promising strategy to enhance radiosensitivity in various human cancers.

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INHIBITION OF CLONOGENIC TUMOR GROWTH: A NOVEL FUNCTION OF SMAC CONTRIBUTING TO ITS ANTICANCER ACTIVITY

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Smac (second mitochondrial activator of caspases) is a mitochondrial protein that is released from mitochondria during apoptosis. Smac is described to promote apoptosis by binding and antagonizing IAPs (Inhibitor of Apoptosis Proteins). While Smac has been described to sensitize for apoptosis, its effect on tumor cell viability in the absence of apoptotic stimuli has remained unclear. Here, we report that Smac inhibits clonogenic tumor cell growth by blocking random migration and proliferation and by enhancing apoptosis in a cell density- and cell type-dependent manner in SH-EP neuroblastoma cells. Inhibition of clonogenic survival by overexpression of full-length or processed Smac strictly depended on low cell density and was reversible by replatement experiments at high density. We discovered that Smac inhibits cell motility and random migration at low cell density. In addition, Smac enhanced apoptosis and inhibited protein expression, but not mRNA synthesis of XIAP, survivin and other short-lived proteins (FLIP, p21) at low cell density, indicating that Smac globally inhibited protein expression. Also, Smac inhibited proliferation and increased polynucleation with no evidence for polyploidy, cell cycle arrest or senescence indicating that Smac impaired cell division. Interestingly, inhibition of clonogenic capacity by Smac occurred independently of its apoptosis promoting activity. By demonstrating that Smac restrains clonogenic growth, our findings may have important implications for control of tumor growth and/or its metastatic spread. Thus, Smac agonists may be useful in cancer therapy, e.g. for tumor control in minimal residual disease.

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BETULINIC ACID AS NEW ACTIVATOR OF NF- κ B: MOLECULAR MECHANISM AND IMPLICATIONS FOR CANCER THERAPY

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Nuclear factor- κ B (NF- κ B) is an important regulator of cellular stress-induced transcriptional activation. We previously found that Betulinic acid (BetA) triggers apoptosis in cancer cells by disrupting the mitochondrial membrane and by increased production of reactive oxygen species. Since oxidative stress may activate NF- κ B, we investigated the effect of BetA on NF- κ B activation. Here, we provide for the first time evidence that BetA activates NF- κ B, which, surprisingly, promotes BetA-induced apoptosis. Of note, activation of NF- κ B in response to treatment with BetA was observed in a variety of tumor cell lines derived from neuroblastoma, glioblastoma or melanoma. BetA induced DNA-binding of NF- κ B complexes consisting of p50 and p65 (RelA)-subunits. Accordingly, nuclear translocation of p65 upon addition of BetA was observed by immunofluorescence microscopy. Activation of NF- κ B by BetA involved an increase in IKK activity, phosphorylation of I κ B at serine 32/36 and subsequent decrease of I κ B protein level demonstrating that NF- κ B activation by BetA involved the IKK complex. Also, BetA induced NF- κ B-mediated transcription, which preceded BetA-induced apoptosis. Notably, inhibition of NF- κ B by different chemical inhibitors of NF- κ B (MG-132, PDTC, sul-fasalazine), which suppressed NF- κ B activation upon BetA treatment, attenuated BetA-induced apoptosis. Most importantly, specific NF- κ B inhibition by either transient or stable expression of an I κ B superrepressor mutant inhibited BetA-induced apoptosis pointing to a proapoptotic function of NF- κ B in BetA-triggered cell death. Thus, our findings that activation of NF- κ B by BetA promotes BetA-induced apoptosis provide novel insight into the mechanism of action of BetA and have important implications for the use of NF- κ B inhibitors in BetA-based treatment protocols.

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FLOWCYTOMETRIC QUANTIFICATION OF DRUG INDUCED MITOCHONDRIA- AND CASPASE-MEDIATED APOPTOSIS SIGNALING IN PRIMARY LEUKEMIA CELLS

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Background. Defects in apoptosis signaling are involved in leukemogenesis and may be responsible for drug resistance and treatment failure. Analysis of apoptosis pathways in primary leukemia cells for assessment of drug sensitivity or resistance has been hampered by the lack of convenient methods for measurement of activated apoptosis signaling. **Aims.** Quantification of key apoptosis signaling events such as caspase activation and mitochondrial alterations for analysis of apoptosis and drug resistance in primary leukemia cells *in vitro* and *in vivo*. **Methods.** We developed and characterized a flow cytometric method for combined measurement of caspase activation by cleavage of (Asp)²-Rhodamine 110 (D2R) and alterations in the mitochondrial membrane potential in living cells. The signal of the released Rhodamine can be measured in combination with the mitochondria membrane potential sensitive dye CMXRos by flowcytometry. **Results.** Upon apoptosis induction, in cell lines and in primary leukemia cells, the released Rhodamine shows a strong signal in living cells. No such signal is obtained in post-apoptotic cells, indicating that the Rhodamine substrate measures an early and specific step of apoptosis signaling. The derivative of D2R, (Z-DEVD)²R, also applicable in live cells, proved to specifically measure activity of recombinant caspase-3, -7 and -10. In Jurkat leukemia T-cells and Reh leukemia B-cells, simultaneous measurement of caspase activity and mitochondrial membrane potential in the same cell identified a dif-

ferential induction of mitochondrial dependent and independent caspase activation by different cytostatic drugs. Despite similar induction of cell death, a differential activation of caspases by Cytarabine and Cyclophosphamide could be quantified, indicating that in addition to deficient caspase activation, drug specific differences in activation of apoptosis signaling can be assessed. In primary leukemia cells, flow cytometric analysis with (Z-DEVD)2R specifically detected proficient and deficient caspase activation in T-ALL and B-precursor ALL cells. We could also show complete inhibition of DEVDase activity by ZVAD-fmk in primary leukemia cells. However, cell death was largely unaffected by caspase inhibition, suggesting that caspase independent cell death mechanisms are operative in drug induced leukemia cell apoptosis *in vitro*. In a xenotransplant model for human leukemia, we could quantify drug induced activation of caspases by Cytarabine and Cyclophosphamide *in vivo*. CONCLUSIONS/PERSPECTIVES: Measurement of DEVD2R cleavage in primary leukemia cells permits detection of chemotherapy induced caspase activation. Quantification of cellular caspase activation reveals differential induction of apoptosis signaling by cytotoxic drugs. The marked heterogeneity of drug induced apoptosis signaling observed in primary leukemia warrants further studies on its prognostic value and the underlying molecular mechanisms of drug induced caspase activation.

0481

PROSTATE-APOPTOSIS-RESPONSE-GENE-4 SENSITIZES NEOPLASTIC LYMPHOCYTES TO TRAIL-INDUCED APOPTOSIS

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Failure of neoplastic cells to undergo apoptosis upon stimulation of the extrinsic pathway contributes to the development of chemoresistance. Acknowledging the previously demonstrated deregulated expression of the prostate-apoptosis-response-gene-4 (par-4) in ex vivo cells of patients suffering from acute and chronic lymphatic leukemias, we hypothesized that expression of par-4 influences sensitivity of neoplastic lymphocytes to stimulation with an agonistic TRAIL-antibody. Evaluating this hypothesis we thus show, that par-4-transfected Jurkat cells exhibit a three-fold increase in rate of apoptosis upon incubation with the TRAIL-antibody as compared to their mock-transfected counterparts. Defining the underlying molecular mechanisms we provide evidence, that par-4 promotes activation of the initiator caspase-8. Despite concomitantly upregulating Bcl-2, overexpression of par-4 enhances apoptosis via the mitochondrial pathway by increasing cleavage of Bid and activation of caspase-9. Moreover, expression of par-4 results in an enforced activation of the executioner caspases-6 and -7, whereas caspase-3 remains unaltered, effects observed with a concomitant down-regulation of the inhibitors-of-apoptosis proteins cIAP-1 and XIAP. In conclusion, we here provide first evidence that expression of par-4 augments sensitivity of neoplastic lymphocytes to TRAIL-induced cell death, and describe the impact of par-4 expression on key molecules considered crucial in the induction of apoptosis via the extrinsic pathway.

0482

OCTREOTIDE MODULATES MULTIDRUG RESISTANCE ASSOCIATED PROTEIN EXPRESSION AND INCREASES CHEMOSENSITIVITY OF HUMAN LEUKEMIA CELL LINES TO ADRIAMYCIN

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Background: A major problem in the treatment of leukemia is drug resistance to chemotherapeutic agents that appears at diagnosis or after chemotherapy. as a minimal residual disease. The cellular drug resistance proteins permeability-related glycopro-

tein (P-Gp/MDR-1) and multidrug-resistance associated protein (MRP-1), which function as drug efflux pump, participate in anthracycline resistant mechanisms. Octreotide (OCT) is an eight amino acid peptide, which attains its biological effects on target cells by binding preferentially to high affinity membrane bound G-protein-coupled somatostatin sst2 and, to a lesser extent, to sst3 and sst5 receptors (SS-Rs). The expression of SS-Rs in human lymphoid leukaemia cell lines, in malignant lymphomas and in lymphoproliferative diseases is clearly detectable. However, the effect of OCT on the expression of drug resistance proteins is still unknown. *Aims.* We investigated the *in vitro* effect of OCT alone or in combination with adriamycin (ADR) on the MRP-1 expression in two, and the *in vitro* cytotoxic effect of ADR alone or in combination with OCT in four lymphoid leukemia cell lines and in relation to sst2 and sst5 expression. *Methods.* The *in vitro* effect of OCT alone or in combination with ADR on the MRP-1 expression was assessed in Jurkat and MOLT-4 human lymphoblastic leukemia cell lines by the detection of MRP-1 mRNA. Cells were treated for 48 hours with OCT (1-10 microM) and/or ADR (0.005-7 microM). RNA was extracted from cells by the RNazol method, cDNA amplification was carried out by RT-PCR and evaluation of MRP1 mRNA expression was accomplished by quantification of the electrophoresed specific PCR products by Molecular Imager FX (Biorad). The *in vitro* cytostatic and cytotoxic effects of ADR alone or in combination with OCT on Jurkat, MOLT-4, CCRF-CEM and RPMI-8226 human lymphoid leukemia cell lines were evaluated with the MTT colorimetric metabolic assay. The mean concentrations of each drug that generated 50% or total (100%) growth inhibition (GI50 and TGI, respectively) as well as the drug concentrations that produced cytotoxicity of 50% of the cultured cells (IC50) were calculated by the linear regression method. *Results.* Although OCT or ADR alone did not affect the MRP-1 expression, when cells were treated with OCT in combination with ADR the MRP-1 expression was almost completely downregulated in a dose dependent manner regarding to ADR concentration. OCT did not induce cytostasis or cytotoxicity in any of the concentrations tested and treated cell lines. However, the combinations of OCT with ADR significantly augmented the cytostatic or cytotoxic effects of ADR in all treated cell lines (p<0.001). *Conclusions.* OCT significantly augments the ADR antileukemic activity probably by downregulating the MRP-1 expression. This effect of OCT requires the cytotoxic effect of ADR in a dose-dependent manner. These important observations need further investigation and may prove significant for the development of more effective strategies for the treatment of leukemias or lymphomas. Email address: research@energonbio.com

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ERK5 FORMS ARE MODULATED BY REGULATING PHOSPHORYLATION AND PROTEIN LEVEL IN LEUKAEMIA CELLS UNDERGOING HYPOXIA-INDUCED APOPTOSIS

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1 (*Background*). Hypoxia is a master regulator of haematopoiesis whose effects in leukaemia cell populations are not well characterized yet at either the cell biology or molecular level. 2 (*Aims*). Some molecular aspects of the response to severe hypoxia of myeloid leukaemia cell lines cells have been investigated in this study. 3. (*Methods*) Friend's murine erythroleukaemia (MEL) cells, or the human leukaemia cell lines K562 and HL60 were incubated at 0.1% O₂ in an anaerobic incubator, or in normoxia, and a number of parameters, as described below, were determined at different times of incubation. 4 (*Results*). Hypoxia prevented the MEL cell number increase which occurred in normoxia and determined early and massive apoptosis, as well as cell cycle arrest of surviving cells. The AKT protein, an important pro-survival signal, was cleaved in hypoxia. Hypoxia decreased the intensity and duration of

ERK^{1/2}, p38 and JNK activation occurring in normoxia, without altering the expression of these proteins. On the other hand, hypoxia suppressed the p120 ERK5 constitutive activation and protein expression, unchanged in normoxia. ERK5 mRNA was not decreased in hypoxia. Phosphorylation of a p82 ERK5 form was also abrogated in hypoxia, although protein level massively increased, but not in normoxia. This down-modulation was also observed in HL60 and K562 cells undergoing hypoxia-induced apoptosis. The disappearance of p120 ERK5, never reported before, and the dephosphorylation of p82 ERK5 were prevented by the treatment with z-VAD, a pan-caspase inhibitor. 5 (Conclusions) The results of this study are consistent with a role of ERK5 as a pro-survival signal which is suppressed in leukaemia cells undergoing hypoxia-induced apoptosis.

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INVOLVEMENT OF PML-RAR α ONCOPROTEIN IN THE REPRESSION OF P-GP FUNCTION IN ACUTE PROMYELOCYTIC LEUKEMIA

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Multidrug resistance (MDR) phenotype, mediated by permeability glycoprotein (P-gp), is the best characterized mechanism of resistance to chemotherapy in acute myeloid leukemia. P-gp, encoded by MDR1 gene is low expressed in acute promyelocytic leukemia (APL) cells. The PML-RAR α fusion protein, present in APL, is thought to exert its leukemogenic activity through the formation of repressor complexes containing nuclear co-repressors and histone deacetylases (HDAC), leading to aberrant transcription. Recent studies suggested that MDR1 gene expression is regulated by epigenetic mechanisms involving HDAC and DNA methyltransferases. Therefore, it is conceivable that the PML-RAR α oncoprotein influences MDR1 expression. Here, we evaluated P-gp function in hCG-PML-RAR α transgenic mice (PML-RAR-TM), which develop APL after a long pre-leukemic phase, and in blasts of human APL. P-gp activity was evaluated using the rhodamine (Rh123) efflux assay by flow cytometry. Rh123 fluorescence was measured in CD117+ bone marrow cells from 22 PML-RAR-TM before and after developing APL. Age-matched wild-type littermates were studied as controls. Among pre-leukemic PML-RAR-TM, P-gp function was reduced in comparison to matched controls (30.6 \pm 3.0% versus 38.6 \pm 2.4%, p=0.015). Moreover, P-gp deregulation increased with age, being unaltered in PML-RAR-TM younger than 8 months, but significantly lower in PML-RAR-TM older than 8 months, compared to matched controls (29.1 \pm 3.0% versus 40.1 \pm 2.8%, p=0.003). In addition, among leukemic mice, P-gp function was significantly lower than in the pre-leukemic counterparts (10.11 \pm 11.16% and 30.6 \pm 3.0%, respectively; p=0.025). Western blot analysis confirmed that the decrease of P-gp function was due to lower protein expression. Treatment of PML-RAR-TM blastic cells *in vitro* with ATRA at 10⁻⁶ M for 48 hours increased P-gp function in comparison to non-treated leukemic cells (10.9 \pm 2.0% versus 6.5 \pm 1.3%, p=0.004). In order to explore the involvement of HDAC and DNA methyltransferases in MDR1 expression in APL, we evaluated P-gp function in APL blasts of 7 patients, cultured with ATRA at 10⁻⁶M, HDAC inhibitors, TSA (0,1 μ M) and PB (1mM), and methylation inhibitor 5-aza-2'-deoxycytidine (5-AZA, 1 μ M). P-gp function was evaluated after 24, 48 and 72 hours. No differences in P-gp function were observed in 24 hours of culture for any of the drugs. ATRA partially increased P-gp function at 48 hours (27,26 \pm 12,7% versus 13,16 \pm 11,5% for ATRA and control respectively; P<0.02) and 72 hours (20,66 \pm 4,7% versus 8,61 \pm 7,23% for ATRA and control respectively; P<0.01). However, neither HDAC inhibitors nor methylation inhibitor exerted any influence on P-gp function. These results show that suppression of MDR1 expression precedes overt acute leukemia in PML-RAR-TM and suggest that PML-RAR α oncoprotein is involved in the downregulation of the MDR1 gene in both human and transgenic models of APL.

ATRA therapy increases P-gp function in APL blast cells, but not HDAC or methylation inhibitors alone, suggesting that other epigenetic mechanisms are involved in the regulation of MDR1 expression in APL.

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DEREPRESSION OF THE LYSOZYME GENE BY DEMETHYLATION WITH 5-AZA-2'-DEOXYCYTIDINE (DECITABINE) IS INDEPENDENT OF HISTONE DEACETYLASE INHIBITION IN AML1/ETO POSITIVE KASUMI-1 CELLS

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In acute myeloblastic leukemia with t(8;21) the AML1/ETO fusion gene acts as a repressor by mediating histone deacetylase (HDAC) activity to targets of wild-type AML1. Recently Klisovic et al. proposed direct interactions between AML1/ETO and DNA methyltransferases (DNMT) (Cancer Res. 65 (4):1277-84. 2005). The human lysozyme (LZM) gene (containing five consensus binding sites for AML1/RUNX1 in its 5' flanking region) has both a closed chromatin structure and is highly methylated in immature myeloid cells. In a U-937 cell model, low-level LZM transcription is further repressed following induction of AML1/ETO protein. We now addressed whether this AML1/ETO-mediated LZM gene repression may independently be antagonized by inhibition of DNMTs by 5-aza-2'-deoxycytidine (Decitabine, 5-azaCdR) and HDACs by Trichostatin A (TSA). Analyses of both the 5' region and exon 4 CpG island of the LZM gene in AML1/ETO-positive Kasumi-1 cells by Southern blot and bisulfite sequencing showed incomplete methylation. LZM mRNA levels in Kasumi-1 cells but not in several AML1/ETO-negative myeloid cell lines were specifically upregulated both with 5-azaCdR and TSA. Sequential treatment with different concentrations of 5-azaCdR followed by TSA showed no synergistic effects, but instead revealed a slightly inhibitory effect compared to 5-azaCdR alone. Marked regional demethylation was noted after treatment of Kasumi-1 with 5-azaCdR but not TSA, and was not a sequela of differentiation, since expression of CD34, myeloperoxidase, CD33/13, CD11b, and CD14 were unaltered. By 'MspI protection' assay, increased chromatin accessibility within the 5' LZM gene was observed concomitantly with 5-azaCdR-induced demethylation. In contrast, TSA treatment had no effect on MspI accessibility, but resulted in increased acetylation of histones H3 and H4 both in the 5' flanking region and exonic CpG island. In conclusion, antagonization of repressive activity in an AML1/ETO leukemia model, but not AML1/ETO negative myeloid cells was independently achieved by inhibition of DNA methylation and of histone deacetylation. The lack of a typical CpG island in the 5' flanking region may be responsible for these independent effects. Further studies are ongoing to test whether AML1/ETO protein recruits methyltransferase activity to the LZM gene, leading to hypermethylation of its CpG-rich regions.

Autologous transplantation

0486

PATIENTS UNDERGOING 2ND MOBILIZATION FOLLOWING A FAILED PERIPHERAL BLOOD STEM CELL MOBILIZATION: FACTORS INFLUENCING A SUCCESSFUL OUTCOME

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Background. A significant proportion of patients fail to harvest following peripheral blood (PB) stem cell mobilization. Little is known about the outcome following a second mobilization

attempt and the optimal priming regimen has not been established. *Aims.* To assess the factors influencing successful outcome after a second stem cell mobilization. *Methods.* 36 patients with hematological malignancies (multiple myeloma n=9, follicular lymphoma n=8, diffuse large B cell lymphoma n=7, leukemia n=6, mantle cell lymphoma n=2, Hodgkin's disease n=2, amyloid n=1, T cell NHL n=1) failing PB mobilization between 1999 - 2004 proceeded to a second harvest. Data was collected on number, type and timing of previous therapy as well as renal function. Harvest data included type and timing of primes, harvest yield and same day PB CD34+ counts. *Results.* The first prime was cyclophosphamide/G-CSF in 27 (75%), G-CSF alone in 2 (6%) and disease specific therapy in 7 (19%). Second prime was cyclophosphamide/G-CSF in 17 (47%), G-CSF alone in 10 (28%) and disease specific therapy in 9 (25%). Median lines of previous therapy were two (range 1-3). 33 patients had exposure to alkylating agents and 6 to platinum compounds. 14 (39%) achieved an adequate collection ($>2 \times 10^6$ CD34/kg) after 2 mobilizations. Successful reharvest primes were cyclophosphamide/G-CSF in 9/17 (37%), ESHAP/G-CSF in 3/6 (50%) and G-CSF alone in 2/10 (20%). More lines of treatment, alkylating agents and platinum did not increase the reharvesting failure rate. Time from last chemotherapy to second harvest and from first to second harvests was shortest in successful harvests (median 16 versus 19.5 weeks and 47 versus 86 days respectively) but the difference was not statistically significant. There was renal impairment in 10 (28%) but only 2 (14%) of successful mobilizers. Of patients not harvested on first mobilization due to poor PB CD34 counts (n=21), 17 (81%) still failed after 2. However if $>1 \times 10^6$ /kg was harvested on the first occasion (n=9), 7 (78%) achieved a total $>2 \times 10^6$ /kg after the second mobilization. To assess whether PB CD34/microlitre predicted successful harvesting at second mobilization, PB counts were compared with that days harvest CD34 count/kg. Linear regression analysis showed a highly significant association at second harvest ($R=0.95$, $p=0.0000$). *Conclusions.* The amount of prior therapy and length of delay before second mobilization did not significantly affect harvest yield. Renal impairment and total failure at first harvest were associated with poor outcome. Successful reharvesting was most common using cyclophosphamide/G-CSF or ESHAP/G-CSF.

0487

OPTIMAL TIMING OF AUTOLOGOUS PERIPHERAL BLOOD STEM CELL HARVEST FOLLOWING PRIMING WITH CYCLOPHOSPHAMIDE AND G-CSF: SIGNIFICANT IMPROVEMENT IN OUTCOME HARVESTING DAY 9 COMPARED WITH DAYS 6, 7, 8 OR 10

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Background. The increasing role of high dose therapy and autologous stem cell rescue has led to increasing demand for stem cell harvests. The association between peripheral blood (PB) CD34+ count and harvest yield has been established for a decade. By waiting for adequate PB CD34+ counts before harvesting, the number of inadequate harvests has been reduced. However the optimal harvesting schedule is yet to be delineated. Booking a harvest too early after priming will result in more attendances for blood counts and ineffective use of apheresis machine time; booking too late will result in more patients missing the peak in circulating PB progenitors resulting in more failed harvests. *Aims.* To determine the optimal time to plan a PB stem cell harvest following cyclophosphamide/G-CSF priming. *Method:* During 2003-2004, 168 adult patients were primed using low dose cyclophosphamide: 2g/m² with G-CSF 5-10µg/kg/day (n=140), 1.5g/m² with G-CSF 5-10µg/kg/day (n=24) and 3g/m² with G-CSF 10µg/kg/day (n=4), for hematological malignancy (MM n=88, DLBL n=25, FCL n=22, HD n=15, leukemia n=10, T-cell NHL n=6, MCL n=2). Patients underwent continuous flow leukapheresis when the PB CD34+ cell count was $>10/\mu\text{L}$. The time from prime to harvest and

mobilization efficiency were analyzed. *Results.* 29 (17%) were not harvested due to poor PB CD34+ counts and 9 harvests (5%) were unsuccessful (total yield $<2 \times 10^6$ CD34+/kg). 130 (77%) patients were successfully harvested (median CD34+ cell dose 4.1×10^6 /kg (range 2.1-14.5)); 60% required one harvest, 34% required 2, 6% required 3. First attendance varied from day 6-10 post-priming and of patients successfully harvested, 15 (11%) first attended on d6, 66 (51%) on d7, 9 (7%) on d8, 27 (21%) on d9 and 13 (10%) on d10. Patients actually harvested (as a % of those attending on a given day): d6 n=0 (0%), d7 n=9 (9.9%), d8 n=43 (41%), d9 n=72 (68%), d10 n=41 (60%) and d11 n=22 (58%). The minimum harvested progenitor threshold was achieved in a single apheresis on d7 in n=3 (3.3%), d8 n=31 (30%), d9 n=53 (50%), d10 n=16 (24%) and d11 n=4 (11%). The median number of days attending when first attendance was d6, 7, 8, 9 and 10 was 4, 3, 2, 1 and 2 respectively and the median total harvest CD34+ count/kg was 3.3, 3.9, 3.4, 4.5 and 4.8 respectively. The failure rate for those first attending d6-8 was 23-38% but only 3.6% and 24% when first attending days 9 and 10. Patients first attending day 9 demonstrated a high total harvest yield, achieved with the fewest visits and with the least chance of failing to harvest. *Conclusions.* Booking a harvest at the optimal time after a chemotherapy prime can complement the use of PB CD34+ counts in improving outcome. Our results indicate that after cyclophosphamide/G-CSF, there is no benefit in patients attending before day 9. Harvesting from day 9 will reduce laboratory and staffing costs and by increasing efficiency will help shorten waiting times for transplantation programs.

0488

ERYTHROPOIETIN-ALFA IN PATIENTS UNDERGOING UNRELATED STEM CELL TRANSPLANTATION

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Background. Reduction of RBC transfusion rate in anemic stem cell transplant patients can be achieved by using recombinant human erythropoietin (EPO) as demonstrated in several randomized trials. However, there is no randomized study examining exclusively patients transplanted with an unrelated donor. *Aims.* The primary aim of our controlled, randomised clinical trial with 90 patients (ITT study population) was to demonstrate a reduction in RBC transfusion rate in unrelated transplant patients receiving EPO compared to control patients. Secondary goals were the duration of the RBC transfusion period and the number and duration of platelet transfusions in each study group. *Methods.* Patients in the EPO group received Erypo® 10000 I.E. daily from the day of transplant (day 0) to day +10, afterwards 3x/week until hemoglobin reached ≥ 9 g/dL for 7 consecutive days without RBC transfusion. The maximum duration of EPO therapy was restricted to day +60 resp. discharge from hospital. *Results.* Overall, we randomized 75 patients (recruiting stopped after interim analysis) and are presenting data of 67 evaluable (per protocol) patients (EPO: 30; control: 37) together with the respective data of the 46 patients surviving the study period of 60 days (EPO: 21; control: 25; data after slash). The two groups were balanced with regard to gender, age, HLA matching, disease except for kind of conditioning (significantly more patients with RIC in the EPO group). Median hemoglobin (Hb) was 8.9/9.8 (EPO) compared to 8.9/9.0 g/dL (control) at time of transplantation, and 8.5/8.7 (EPO) vs. 8.3/8.5 (control) g/dL at the end of the study ($p = \text{n.s.}$). 11 out of 26 pts (42%) resp. 11 out of 17 (65%; surviving patients) treated with EPO reached Hb ≥ 9.0 g/dL for more than 7 days without transfusion (in 4 other pts, Hb never dropped below 9.0 g/dL), and in the control group 12 out of 37 pts (32%) resp. 10 out of 25 (40%; surviving patients) ($p = \text{n.s.}$). The number of median RBC and also platelet transfusion units were comparable in both groups: RBC units 8.5/8.0 (EPO) vs. 8.0/8.0 (control), platelet

units 6.5/5.0 (EPO) vs. 7.0/7.0 (control) ($p = n.s.$). The transfusion periods (RBC, platelets) were numerically shorter in EPO patients, but the higher percentage of patients with RIC (thus less toxicity) has to be considered for this group. There was a trend for faster neutrophil engraftment with EPO pts (mean 15.5/15.7 days) than with control pts (18.2/18.0 days) ($p = 0.04/0.09$). However, this effect was also due to more patients with RIC in the EPO group as shown by two-factor analysis of variance. **Conclusions.** Our study demonstrated no significant differences between the two study groups with regard to the primary and secondary study goals. The inadequate EPO response is perhaps due to the high incidence of transplant-related complications observed in our patients (e.g. GvHD grade 3+4: 50% EPO vs. 29% control). In addition, a benefit from EPO therapy for transplant patients might be higher if EPO therapy is initiated at day 30-35 after transplant as suggested by recent literature data.

0489

HIGH DOSE CHEMOTHERAPY (HDCT)+AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN PATIENTS WITH MULTIPLE SCLEROSIS (MS): TREATMENT STRATEGIES AND OUTCOMES

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Objectives: HDCT+ASCT is a new and promising therapy for MS patients. At present, the information about its efficacy in MS patients at different disease stages is lacking. According to our concept there are 3 strategies of HDCT+ASCT depending on disease stage: early, conventional, and salvage. Evaluation of both clinical and patient-reported outcomes in MS patients undergoing HDCT+ASCT is worthwhile. The aim of this research was to study treatment outcomes in MS patients with different strategies of HDCT+ASCT. **Methods.** 17 patients with MS were included in the study (mean age - 32.3, SD - 6.6; male/female -4/13). Median EDSS at base-line was 6.0 (range 2.0 - 7.5). The median follow-up duration was 18 months (range 3 - 60 months). All of the patients have previously undergone conventional treatment. Neurological and QoL evaluation was provided at baseline, at discharge, 3, 6, 9, 12 months, and then every 6 months after HDCT+ ASCT. MRI was conducted at baseline, at 6, 12 months, and at the end of follow-up. Clinical improvement was defined as a decrease in the EDSS score by at least 0.5 points on two consecutive visits 6 months apart as compared with baseline; disease stabilization - as no change in EDSS score during follow-up; disease progression was defined as an increase by at least 0.5 points after 6 months and/or appearance of new lesions on MRI. QoL was assessed by FACT-BMT and FAMS. QoL response was evaluated by the method of integral profiles using Integral QoL index. **Results.** Eleven (73.3%) out of 15 patients with follow-up more than 6 months experienced a clinical stabilization or improvement. Three patients showed significant improvement in EDSS (by more than 1.0 point), 2 patients improved by 1.0 point, and 3 patients - by 0.5 points on EDSS. Three cases remained stable. All of the patients with clinical stabilization and improvement exhibited negative MRI scans. One patient continuously worsened and died 3 years after the transplantation. Three other patients worsened by 0.5 points on their EDSS. Two out of 17 patients underwent early transplantation (EDSS at base-line was 2 and 3.5) and they showed both clinical (EDSS decreased by 0.5 points) and QoL improvement. One patient underwent salvage transplantation (female, 49 years old; secondary progredient type; base-line EDSS 7.5; follow-up 48 months). As a result, signifi-

cant clinical improvement (EDSS decreased from 7.5 to 6.0) and excellent QoL response were achieved at the end of follow-up. Analysis of QoL response showed that at one year after HDCT+ASCT and during follow-up the majority of patients with clinical stabilization and improvement exhibited an average or excellent QoL response. **Conclusions.** HDCT+ASCT in MS patients resulted in clinical stabilization and improvement in 73.3% of patients under observation. In addition, dramatic improvement of QoL took place. Thus, the results obtained demonstrate feasibility of early, conventional, and salvage HDCT+ASCT in MS patients; further studies are needed to confirm the above concept.

0490

PLASMACYTOMA RELAPSES IN THE ABSENCE OF SYSTEMIC PROGRESSION POST HIGH DOSE THERAPY FOR MULTIPLE MYELOMA

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Background. Autologous (ASCT) and allogeneic stem cell transplantations (alloBMT) are well-established therapies for multiple myeloma. However, patients continue to relapse at a constant rate. The patterns of MM relapse after high dose treatment (HDT) with stem cell support are very heterogeneous. The incidence of plasmacytoma relapses in the absence of myeloma progression is unknown after allogeneic transplantation, although a rate of 7% of extramedullary relapses after ASCT has been reported by the Spanish Myeloma Registry. **Aims.** The aim of this retrospective analysis was to evaluate the incidence of plasmacytoma relapses in the absence of systemic progression post HDT for myeloma patients and review their clinical course. **Patients/Methods.** The medical records of 147 patients with MM who underwent ASCT were reviewed. Prior to autograft 81% of cases had received only one regimen of chemotherapy. Most patients were treated with VAD or VAD-like regimens. At the time of transplant only 5% of patients were in CR. The vast majority had achieved a partial remission (PR) with previous chemotherapy. The median time interval between diagnosis and transplant was 10 months. At the same time, 16 patients underwent an alloBMT. Seven patients underwent a conventional alloBMT, while 9 patients received a reduced intensity conditioning (RIC) transplant. The appearance of plasmacytoma was detected by imaging techniques (CT or MRI), and when possible confirmed by biopsy. **Results.** Fourteen patients out of 147 who underwent ASCT (9.5%) and one patient out of 16 who underwent an alloBMT (6%) relapsed as plasmacytoma only. Thirteen patients were males and the median age was 41 years. Three patients had light-chain myeloma and the other 12 patients had IgG or IgA MM. Eleven patients (73%) had evidence of lytic disease at diagnosis. One patient had a plasmacytoma at S1 at initial diagnosis. Eleven out of 15 patients (73%) presented with Durie-Salmon stage III disease at diagnosis. None had extramedullary disease prior to transplantation. The median time from SCT to plasmacytoma relapse was 24 months. The sites of plasmacytoma included bone, skin, skull, rectum and testicles. Seven patients had bone plasmacytoma and 8 patients soft tissue plasmacytoma. No relationship was noted between sites of original lytic lesions at diagnosis and sites of bone plasmacytoma at relapse. Five patients were treated with local radiotherapy, while seven patients received a combination of radiotherapy and chemotherapy or thalidomide, and two patients received chemotherapy alone with or without thalidomide. The recipient of alloBMT who relapsed with bone plasmacytoma was initially treated with VAD-chemotherapy and local radiotherapy followed by a mini-allograft from the

original donor. Eleven patients died at a median of 10 months following diagnosis of the plasmacytoma. Four are still alive, 12-20 months post-plasmacytoma diagnosis. *Conclusions.* These cases of unconventional disease recurrence are likely to be seen due to sub-clinical seeding of tumour cells suggestive of the presence of an extramedullary clone of plasma cells with a high degree of chemoresistance. Our results and the available data from the literature suggest that optimal therapy for patients with isolated plasmacytoma relapse has not been defined yet.

0491**PEG-FILGRASTIM VS FILGRASTIM AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION: A CASE-CONTROL STUDY**

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Background. Myeloid growth factors are currently recommended by ASCO guidelines as adjuvant agents after autologous peripheral blood stem cell transplantation (PBSCT) for shortening the period of severe neutropenia and reducing the related risk of life-threatening infections. Peg-filgrastim is a novel granulocyte-colony stimulating-factor (G-CSF) characterized by prolonged activity and unique mechanisms of clearance. *Aims.* To compare safety, efficacy and costs of G-CSF (filgrastim) and peg-filgrastim and to verify the potential advantages of peg-filgrastim in the specific setting of recovery after PBSCT. *Methods.* We investigated the effects of a single s.c. injection of peg-filgrastim (6 mg) on day 3 in 47 patients (36 multiple myelomas, MM; 7 non-Hodgkin's lymphomas, NHL; 4 Hodgkin's disease, HD), who received autologous PBSCT. Mean age was 55.4 years (range 23-66). In 34 patients PBSCT was performed as consolidation in first complete or partial remission, while 13 patients received the procedure as salvage treatment for chemo-sensitive disease. Conditioning regimens were intermediate/high dose (100-200 mg/sqm) melphalan in MM and BEAM protocol in NHL and HD. For comparison, we selected a group of 47 historical controls matched for age, type and status of disease, conditioning regimen, and infused number of CD34+ stem cells, who had received standard daily s.c. administration of filgrastim (300 mcg/d) from day 5 to neutrophil recovery after autologous PBSCT. *Results.* No serious adverse events occurred in this study. Tolerability of peg-filgrastim and filgrastim was equivalent. There was no difference (p n.s.) between the two groups (peg-filgrastim vs filgrastim) in terms of median time to neutrophil > 0.5 x 10⁹/L (10+/-1.3 vs 11+/-2.4 d), hemoglobin recovery > 10 g/dL (12.5+/-6.6 vs 14+/-4.8 d), days of febrile neutropenia (1+/-2.5 vs 1+/-2 d) and number of red cell (0+/-1.6 vs 1+/-1.8 units) or platelet(1+/-1.6 vs 1+/-1.8 units) transfusions. Likewise, the median number of days with severe neutropenia (< 0.5 x 10⁹/L and < 200 x 10⁹/L) was also not statistically different(5+/-1.8 vs 5+/-2.1, and 3.4+/-1.7 vs 3+/-2.4, respectively). Interestingly, the median time to platelet > 20 x 10⁹/L (11+/-6.6 d, range 0-23 vs 14+/-5.1 days, range 3-28, p < 0.03) was significantly shorter in peg-filgrastim group. The median number of filgrastim injections in controls was 11 (range 7-20), so that the cost of this drug for single patient was 946 euros, as compared to 910 euros for patients receiving peg-filgrastim. *Conclusions.* Peg-filgrastim and filgrastim have comparable safety, efficacy and costs in patients affected by hematological malignancies who undergo autologous PBSCT. The reduced period of thrombocytopenia observed with peg-filgrastim requires to be confirmed and further investigated in larger studies.

492**A PROSPECTIVE RANDOMIZED TRIAL OF TWO DOSE-INTENSIVE MELPHALAN REGIMENS (100 VS 200 MG/MQ) IN MYELOMA NEWLY DIAGNOSED PATIENTS: AN INTERIM ANALYSIS**

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Several trials have shown the superior impact of high-dose melphalan (usually 200 mg/m², MEL200) versus standard therapy in myeloma patients. Intermediate-dose melphalan (100 mg/m², MEL100) is also superior to the standard dose, but has not been clinically compared with MEL200 in a randomized study. In our last case-matched study, we demonstrated that MEL100 was less toxic than MEL200, MEL100 was inferior to MEL200 in terms of EFS but not in terms of OS. We now compare the efficacy and toxicity of MEL100 and MEL200 in a prospective randomized trial. AIMS The end points of the study were: response, EFS, OS and toxicity. All patients were untreated, with measurable disease and aged < 65. The Southwest Oncology Group (SWOG) diagnostic criteria and Durie and Salmon staging system were used. Exclusion criteria were prior treatment for myeloma, abnormal cardiac function (systolic ejection fraction <50%), respiratory disease (vital capacity or carbon monoxide diffusion < 50% of normal), abnormal liver function (serum aminotransferase value >2.5 of normal), abnormal renal function (serum creatinine > 3 mg/dL), HBV, HCV, or HIV positivity, concomitant cancer or psychiatric disease. The institutional review board approved the protocol and written informed consent was obtained from all patients. The MEL100 regimen included: 2 DAV debulking courses (dexamethasone - doxorubicin [adriamycin]- vincristine; adriamycin 50 mg/m² day 1, vincristine 1 mg day 1, dexamethasone 40 mg days 1, 2, 3, 4, each course repeated every 28 days), cyclophosphamide 4 g/m² plus G-CSF and subsequent leukapheresis, double MEL100 with stem cell support. The MEL200 regimen was as MEL100, but the double autografts were conditioned with MEL200. An interim analysis has been planned after the first 200 newly diagnosed myeloma patients, median age 58, range 33-65, entered the study, between January 2002 and January 2005. At present, 83 patients have a follow-up superior than 1 year and have completed the entire program: 40 in MEL100 arm and 43 in MEL200 arm. Patient characteristics were similar in both groups (MEL100 and MEL200 respectively): median age 58 vs 59 yr, patients > 60 yr 48% vs 51%, Durie-Salmon stage II 31% vs 31%, Durie-Salmon stage III 64% vs 63%, Durie-Salmon stage B 5% vs 6%, median β_2 -microglobulin 2.4 vs 2.6 mg/L, median Hb 10.4 vs 10.8 g/dL, median serum calcium 2.39 vs 2.52 mmol/L, median albumin 3.56 vs 3.36 g/dL, median plasma creatinine 0.8 vs 0.8 mg/dL. An interim analysis is ongoing and will be finished in April 2005, the results of this analysis will be presented.

0493**PEGFILGRASTIM SUCCESSFULLY MOBILISES CD41+ MEGAKARYOCYTE PROGENITOR CELLS INTO THE PERIPHERAL BLOOD IN SUBJECTS WITH SOLID TUMOURS**

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Stem cell transplantation with cytokine-mobilised peripheral blood (PB) is characterised by early engraftment and platelet recovery compared to bone marrow transplantation. The number of megakaryocyte (MK) progenitors infused correlates with platelet recovery. We have shown in a subset analysis of 31 chemotherapy-naive patients with advanced malignancies ran-

domised to daily filgrastim or pegfilgrastim 6mg, 12mg or 18mg (Willis, EBMT 2005) that pegfilgrastim 18mg mobilised significantly more MK progenitors into the PB than filgrastim 10 mcg/kg/day. In this study, we compared the number of CFU-MK identified by immunohistochemical staining for GPIIb/IIIa (CD41a) antigen against MK progenitors identified by FACS analysis (CD41a/ CD34 coexpression) in the pegfilgrastim 18mg and filgrastim 10 mg³/kg/day groups. Mononuclear cells isolated from PB by Ficoll-Paque density centrifugation were cultured, using a collagen-based assay, and colony-forming unit MKs (CFU-MKs) were identified by immunohistochemical staining for the GPIIb/IIIa (CD41a) antigen and quantified. Labelled MNC were analysed by FACS for CD41a/CD34⁺ coexpression. Peak CFU-MKs (count/mL blood) were analysed using the non-parametric Mann-Whitney-Wilcoxon's test comparing pegfilgrastim 18mg treatment group with filgrastim (Table).

Peak (days 3-7)	Filgrastim CFU-MK	Pegfilgrastim CFU-MK	Filgrastim CD41a/CD34 ⁺	Pegfilgrastim CD41a/CD34 ⁺
Dose	10mcg/kg/day	18mg	10mcg/kg/day	18mg
N	6	7	6	6
Mean (SE)	4555 (1359)	11764 (2366)	0.028 (0.022)	0.123 (0.023)
P-value		0.024		0.438

Mean peak levels of CFU-MK progenitors in the pegfilgrastim 18 mg group showed a 560-fold increase from mean baseline PB levels (20±7, n=26), compared to a 230-fold increase in filgrastim subjects from baseline. Higher peak levels of CFU-MKs were seen in the pegfilgrastim 18 mg group compared to filgrastim 10mcg/kg ($p=0.024$). Mean peak levels of CD41a/ CD34⁺ progenitor cells identified by FACS analysis showed a 12.3 fold increase from mean baseline (0.02±0.02, n=12), compared to a 5.8-fold increase in the filgrastim subjects from baseline. This difference was not statistically significant ($p=0.438$). Pegfilgrastim appears to stimulate the transendothelial migration of MK progenitor cells from the bone marrow into the PB more efficiently than filgrastim. If this translates to a reduction in platelet transfusion requirements, then the finding is of therapeutic importance. CD41a/CD34⁺ FACS analysis, although less sensitive than the immunohistochemical staining culture-based assay, reflects MK mobilisation and is therefore a practical way of predicting megakaryocyte progenitor cell numbers in the clinic.

0494

PEGFILGRASTIM ALONE AND IN CONJUNCTION WITH CHEMOTHERAPY SUCCESSFULLY MOBILISES PERIPHERAL BLOOD PROGENITOR CELLS

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Growth factors are used alone or in conjunction with myelosuppressive chemotherapy to induce peripheral blood progenitor cell (PBPC) mobilisation. We evaluated PBPC mobilisation with 3 different doses of pegfilgrastim (6 mg, 12 mg and 18 mg), either alone or in combination with chemotherapy. Chemotherapy naive subjects with solid tumors were randomised in Cycle 0 (cytokine alone) to receive a single dose of 6, 12 or 18 mg pegfilgrastim or 10 mg³/kg filgrastim on day's 1-7 or until ANC $\geq 7.5 \cdot 10^9/L$. Two weeks later in Cycle 1 (cytokine plus chemotherapy), subjects received pegfilgrastim at the same dose as in the previous cycle or daily 5 mg³/kg filgrastim 24 hours after administration of carboplatin AUC 6 (1 hour IV infusion) and paclitaxel 175 mg/m² (3 hour IV infusion). Sixty-one subjects were randomised into the study. In Cycle 0 the mean peak CD34⁺ cell count was similar in the filgrastim 10 mg³/kg and pegfilgrastim 6mg groups [4.51 and 4.24 $\cdot 10^4/mL$ ($p=0.834$), respectively] and was significantly higher, compared to filgrastim 10 mg³/kg, in the pegfilgrastim 12 mg and 18 mg groups [8.18 ($p=0.034$) and 9.90 $\cdot 10^4/mL$ ($p=0.006$), respectively]. In Cycle 1 mean peak CD34⁺ cell counts were similar in the fil-

grastim 5 mg³/kg and pegfilgrastim 6mg groups (1.19 and 1.18 $\cdot 10^4/mL$ ($p=0.989$), respectively) and slightly higher in the pegfilgrastim 12 mg and 18 mg groups (1.45 ($p=0.692$) and 3.14 $\cdot 10^4/mL$ ($p=0.043$), respectively).

Table 1. Peak total colony forming units (count/mL blood).

	Filgrastim	Pegfilgrastim		
		6mg	12mg	18mg
Cycle 0				
N	12	13	14	11
Mean (SE)	7449 (1357)	6296 (1809)	13031 (5882)	14867 (3185)
Cycle 1				
N	14	12	14	13
Mean (SE)	2975 (1037)	4562 (2454)	9110 (1342)	5758 (1468)

Colony forming units and long-term culture initiating cell assays confirm both the short and long term engraftment capacity of these cells. The incidence of serious adverse events was low and comparable between groups. A dose response was observed with both pegfilgrastim alone and with pegfilgrastim plus chemotherapy and suggests that there is an increased potential for mobilizing greater numbers of PBPC's with increasing doses of pegfilgrastim.

0495

MONITORING OF CARDIOTOXICITY DURING HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN ACUTE LEUKEMIA

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Cardiotoxicity is a well-known and serious complication of treatment in haematology. Anthracyclines (ANT) and high-dose chemotherapy represent the greatest risk. Various methods have been recommended for cardiotoxicity monitoring. Biochemical markers of functional and structural myocardial damage have been gaining ground in this field. *Aims.* Assessment of cardiotoxicity during haematopoietic stem cell transplantation (HSCT) in acute leukemia with biochemical markers-N-terminal pro brain natriuretic peptide (NT-proBNP) and cardiac troponin T (cTnT). NT-proBNP is a marker of cardiac dysfunction, values below 125 pg/mL are considered normal and allow to rule out heart failure. *Methods.* Nineteen adult patients with acute leukemia (16 AML, 3 ALL) were studied. They were 13 males and 6 females with a mean age 42.8±10.0 years (range: 22-56). The patients were pretreated with ANT in the cumulative dose of 432.1±77.3 mg/m². Preparative regimen (PR) consisted of Busulphan 16 mg/kg and Cyclophosphamide 120 mg/kg (HD-CF) in 13 patients, total body irradiation (TBI) 12 Gy and HD-CF in 6 patients. Ten patients underwent allogeneic HSCT, 9 autologous HSCT. Serial measurements of plasma NT-proBNP values were performed the day before PR, the day after PR, the day after HSCT and at the time of bone marrow recovery (after circa 14 days). Plasma cTnT concentrations were measured the day before PR and the day after HSCT. *Results.* The day before PR, mean plasma NT-proBNP value was 106.3±55.7 pg/mL (slightly elevated in 4 patients). The mean NT-proBNP value increased to 426.1±391.5 pg/mL (elevated in 12 patients) after completion of PR. After HSCT, a further increase to 847.6±780.6 pg/mL (elevated in 14 patients) was observed. At the time of bone marrow recovery, the mean NT-proBNP value was 330.8±236.8 pg/mL, values remained elevated in 12 patients. The differences were statistically significant in comparison with the baseline NT-proBNP value ($p < 0.01$). The NT-proBNP elevations were more pronounced in patients with cumulative doses of ANT above 450 mg/m² ($p < 0.05$), in patients with PR containing TBI and HD-CF ($p < 0.05$) and in patients undergoing allogeneic HSCT ($p < 0.05$). Associations between changes in NT-proBNP values and gender or age were not significant. In all patients, plasma cTnT concentrations were negative at the baseline and after HSCT. *Conclusions.* Our results

suggest that administration of PR and HSCT is in most acute leukemia patients associated with acute neurohumoral activation (significant rise in NT-proBNP). In our study, NT-proBNP remained elevated in 12 (63.2%) patients at the time of bone marrow recovery. These persistent NT-proBNP elevations indicate subclinical cardiotoxicity (risk for development of heart failure) and require further follow-up. NT-proBNP elevations were significantly more pronounced in patients with higher cumulative doses of ANT and in patients with PR containing combination of radiotherapy and high-dose chemotherapy. These therapeutic procedures seem to be more cardiotoxic and not very appropriate for patients with cumulation of risk factors for cardiotoxicity. Negative plasma cTnT concentrations show no detectable damage of myocyte structure during HSCT.

0496

CELL DOSE IS THE MAJOR FACTOR FOR RESPONSE TO DLI IN CML

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Donor lymphocyte infusion (DLI) is an effective treatment for patients with CML in relapse after allogeneic stem cell transplantation. However, the optimal cell dose for treatment remains to be identified. To address the question we have investigated the factors affecting the dose required to achieve remission (effective cell dose, ECD). We analyzed 68 patients treated with escalating DLI, thirty patients were in molecular, 20 in cytogenetic, 18 in hematologic relapse (14 in chronic and 4 in accelerated phase). Patients transplanted from a sibling (Sib) donor received 10^7 5×10^7 10^8 donor CD3 cells/Kg. Patients receiving transplant from an unrelated (VUD) donor received 10^6 - 10^7 5×10^7 10^8 donor CD3 cells/Kg. Sixty-three patients achieved molecular remission (93%). Acute GvHD grade II-III occurred in 13 patients (19%). Extended chronic GvHD occurred in 12 patients (18%) and in 9 of these followed acute GvHD; none of the patients died of GvHD. Median time from ECD to remission was 3.9 months (1.4-24.3). The ECD was influenced by the disease stage at time of DLI: 10^6 - 10^7 was sufficient to restore molecular remission in 69% of patients in molecular or cytogenetic relapse but only in 36% of patients in hematologic relapse ($p=0.022$). The ECD was also influenced by donor type: 73% of VUD recipients responded to a dose equal or less than 10^7 compared to 46% of Sib recipients ($p=0.031$). Five patients failed to achieve molecular remission; among these three progressed to blastic phase. In one case, a dose higher than the maximum dose scheduled was administered (5×10^8 CD3 cells/Kg) and the patient then achieved molecular remission. Accordingly, we observed a significant correlation between the cell dose and the number of responders, suggesting that DLI is subject to a dose-response effect. We conclude that ECD correlates with disease stage and donor type. We suggest that, for best efficacy the number of donor lymphocytes should be escalated indefinitely.

Bleeding disorders (congenital and acquired) II

0497

ACQUIRED HAEMOPHILIA-REPORT OF FIVE CASES

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Acquired haemophilia is a rare disorder caused by auto antibodies to factor VIII. In a half of cases there is an association with other medical conditions or previous drug usage. The most common is bleeding into the skin or muscles. However, during the course of the illness, bleeding may occur at any site. About 20% of patients die of disease. *Case report.* We present five cas-

es treated at our institution in the period 1990- 2004. There were three females and two males, age 21-74, median age 53 years. There were two cases of postpartum acquired haemophilia, one case in association with bullous pemphigoid, and two cases without any association with previous medical conditions or drugs. The level of factor VIII: C was 0, 01-0,03 U/mL. The inhibitor level was 2,25-540 BU. One case, 74 years old male was lupus anticoagulant positive. All cases presented with significant haemorrhage in the form of subcutaneous haematomas. A postpartum case with inhibitor level of 540 BU presented with severe life threatening intraabdominal haemorrhage which needed surgical intervention. There was a case of severe life threatening haemorrhage in the region of the neck which needed urgent tracheotomy, and a case of bleeding after dental extraction and haematuria in connection with kidney stone. All patients received combined immunosuppressive treatment with prednisone and cyclophosphamide or azathioprine. The plasmapheresis was done in four patients. Two patients received high dose Ig treatment. APCC, and rVIIa treatment were applied each in one patient, both successfully. A disappearance of factor VIII antibodies happened in 3 cases with inhibitor level of 2,25 to 64 BU, and the persistence of factor VIII antibodies in spite of treatment was in two cases. It was the postpartum case with inhibitor level of 540 BU, and the case with inhibitor level of 3,2 BU which was lupus anticoagulant positive. The both patients with resistant disease died, in first case the cause of death is not known, and the other one died from acute heart failure after episode of severe haemorrhage in the leg. *Conclusions.* Acquired haemophilia is a rare disease which presents with significant bleeding, very often in severe, life threatening form. In the case of persistent disease the prognosis is unfavorable.

0498

ASSESSMENT OF PROLIFERATION AND DIFFERENTIATION OF MEGAKARYOCYTIC PROGENITORS IN HCV CHRONICALLY INFECTED THROMBOCYTOPENIC PATIENTS

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Thrombocytopenia is a major hematological disorder commonly observed in patients with chronic liver diseases induced by variable etiologies, to date, its underlying mechanism remains controversial. The aim of the present study was to assess the *in vitro* proliferation and differentiation of the megakaryocytic progenitors of the purified CD34+ bone marrow cells in thrombocytopenic HCV chronically infected patients, using short term megakaryocytic culture, in addition to assessment of serum thrombopoietin (Tpo) level. *Methods.* Thirty chronic hepatitis C (HCV-PCR positive) thrombocytopenic patients were included; in addition to twenty healthy individuals as controls. Bone marrow CD34+ cells purified by indirect labelling and then cultured in a collagen based Mk assay kit (MegCult-C, StemCell Technologies, Canada). The cultures were fixed on day 12, and the Mk colonies were identified by the alkaline phosphatase anti-alkaline phosphatase technique using the monoclonal antibodies CD41 (GP IIb/IIIa). The slides were scored for Mk colony forming units (CFU-Mks) (3-20 and >20 cells), Mk burst-forming units (BFU-Mks) (>50 cells) and mixed colonies. *Results.* Serum thrombopoietin level showed statistically significant elevation in thrombocytopenic HCV chronically infected patients in comparison to the control group, (456.8 ± 212.9 pg/mL versus 62.3 ± 14.9). *Results.* of the short term megakaryocytic culture as an indirect index of megakaryopoiesis showed that there is an evident depression in the burst forming unit megakaryocyte (BFU-meg), the colony forming unit megakaryocyte (CFU-meg), the total Mk colony formation (per 2.5×10^6 CD34+ cells) in thrombocytopenic HCV chronically infected patients (17=31, range 5-58) was lower than the healthy control group (91= 44 range 23 -153). Correlation studies showed that there was a positive correlation between the number of CFU-meg and the platelet count, a negative correla-

tion between serum Tpo level and the number of CFU-meg, and a negative correlation between serum Tpo level and the platelet count, a positive correlation between serum Tpo level and prothrombin time, AST, Albumin and the Child Pugh score of liver disease. **Conclusions.** Taken collectively, we could conclude that thrombocytopenia in chronic hepatitis C viral infection, could be a result of bone marrow megakaryocytic hypoplasia due to suppression of the proliferative and differentiative capabilities of megakaryocyte progenitors which could reflect CD34+ stem cells defect possibly due to infection of these cells by HCV previous described. It is also associated with high levels of circulating thrombopoietin which could indicate an intact competent hematopoietic response of the thrombopoietin in thrombocytopenic HCV chronically infected patients

0499

SPECTRUM AND PREVALENCE OF VON WILLEBRAND DISEASE FROM WESTERN INDIA

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Von Willebrand disease (VWD) is one of the most common inherited bleeding disorders in the west. Scattered studies from India show a prevalence of approximately 10% of VWD among the hereditary bleeding disorder cases. VWD therefore remains an under diagnosed entity in India. Furthermore, the prevalence of different subtypes of VWD is also not known which is essential for a proper management of these cases. The present study was thus undertaken to know the prevalence of VWD in our country and also its various subtypes. **Methods.** Seven hundred and ninety six patients presented with various bleeding manifestations were analysed in the present study. The initial screening and confirmation tests for the diagnosis of VWD included Bleeding time (BT), screening coagulation tests i.e. Prothrombin Time PT, APTT, TT), factor VIII: C assay, ristocetin induced platelet aggregation (RIPA) and VWF antigen estimation. VWF multimer analysis, Ristocetin cofactor activity (RCOF), VWF collagen binding assay (VWF CBA), factor VIII: VWF binding assay were also done to classify and subtype these VWD cases. **Results.** The patients were sub typed as per the ISTH criteria. Out of the 796 patients screened, 58 were diagnosed as VWD. Out of the 15 families with a positive family history of bleeding, 26 additional cases were diagnosed as VWD. Majority of the patients were type 3 (60%) with severe clinical manifestations. 18% of type 1 VWD patients were detected in this group while the prevalence of the qualitative variants of VWD i.e. type 2 VWD was found to be 19% and the prevalence of various subtypes were type 2A (8%), type 2B (5%), type 2M (1%), type 2N (5%). **Interpretation & Conclusions.** The very high prevalence of type 3 VWD and a low incidence of type 1 VWD which is in contrast to the western reports suggests the low awareness of the disease as also the under diagnosis of the mild cases in our country.

0500

BLEEDING PROPHYLAXIS IN CHILDREN WITH HAEMOPHILIA A/B USING PLASMA-DERIVED CONCENTRATED IMMUNATE/IMMUNINE-THE SAFE WAY TO IMPROVE QUALITY OF LIFE

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Bleeding prophylaxis using coagulation factor concentrates is a standard method for the treatment of patients with haemophilia usually leading to reduction in bleeding episodes. It also improves the patient's quality of life. **Aims.** The aim of this

project was to evaluate the influence of prophylactic treatment with coagulation factor concentrates on joint motility which is most often affected in patients with haemophilia as well as on the musculoskeletal system as a whole. We focused in particular on the possible development of target joints and monitored whether this treatment led to a decrease in the number and severity of bleeding episodes and frequency of hospital admissions. We also investigated the possible development of inhibitor (antibody) against FVIII or FIX. Patients and **Methods.** 28 children with severe haemophilia A or B aged 9 months to 18 years from four paediatric haemophilia centres in the Czech Republic were included in the study. All received prophylactic administration of high purity plasma derived concentrates of FVIII/FIX manufactured by Baxter AG Vienna, Austria Immunate/Immunine. The study was prospective. The patients were examined in local centres in 5 out-patient visits. We focused on the number and severity of bleeding episodes and on the status of the musculoskeletal system using repeated goniometric measurements. We noted whether any bleeding episode resulted in hospital admission. This aside, we also evaluated blood samples for possible FVIII/FIX inhibitors and we tested the viral safety of concentrates by repeated testing for blood-borne infections (HAV, HBV, HCV and HIV). A questionnaire designed to evaluate quality of life (QoL) was given to both patients and their parents during each visit. **Results.** Despite the relatively small number of patients we found unambiguous improvement in the musculoskeletal system in individual patients and in the cohort as a whole. In all but one patient a reduction or at least no worsening in the number and severity of bleeding episodes was found and no target joint developed over the study period. We also failed to detect any inhibitor development or changes in the serological tests mentioned above. **Conclusions.** Bleeding prophylaxis in children with severe haemophilia A or B by treatment with concentrates Immunate/Immunine, can be considered safe and effective. It leads to improvement in motility and increase in the quality of life. Moreover, this method of treatment, according to the literature, probably prevents serious health problems including complicated and costly orthopaedic surgical interventions in adulthood.

0501

ASSESSMENT OF BONE MARROW MEGAKARYOCYTES ULTRASTRUCTURAL CHANGES IN CHRONIC HCV THROMBOCYTOPENIC PATIENTS

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To date, underlying mechanism of thrombocytopenia observed in patients with HCV related chronic liver diseases remains controversial. We presented for the first time, data on the ultrastructures changes (By Transmission Electron Microscopy) of Megakaryopoiesis in Chronic HCV infected Thrombocytopenic patients. Aim of the present study was to assess the ultra-structural megakaryocytes changes (by transmission electron microscopy) in thrombocytopenic HCV chronically infected patients. **Methods.** Twent chronic HCV (HCV-RNA positive by RT-HCV-PCR) thrombocytopenic patients were included; in addition to ten healthy individuals as controls. They were subjected to bone marrow cytological assessment, in addition to assessment of the bone marrow megakaryocytes ultra-structural changes by the transmission electron microscopy (Philips 201, Philips, Eindhoven, The Netherlands): Fresh bone marrow cells were washed in RPMI 1640, pelleted, fixed in 2% glutaraldehyde in 0.1 M phosphate buffer for 24 hours at 4°C. Cells were dehydrated, osmicated, and embedded in Epon 812 according to routine procedures. Semithin sections (1 to 0.5 µm) were inspected light microscopically to select megakaryocytes. **Results.** demonstrated that, in all patients more than 50% of the bone marrow megakaryocytes were damaged, showing ultra-structural abnormalities: condensation in chromatin, the nuclear envelope was indented and inconspicuous, but containing nuclear pores, dilatation of the rough endoplasmic reticulum, opacity of the mitochondria, ill development of Golgi apparatus, reduction or disappearance

in \square granules, the demarcation membranes were dilated with loss of their beaded appearance, irregularly distributed which may account for the production of platelet with variable sizes retardation in platelet production, in the undamaged cells platelet territories had not reached the peripheral zone, also production of platelet without organelles or granules were detected. Some of the damaged cells showed virus like particles having a diameter of 45 nm on average, under high resolution these particles appeared electron dense core with membrane envelop having surface projections. In conclusion: The previous ultrastructural changes could explain a deleterious effect of the HCV on the infected megakaryocytes with subsequent defective platelet production.

0502

INTRON 1 FACTOR VIII GENE INVERSION IN A POPULATION OF TURKISH HEMOPHILIA A PATIENTS

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Background. Haemophilia A (HA), the deficiency of coagulation factor VIII (FVIII), is the most common, sex-linked inherited bleeding disorders. The disease is caused by a wide range of heterogeneous mutations in the FVIII gene and leads to a partial or total deficiency of FVIII protein activity. **Aims.** In this study we aim examined 61 unrelated patients with HA (37 severe types, 17 moderate types, 7 mild types; Median age 26 [3-58]) to identify the incidence of intron 1 FVIII gene inversion in Turkish population. **Methods.** Total genomic DNA was extracted from peripheral blood leucocytes by the proteinase K method. A detailed family history of the disease and the inhibitor status were also obtained. We performed two amplification under polymerase chain reaction (PCR) conditions suggested by Bagnall et al. One reaction contained tree primers: int1h-2F, int1h2R and 9F. These PCRs able to differentiate wild type from the patients with inversions and the patients with inversions from carriers. The amplified segments were readily separated on a 2% agarose gel. **Results.** In this study, inhibitor was detected in 12 (19.6%) patients, arthropathy was detected in 45 (73.7%) patients and family history was detected in 37 (59.6%) patients. According to the result of DNA analysis of sixty one Haemophilia A patients, intron 1 inversion of FVIII gene was not found. A mutant patients DNA sample obtained by Dr. Un was used as a positive control in every amplification. **Conclusions.** Intron 1 inversion of FVIII gene was not investigated in Turkish haemophilia A patients so far, whereas this inversion was detected 1.7-5% in Italian, 5% in Spanish, 4.3% in Czech Republic, 1.8-3.8 in UK, 1.5% in Iranian and 3.8 in Indian haemophilia A patients. Although the number of haemophilia A patients is low, the results of this study have the first data about Turkish population. Further studies will be required to show intron 1 inversion of FVIII gene in larger populations.

0503

CHIMERIC CD20-ANTIBODY RITUXIMAB IN FOUR CASES OF ACQUIRED HEMOPHILIA A

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Acquired hemophilia A is a rare bleeding disorder but often life-threatening. There exists no standardized therapeutic course, neither to control bleeding, nor to eliminate the inhibitor. We report on four cases of acquired hemophilia A, we used the chimeric CD20-antibody rituximab as second line or salvage therapy. First case: Patient 1, a 66-year-old man, relapsed six months after first complete remission of acquired hemophilia A with mild spontaneous cutaneous bleeding, aPTT 73 sec, FVIII 2%, inhibitor 1 BU. At this time he was still on ther-

apy with prednisolone 10mg abs. per day and cyclophosphamide 1,5 mg/kg per day. We applicated weekly infusions of rituximab 375mg/m² for 1 month and prednisolone 2 mg/kg per day for 10 days, then we stopped both. There was no more bleeding and 1 week later FVIII-activity was increased to 16%, further follow-ups showed continuous rising values. Five months later we found a second complete FVIII recovery. Second case: But 1 year later patient 1 had a relapse with mild spontaneous cutaneous bleeding again. This time we gave only a single shot of rituximab 375mg/m². Third complete remission appeared only 4 weeks later and still lasts for 1 year. Third case: Patient 2 is a 92-year-old man with spontaneous musculocutaneous bleeding because of acquired hemophilia A, aPTT 60 sec, FVIII 1%, inhibitor 15 BU. Due to supportive blood transfusions we started therapy with prednisolone 2 mg/kg and cyclophosphamide 1,5 mg/kg per day. Because there was no clinical or laboratory response, prednisolone and cyclophosphamid was stopped. We added one course of rituximab 375mg/m² and soon bleeding tendency disappeared. Two weeks later there was a complete FVIII recovery and no more inhibitor. There is no relapse for 1 year now. Fourth case: The diagnosis of acquired hemophilia A was established in the case of a 75-year-old man, patient 3, suffering of serious spontaneous musculocutaneous bleeding, aPTT 98 sec, FVIII 1%, inhibitor 140 BU. Beside a substitution of 227.000 I.E. of factor eight inhibitor bypassing activity (Feiba[®]), we initiated inhibitor elimination with prednisolone and cyclophosphamid both 2 mg/kg per day. The patient got a hyperglycemia and a psychotic crisis. We stopped prednisolone at once and started weekly infusions of rituximab 375mg/m² for 1 month. There was no more bleeding, but inhibitor level decreased slowly and normalized at least 1 year later. In none of the cases rituximab caused problems during applications or related complications until now, especially no infectious. We conclude the chimeric CD20-antibody rituximab is a safe and effective agent in therapy of acquired hemophilia A.

0504

APPLICATION OF CONJOINT ANALYSIS TO ELICIT PREFERENCES IN HEMOPHILIA CARE: CONAN STUDY

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Hemophilia care entails the absorption of a huge amount of human and economic resources and influences patients' quality of life. Moreover, in contrast with the traditionally limited patient involvement in the choice of treatment products, the treatment of hemophilia is peculiar in that involves a complex interaction between different parties-such as patients, physicians, policy-makers-carrying their own set of preferences. Aim To establish preferences in haemophiliacs, physicians and pharmacists as they relate to haemophilia treatment. **Methods.** Preferences of patients, physicians and pharmacists toward perceived viral safety, risk of inhibitor development, infusion frequency during prophylaxis, pharmaceutical dosage form, distribution modes and price were evaluated by conjoint analysis, using a discrete choice experiment. This technique shows how persons are willing to trade between different constituents of health care and thereby estimates their relative importance. Relationship between sociodemographic and/or clinical characteristics of patients and their preferences were evaluated. **Results.** Overall 178 patients, 69 physicians and 58 pharmacists completed the study. Patients, physicians and pharmacists displayed preferences 1) similar in direction and strength for risk of inhibitors and frequency of prophylaxis, 2) similar in direction, but not in strength for perceived viral safety and price, with patients showing lower strength compared to physicians and pharmacists, and 3) dissimilar in direction and/or strength for dosage form, which tested important only for pharmacists

and distribution mode, which tested important for patients and physicians only. Perceived viral safety and price tested important for all groups, but the strength of the preference was higher for pharmacists and physicians compared to patients. Regarding segmentation, the displayed preference on viral safety was greater for moderate haemophiliacs compared to severe haemophiliacs and was greater for patients in recombinant treatment to plasma derived. Compared to moderate haemophiliacs, severe haemophiliacs placed a higher value on the decrease in the risk of developing inhibitors and the value associated with infusion frequency was also much greater in severe haemophiliacs. The employment status played an important role on marginal effect of product distribution modes: employed patients placed a higher value to the level *home distribution* and *community pharmacy distribution* compared to self-employed and unemployed patients. Our study provides evidence of the differences between different stake-holders in the preferences toward hemophilia replacement therapy, indicating that different opinions should be taken into account when planning optimal care. In particular, patients are more focused on the process attributes respect to the other respondents and it indicates that, despite the outcomes are generally relevant, often the process is as relevant as outcomes attributes for patients. In addition, by stratifying the patients we can identify some characteristics that are very important for the interviewees. As a consequence, improvements in the patients' perception of the quality of health care (i.e. values) may be achieved at little or no cost. Conjoint analysis and discrete choice experiments are feasible and useful even in rare and particular diseases like hemophilia.

0505

DETECTION OF PLASMA CYTOKINES (TNF- α , IL-2, IL-6, IL-8) IN VENEZUELAN PATIENTS WITH DENGUE FEVER (DF) AND HEMORRHAGIC DENGUE FEVER (HDF)

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Dengue fever is an acute viral disease transmitted by the mosquito *Aedes aegypti*. In around 30% of the patients, the disease progress towards the haemorrhagic form, which may be potentially life-threatening. The disease is highly prevalent in several regions of Asia and America. In Venezuela, dengue fever has become endemoepidemic, constituting a severe problem of public health. The pathogenesis of the haemorrhagic form of the disease is far from clear, although several cytokines are believed to play an important role. *Materials and Methods.* During the 2004 epidemic break of dengue fever (DF) in Venezuela, forty two (42) patients, whose age ranged from 9 to 76 years, were admitted at Clínica El Avila (Caracas, Venezuela) with clinical and laboratory diagnosis of DF. At the time of admission (usually 3-4 days from the beginning of the symptoms), besides the clinical laboratory samples to evaluate routine haematological parameters, coagulation tests (prothrombin time, PTT, thrombin time, fibrinogen and fibrin degradation products, plasminogen and antithrombin), blood chemistry (BUN, creatinine, transaminases), a blood sample was taken to determine plasma concentration of four different cytokines: IL-2 (interleukin-2), IL-6, IL-8 and TNF- α (tumor necrosis factor- α). These were quantified with an ELISA assay using a commercial kit (Quantikine®, R & D Systems, Minneapolis, MN, USA). Twenty (20) apparently healthy blood donors served as normal controls. *Results.* Of the 42 patients with DF, 13 patients (31%) showed laboratory evidence of haemorrhagic dengue fever (HDF) (platelet count below 100.000/ μ L and signs of haemoconcentration indicated by an haematocrit greater than 20% of normal value); of these, 6 patients (46.2%) developed petechiae, purpura and severe thrombocytopenia with platelet count below 20.000 / μ L, requiring administration of plasma components or platelet transfusions. No cases of dengue shock syndrome were observed. Concentrations of TNF- α were found to be signifi-

cantly increased in 14 patients (33.3%) when compared to normal controls. The increase in TNF- α concentration was positively correlated with the severity of thrombocytopenia. IL-6 was increased only in 6 patients (14.3%), most of them with the severe form of the disease. IL-2 and IL-8 concentrations were not significantly different from those of the normal controls. *Conclusions.* These data suggest that during the development of DF and HDF circulating proinflammatory cytokines such as TNF- α and IL-6 play an important role in the pathogenesis and severity of the disease.

0506

ACQUIRED HEMOPHILIA IN PATIENT WITH INTERFERON- α TREATMENT FOR HEPATITIS C VIRUS INFECTION

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Acquired Hemophilia (AH) is a rare bleeding disorder, caused by an autoimmune depletion of Factor VIII C (F VIII:C), due to specific inhibitor. The inhibitor may occur in association with pregnancy or post-partum status, autoimmune diseases, medication, solid tumors, hematologic malignancies, infections and dermatological conditions. However, up to 46% of cases have no identifiable underlying disorders. The association between formation of factor VIII inhibitors and interferon- α treatment in patients with chronic hepatitis C, is extremely rare, and only one case, to our knowledge, has been described. Treatment consists of two objectives: permanent inhibitor suppression and management of the acute bleeding episode, but no general consensus exist on the best therapeutic approach. Several investigations suggest that oral cyclophosphamide and prednisone, without FVIII therapy, may be useful in patients with high titre inhibitor. We report a case of AH associated with interferon- α treatment for chronic hepatitis C virus infection, treated with oral immunosuppressive therapy and activated recombinant FVII (rFVIIa NovoSevenR) A 58-year old man, with a 10-years history of chronic hepatitis C virus infection and six months peginterferon- α 2a and Ribavirine treatment, developed spontaneous soft tissue hemorrhages especially on right leg and on tongue, with acute and severe anemia (Hb 8 gr/dL), although platelets number was normal (Plt 202X10⁹). His family history was negative for hemorrhagic diathesis. HCV-RNA was elevated (350.000 copy/mL). Coagulation assay showed a normal prothrombin time and fibrinogen levels and a prolonged activated partial thromboplastin time (APTT 73"-n.v. 34"). FVIII C level was < 1% (n.v. 60-150); lupus anticoagulant's research was negative. An antibody direct against FVIII C was found at high titre (130 BU/mL). A diagnosis of AH was made and oral immunosuppressive therapy with prednisone 1mg/Kg/die, cyclophosphamide 100mg/die, and rFVIIa (NovoSevenR) at dose of 90 μ g/kg every three hours for two days, was started. APTT, level of FVIII and inhibitor was measured every 1-week. APTT gradually returned to normal value, inhibitor level decreased, whereas FVIII levels increased and returned to normal value after 4 weeks (Tab.1). One month later, hemorrhagic diathesis disappeared and Hb increased (14,1 gr/dL) without blood transfusions. Cyclophosphamide was stopped after 4 weeks and prednisone was gradually tapered off after 3 months. In patients with acute and chronic hepatitis C virus infection has been hypothesized a dysregulation of the immune system that may favour the development of an abnormal lymphoid clone and in our case, probably, autoantibodies against FVIII. In conclusion our observation illustrates high titre inhibitor-AH associated with peginterferon- α 2a treatment for chronic hepatitis C virus infection, successfully treated with oral prednisone, cyclophosphamide and rFVIIa. Causal relationship between interferon- α treatment for chronic hepatitis C virus infection and AH remains speculative. Although the clinical course is not predictable and inhibitor may disappear spontaneously, in some cases, with high titre inhibitor associated, combined therapy with prednisone, cyclophosphamide and rFVIIa may be sufficient to suppress inhibitor and to arrest bleeding.

0507**RITUXIMAB FOR TREATMENT OF RECURRENT THROMBOTIC THROMBOCYTOPENIC PURPURA**T.C. Bortolheiro, N.S. Castro, R.D. Cançado, C.S. Chiattonne
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Thrombotic thrombocytopenic purpura (TTP) is a rare and severe disease, characterized by thrombocytopenia, microangiopathic anemia, fever, renal failure, and neurologic manifestations, due to the deposition of the von Willebrand factor-platelet-rich hyaline thrombi in the arterioles and capillaries. The use of plasma exchange has decreased the mortality rate by TTP from almost 100% to 10-30%. Relapse occurs in more than 1/3 of the cases and a subset of patients develop multiple relapses or chronic disease, requiring innumerable sessions of plasma exchange which is costly and associated with many adverse reactions such infections, thrombosis and transfusion reactions. Methodes We describe a patient, diagnosed with TTP in 1996, who failed to achieve sustained remission with plasma exchange, steroids, vincristine and remained in remission after a splenectomy, performed in 1997. The patient relapsed in 2003 and resumed plasma exchange, with progressive improvement of cytopenias. When the plasma exchange was reduced to day-in-day-out, the platelets decreased and the LDH increased. We again tried steroids and vincristine, without response. Then we started with rituximab, 375 mg/m² weekly, during four weeks. She required plasma exchange only during the first two weeks. After the second dose of rituximab, the plasma exchange was discontinued and she remained in complete remission for 9 months. Subsequently, she was admitted to the hospital with femoral thrombosis, with normal LDH and a normal platelet count. On the fortieth day of thrombosis treatment, the TTP relapsed. She refused the plasma exchange and started with rituximab, with the same schedule and achieved complete remission in two weeks, sustained until now (11 months). *Conclusions.* We compared this case with the 8 previously-reported cases of TTP treated with rituximab, and we concluded that rituximab was demonstrated to be very effective in treating TTP, without collateral effects, although randomized clinical trials are required before sanctioning the use of rituximab alone or in combination with plasma exchange for the treatment of TTP.

0508**REGIONAL MOLECULAR DATABASE IN HAEMOPHILIA A IN HUNGARY**A.N. Nagy¹, W. Schröder², F.H. Herrmann², B. Meng¹, H. Losonczy¹, M. David¹, M. Kecskes¹¹*Pécs University, Faculty of Medicine, Pécs, Hungary;*²*Institut für Humangenetik, GREIFSWALD, Germany*

According to the statistics till now 893 patients with hemophilia A were registered in Hungary. *Aims.* Aim of our study to organise genetic examination of haemophilia A patients and their family members, in order to determine carrier state of women. Our goal was to create a database which could serve as a basis of the carrier screening and prenatal diagnosis. We studied up to now 74 affected members of 61 independent family with haemophilia A (50 families with severe, 8 families with moderate and 3 families with mild haemophilia A). Six out of 74 patients (8,1%) showed the presence of inhibitors. We studied Intron 22 inversion by Southern blotting method. For carrier diagnosis three extragenic RFLP's (Taq I/St14.1, Bgl II DX13, Xba I/DXS115) and two intragenic RFLP's (Hind III/int 19 and Xba I/int 22) have been characterised. Twenty two family (36,1%) had a type I (distal) factor VIII intron 22 inversion, and 6 (9,8%) showed a type II (proximal) inversion. In 1 case, the molecular analysis showed intron 1 inversion (1,6%). The detection of the intron 22 inversion state was informative for 19 females. In 32 families (52,4%) no inversion could be detected. For those families we offered an indirect genomic diagnosis based on RFLP analysis for carrier screening and this indirect

diagnosis was performed in nine case the carrier state could be confirmed, and in 7 case excluded. At least one marker was informative for all carriers (an intragenic marker for 6 times an extragenic marker only for 3 times). *Conclusions.* The prevalence of inversion in our study is similar to the data stated in literature. The inversion screening enables a secure identification of female carriers in the appropriate families. Indirect diagnosis with a high degree of heterozygosity of women is a good basis for carrier detection and genetic counselling, as far as a sequence analysis cannot be offered for all families.

0509**MOLECULAR TESTING OF HEMOPHILIA A IN MOLDOVAN PATIENTS**N. Sirocova¹, S. Nakaya², A.R. Thompson³¹*National Center of Reproductive Health, CHISINAU, Moldova;*²*Puget Sound Blood Center and, SEATTLE, WA, USA;* ³*Puget Sound Blood Center, SEATTLE, WA, USA*

Hemophilia A is an X-linked bleeding disorder which results from heterogeneous mutations in factor VIII (FVIII) genes with the incidence of 1 in 5,000 males. Approximately 45% of severely affected hemophilia A patients have an intron 22-A gene inversion. In addition 2-5% of severe cases result from an intron 1-5' inversion. Other cases have a variety of mutations throughout the gene. The aim of this study was to determine disease causing mutations using long range PCR technique, heteroduplex and sequence analyses. Presented here are the results of a molecular study of 19 patients (18 severe and 1 moderate) with hemophilia A. DNA samples were prepared by standard phenol-chloroform procedure. Long range PCR was used for detection of intron 22 inversions. Patients negative for intron 22 or intron 1 inversions were studied by heteroduplex analysis of FVIII's 26 exons with following sequencing. All mutations were confirmed by studying a carrier, an affected sibling or repeat studying of the proband. Of 18 severe hemophilia A patients, 8 (44%) have an intron 22-A gene inversion and one has an intron 1-5' inversion. One has a deletion of exon 26 that does not include the intron 25 BglII polymorphic site. Other families have 4 frameshift, 1 splice junction and 4 missense mutations. Of these 9, 6 are novel including: Pro130 (DC.CCCT-CCCT), Ile1790 (DT ATT-AT), e23 (-2) ag-gg, Gly455Trp, Asn1805Ile and Tyr1837Cys. Using these data we have identified carrier status. Ten of 18 patients represent sporadic case. All mothers of these patients were diagnosed as carriers. From female relatives in families with hemophilia history 2 were found as carriers and 2 were normal.

0510**CHARACTERIZATION OF P53 AND ATM ABNORMALITIES IN B-CLL PATIENTS**

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Defects in p53 gene confer the inferior prognosis for B-CLL patients together with inactivation of p53 regulatory kinase ATM and non-mutated IgVH locus. Inactivation of ATM seems to be a likely alternative to defects in p53 and seems to highly coincide with non-mutated IgVH. The defects in p53 do not correlate so closely with IgVH germ-line status and require further, more detailed investigation. The aims of the present study were to analyze the defects in ATM and p53 locuses in a large cohort of B-CLL patients and to determine their relationship to IgVH status. We analyzed the status of p53 gene in 139 patients diagnosed with B-CLL of all stages (both treated and untreated) using functional analysis in yeasts (FASAY; to our knowledge used for the first time in B-CLL samples) and monitoring of protein expression by Western-blotting. Cytogenetic data con-

cerning the *p53* (LSI p53 17p13.1) and ATM (LSI ATM 11q22-q23) were available for two thirds and one half of the analyzed patients, respectively. All the identified mutations in the *p53* gene were determined by direct sequencing from yeast colonies harboring mutated phenotype. We have used PCR and direct sequencing to analyze the IgVH rearrangements and mutation status. Deletion of ATM was detected in 25% of patients, inactivation of *p53* occurred in 15,8% of patients and defects in these two genes were mutually exclusive in all the cases. The deletion of one and mutation of the second allele was the most frequent type of *p53* inactivation. The *p53* protein expression correlated well with point mutations or small deletions in the gene, since all these patients expressed considerably higher amount of protein than any but one sample with wild-type *p53*. Except of 11 missense mutations we detected also 2 small deletions, 2 alternatively spliced isoforms and 1 frameshift mutant. One third of tested samples (n=105) exhibited mutated IgVH locus, using 98% cut-off level. Deletion of ATM locus and inactivation of both alleles of *p53* occurred almost exclusively in patients with non-mutated IgVH (12/12 for ATM and 11/12 for *p53*). Situation is not so clear for patients with either deletion or mutation of *p53*, since to the contrary more patients (5/8) harbored mutated IgVH. 5. Summary/conclusions: We confirm in a large cohort of patients that defects in ATM and *p53* locuses are mutual alternatives and that ATM inactivation coincides strictly with non-mutated IgVH. While it seems that inactivation of both alleles of *p53* also occurs markedly more frequently in patients manifesting non-mutated IgVH, it is not so obvious for (relatively rare) patients with inactivation of only one allele of *p53*.

Supported by grants MSMT No.1K04017 and Elpida-Nukleus.

Chronic lymphoblastic leukemia and related disorders - Biology

0511

IMMUNOGLOBULIN (IG) HEAVY AND LIGHT CHAIN CDR3 JUNCTIONAL FEATURES IN CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL) VS. MULTIPLE MYELOMA (MM)

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The purpose of highly diverse CDR3 regions in all antigen receptors is to provide antigen specificity. As previously shown in autoreactive cells, certain CDR3 features may provide high-affinity binding even without extensive somatic hypermutation (SHM): thus, long CDR3s have been associated with self-reactive or polyreactive antibodies. CLL IG repertoire is restricted and also characterized by the existence of subgroups with shared, 'stereotyped' CDR3s. In the present study, we examined HCDR3 and KCDR3/LCDR3 features in 291 CLL patients and 95 MM patients. In the CLL group, 182/291 IGHV-D-J, 94/192 IGKV-J and 47/99 IGLV-J sequences were less than 98% homologous to germline ('mutated'); all MM sequences were mutated. HCDR3 median lengths in CLL vs. MM were, respectively, 16 and 15 aminoacids (range: 6-29 and 6-28, respectively); longer HCDR3s were observed in unmutated CLL sequences, especially those utilizing IGHV1 subgroup genes. The IGHJ6 gene was used in 29/182 (16%) mutated vs. 43/10⁹ (39%) unmutated CLL sequences and in 12/72 (16%) MM sequences. In the mutated CLL subgroup, frequent IGHJ6 usage was observed in 17/56 (30%) IGHV4-utilizing sequences; furthermore, IGHJ6 was used in 6/9 IGHV4 CLL mutated sequences with evidence for selection by antigen. IGHD2 and IGHD3 subgroup genes were used with similar frequencies in either CLL or MM. IGHD genes were predominantly read in reading frame (RF) 2 in both

CLL and MM sequences; however, IGHD5 and IGHD6 genes in CLL and IGHD2 genes in MM were mostly read in RFs 1 or 3. N nucleotides were detected in 281/291 CLL sequences (96%/median, 15; range, 1-52) and 71/72 MM sequences (98%/median, 15; range, 1-58). KCDR3 median length was 9 aminoacids in both CLL and MM (range: 6-12 and 5-11, respectively); longer KCDR3s (median, 10 aminoacids) were observed in unmutated CLL sequences utilizing IGKV1 and IGKV3 subgroup genes. IGKJ3-5 gene usage was observed with a similar frequency (~25%) in either CLL or MM. Evidence for TdT activity was identified in 66%/42%/40% of unmutated CLL/mutated CLL/MM sequences. LCDR3 median length was 11 aminoacids in both CLL and MM (range: 8-13 and 8-12, respectively); shorter LCDR3s (median, 10 aminoacids) were observed in CLL sequences utilizing IGLV2 subgroup genes, while longer LCDR3s were found in IGLV3-21 CLL sequences (12 aminoacid-long LCDR3s in 11/18 CLL vs. 0/8 MM IGLV3-21 rearrangements). IGLJ1 gene usage was more frequent in CLL (frequency: 21% for both mutated and unmutated sequences) than MM (14%). N nucleotides were identified in 44%/42%/40% of unmutated CLL/mutated CLL/MM sequences. In conclusion, CLL CDR3 regions often exhibit features associated with 'natural' autoantibodies produced early in B cell development, e.g. increased usage of IGJ6 and IGHD2/3/6 subgroup genes (which provide important aromatic side chains); increased frequency of IGLJ1; increased length (both in heavy and light chains), especially among unmutated sequences. Finally, the distinctive features of CLL CDR3 regions allude to recognition of individual, discrete antigens or classes of structurally similar epitopes, which, in the unmutated subset of CLL, could exert pressure for maintaining germline configuration.

0512

SURFACE EXPRESSION OF VEGF BY CLL CELLS CORRELATES WITH INTRACELLULAR BCL-2 AND KI67 EXPRESSION AND SUGGESTS THAT VEGF MAY CONTRIBUTE TO CLL GROWTH AND SURVIVAL IN AN AUTOCRINE MANNER

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B-cell chronic lymphocytic leukemia (CLL) is a malignant disorder, characterised typically by accumulation of long-lived B lymphocytes, with low proliferation rate but an increased resistance to apoptosis. Patients with CLL have an extremely variable clinical courses and newer prognostic markers, such as CD38 and ZAP-70 protein expression, may predict which patients in early stage CLL are likely to progress. Increased angiogenesis and vascular endothelial growth factor (VEGF) may be important in the pathophysiology of B-CLL and we have previously shown a strong correlation between expression of CD38 and VEGF on CLL cells. 2.Aims. In the present study, we explored the relationship between VEGF surface expression by CLL cells and CD38 and ZAP-70 expression, intracellular levels of the cell proliferation marker, Ki67 and of the anti-apoptotic protein, bcl-2, as well as Binet stage, time from diagnosis, complete blood count and serum LDH, β 2 microglobulin and CRP. 3.Methods. Peripheral blood samples were taken, following informed consent, from 24 consecutive CLL patients (13 Binet stage A, 6 stage B, 5 stage C disease). 12/24 patients had received chemotherapy but not for at least 4 weeks prior to the study. Immunophenotyping was performed by triple color flow cytometry (Coulter EPICS-XL) on peripheral blood CD19+, CD5+ lymphocytes. 4.Results. Median percentage (with range) of CLL cells positive for surface expression of VEGF was 47.4% (1.2%-84%), compared to 6.6% on B cells from normal controls (p=0.001, Spearman's correlation test). Median percentage surface expression of CLL cells positive for CD38 and ZAP 70 was 2.1 (0.2-70.8) and 4.7 (0.3-33.3) respectively, with positive correlation between the 2 measures (p<0.05). Median percentage intracellular expression of Ki67 and of BCL-2 by the CLL cells was 12 (0.2-56.1) and 94.4 (0.6-99.8) respectively. Percent-

age surface expression of *VEGF* by the malignant cells correlated with disease stage ($p=0.0135$), time from diagnosis ($p<0.05$), intracellular *bcl-2* expression ($p<0.001$) and *Ki67* expression ($p<0.05$). However, *VEGF* surface expression failed to show a significant correlation with either *CD38* or *ZAP70*. No significant correlation was found between either *Ki67* or *BCL-2* and clinical stage. Lymphocyte doubling time had a positive correlation with lymphocyte count but no significant correlation with percentage expression of *VEGF*, *CD38*, *ZAP70*, *BCL-2* or *Ki-67* by the CLL cells. 5.Summary/conclusions.The demonstration of significant correlation between surface expression of *VEGF* and intracellular expression of both *bcl-2* and *Ki-67* by CLL cells in this study supports the hypothesis that, as well as stimulating bone marrow angiogenesis, *VEGF* may contribute to both B-CLL growth and survival in an autocrine manner. The correlation between *VEGF* expression by the malignant cells and both disease stage and time from diagnosis adds further support for a role of *VEGF* in B-CLL progression. Our data provide further evidence in support of the use of *VEGF/VEGFR* pathway inhibitors in CLL, either in combination with conventional chemotherapy or possibly as single agent therapy to delay or prevent disease progression in patients with early stage CLL and poor prognostic features.

0513

LEVELS OF CYTOMEGALOVIRUS-SPECIFIC CD8+ T CELLS ARE MARKEDLY EXPANDED IN PATIENTS WITH B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA (B-CLL) AND EXHIBIT RESTRICTED TCR VBETA USAGE

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B-cell chronic lymphocytic leukaemia is associated with the development of immunosuppression. Abnormalities in the phenotype and function of T cells have been reported in patients with B-CLL including absolute CD8 lymphocytosis, inversion of CD4/CD8 ratio, high expression of activation markers and increased number of highly differentiated T cells with a CD28-CD57+ phenotype. Significant overexpression of some of Vbeta families has been reported in CLL patients. Cytomegalovirus (CMV) infects the majority of the human population in their lifetime and stimulates the generation of a vigorous CMV-specific immune response. The magnitude of the CD8+ response is often increased in patients with immunosuppression, which may be a response to subclinical episodes of viral reactivation. Cytomegalovirus (CMV) is most likely to be involved in the clonal expansion of T cells in B-CLL. We studied the frequency of CMV-specific CD8+ T cells in patients with B-CLL. Blood samples were collected from 73 patients (45 CMV seropositive). CMV-specific CD8+ T cells were identified by tetramer staining using seven types of CMV tetramers. The frequency of CMV-specific CD8+ T cells was up to 32.2% of CD8+ T cells. Patients had detectable NLV-specific CD8+ T cells up to 19.2% with the average frequency of 4.0% relative to 1.4% for controls ($p=0.03$). For TPR response the average was 7.8% for patients compared to 4.1% in controls. The frequency of CD57+CD28-phenotype in the CD3+CD8+ population was significantly higher in CMV seropositive patients compared to CMV seronegative patients ($p<0.0001$). TCR Vbeta expression was studied using monoclonal antibodies. CMV-specific CD8+ T cells showed restricted TCR Vbeta usage and the most expanded TCR Vbeta families within the CD8 population were CMV-specific. In conclusion, CMV-specific CD8+ T cells are significantly higher in CMV positive B-CLL patients than elderly controls. This level of immune response is likely to be required to control viral reactivation. The most expanded TCR Vbeta families within the CD8 population in B-CLL appear to be CMV-specific.

0514

DIFFERENT ISOFORMS OF PAX-5 (BSAP) REGULATE EXPRESSION OF AID IN NORMAL AND CHRONIC LYMPHOBLASTIC LEUKEMIA B-CELLS (CLL)

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Activation induced cytidine deaminase (AID) plays a key role in somatic hypermutation (SHM) and class switch recombination (CSR). However, the mechanisms accounting for AID action and control of its expression remain unclear. Recent work demonstrated that the B-cell specific activator protein (BSAP), encoded by the Pax-5 gene, an essential factor in B cell development, up-regulates AID expression, whereas *Id-2* and *prdm-1* (Blimp-1) down regulate AID expression. In a recent work we have shown, that in contrast to normal circulating B-cells, AID transcripts are expressed constitutively in CLL patients undergoing active CSR, but interestingly this expression occurs in unmutated CLL B-cells. These data favour the view that AID protein may act differentially on CSR and SHM pathways (Oppezzo et al, *Blood*, 2003) but the role-played by AID in both processes remains to be elucidated. To define the role of Pax-5, *Id-2* and *prdm-1* genes in the regulation of AID expression in normal and CLL B cells. To gain insight into the molecular mechanisms underlying AID expression and CSR, we analyzed by RQ-PCR the expression level of Pax-5, *Id-2*, *prdm1* and AID genes in normal human B-cells and CLL B-cells upon CD40 ligand stimulation, which induces expression of AID. Protein expression of BSAP and AID was analyzed by electrophoretic shift mobility assay (EMSA) and western-blot. We have studied the role of Pax-5, *Id-2* and *prdm-1* genes in regulation of AID expression and CSR in 6 normal and 54 CLL patients (27 with constitutive AID expression). Our results show that AID expression in CLL B-cells is tightly regulated by the differential expression of Pax-5 gene. The expression of AID proteins is associated with strong expression of only complete form of the Pax-5 gene (Pax-5a), whereas absence of AID is consistently associated with a reduction of Pax-5a mRNA and the appearance of a second spliced form with a complete deletion of the eighth exon (Pax-5/deltaEx8). Similar results were found before and after CD40L+IL-4 stimulation in normal and CLL B-cells without expression of AID. Since the deleted form lacks an important fragment of the auto-regulatory activation domain while conserving the inhibitory region, we determined by using EMSA followed by Western Blot with specific anti-BSAP and AID antibodies whether the appearance of this isoform could play a role in AID expression. Our results demonstrated that both isoforms of BSAP are able to bind to the AID-promoter region, suggesting that the spliced Pax-5/deltaEx8 isoform could compete with the complete Pax-5a isoform for the access to the AID promoter. In addition our results demonstrated that AID expression correlates with a decrease in *Id-2* and *prdm-1* transcripts.

0515

BIOSYNTHESIS AND BIOLOGICAL ROLE OF LEUKOTRIENE B4 IN CHRONIC LYMPHOBLASTIC LEUKEMIA

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Leukotrienes (LT) are biologically active metabolites of arachidonic acid. In contrast to prostaglandins, which are produced by almost all type of cells, formation of leukotrienes from arachidonic acid is restricted to a few cell types in the human body. Biosynthesis of leukotrienes occurs mainly in myeloid cells and B lymphocytes. Microarray studies have demonstrated an abundant expression of 5-lipoxygenase (5-LO) in B-cell chronic lymphocytic leukemia (B-CLL) cells. *Aims.* Study the biosynthesis of

LTB4 in B-CLL and the effects of leukotriene biosynthesis on the activation of B-CLL cells. **Results.** B-CLL cells produced LTB4 after activation. Studies on the expression of the high affinity receptor for LTB4 (BLT1) by flow cytometry analysis showed that the receptor was expressed, to a varying degree, in all investigated B-CLL clones. The drugs BWA4C (a specific 5-LO inhibitor) and MK-886 (a specific 5-LO activating protein inhibitor), at a concentration of 100 nM, markedly inhibited CD40-induced DNA synthesis and CD40-induced expression of CD23, CD54 and CD150. Addition of exogenous LTB4 almost completely reversed the effect of the inhibitors on DNA synthesis and antigen expression. The effects of leukotriene biosynthesis inhibitors on the expression of several genes will also be discussed. **Conclusions.** The results of the present study suggest that leukotriene biosynthesis inhibitors may have a therapeutic role in B-CLL.

0516

TCRGD+ LARGE GRANULAR LYMPHOCYTE PROLIFERATIONS REFLECT THE SPECTRUM OF NORMAL TCRGD+ T-CELLS IN PERIPHERAL BLOOD

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Expansion of large granular lymphocytes (LGL) is a type of lymphoproliferative disorder, which ranges from reactive expanded activator T-cells to T-cell leukemia and shows variable presentation and clinical course. In T-LGL leukemia the lymphocytes are clonal in nature as evidenced by clonally rearranged TCR genes. The vast majority concern TCR $\alpha\beta$ + T-LGL lymphoproliferations. Much less is known about the characteristics and pathogenesis of TCRgd⁺ cases. **Aims.** To determine the clinical behavior and hematological features of persistent TCRgd⁺ large granular lymphocytosis and define the marker expression of these abnormal cell populations. In addition, we aimed to get more insight into the molecular pathogenesis of TCRgd⁺ T-LGL leukemia. In the present study we evaluated the clinical data of a series of 49 patients with persistent mature TCRgd⁺ T-cell proliferations in peripheral blood and/or bone marrow, including a large number of well defined TCRgd⁺ T-LGL leukemias (n=39). We performed an extensive immunophenotypical analysis using a T/NK-cell antigen panel and a Vg/Vd antibody panel. BIOMED-2 PCR-based GeneScan and sequence analysis of TCR gene rearrangements was performed in all peripheral blood (PB) and/or bone marrow samples. **Results.** TCRgd⁺ T-LGL leukemia is similar to TCRab+ cases in clinical presentation and both are characterized by an indolent clinical course. Patients appear to be symptomatic in 51% of cases and the main presenting manifestations are chronic neutropenia (49%), anemia (23%), and thrombopenia (8%). Associated autoimmune diseases are present in 26% of cases. Rheumatoid arthritis (RA) is the most frequent autoimmune manifestation (13%). Multiple associated hematological disorders are found in 21% of cases. CD3+ TCRgd⁺ leukemic LGL cells were predominantly positive for CD8, CD57, Vg9 and Vd2. CD16, CD56, CD11b and CD11c were variably expressed. Other cases were positive for Vd1 and Vg other than Vg9. Sequence analysis of clonally rearranged TCRG and TCRD genes demonstrated that the majority of the patients had a leukemic LGL cell population expressing a TCR consisting of a V α 9-J α 1.2- α 1 chain and a V β 2-J β 1- β chain. Overall, the gene segment usage and Vg/Vd specificity of the T-cell proliferations reflect the spectrum of normal TCR $\alpha\beta$ + T-cells in PB. Besides we found the invariant T nucleotide at the relative second position of the Vd2-Jd1 junctional regions, to be present in almost all Vg9/Vd2 T-LGL proliferations. This invariant T nucleotide leading to a neutral amino acid is considered to reflect selected peripheral T-cells. Based on their immunophenotypic and immunogenotypic features TCR $\alpha\beta$ + T-LGL proliferations seem to reflect their normal PB TCR $\alpha\beta$ + T-cell counterparts. The presence of a

unique selection determinant in a large fraction of TCRgd⁺ LGL leukemia populations might indicate a role for antigenic stimulation of peripherally selected TCRgd⁺ T-cells in the pathogenesis of this disease.

0517

FLOW CYTOMETRIC ANALYSIS OF THE TCR V β REPERTOIRE IN MATURE T-CELL EXPANSIONS

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It has been recently shown that the immunophenotypic analysis of the T-cell receptor (TCR) V β repertoire has a good sensitivity and specificity in defining the monoclonal or polyclonal nature of T-cell expansions. **Aims.** To investigate the TCR V β repertoire by flow cytometry in a series of mature T-cell expansions and its diagnostic value in predicting clonality, compared to polymerase chain reaction (PCR) TCR rearrangement analysis. We selected patients with either an absolute mature T-cell lymphocytosis (absolute lymphocyte count: median 3.5x10⁹/L; range 0.2-119) and/or the presence of an aberrant immunophenotypic profile (i.e. intensity and/or pattern of antigen expression). Nineteen cases were evaluated, 12 males and 7 females, with a median age of 61 years (range 24-71). Morphological evaluation of peripheral blood smears (all cases) and bone marrow/lymphnode histology (6 cases), revealed expansion of LGL in 11 cases, T-NHL in 3, mycosis fungoides in 1 and an uncertain diagnosis in 4 cases. Peripheral blood cells were characterized using four-color immunostaining with the following T-cell markers: CD3, CD2, CD4, CD5, CD8, CD16, CD56, TCR α/β , TCR α/γ , HLA-DR, CD38, CD7, CD158-a, CD158-b, CD1-a. The T-cell expansion was CD4+ in 3 cases and CD8+ in 12; the other 4 cases showed a normal CD4/CD8 ratio. The TCR V β repertoire was evaluated by flow cytometry using the TCR V β kit, a conjugated monoclonal antibody panel against 24 members of 19 families of variable regions of the TCR β chain (Beckman-Coulter Immunotech, Marseille, France). These were combined with CD3 and either CD4 or CD8 antibodies. In all cases, TCR analysis gene configuration rearrangement was assessed by PCR using three different primers forward (V2-8, V9, V10-11) and two primers reverse (J1/2 and JP1/JP2) to amplify the variable regions of the TCR β gene. **Results.** In 13/19 samples, the immunophenotypic analysis identified a reactivity with one of the V β monoclonal antibodies in 52% (range 28-80%) of the total T-lymphocytes. In 2/19 patients, T cells showed a complete lack of any V β antibody reactivity. No case with more than one V β antibody dominant reactivity was found. The following families were identified: V β 5.3 and V β 14 in the CD4+ T-cell expansions; V β 2 (2 cases), V β 3, V β 4 (2 cases), V β 5.1, V β 7.1, V β 13.2, V β 13.6, V β 16, V β 21.3 in the CD8+ T-cell expansions. The latter 15 cases showed a clonal TCR rearrangement detected by PCR analysis. In the remaining 4/19 cases, where the pattern and frequencies of V β antibody positivities by flow cytometry were within the normal ranges, PCR analysis showed a polyclonal TCR gene rearrangement. **Conclusions.** Our findings underline the value of flow cytometry in predicting T-cell clonality with high sensitivity and specificity. Extended immunophenotypic analysis allows a rapid screening within T-cell subsets and the quantitation of the V β -restricted cells, representing a useful tool for diagnosis and for the monitoring of pathologic clones.

0518**STRIKINGLY HOMOLOGOUS VH3-21/VL2-14 GENE REARRANGEMENTS IN CHRONIC LYMPHOBLASTIC LEUKEMIA DESPITE GEOGRAPHICAL ORIGIN**

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We recently reported a new subgroup of chronic lymphocytic leukemia (CLL) utilizing the immunoglobulin (Ig) VH3-21 gene, where many cases display homologous heavy chain complementarity determining regions 3s (HCDR3s) and predominant lambda light chain expression with biased VL2-14 gene usage. The restricted Ig gene features in the VH3-21+ CLLs may indicate that antigen selection plays a role in CLL development. Furthermore, in contrast to other CLLs, VH3-21+ cases show poor prognosis regardless of VH gene mutation status. However, so far these findings have mainly been documented in Swedish CLLs. *Aims.* Our aims were (1) to investigate whether VH3-21+ CLLs have similar molecular characteristics independent of geographical origin and (2) to study prognosis in these patients in an extended material. *Methods.* We analyzed the composition of the heavy and light chain Ig gene rearrangements by PCR amplification and sequencing in a joint material of 87 VH3-21+ CLLs including cases from Sweden (n=32), Germany (n=37), Italy (n=10), Finland (n=3), USA (n=3) and Australia (n=2). VH sequences with less than 98% homology to the germline sequence were considered as mutated. The HCDR3 length was calculated between codon 95-102 and the light chain CDR3 (LCDR3) between codon 89-97 as described by Kabat et al. Survival data was available in 60 cases and survival analysis was performed by Kaplan-Meier and log-rank test. Result Of the 87 VH3-21 gene rearrangements, mutated VH genes (mean mutation frequency 3.8%) were shown in 56 (64%), whereas the remaining 31 (36%) had unmutated VH genes. We could verify the previously reported short and homologous HCDR3s (7 codons) in 51 of 87 VH3-21+ cases (59%) and many of these also showed the HCDR3 amino acid motif, DANGMDV. Moreover, a highly biased usage of the VL2-14 gene (65 of 87 cases, 75%) was found with a considerably restricted LCDR3 sequence, QVWDS (S/G)SDHPWV. In 46 of the cases (53%), combined VH3-21/D-/JH6 and VL2-14/JL3 rearrangements were demonstrated and restricted HCDR3/LCDR3 was detected in cases from all countries included. The overall survival was poor in this cohort with a median survival of 83.6 months, with no significant difference depending on mutation status. In addition, survival analysis did not reveal any difference in overall survival for cases with homologous or non-homologous HCDR3s or for cases with or without VL2-14 usage. Summary Our data confirms poor outcome in VH3-21 utilizing CLLs irrespective of mutation status and reveals presence of highly restricted B-cell receptors in VH3-21+ CLL independent of geographical origin. The fact that very similar VH/VL rearrangements can be found in different countries strengthens the theory of antigen selection through recognition of a common antigen epitope, especially considering the extremely low probability that similar VH/VL rearrangements occur at random. The current theory is that the antigen (s) most likely promotes proliferation of the VH3-21/VL2-14+ cells thus increasing the risk of transformation. However, to date the potential antigen (s) are unknown and further research into finding these is now necessary.

0519**PRAME IS A MEMBRANE PROTEIN ABERRANTLY EXPRESSED IN CHRONIC LYMPHOBLASTIC LEUKEMIA AND MANTLE CELL LYMPHOMA**

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PRAME (Preferentially Expressed Antigen in Melanoma) is expressed at low levels by normal adrenal, ovarian and endometrial cells. In contrast, its expression has been demonstrated at high levels in acute leukemias and multiple myeloma. We have reported that PRAME is also expressed in lymphoproliferative diseases and its transcripts were detected in 26 out of 58 patients with chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). Nevertheless, the expression of PRAME protein in normal and neoplastic tissues is unknown. In order to address this question, we produced a monoclonal antibody (MoAb) against PRAME protein. A 562-nt fragment of the PRAME cDNA was cloned in pCR2.1-TOPO vector and subcloned into a pET24a expression vector, and the 196-amino acid peptide including the immunogenic 9-peptide was expressed in BL21 (DE3) cells. The MoAb against PRAME was generated from splenocytes from mice immunized with the purified peptide. *Material and Methods.* We analyzed PRAME expression by flow cytometry in samples of mononuclear cells (MNC, n=15), purified CD19 cells (CD19+, n=15), from different healthy blood donors and in peripheral blood (PB) samples from 26 CLL and 7 MCL patients. In order to evaluate which PB cells expressed PRAME, we used a panel of MoAbs (CD19, CD3, CD16/56, CD11b/33). *Results.* PRAME positive cells corresponded predominantly to B-lymphocytes and represented less than 20% of normal MNC. In contrast, 25 out of 26 CLL and 6 out of 7 MCL cases presented more than 20% of PRAME+ cells [mean 59% (range:20-95%) and 75% (20-94%) for CLL and MCL, respectively]. To quantify PRAME expression we evaluated PRAME Specific Antibody Binding Capacity (SABC) by a quantitative flow cytometry (QFC) method. The mean values of SABC were of 10,339 (range 3,075 to 24,665) and of 14,191 sites per cell (range 5,059 to 20,679) for CLL and MCL, respectively. In contrast, in normal MNC and CD19+ lymphocytes, SABC mean values were 1,688 (range 1,628 to 1,781) and 1,908 (range 543 to 6,402) sites per cell, respectively. Even in the two cases of CLL and MCL that had less than 20% PRAME+ cells, PRAME SABC was 3,075 and 12,347 sites per cell respectively, therefore significantly higher than the observed in normal PB lymphocytes. To evaluate the sensitivity of the QFC method, we established a cut off value for PRAME SABC based on the highest value detected in normal MNC cells and then serially diluted tumor MCL cells marked with PRAME in normal PB lymphocytes. Based exclusively on PRAME expression, we were able to detect neoplastic MCL cells up to a 1:1000 dilution. Finally, we have demonstrated by in situ immunofluorescence that PRAME is expressed as a membrane protein. *Conclusions.* Antigenic approach revealed that PRAME is a transmembrane protein expressed in low levels in normal B lymphocytes and strongly and aberrantly expressed in the neoplastic clone of CLL and MCL. This finding supports the suggestion that this antigen may be further explored as a target for diagnostic, minimal residual disease detection and for therapeutic approaches.

0520

SERIAL ANALYSIS OF GENE EXPRESSION (SAGE) OF PURIFIED CLL CELLS REVEALS SIMILAR PROFILES DESPITE IGH MUTATIONAL STATUS, AND ALTERED APOPTOSIS-RELATED GENES

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Chronic lymphocytic leukemia (CLL) comprises two subtypes of the disease that are characterized by important outcome differences observed between patients expressing either mutated (MUT) or unmutated (NAIVE) immunoglobulin (Ig) genes. We analyzed the gene expression of the malignant cells to identify basic molecular mechanisms related to the disease using Serial Analyses of Gene Expression (SAGE), an approach thus far not reported in this disease. **Materials and methods.** Peripheral blood was collected from three CLL patients with MUT and three cases of NAIVE Ig genes, in Binet stage A and without treatment. Clonal CD19+ lymphocytes were separated by positive magnetic cell sorting (MACS), and two SAGE libraries were constructed. **Results.** For the MUT library, a total of 104,057 tags were obtained by sequencing, of which 15,287 were unique tags that matched known genes, whereas for the NAIVE library we sequenced a total of 100,260 tags, of which 14,810 were unique tags referred to a UniGene cluster. We used the SAGEstat software, which applies Z statistics, to compare differences in the transcriptional profiles between MUT and NAIVE libraries, and others available SAGE libraries such as normal CD19+ lymphocytes, PB leukocytes, CD34+ hematopoietic cells and mesenchymal stem cells. The comparison between the two subtypes of CLL (MUT x NAIVE) revealed only 27 genes with significant ($p < 0,001$ and fold change higher than 20X) differences: genes such as HLADRb5, SIAT6, RPS9, and PDE7A, ANXA2, KRT17, S100A2, COL1A1, CDH12, were exclusively (or highly expressed) in MUT and NAIVE CLL libraries, respectively. In contrast, the comparison of the transcriptional profiles from CLL with that of CD19+ normal lymphocytes revealed that transcripts of ILR4, IL24, regulator of Fas-induced apoptosis (TOSO) and fibromodulin were exclusively or highly represented in CLL, whereas jun, jun-B, IL-8 and egr-1, among others, were absent or poorly represented in CLL. To corroborate SAGE results, observed differences in gene expression levels were validated by semi-quantitative RT-PCR of the same six cases of CLL, 3 cases of mantle cell lymphoma (MCL), normal CD19+ cells obtained from a healthy blood donor and one normal bulk bone marrow, for 7 different genes: HLADRb5, SIAT-6, SURF-5, ILR4, FMOD, IL24 and IL8. **Conclusions.** This new approach to evaluate the gene expression revealed that both CLL subtypes have very similar profiles, probably because of the similarity of the cell origin and DNA damage in both subtypes. The differences of the gene expression levels observed between the CLL malignant cell and its normal counterpart are related to the deficiency of apoptosis and cell adhesion observed in CLL lymphocytes. Our findings strengthen the demonstration of the similarity of the profiles of the two subtypes, and the deregulation of expression of apoptosis and cell adhesion molecule genes that are probably involved in the genesis of the leukemic phenotype obtained by other approaches, and reveal novel genes involved in these pathways. rodrigo@pegasus.fmrp.usp.br

0521

DISTINCTIVE GENE EXPRESSION PATTERN IN VH3-21 UTILIZING B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA

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The usage of the immunoglobulin (Ig) VH3-21 gene is associated with poor prognosis in B-cell chronic lymphocytic leukemia (B-CLL) despite VH gene mutation status. Many VH3-21+ patients also display restricted heavy and light chain Ig gene rearrangement features, implying a role of antigen selection in disease development. **Aim.** We wanted to investigate whether VH3-21+ B-CLLs have a distinct gene expression profile compared to Ig-mutated and Ig-unmutated cases utilizing other VH genes, as well as to identify genes that might reflect disturbed regulatory pathways. **Methods.** We applied the Affymetrix GeneChip technique to 15 VH3-21+ and 24 non-VH3-21 samples (11 with unmutated and 13 with mutated VH genes). Supervised clustering methods with different algorithms were used to identify genes that gave a distinction between VH3-21+ and non-VH3-21 samples. **Results.** A marker analysis revealed that VH3-21+ and non-VH3-21 B-CLLs were clearly separated and at least 1000 markers for each group were significant at the 5% level. A three-group comparison, including all three subgroups in one marker analysis, demonstrated that the VH3-21 group was more clearly and significantly distinguished from the Ig-unmutated/mutated groups than either of these compared to each other. Using two different algorithms for sample discrimination, eg. Weighted Voting and Linear Discriminant Analysis, a 90-100% correct distinction was revealed between VH3-21+ and non-VH3-21 B-CLLs. Amongst the highest ranked upregulated genes in the VH3-21 group, several are involved in the regulation of transcription and cell cycle control, eg. PP5, E2F2, SMARCD1, GEF1, TYMS, HMGA1 and EIF4G1, which may provide cells with an increased global, or specific transcription activity as well as a higher general proliferative drive. Among the downregulated genes in the VH3-21 group, there are several examples of genes involved in repression of transcription and negative control of cell cycle progression eg. SHARP, SAP18, TBLIX, MXI-1, CDKN1B, CDKN2D and MADH2. Genes that were upregulated or downregulated in VH3-21+ B-CLL were categorized into functional Gene Ontology categories. The categorization indicated an increased growth and cell proliferation among the VH3-21+ samples. Representative Gene Ontology categories, such as 'regulation of cell cycle', 'DNA replication and chromosome cycle' and 'kinase activity', contain twice as many genes than expected that are involved in these processes, and thus indicate that these enriched functional categories might be of importance for the more aggressive phenotype in VH3-21+ tumors. **Summary/Conclusions.** We have shown that the VH3-21+ group has a distinctive expression profile compared to Ig-unmutated and Ig-mutated CLLs, and that different class discriminator models can be applied to accurately classify a sample as being VH3-21+ solely based on gene expression data. Among the highest ranked up and downregulated genes, several were involved in regulation of DNA replication/cell cycling as well as transcriptional activation and kinase activity. It is thus tempting to speculate that these dysregulated genes could reflect a more proliferative phenotype in VH3-21+ tumors, which is ultimately expressed as a clinically more aggressive disease.

0522**INTACLONAL DIVERSIFICATION IN B-CLL**

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The monoclonal Ig expressed on B CLL cells was originally considered to be identical in all cells of the leukemic clone. However, intraclonal VDJ gene diversification was recently reported in B CLL cells using SSCP analysis and sequencing of Ig PCR products exhibiting altered mobility. We investigated the presence of intraclonal diversification using a different approach, by cloning monoclonal VH/DH/JH PCR products from B CLL patients and sequencing a large number of cloned PCR products. We also wanted to identify the criteria between true intraclonal diversification and polymerase mediated base errors during amplification. We extracted DNA from blood from 10 CLL patients. PCR was performed with leader/FR1 VH family specific primers and a consensus JH primer. Forty cycles of amplification with Taq platinum polymerase was used and monoclonal PCR products were ligated to TOPO TA vector. Twenty to 70 insert containing clones were sequenced from both orientations. Polymerase error was calculated by amplification for 40 cycles of a plasmid containing a PCR Ig insert, followed by cloning of the amplified PCR product and sequencing of 26 clones from both orientations. Polymerase mediated base error was calculated at 4.4×10^{-4} . Experiments were repeated twice from the same DNA, to compare the sequences obtained from the 1st and 2ed experiment and set the standards for assigning true diversification. Base differences that were found to be represented in more than two out of 20 clones in a patient were found again when the experiment was repeated. Therefore these base differences were assigned as true intraclonal diversification. This was observed in 4 out of 10 patients (40%). Three of these patients were mutated and one unmutated in their Ig sequence. Base point mutations in all analyzed clones that were unique in one only of the clones examined were observed at the polymerase mediated error rate. Further more, unique base substitutions observed in a experiment were not identified again in a second independent experiment with the same DNA as template. Therefore base substitutions observed in one out of 20 clones only should be assigned as polymerase errors. In one Ig mutated patient with intraclonal diversification clones with evolving diversified pattern were observed and verified again. In addition to intraclonal diversification observed in FR and CDR (FRs>CDRs)regions, true intraclonal diversification was verified at the Ig leader sequence in one patient. In conclusion, intraclonal diversification in one or more bases in a given Ig sequence must be considered if identical base substitutions are observed in more than two clones in a patient (provided at least 20 clones are sequenced). If this is not met, base substations probably represent polymerase mediated errors. The mechanism (s) responsible for true intaclonal diversification is largely unknown but we show that it can also target the leader non coding Ig sequence.

0523**GENE EXPRESSION PROFILING OF SPORADIC T-CELL PROLYMPHOBLASTIC LEUKAEMIA IDENTIFIES TWO DISTINCT GROUPS**N. Osuji¹, I. Del Giudice², E. Matutes³, V. Brito-Babapulle³, D. Gonzales³, D. Catovsky³¹Mayday University Hospital NHS Trust, Surrey, UK; ²Dipartimento di Biotecnologie, Rome, Italy; ³Institute of Cancer Research, Surrey, UK

Sporadic T-cell prolymphocytic leukaemia (T-PLL), a rare post thymic disorder classically presents with marked leukocytosis, hepatosplenomegaly and generalised lymphadenopathy, and shows a rapid progressive course with median survival of 7 months. Initially indolent presentation has also been described. Morphologically, circulating prolymphocytes are small to medi-

um sized with basophilic cytoplasm which shows bleb like protrusions. The nucleus may be smooth or markedly irregular and often contains a visible nucleolus. Small cell variants (20%) and sezary-like variants (5%) are also described, the latter showing markedly irregular, cerebriform nuclear outline. Whilst the most common phenotype reported is CD4+, CD8- (60%), co-expression of CD4 and CD8 is seen in 25% of cases and a CD4-, CD8+ phenotype in 15%. Cytogenetic findings are informative with inversion or reciprocal translocations of chromosome 14 involving breakpoints at q11 (TCR α /beta) and q32.1 (TCL1 and TCL1b) seen in up to 80% of cases. Other common cytogenetic findings include abnormalities of chromosomes 8, translocation (X;14) (q28;q11) which involves the MTCP1 gene, a homolog of TCL1 and, mutations in the ATM gene on 11q23. Although frequent, no abnormality has proven pathognomonic of this disorder. We evaluate the ability of gene expression profiling to sub-classify T-cell prolymphocytic leukaemia (T-PLL) and focus on genes potentially involved in pathogenesis and/or response to treatment. *Methods.* Total RNA (11-20micrograms) was extracted from peripheral blood mononuclear cells of twenty-two samples containing over 92% purity of malignant cells as determined by flow cytometry. Samples were analysed using the Human U133 PLUS2 oligonucleotide genechip which tests for the expression of >30,000 genes. Microarray experiments were performed by MRC geneservice (UK HGMP Resource Centre). *Results.* Using an unsupervised analysis algorithm, expression profiling allowed partitioning into two distinct groups distinguished by over 1000 differentially expressed genes, which yielded zero or low misclassification rates. These groups showed no correlation with a priori defined labels of morphology, phenotype and/or karyotype, however, differential response to alemtuzumab therapy was observed between the groups. Using next nearest centroid analysis, a sub-group of 39 genes was identified which most efficiently differentiated these groups. This included the apoptosis regulatory genes of the TNF receptor family (LMNA, ARTS-1), members of the RAS oncogene family (RAB10) and transcription factors (ATF-3, IRF2). Gene expression profiling reveals two distinct sub-groups within T-PLL and suggests possible variation in response to alemtuzumab therapy between these groups. Validation work is on going.

0524**NON-RANDOM NATURE OF THE T CELL RECEPTOR RESTRICTION IN LARGE GRANULAR LYMPHOCYTE LEUKEMIA**M.W. Wlodarski¹, C. O'Keefe¹, A. Risitano², A. Rodriguez¹, J. Maciejewski¹¹Cleveland Clinic Foundation, Cleveland, OHIO, USA; ²University of Naples, Naples, Italy

Large granular lymphocyte leukemia (T-LGL) is a clonal lymphoproliferation of cytotoxic T cells (CTL) associated with single/multi lineage cytopenias. Clonal CTL in LGL leukemia while apparently dysregulated, retain some physiologic properties of normal antigen specific effector CTL thus resembling an exaggerated response to an immunodominant antigen. It is likely that hematopoietic progenitors are the targets in this process. The most variable portion of the T-cell receptor (TCR), the variable B-chain (VB) CDR3 region, can serve as a molecular signature (clonotype) of a T cell clone. Significant clonal expansions lead to over representation of entire TCR VB chains. Expansions of an individual CTL clones are amenable to sequence analysis of CDR3 regions. We hypothesized that CTL proliferation in LGL leukemia develops not randomly but in the context of an autoimmune response. LGL clonotypes can serve as markers of immune/hematologic response and if common antigens are present, homologies in the structure of CTL clonotypes will be found. The detection strategy for immunodominant clonotypes included following steps: flow cytometric VB typing and CD8+ cell sorting, VB-specific or VB-multiplex RT-PCR followed by size spectratyping of CDR3 amplicons, transformation of cloned PCR products into E.coli and colony sequencing. We studied the TCR VB CDR3 repertoire in 75 patients with

suspected LGL leukemia. Among 1280 analyzed sequences, we isolated 86 pathologically expanded clonotypes in 60 patients (22 patients carried more than one clonotype). In most cases, the over-represented VB families were >20% of CD8+ population. Clonotypic frequency was 65±30% for a given VB family or 35.4±31% of all CD8+ cells. By comparison, the most expanded clones in healthy controls constitute only 15% of a single VB family or 0.7% of the CTL repertoire. For 7 patients followed serially either by sequencing or clonotype specific PCR, a marked decrease in the frequency of the immunodominant clone was observed with successful therapy. Most significantly, we detected identical immunodominant clonotype in two LGL patients. Interestingly, high homology was also found between expanded clonotypes in two patients and nonexpanded clonotypes in other patients. In contrast, the physiological clonal CTL repertoire is highly diverse and we were not able to detect any significant clonotype homologies in 26 healthy controls. The finding of identical or highly homologous clonotypes in several LGL patients with similar HLA background suggests a non-random clonal evolution in T-LGL possibly driven by a common antigen the presence of common target antigens and supports the non-random nature of the clonal evolution in LGL. This possibly occurred in the context of initially polyclonal immune response directed against a common antigenic target. Clonotypic sequences can be applied to monitor the disease course in LGL but such an approach may also be used in other CTL mediated diseases. Finally, clonotypic sequences can be utilized for the design of anti-idiotypic vaccines.

Chronic myeloid leukemia II

0525

ADENOVIRUS MEDIATED HUMAN β INTERFERON GENE TRANSFER INTO HUMAN MESENCHYMAL STEM CELLS FOR GENE THERAPY IN CHRONIC MYELOGENOUS LEUKEMIA

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For developing gene therapy for chronic myelogenous leukemia (CML), in this study, we evaluated the feasibility of using CML patient autologous bone marrow stromal cells (BMSCs) as a target cell population and studied the efficiency of recombinant adenovirus mediated human β interferon (hIFN- β) gene transfer into BMSCs. Using adenoviral vector AdV/LacZ, the transduction efficiency was 80.7±6.8% at a MOI of 50. Ad/hIFN- β is a E1-deleted, replication-deficient adenoviral serotype 5 vector encoding a 501 bp-containing human IFN- β cDNA. We studied the *in vitro* expression of hIFN- β in human BMSCs following transduction with Ad/hIFN- β . On transduction of BMSCs at a MOI of 50, the expression and secretion of hIFN- β were achieved as high as 5492±660~50647±4049 ng/10⁶ cells per 24 hours over the course of 3 weeks. We further studied the effects of hIFN- β produced by transduced BMSCs on the proliferation of the human leukemia cell line K562 cells *in vitro*, the proliferation of K562 cells was markedly inhibited in the experimental groups after 5 days of culture, as compared with the other two control groups. We also found that the percentage of K562 cells in the G1 phase of cell cycle can be increased by treatment of hIFN- β produced by transduced BMSCs, but the percentage of K562 cells in the S phase of cell cycle can be decreased in the same time. Apoptosis rate in the experimental groups was 30.8±8.5%, as compared with the other two control groups (5.6±1.3% and 5.5±0.8%, respectively) ($p < 0.01$). Our results indicate that hIFN- β gene engineered autologous BMSCs of CML donors could be successfully established and that local production of hIFN- β is sufficiently to markedly inhibit the proliferation of K562 cells and induce apoptosis of K562 cells *in vitro*, suggesting an important potential use in the clinical gene therapy of CML.

0526

IS NON-MYELOABLATIVE TRANSPLANT SUFFICIENT TO CURE CHRONIC MYELOID LEUKEMIA?

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Non-myeloablative stem cell transplantation (NST) has been studied as a safer approach for patients ineligible for conventional myeloablative transplant in recent years. NST is more dependent on graft-versus-leukemia effect, which is known to be powerful in patients with chronic myeloid leukemia (CML). Toxicity of NST is relatively low, but long-term efficacy of NST is unclear. We studied group of CML patients after NST with aims to evaluate toxicity, achievement and persistence of complete chimerism, complete molecular remission (CMR), and survival. We performed a retrospective analysis of 18 CML patients, who underwent NST in our centre from June 1998 to September 2004 (peripheral blood stem cells in all cases, 17 from matched sibling donor, 1 from matched unrelated donor). The conditioning consisted of fludarabine (30 mg/m²/day; 5 days), busulfan (total dose 8 mg/kg; 2 days); and ATG Fresenius (10 mg/kg/day; 4 days). Seventeen patients were in the first chronic phase before NST, one in the accelerate phase. Median age of patients was 50 years (range 15-59). Cyclosporine was used for graft-versus-host disease (GvHD) prophylaxis. Kinetics of minimal residual disease (MRD) was monitored by quantitative real-time reverse-transcriptase polymerase chain reaction (RQ-RT-PCR) and by competitive nested RT-PCR. **Results.** All patients engrafted, neutrophil recovery occurred at median of 17 days. There was no transplant-related mortality. No significant non-hematological toxicity (WHO grade 3 or 4) was observed. The incidence of acute GvHD was 56% (grade I-II in 6 cases, grade III-IV in 4 cases), chronic GvHD developed in 61% of patients (extensive in 6 cases, limited in 5 cases). On day +30, +60, and +100, complete chimerism was achieved in 22%, 44%, and 50% of patients, respectively. From 18 evaluated patients, complete chimerism was observed in 16 patients with median of 96 days (range 14-371). Hematological remission only and complete cytogenetic remission only was found in two patients (11%), and in 4 patients (22%), respectively. Twelve patients (67%) achieved complete molecular remission (CMR) in median of 135 days after transplant (range 56-449). All six patients without CMR underwent additional therapy (donor lymphocyte infusion (DLI) in 4 cases, imatinib in 2 cases), four patients achieved CMR after this therapy. Five patients (28%) relapsed in median of 25 months after transplant (range 5-46), there were 3 molecular relapses and 2 hematological relapses. Patients with relapses were treated by DLI (3 cases) and by imatinib (2 cases). On January 2005, with median follow up 26 months from transplant (range 4-79), 17 from 18 patients were alive, 14 patients were disease free (78%). One patient died from extensive chronic GvHD with sepsis after DLI. Nonmyeloablative transplantation after Flu-Bu-ATG conditioning has good tolerance with minimal acute toxicity and no transplant related mortality. However, some CML patients are not cured (no achievement of CMR in 33% of patients), and relapses are detected after transplant (in median of 25 months 28% of patients relapsed). Therefore, long-term, regular monitoring of all patients and additional therapy in some cases are absolutely necessary after NST.

0527

DIFFERENTIAL CONTROL GENES IN BCR/ABL MONITORING BY REAL-TIME RT-PCR

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Monitoring of BCR-ABL transcript level is a very important tool to follow up response to treatment in patients with chron-

ic myeloid leukemia (CML). Therefore a standardized real-time RT-PCR protocol has been recently estimated (Leukemia, 2003;17:2318). Ten years ago we estimated our own protocol based on competitive RT-PCR with competitor addition prior to RNA extraction, which enabled control for variables in RNA extraction and cDNA synthesis (Leukemia, 1998;12:1303). The method was validated and accredited by the Czech Accreditation Institute, full member of the European Accreditation Organization. Ten years of clinical practice proved the great ability of the method to characterize the disease status and to be of high predictive value. At present, in order to use standardized real-time RT-PCR but keep continuity of the quantification, we have compared our competitive method with standardized real-time RT-PCR from Europe Against Cancer (EAC) Program (Leukemia, 2003;17:2318). In this study we concentrated on testing of control gene effects on BCR-ABL monitoring during CML therapy. As control genes we used total ABL, GUS (β -glucuronidase) and B2M (β -2-microglobulin) which were recommended in EAC protocol (Leukemia, 2003;17:2474). We tested 1) linearity of BCR-ABL measurements by quantifying serial dilutions of competitors - in our case lysates of K562 and BV173 cells, 2) expression stability of control genes during therapy and 3) we compared kinetics of BCR-ABL by competitive method and by real-time RT-PCR with the three control genes. The last two tests were performed in 10 CML patients during treatment (146 samples). Amplification and data analysis were performed using Rotor gene 3000 instrument and Corbett Research Software 6.0.16 (Corbett Research, Sydney, Australia). The results were compared with cytogenetic, hematologic and clinical data. Linearity of BCR-ABL measurements was confirmed for B2M and GUS control genes but not for the total ABL. As expected from relation BCR-ABL/ABL + BCR-ABL, the linearity was not kept at high BCR-ABL levels. Similarly, serial examinations during therapy using total ABL control gene revealed that the tumor load was underestimated at high BCR-ABL levels and did not correlate with hematologic examinations. ABL control gene prevented the detection of BCR-ABL overexpression which we found to be an important prognostic factor for hematologic relapse. The B2M gene was found as the most stably expressed gene during therapy. BCR-ABL levels related to B2M very closely correlated with results of the competitive RT-PCR. GUS expression was rather unstable during therapy in some cases. In conclusion, from the three control genes recommended by EAC protocol we found B2M as the most convenient for sequential analyses of CML patients. On the contrary, we do not consider the total ABL, even though widely used, as an appropriate control gene for BCR-ABL quantification. The study was supported by grant NC/7550-3 from the Internal Grant Agency of Ministry of Health of the Czech Republic.

0528

THE CML-SPECIFIC ONCOGENE BCR/ABL INDUCES EXPRESSION OF HISTIDINE DECARBOXYLASE (HDC) AND THE SYNTHESIS OF HISTAMINE IN LEUKEMIC CELLS

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The numbers of basophils are typically elevated in patients with chronic myeloid leukemia (CML) and characteristically increase during disease progression. Histamine produced by basophils is highly upregulated in CML. We examined the biochemical basis of production of histamine in CML cells and analyzed the effects of the CML-related oncoprotein BCR/ABL on histamine generation. In addition, we asked whether histamine production in leukemic cells is associated with abnormal growth of CML cells. *Methods.* Expression of histamine and of histidine decarboxylase (HDC), the major enzyme involved in histamine synthesis, was examined in primary CML cells obtained from patients with chronic phase (CP) or accelerated phase with

basophilia (AP), in the CML-derived cell lines K562 and KU812, and in Ba/F3 cells inducibly expressing BCR/ABL on exposure to doxycycline (TonB.210-X cells). Expression of HDC mRNA was determined by RT-PCR and Northern blotting, and the levels of histamine by RIA. Signaling pathways contributing to Bim expression in leukemic cells were examined by pharmacologic inhibitors of MEK (PD98059), PI3-kinase (LY294002), and mTOR (rapamycin). To explore the role of histamine as a potential autocrine growth regulator in CML cells, culture experiments using histamine and histamine receptor (HR) antagonists (HR1=loratadine and HR2=cimetidine) were conducted. *Results.* In all patients with CML-AP, high levels of HDC mRNA and of histamine were detectable, whereas CML cells in CP expressed only low amounts of HDC mRNA and of histamine. Similarly, HDC mRNA and histamine were detectable in the basophil-committed CML cell line KU812, but not in the CML cell line K562. Exposure of primary CML cells or KU812 cells to the BCR/ABL tyrosine kinase inhibitors imatinib (1 μ M) and AMN107 (100 nM; Novartis Pharma AG) decreased the levels of histamine and the expression of HDC mRNA in BCR/ABL-transformed cells. Moreover, BCR/ABL was found to promote the expression of HDC mRNA and to increase the levels of histamine in TonB.210-X cells. The BCR/ABL-induced synthesis of histamine in Ba/F3 cells was blocked by LY294002 (PI3 kinase inhibitor), but not by PD98059 (MEK inhibitor) or rapamycin (mTOR inhibitor). The BCR/ABL-induced increase in histamine in TonB.210-X cells was neither accompanied by morphologic signs of basophil differentiation nor by upregulation of other basophil-related differentiation antigens. Neither histamine nor the H1R and H2R antagonists showed an effect on growth of BCR/ABL-transformed cells. Our data show that the CML-specific oncoprotein BCR/ABL induces the synthesis of histamine in leukemic cells.

0529

ANTILEUKEMIC ACTIVITY OF ARSENIC TRIOXIDE IS ENHANCED BY CO-TREATMENT WITH THE GLUTATHIONE-DEPLETING AGENT L-BUTHIONINESULFOXIMINE IN IMATINIB SENSITIVE AND RESISTANT CML CELLS

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Current studies investigate arsenic trioxide (ATO, Trisenox[®]) as a potential drug to optimize imatinib based treatment of chronic myelogenous leukemia (CML). However, preliminary clinical data reveal only moderate activity of ATO in CML (imatinib sensitive or resistant). *Aims.* ATO sensitivity is inversely related to the intracellular Glutathione-content (GSH) in various malignancies. To investigate whether GSH affects ATO sensitivity in CML cell lines, we determined the antiproliferative activity of ATO in correlation to intracellular GSH-levels. Furthermore, we sought to sensitize CML cells to ATO by GSH depletion using L-buthioninesulfoximine (BSO) and studied Bcr-Abl specific activity of both agents. *Methods.* MTS-proliferation assays were performed to determine the concentration of ATO needed to induce 50% cellular growth inhibition (IC50). Cell lines used were the imatinib sensitive CML blast crisis lines AR230-s, KCL22-s, Lama-s as well as the imatinib resistant AR230-r1, KCL22-r1, Lama-r1. Intracellular GSH-levels were measured using a commercially available kit. Protein content was analyzed using the Bradford method. GSH-levels are given in nmol/mg of whole cellular protein. ATO toxicity was analyzed using trypan blue exclusion and flow cytometric analysis of apoptosis (Annexin/PI-method). Bcr-Abl specific effects were investigated using immunoblotting. MTS-proliferation assays indicated a cell type dependent activity of ATO with IC50-values ranging from 1.2 \pm 0.2 μ M (Lama-s) up to 6.9 \pm 1.4 μ M (AR230-r1). The most sensitive Lama-s cells expressed low GSH-levels (13.8 \pm 3.0 nmol/mg), highly resistant AR230-r1 cells revealed a 4-fold increase in GSH-content (51.7 \pm 15.7 nmol/mg).

Imatinib resistance was not associated with significant GSH-content modulation when compared to the imatinib naïve counterparts (34.8±10.6 nmol/mg [AR230-s] vs 51.1±15.7 nmol/mg [AR230-r1]; (43.3±10.0nmol/mg [KCL22-s] vs 36.4±10.9 nmol/mg [KCL22-r1]; 13.8±3.0nmol/mg [Lama-s] vs 11.4±0.3 [Lama-r1]). Treatment of AR230-s cells with 100 µM BSO for 12 h led to significant downregulation of cellular GSH (36.3±13.4 nmol/mg [control] vs 11.6±6.1 nmol/mg [BSO]). Treatment with BSO did not affect cellular viability nor induced apoptosis. Subsequent co-treatment of AR230-s cells with ATO (1 µM) and BSO (100 µM) for 24 h reduced viability to 32% compared to untreated cells, whereas treatment with 1µM ATO alone did not affect viability. Flow cytometric analysis of apoptosis reflected viability data with a 5.5 fold increase of apoptotic cells in the combined treated fraction. Similar data were generated using AR230-r1, KCL22-s and KCL22-r1 cells. Combination treatment exerted rapid downregulation of Bcr-Abl protein (41% of control) after 24 h exposure, whereas ATO or BSO alone did not affect Bcr-Abl levels. In primary cells derived from patients resistant to imatinib (n=4), 72h exposure to 1 µM ATO weakly affected viability, however, a significant decrease of cell viability was observed in the combined treated fraction (range:37-82% [ATO+BSO] vs (range:14-30% [ATO])). **Conclusions.** GSH-depletion by BSO sensitizes imatinib-sensitive and -resistant CML cell lines and primary cells to ATO induced cytotoxicity. Combination treatment shows Bcr-Abl specific activity demonstrated by Bcr-Abl downregulation. Therefore, GSH-depletion may be a means to improve the antileukemic activity of ATO in imatinib-based treatment of CML.

0530

GENE EXPRESSION ANALYSIS IDENTIFIES GENES THAT ALLOW PREDICTION OF RESPONSE TO α -INTERFERON IN CHRONIC PHASE CML PATIENTS

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α -interferon (INF) was the first drug to significantly prolong survival in chronic myeloid (CML) and has been extensively used to treat CML patients over the past years. Today, in the Imatinib era INF still has a role as additional agent in patients with a suboptimal response to Imatinib. However, not all patients will respond to INF and the reasons for this heterogeneity are largely unknown. The aim of the study was to predict response to INF in diagnostic samples. **Methods.** Microarray-based gene expression analysis enables the study of thousands of genes in one single experiment and has been shown to be a useful tool to assign samples to specific categories. Gene expression profiles in diagnostic samples from 15 CML chronic phase patients were analyzed using cDNA microarrays with 7458 genes. Blood samples were collected at diagnosis and all patients were subsequently treated with α -interferon. Seven patients were responders, defined as patients achieving a complete or major cytogenetic response at 12 months or earlier after initiation of interferon. Eight patients that had no cytogenetic response at 6-18 months were regarded as non responders. A list of 61 genes differentially expressed in both sample groups was generated by sorting those genes that showed the largest difference in median expression between the groups. The top 20 differentially expressed genes were subsequently used in an 'all pair' selection procedure to identify those genes that are highly predictive of response to INF. The genes selected by this procedure were *LTF*, *PRG2*, *JARID1A*, *NRGN*, *RNASE2* and *DEFA4*. The accuracy of these genes in predicting response to INF was determined in a *leave one out cross validation* procedure and determined to be 0,13. Our results suggest that it might be possible to use microarray-based gene expression analysis to predict future treatment outcome in CML diagnostic samples.

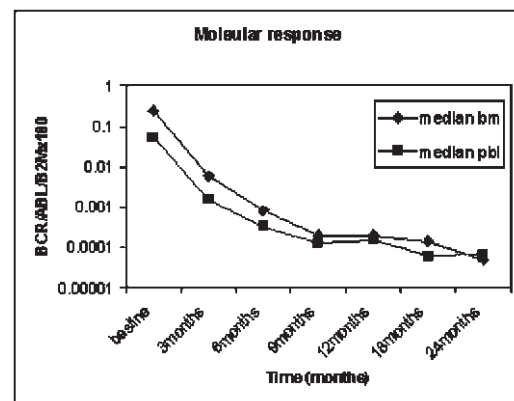
0531

MOLECULAR RESPONSE TO A IMATINIB PLUS α -INTERFERON THERAPY IN NEWLY DIAGNOSED, EARLY CHRONIC PHASE CHRONIC MIELOID LEUKEMIA

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We sought to determine dynamics of BCR/ABL mRNA expression levels in 76 patients with early chronic phase chronic myeloid leukemia (CML) during Imatinib and α -Interferon therapy. All patients received a standard dose of Imatinib (400 mg/d), while α -interferon scheduled dose was 50 mg/wk, 100 mg/wk and 150 mg/wk in the 3 different cohorts. The response was monitored by cytogenetics from bone marrow metaphases and molecular response was assessed by RT-PCR (TaqMan) from bone marrow and peripheral blood samples. We expressed molecular response as the ratio between BCR/ABL and α 2-microglobulin x 100 and the lowest level of detectability of the method.



A complete cytogenetic response was achieved in 53 patients after a median observation time of 12 months, while in other 10 patients a partial cytogenetic response was obtained, for an overall major cytogenetic response rate of 63 of 76 (83%). After 12 months we observed a progressive decrease of the amount of the BCR/ABL transcript in the patients who achieved a complete cytogenetic response. The reduction of the BCR/ABL transcript levels that we observed in this group of patients was about 3 logs. In the patients who had achieved a complete cytogenetic response, the molecular response was assessed also after 18 and 24 months. So we observed that 18 months after the first dose of Imatinib and α -interferon 70% of patients were still in complete cytogenetic response. At 24 months the median value of BCR/ABL transcripts continues to decrease about another 1log, as shown in Figure 1. Although there had not been observed any differences between the 3 cohorts our conclusions are that treatment with Imatinib in newly diagnosed CML patients is associated with a rapid decrease of BCR/ABL levels and that the BCR/ABL transcripts continues to decrease after 2 years of Imatinib therapy.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L.

0532**THE EFFECT OF A HISTONE DEACETYLASE INHIBITOR ON A IMATINIB RESISTANT SUBCLONE OF CML CELLS**Y. Zimra¹, M. Shaklai¹, V. Mirkin¹, I. Beliaev¹, E. Rabizadeh²¹Beilinson Hospital, PETHACH TIKVA, Israel; ²Beilinson hospital, PETHACH TIKVA, Israel

Background. Chronic myeloid leukemia (CML) is a stem cell disorder caused by a constitutively activated tyrosine kinase, the Bcr-Abl oncoprotein. An inhibitor of this tyrosine kinase, imatinib mesylate, is rapidly becoming the first-line therapy for CML. However, the development of resistance to this drug is a frequent setback, particularly in patients in advanced phases of the disease. Several mechanisms of resistance have been described, the most frequent of which are amplification and/or mutations of the BCR-ABL gene. To overcome resistance, several approaches have been studied *in vitro* and *in vivo*. Some studies including ours, have shown that histone deacetylase inhibitors (HDI) act synergistically or additively with Imatinib in cytotoxic induction of the BCR-ABL-translocation-bearing cell lines K562, a model of CML. Butyric acid (BA) is a known histone deacetylase inhibitor. Pivaloyloxymethyl butyrate (PIVANEX) a BA derivative developed and tested in our laboratory. PIVANEX was shown to induce histone hyperacetylation, apoptosis, differentiation and reduction in BCR-ABL protein levels in the K562 cell line. **Aims.** to study the effect of PIVANEX on K562 Imatinib resistant subclone. **Methods.** In order to prepare this resistant subclone, K562 cells were cultured in the presence of low and gradually increasing concentrations of Imatinib until satisfactory Imatinib resistance was achieved. The cells were cultured in the absence or the presence of PIVANEX, Imatinib and combinations of the two at different concentrations. **Results.** Herein we show that the IC₅₀ value of the K562 resistant subclone was 30 times greater than that of K562 cells after exposure to Imatinib. The Imatinib resistant cells however, had similar BCR-ABL protein levels. PIVANEX had caused viability reduction, apoptosis and caspase activation in the Imatinib resistant cells. Treatment of these cells with the combination of Imatinib and PIVANEX was additive in most cases. But preliminary data could not demonstrate changes in the levels of BCR-ABL protein. More studies should be performed to investigate the effect of PIVANEX and its combination on these resistant cells. Its possible that PIVANEX affect the resistant cells by inducing apoptosis via mechanism which does not involve BCR-ABL protein. **Summary/conclusion:** Studies on the resistant cell line will offer a beneficial tool for further investigations of Imatinib resistance in CML patients and development of a novel treatment.

0533**POOR RESPONSE TO IMATINIB THERAPY IN CHRONIC MYELOID LEUKAEMIA (CML) EXPRESSING VARIANT BCR-ABL TRANSCRIPTS**

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In CML, a series of studies have established that imatinib will produce complete cytogenetic remission (CCR) in approximately 80% of newly diagnosed cases, in about 50% of chronic phase patients who have failed interferon, and in 10-20% of patients with more advanced disease. At least 98% of CML patients express either one or both of e13a2 (b2a2) or e14a2 (b3a2) BCR-ABL fusion transcripts. These arise from breakpoints in the major breakpoint cluster region of BCR. Variant transcripts arising from BCR breakpoints in regions other than the major breakpoint cluster region are rare. The e19a2 fusion transcript has been associated with chronic neutrophilic leukaemia (CNL) and a more indolent clinical course. Fifteen cases with fusion transcripts lacking ABL exon 2 (i.e. e13a3, e14a3 or e1a3) have been described in CML, and e6a2 is even less common. There are no data currently available on the efficacy of imatinib in patients with BCR-ABL transcripts other

than e13a2 or e14a2. From October 2000, 155 cases of CML have been molecularly screened at our institution. 151 expressed one or both of the major breakpoint cluster region transcripts e13a2 or e14a2. Here, we report the effect of imatinib in the remaining 4 cases expressing a variant transcript. Case 1: A 57 year old lady presented in newly diagnosed chronic phase CML, and was found to express the e13a3 BCR-ABL fusion transcript. She was treated with imatinib, 400mg daily, and achieved CCR and transient molecular negativity within 11 months of therapy. Two months later, she developed extramedullary and then molecular and cytogenetic relapse, with evidence of a BCR-ABL kinase domain mutation T315I. She died from refractory disease 18 months from commencing therapy. Case 2: A 61 year old man was diagnosed with chronic phase CML and the e19a2 transcript was detected. He had no haematological response to interferon, and commenced imatinib, 400mg, 34 months after diagnosis. He had no cytogenetic response, and progressed to blast crisis 7 months after commencing imatinib. Case 3: A 65 year old man presented in blast crisis, and was found to express the e6a2 fusion transcript. Despite imatinib treatment at 600 mg daily, he had no haematological response, and died within 6 weeks of refractory disease. Case 4: A 52 year old lady presented with accelerated phase CML (peripheral blood blast count 13%). She failed interferon therapy, but then progressed to lymphoid blast crisis, which was successfully treated by chemotherapy and autografting. The e19a2 transcript was detected. She then commenced imatinib 600mg daily. This gave haematological but no cytogenetic response for 48 months, before developing extensive marrow fibrosis. To our knowledge, this is the first report of the effects of imatinib in patients with variant BCR-ABL transcripts. Overall, none of these four cases achieved CCR with imatinib, and 3 have died. At present, we suggest caution in extrapolating the excellent results of imatinib therapy in major breakpoint CML to patients with variant transcripts. More clinical reports on the outcome of imatinib therapy are required in patients expressing variant transcripts.

0534**IMPROVEMENT OF FASTING BLOOD GLUCOSE IN DIABETIC PH+ CML PATIENTS TREATED WITH IMATINIB**M. Breccia¹, M. Muscaritoli², M. D'Andrea², F. Gentilini², R. Latagliata², G. Alimena²¹University 'La Sapienza', ROME, Italy; ²University 'La Sapienza', ROME, Italy

Imatinib can be easily and safely administered to CML patients with type 2 diabetes. We report on our experience on 8 diabetic Ph+ CML patients, in 7 of whom an improvement of fasting glucose (FG) in concomitance with attainment of a complete cytogenetic response (CCR) was observed; the FG improvement allowed to a consequent reduction of oral antidiabetic drugs or insuline dosage. Three patients were males and 5 females, median age 66 years (range 57-70). Five patients were in chronic phase (CP) (4 were resistant to prior IFN therapy and 1 was previously untreated) and were given imatinib at 400 mg/d; 3 patients were in accelerated phase (AP), all were pre-treated with IFN, and were given imatinib at 600 mg/d. All patients had been diagnosed as being diabetic from 3 (1 patient) to more than 10 years before CML onset. Four patients were using insulin and 4 were on antidiabetic oral drugs. Before starting imatinib, median glucose level was 250 mg/dL (range 160-330). At 3 months, 7/8 patients reached CCR and median FG level was 110 mg/dL (range 96-140), with allowed reduction of antidiabetic drugs. At 12 months, all 7 patients maintained the CCR and a good glicemic control (median FG 108 mg/dL, range 89-130). In one of these responding patients, we monitored also the glycosilated haemoglobin (HbA1C) fraction and the plasma insuline level all along the imatinib therapy. We found a progressive reduction of HbA1C (8% at 6 months and 5% at 12 months) with respect to before starting imatinib (HbA1C 12% and plasma insulin level 25 UI) with stable values of plasma insulin dosage (24 UI). At the time of this writing all patients have reached 18 months of therapy and 7/8 still maintain CCR

and a good glycaemic control (median FG 105, range 90-125), while 1 AP patient who was resistant to imatinib, never obtained good blood glucose control. The mechanisms through which imatinib could improve FG in our diabetic CML patients is presently yet not understood. A direct effect of imatinib on key enzymes of glucose metabolism has been demonstrated *in vitro* (Boren et Al), and it is conceivable that at therapeutic concentrations *in vivo* it may act not only on bcr/abl tyrosine kinase, but also on intracellular pathways involved in the action of insulin on peripheral cell receptors. Insulin resistance in type 2 diabetes is mediated by a reduction of insulin receptor substrates (IRS) or through an elevated fatty acid (FFA) concentrations. It is possible that imatinib may exert its effect on glucose metabolism *in vivo* by reducing FFA or by interfering with potentially disrupted molecular mechanisms downstream the IRS in the diabetic cells. In conclusion, a possible role of imatinib on glucose metabolism with possible implication in the imatinib resistance is suggested, which deserves further investigations, both in diabetic and non-diabetic CML patients.

0535

SUDDEN BLASTIC CRISIS (BC) IN CML PH+ PATIENTS IN COMPLETE CYTOGENETIC REMISSION (CCR) INDUCED BY IMATINIB

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Only rare cases of sudden BC occurring in Ph+ CML patients in CCR induced by imatinib have been reported. We describe here 6 cases that, while in CCR on imatinib, developed a sudden (BC) and compare the presenting features of these patients with those of patients who developed BC while in resistant disease. In a period of 4 years we observed a total 164 CP-CML patients who were treated with imatinib at the standard dose of 400 mg/d after resistance/intolerance to IFN (100 patients) or as first line therapy (64 patients). Among a total of 11 patients (6.7%) with a blastic evolution, 6 presented a sudden BC, which occurred after the early detection (3 months) of CCR. Five patients were males and 1 was female, median age 55 years (range 40-74). According to Sokal score, 3 patients were low and 3 patients intermediate risk. Only in 1 patient the karyotype analysis had revealed at diagnosis a deletion of 9 (q). Molecular analysis had showed a b3a2 fusion transcript type in 5 patients and b2a2 type in 1 patient. Four patients were pre-treated with IFN and hydroxyurea and in 2 patients imatinib was the first line therapy. Median duration of CP was 35.5 months (range 12-111), median duration of imatinib therapy was 9 months (range 5-15). All patients had obtained complete haematological remission between the first and the third week and CCR at 3 months. BC morphological and phenotypic characterization showed a myeloid subtype in 3 patients (2 had an initial extramedullary localization), B-lymphoid in 2 patients and biclonal in 1 patient. Cytogenetic analysis at the time of SBC revealed a clonal evolution in 1 patient (double Ph+), 100% Ph+ positive cells in 1 patient, a Ph+/Ph- cell mosaicism in 2 patients, a normal Ph- karyotype in 2 patients. In 1 patient a M351T mutation of the catalytic domain was detected with DHPLC analysis. At the time of this writing only 2 patients are alive (1 at 8 mo.s after a cord blood transplantation and 1 at 19 mo.s after intensive chemotherapy). As compared to the features of the 5 patients who developed BC after imatinib resistance, we observed a preponderance of male sex, low risk score, b3a2 fusion transcript type and lymphoid phenotype in patients with SBC, this indicating possible existence of biological and clinical heterogeneities between the two patients subgroups. Moreover, in 4/6 SBC patients the first sign of evolution was a sudden drop of WBC counts, in the absence of other clinical and/or haematological abnormalities. In conclusion, our observation stresses on the need for continued monitoring of patients under imatinib treatment, with careful attention to be addressed to minor modifications of peripheral blood count; the need to find strategies for eradication of leukemic residual cells is further emphasized.

0536

A NEW ABL KINASE INHIBITOR (AMN107) HAS IN VITRO ACTIVITY ON CHRONIC MYELOID LEUKAEMIA (CML) PH+ CELLS RESISTANT TO IMATINIB

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Imatinib mesylate (Novartis Pharma), an inhibitor of the bcr/abl tyrosine kinase, has rapidly become the first-line therapy for CML. Imatinib has proved remarkably effective at reducing the number of leukaemia cells in individual CML patients and promises to prolong life substantially in comparison with earlier treatments. However, in patients in advanced phases of the disease the development of resistance to this drug is a frequent setback. Therefore, new inhibitors of bcr/abl are needed. Very recently, a new bcr/abl inhibitor, AMN107 (Novartis Pharma), has been developed. We have tested AMN107 on human leukaemia cell lines and on blasts isolated from imatinib-resistant CML patients. After a 24 h incubation, AMN107 (10 nM) blocked K562 cells in the G1 phase of the cell cycle. To obtain the same effect with imatinib, a 200 nM concentration was required. AMN107 had no effect on cell cycle progression of bcr/abl-negative cell lines such as HL60 and NB4, even if the concentration was raised to 500 nM. After 48 h incubation, AMN107 (10 nM) was capable of inducing a massive apoptosis of K562 cells whereas, once again, 200 nM imatinib was required to obtain the same effect. Western blot analysis with phosphospecific antibodies revealed that in K562 cells AMN107 (50 nM) markedly down-regulated autophosphorylation of bcr/abl Tyr177 and Tyr412, whereas autophosphorylation of Thr735 was unaffected. In contrast, imatinib even if used at 200 nM, did not diminish phosphorylation of either bcr/abl Tyr177 or Tyr412. This finding seems particularly important because recent evidence has demonstrated that the signalling pathway emanating from Tyr177 plays a major role in the pathogenesis of CML. Indeed, phosphorylated Tyr177 forms a high-affinity binding site for the SH2 domain of the adapter Grb2. The main effectors of Grb2 are Sos and Ras, however Grb2 also recruits the scaffolding adapter protein Gab2 to bcr/abl via a Grb2-Gab2 complex, which results in activation of phosphoinositide 3-kinase (PI3K)/Akt and Erk signaling networks. Consistently, we found by immunoprecipitation decreased levels of bcr/abl-associated Gab2, Grb2, and p85 regulatory subunit of PI3K in AMN107-treated cells. AMN107 treatment of K562 cells also caused a reduction of STAT5, cCBL, CRKL, and Akt phosphorylation levels, as well as Bcl-XL expression. AMN107 (5 mM for 24h) significantly increased the apoptosis rate of CML blasts isolated from patients resistant to imatinib. Therefore, AMN107 might represent a new bcr/abl selective inhibitor useful for overcoming imatinib resistance.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2004, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L. grants.

0537

ASSESSING BCR-ABL IN ERYTHROID AND MYELOID CELLS BY MGG-FISH IN CML- EQUAL EXPRESSION AT DIAGNOSIS AND DURING EARLY TREATMENT BUT PREDOMINANCE OF ERYTHROID INVOLVEMENT AFTER LONG-TERM INF TREATMENT

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Chronic myeloid leukemia (CML) is a stem cells disorder involving cells of erythroid as well as myeloid origin. The typical Philadelphia (Ph) chromosomal aberration, resulting in a fusion of BCR and ABL genes translating into the constitutively activated Bcr-Abl tyrosine kinase, can be studied by fluorescence in situ hybridization (FISH). We have combined FISH

with classical cytomorphology (MGG) on the same sample slides, to assess the penetration of BCR-ABL in erythroid and myeloid interphase bone marrow cells. Samples from 35 CML patients (19M/16F, median age 58 years, range 20-81 years) were examined utilizing a dual color DNA probe (LSI BCR/ABL ES Dual Color Translocation Probe, Vysis, IL). Twelve patients were investigated at diagnosis, 23 patients after 1-166 (median 39) months of treatment, mainly with either α -interferon (INF, n=10) or hydroxyurea (n=14). The mean penetration of BCR-ABL positive clone was similar in granulopoietic and erythropoietic cells [54.8% (30.6) v 55.3% (30.3); p=0.167]. Studying individual patients, there was a significant correlation between the percentage of Ph+ granulopoietic and erythropoietic bone marrow cells ($r^2=0.60$, $p<0.001$). This relationship was observed among both newly diagnosed and earlier treated patients. When clinical and hematological features were compared between earlier treated patients with an overweight of BCR/ABL positive erythropoietic and granulopoietic bone marrow cells, respectively, it appeared that duration of INF therapy was the only significant difference between the two groups. Patients that had been treated with INF for 2 years or longer, had significantly lower mean (SD) percentage of BCR/ABL positive granulopoietic cells compared to those treated with INF for a shorter period or not at all [20.2% (27) v 62.2% (21); p=0.0004]. In contrast, no significant effect from INF therapy on the erythropoietic bone marrow cells was observed [33.6% (31) v 56.1% (32); p=0.11]. In 3 cases, all or nearly all of the remaining BCR/ABL positive bone marrow cells apparently belonged to the erythropoiesis. The finding that erythropoietic and granulopoietic cells in newly diagnosed CML cases are equally involved in the leukemic clone is of interest, considering the dominance of granulopoietic cells at disease presentation. It suggests that the BCR-ABL gene expression affects the two cell lineages in different ways, obviously favouring a dominance of the myeloid phenotype. Furthermore, it seems that erythropoietic cells are less affected by long-term INF therapy than granulopoietic cells, suggesting that therapeutic responses may differ between cell lineages. It remains to be determined if a similar lineage related response pattern can be found also in imatinib treated cases.

0538

THE APOPTOTIC RELATED GENE 'BAD' PREDICTS THE RESPONSE TO IMATINIB TREATMENT IN CHRONIC MYELOGENOUS LEUKAEMIA

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Background. Chronic myelogenous leukaemia (CML) is characterized by the bcr-abl fusion protein which exerts its pathogenic effect by enhancing cell survival and by increasing proliferation. It has been demonstrated that this fusion protein affects apoptosis by phosphorylation of the RAS-pathway members. Imatinib mesylate (Glivec®), an inhibitor of the bcr-abl tyrosine kinase has proven highly effective in treatment of CML. However, some patients have a primary or acquired resistance to imatinib therapy. **Aims.** In an attempt to find markers predicting the response to bcr-abl tyrosine kinase inhibition, we analysed the expression of the apoptosis related genes BCL-XI, BCL-Xs and BAD before and during imatinib therapy. **Methods.** Thirty-six CML patients commencing imatinib treatment in first chronic phase were included in the study; 28 patients were newly diagnosed and 8 were interferon intolerant/refractory patients in late chronic phase. Every third months the amount of bcr-abl transcripts were analysed on blood and bone marrow samples by real-time PCR. Based on whether they obtained a bcr-abl transcript reduction of 3 log (base 10) or not, after 12 months imatinib treatment, the patients were divided in two groups: (i) good responders (n=15) and poor responders (n=21). Among the 21 poor responders, 7 had developed imatinib resistance

defined as no major cytogenetic response after 12 months treatment. The mRNA expression of BCL-XI, BCL-Xs and BAD were measured on peripheral blood mononuclear cells by real-time PCR using the TaqMan system. **Results.** Prior to imatinib treatment, the BCL-XI expression was significantly higher in patients in late chronic phase compared to newly diagnosed patients ($p=0.009$), while there was no difference in their bcr-abl transcript levels. The BAD gene expression before start of treatment was significantly higher among the 'poor responders' compared to the 'good responders' ($p=0.015$). After 3-12 months imatinib treatment the 'good responders' displayed a significant increase in BCL-XI and BAD gene expression, while no significant changes were seen among the 'poor responders'. **Conclusions.** Although the BCL-XI gene expression did not correlate with the amounts of bcr-abl transcripts, higher expression of BCL-XI in the late chronic phase patients might reflect a more advanced disease. Also, it is likely that anti-apoptotic and pro-apoptotic members of the BCL2 family is affected by imatinib therapy in CML. We suggest that the BAD gene expression might be useful for predicting patients that will have a major molecular response to imatinib therapy. Dr Yuan Wei is a Ph.D. student from Department of Internal Medicine, Qilu Hospital, Shandong University, P.R. China, performing her work at Sahlgrenska University Hospital, Gothenburg, Sweden

0539

CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB MESYLATE SHOW INCREASED BONE MARROW LYMPHOCYTE NUMBER

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Background. Morphologic bone marrow changes such as marked reduction of granulopoiesis, quantitative normalization of erythropoiesis, and significant decrease in megakaryocytes with the reappearance of normal-sized forms have been shown in patients with chronic myelogenous leukemia (CML) during prolonged imatinib treatment. However effects of the drug on other haematopoietic lineages have not been described until now. **Patients and Methods.** All 572 bone marrow aspirates from the 115 imatinib-treated CML patients followed from 06/01/2000 to 06/30/2004 were examined morphologically at sequential time points of the treatment (0, 3, 6 and 12 months), then once a year when complete hematologic response and morphologic marrow remission were achieved. **Results.** The differential count of aspirate smears revealed lymphocytes accounting for 10 to 15 percent of all nucleated cells in 32 patients (27.8%), 16 to 20 percent in 13 patients (11.3%), 21 to 30 percent in 8 patients (6.9%) and 31 to 40 percent in 5 patients (4.3%). Lymphocytes were small size and morphologically mature. Bone marrow infiltration was mainly diffuse. However lymphoid aggregates associated to rare plasma cells were found in 34 patients (58.6%). Lymphocytes appeared at 3 or 6 months after the initiation of therapy in 42 patients (72.4%) and at 9, 12 or 18 months in the remaining. They were persistent during the whole time of treatment. No correlation was shown with lymphocyte count in the peripheral blood. We subsequently compared these results with bone marrow examination performed in the 59 patients undergoing treatment with interferon α alone or in association with Cytosine-arabinoside before receiving imatinib. Of these, only 5 (8.5%) developed lymphocytes in their marrow but their rate was less than 10 percent of the nucleated cells. **Conclusions.** Here we report a significant increase of marrow lymphocytes in 50% of CML patients treated at an effective oral dose of imatinib mesylate, whereas no so high marrow lymphocyte number was observed prior to the initiation of imatinib therapy. Further investigations are now necessary to characterise this lymphoid population and to document its prognostic significance.

Cytogenetics and molecular diagnostics

0540

PROGNOSTIC SIGNIFICANCE OF ADDITIONAL CHROMOSOMAL ABERRATIONS IN CHILDREN WITH ETV6-AML1 POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. and **Aims.** Cryptic translocation t(12;21)(p13;q22) which give origin to the hybrid gene ETV6-AML1 can be found by FISH in approximately 25% of children with acute lymphocytic leukemia (ALL) as the most frequent specific aberration. Despite of the fact that according to the most authors this finding is favorable prognostic sign, ETV6-AML1 positive children can have late relapses. One of the reasons could be high instability of the genome of leukemic cells, which is manifested on chromosomal level by additional aberrations and complex rearrangements. The aim of the study was to evaluate the significance of additional chromosomal aberrations for prognosis of children with ETV6-AML1 positive ALL. **Methods.** For the assessment of ETV6-AML1 fusion gene RT-PCR and/or double target interphase FISH with locus-specific probe (Abbott-Vysisä) were used (200 interphase nuclei analyzed, cut-off level 2.5% tested on controls, standard deviation $\pm 0.5\%$). Karyotypes were analyzed by conventional and molecular-cytogenetic methods. Structural and/or complex chromosomal aberration were proved by FISH by whole chromosome painting probes (Cambioä, Cambridge, UK) and/or by mFISH with the '24Xcyte' probe kit (MetaSystemsä GmbH, Altussheim, Germany). **Results.** We examined 87 children with ALL and ETV6-AML1 fusion gene proved by RT-PCR and/or I-FISH. Most of them are living in the first or second complete remission. Relapse appeared in 17 children (19.5%). Three patients died (two because of relapse and one for treatment complications). In 52 children (60%) we found except t(12;21)(p13;q22) additional chromosomal aberrations, the most frequently trisomy or tetrasomy of chromosome 21 (14 cases), deletion of non-translocated ETV6 allele (14 cases) and/or deletion of 6q (6 cases). In eight children variant translocations of chromosomes 12 and 21 with other partners were found. Complex karyotypes were identified in 39 children in total (45%). Analysis of event-free survival (EFS) revealed significantly shorter survival in patients with additional structural aberrations in ETV6-AML1 positive cells ($p=0.005$). **Conclusions.** Cryptic translocation t(12;21)(p13;q22) can be associated with additional chromosomal abnormalities (deletions, translocations, insertions). In our cohort of patients with ETV6-AML1 positive ALL, karyotypes with additional structural and/or complex aberrations were indicator of poor prognosis.

Supported by grants GACR 301/04/0407, MSM 0021620813 and IGA NC7490.

0541

CHARACTERIZATION OF FUSION PARTNER GENES IN 112 PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MLL REARRANGEMENT

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Background. Chromosomal translocations involving MLL gene result in the fusion of MLL to a variety of partner genes in acute leukemias. Previous studies of MLL rearrangement were mainly based on cytogenetic findings in which the MLL fusion partners were not well characterized. **Aims.** We aimed to (1) systematically analyze the frequency of MLL gene rearrangement [MLL (+)], (2) to characterize the MLL partner genes, and (3) to correlate the MLL fusion transcripts with clinicohematologic features and treatment outcome in *de novo* acute myeloid leukemia (AML). **Methods.** Southern blot analysis was used to screen MLL rearrangement in patients with *de novo* AML. Reverse transcriptase-polymerase chain reaction (RT-PCR) or multiplex nested PCR followed by GeneScan analysis and/or direct sequencing, was used to detect the common MLL fusion transcripts. cDNA panhandle PCR was used to identify the infrequent or unknown MLL partner genes. **Results.** MLL (+) was identified in 112 of 954 patients with *de novo* AML, 96 were adults and 52 were males. The ages of MLL (+) patients ranged between one day and 85 (median 48). The distribution of FAB subtypes in MLL (+) AML was 6 M0, 24 M1, 29 M2, 24 M4, and 26 M5, 1 M6 and 2 M7. The MLL fusion transcripts comprised of 61 (54.5%) partial tandem duplication of MLL (MLL-PTD), 14 MLL-AF9, 9 MLL-AF10, 9 MLL-ELL, 8 MLL-AF6, 4 MLL-ENL and one case each of MLL-AF1, MLL-AF4, MLL-MSF, MLL-LCX, MLL-LARG, MLL-SEPT6 and MLL-CBL. The frequency of MLL-PTD was 7.1% in adult *de novo* AML compared with 0.9% in childhood AML ($P<0.001$). MLL-AF9 and MLL-AF10 were most frequently associated with AML-M5. 11q23 abnormalities were detected in 29 of the 45 non-PTD MLL (+) patients who had cytogenetic analysis; 46 patients with MLL-PTD had chromosomal examination, none had 11q23 abnormalities, 34 had normal karyotypes, and 3 had trisomy 11. Fifty-five adult MLL (+) patients and 15 MLL (+) children received combination chemotherapy. The complete remission rate was 64% for adults and 100% for children. The median remission duration, event-free survival and overall survival of adult MLL (+) AML were 6.0 ± 1.7 months, 7.2 ± 2.2 months, and 11.1 ± 0.6 months, respectively. In adult AML, there were no differences in complete remission rate ($p=0.585$), remission duration ($p=0.405$), event-free survival ($p=0.433$) or overall survival ($p=0.522$) between MLL-PTD and MLL non-PTD groups. Of the 15 pediatric MLL (+) AML, the remission duration, event-free survival, and overall survival were 22.6 ± 7.3 months, 24.3 ± 8.2 months, and 27.3 ± 15.3 months, respectively. **Conclusions.** The frequency of MLL rearrangement was 11.4% in adult *de novo* AML and 14.2% in pediatric AML. cDNA panhandle PCR technology was able to identify all the rare or novel MLL partner genes. MLL-PTD was the most common fusion transcript in adult MLL (+) AML, whereas it was rare in children. Adult MLL (+) patients had a poor outcome with no difference in survival between MLL-PTD and MLL non-PTD groups.

0542

K-RAS MUTATIONS ARE FREQUENTLY ASSOCIATED WITH CHILDHOOD PRO-B ACUTE LYMPHOBLASTIC LEUKEMIA WITH MLL REARRANGEMENTS

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Background. Chromosomal translocations involving MLL gene

occur in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). The association of N-ras or K-ras gene mutations with MLL (+) acute leukemias is not clear. *Aims.* We sought to analyze the association of N-ras or K-ras mutations in patients with MLL (+) ALL and *de novo* AML. *Methods.* Southern blot analysis was used to screen MLL rearrangements in patients with acute leukemias. DNA PCR followed by direct sequencing was performed to detect mutations at codons 12, 13, and 61 of N-ras and K-ras genes. *Results.* Excluding partial tandem duplication of MLL, 87 patients with MLL (+) acute leukemias were examined for Ras mutations. Of 40 MLL (+) children, 20 had pro-B ALL, 4 T-ALL, and 16 *de novo* AML. The 47 adult MLL (+) acute leukemias comprised of 10 pro-B ALL, one T-ALL and 36 *de novo* AML. Of the 20 pediatric MLL (+) pro-B ALL, mutations at codon 12 of N-ras (Gly12Asp) were found in 2 children; at codon 12 of K-ras in 7 (4 Gly12Asp, 2 Gly12Val and 1 Gly12Ala), and at codon 13 of K-ras (Gly13Asp) in another one. None had mutations at codon 61 of N-ras or K-ras. None of the 4 children and one adult with MLL (+) T-ALL carried N-ras or K-ras mutations. All the 10 adult MLL (+) pro-B ALL lacked Ras mutations. The frequency of N-ras mutations in childhood MLL (+) pro-B ALL (2/20) was not different from that in adults (0/10) ($p=0.540$), whereas the frequency of K-ras mutations in children (8/20) was significantly higher than that in adults (0/10) ($p=0.029$). In MLL (+) AML, 6 adults had N-ras mutations (2 Gly12Asp, 2 Gly12Cys, one each of Gly13Arg and Gln61His) compared with 2 in children (one each of Gly12Ala and Gln61Pro) ($p=1.000$). Nine adult MLL (+) AML had K-ras mutations (2 Gly12Asp, 2 Gly12Ala, 2 Gly13Asp, one silent mutation at Gly13 and 2 Gln61His) compared with 2 in children (one each of Gly12Ala and Gly13Asp) ($p=0.466$). One adult harbored both N-ras and K-ras mutations. Taken together, Ras mutations were found in 25% (4/16) of childhood MLL (+) AML and 39% (14/36) of adult MLL (+) AML. In children, the frequency of N-ras mutations in pro-B ALL did not significantly differ from that in AML (2/16) ($p=1.000$), the frequency of K-ras mutations in pro-B ALL (8/20) was higher than that in AML (2/16) but the difference did not reach statistical significance ($p=0.133$). *Conclusions.* Ras mutations were associated with 50% of childhood MLL (+) pro-B ALL and 39% of adult MLL (+) *de novo* AML.

0543

THERAPY-RELATED ACUTE LEUKEMIA AND DNA DOUBLE-STRAND BREAK REPAIR

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Background: The frequency of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) arising after anticancer therapy with DNA-damaging agents such as DNA topoisomerase II inhibitors has increased over the last two decades. However, the factors that predispose to these therapy-related disorders are still poorly defined. *Aims.* Topoisomerase II inhibitors such as etoposide (VP-16), doxorubicin and mitoxantrone, which are widely used for the treatment of human cancers, prevent the enzyme from religating cleaved DNA, thus generating DSB. DSBs constitute a major threat to genome integrity. In mammalian cells, DSBs can be repaired by two major pathways: the Non-Homologous DNA End Joining (NHEJ) pathway and the Homologous Recombination (HR) pathway. It has been reported that DNA double-strand break (DSB) repair by the non-homologous end-joining (NHEJ) pathway is impaired in myeloid leukemia cells. This led us to hypothesize that therapy-related AML (t-AML) or MDS (t-MDS) results from individual differences in the repair of DSBs generated by the treatment. *Methods.* We carried out experiments using six EBV-transformed lymphoblastoid cell lines derived from patients who developed t-AML or t-MDS after being treated with chemotherapy containing topoisomerase II

inhibitors for a first cancer. We also used two EBV-transformed lymphoblastoid B cell lines derived from patients who had also been treated with chemotherapy containing topoisomerase II inhibitors for a first cancer but who did not develop t-AML or t-MDS. *Results.* We analyzed DSB end-joining fidelity in patient and control cells by performing PCR on 4506 bacterial colonies obtained after transformation with repaired plasmids using primers located on either side of the DSB. We found that 2581 out of 3381 plasmids (76%) and 856 out of 1125 plasmids (76%) were accurately joined in the patient and control cell lines, respectively (results from two independent experiments). Thus, there was no difference in the fidelity of rejoining EcoRI-induced DSB between patient and control cells. *Conclusions.* We show here that DSB repair is accurate, *in vivo*, in non-tumoral cells derived from patients who developed t-AML or t-MDS after treatment for a first cancer with DNA topoisomerase II inhibitors. These results indicate that a constitutive defect in the NHEJ pathway is unlikely to predispose to t-AML or t-MDS.

0544

CYTOGENETIC FINDINGS IN IDIOPATHIC MYELOFIBROSIS: STUDY OF 68 CASES

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Idiopathic myelofibrosis (IMF) is a chronic myeloproliferative disorder characterized by bone marrow (BM) fibrosis, extramedullary hematopoiesis and leucoerythroblastic blood count. The disease is recognized as clonal hemopoietic stem cell disorder where fibroblast proliferation is secondary or reactive phenomenon. In recent years, many data on the extreme heterogeneity of survival among IMF patients (pts) have been accumulated. It has been found that cytogenetic abnormalities are indicators of worse prognosis. However, a number of pts with abnormal karyotype varies from 30% to 75% with widely different incidence of specific abnormalities. The objective of our study was to analyze the frequency and type of chromosomal abnormalities in 68 pts (33 females, 35 males, mean age 62 years) diagnosed for well-established IMF. Bone marrow chromosomal analysis was performed according to modified GTG method, described previously as HG-bending. No metaphases were found in 18% (12/68) of pts. Clonal abnormality was manifested in 39% (22/56) of pts, and 61% (34/56) had normal karyotype. Among chromosomal aberrations, typical for IMF, del (13) (q13) was found in one pt, del (20) (q11) in two pts, but partial trisomy 1q has not been found in our group of patients. We have found some new abnormalities in two cases: reciprocal translocation of chromosome 13, and chromosomes 2 and 5 with the same breakpoints on the long arm of chromosome 13 (13q13), respectively. Other findings included anomalies of chromosome 8 acquired trisomy 8 (five cases) or monosomy 8 (one case); derivative of chromosome 18 (two cases), monosomy 18 (one case), deletion of the long arm of chromosome 18 (one case); monosomy 12 (two cases); trisomy 21 (one case); trisomy X (one case) and loss of a Y chromosome (one case). Karyotypes of three patients were complex with aberrations that involved several chromosomes, and some of them mosaicism. In addition, we have found no specific aberrations of IMF: t(2;16) (q31;q24); t(12;16) (q24;q24) (two cases) and near-tetraploidy karyotype (two cases). In conclusion, our group of patients had typical chromosomal aberration of IMF, and other than that, completely new abnormalities as well as complex aberrations of karyotypes in our IMF pts. The impact of these findings on prognosis and survival have to be investigated in larger cohort of pts with IMF.

0545**MICRO-ARRAY CGH AND FISH DETECTION OF CRYPTIC CHROMOSOMAL REARRANGEMENTS IN ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE**

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Background. Genetic diagnosis is critical for personalized treatments of AML. FISH and micro-array CGH are powerful approaches to detect clonal aberrations in malignant hemopathies with normal karyotype. We have recently showed that around 60% of AML cases with normal karyotype have NPM1 mutations and abnormal cytoplasmic sublocalization of nucleophosmin (NPMc+ AML) (Falini et al, *NEJM* 352; 254-266, 2005). NPMc+ AML exclude major chromosomal rearrangements, i.e. AML1/ETO, CBFβ/MYH11, PML/RARA, DEK/CAN, and BCR/ABL1 which are clustering with normal nucleolar sublocalization of nucleophosmin (NPMc- AML). Only 27% of cases with normal karyotype are found in the last subgroup. **Aims.** Our aim is to investigate by FISH and microarray-CGH NPMc- AML with normal karyotype in order to pick up rare cryptic aberrations. An index case is reported. **Methods.** A case of NPMc- AML and normal karyotype was selected by immunohistochemistry and conventional cytogenetics. Absence of AML1/ETO, CBFβ/MYH11, PML/RARA, DEK/CAN, and BCR/ABL1 rearrangements was documented. Microarray-CGH was performed using the Spectral Chip Human BAC array Kit (Spectralgenomics, TX, USA) which is spotted with 3000 BAC clones specific for genomic regions at a distance of 1 megabase from each other. Experiments were performed according to the manufacturer's protocol. Analyses of gains and/or losses of each clone was carried out using a Scanner (GenePix 4000B, Axon Instruments Inc, CA, USA) equipped with a Software for capture (GenePix Pro 6.0, Axon Instruments Inc, CA, USA) and analysis (Spectralware, Spectralgenomics, TX, USA). Metaphase FISH was performed with the following RP1 and RP11 PAC/BAC clones for the 9q33-34 bands: centromere-165P4-282P20-216B9-550J21-167N5-247A12-253N4-65J3-492E3-483H20-409K20-138E2-202H3-88G17-618A20-17L7-1132H120-913J14-888H1-835J22-105C7-143H20-544A12-112N13-telomere. **Results.** Micro-array CGH detected loss of genomic material at 9q34.1 band, and more precisely, at RP11-98H23 and RP11-17O4 clones. The 9q34 deletion was confirmed by metaphase FISH and narrowed between clone 216B9 (centromerically) and clone RP11-544A12 (telomerically). It extends roughly 2.5 megabases. Several putative oncogenes and tumor suppressor genes have been mapped within this region: PKN3, ZDHHC12, TBC1D13, ENDOG, NUP188, SH3GLB2, ABL1. Moreover, loss of the 3'SET (monoallelic deletion of clone RP11-550J21) and of the 5'NUP214/CAN (monoallelic deletion of clone RP11-143H20) indicates a 5'SET-3'NUP214/CAN juxtaposition. **Summary/Conclusions.** Integration of micro-array CGH and FISH allowed us to pick up a cryptic del(9)(q34) and a SET/CAN rearrangement. This case strengthens our hypothesis that the NPMc- AML subgroup is a good candidate to apply microarray CGH and FISH to detect cryptic genomic changes. So far, only one undifferentiated acute leukemia with normal karyotype and SET/CAN fusion has been reported.

Supported by: CNR-MIUR, FIRB, and Fondazione Cassa di Risparmio, Perugia, Italy.

Acknowledgements: PAC and BAC clones were kindly provided by Dr. M Rocchi, University of Bari, Italy and TIGEM, Ospedale San Raffaele, Milan, Italy

0546**A HIGHLY SENSITIVE AND SPECIFIC FLUORESCENCE IN SITU HYBRIDIZATION (FISH) APPROACH FOR THE DETECTION OF T(9;11) (P22-23;Q23) IN ACUTE MYELOID LEUKAEMIA**

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The most frequent partner in translocations involving the MLL gene at 11q23 (3-5% of adult *de novo* acute myeloid leukaemias, dnAMLs) is the AF9 gene at 9p22-23. The detection of these rearrangements is important because a) MLL rearrangements represent a cytogenetic group characterized by low survival rate and poor response to treatment and b) it was reported that MLL/AF9 fusion may carry a less severe prognostic significance as compared with other 11q23/MLL rearrangements. We set up a highly sensitive and specific DNA probe for the detection of t(9;11)(p22-23;q23)-MLL/AF9 fusion by interphase (FISH). A dual colour dual fusion probe was designed encompassing the MLL and the AF9 gene, resulting in the correct identification of the two derivative chromosomes (visible as two fusion signal) as well as of the two normal alleles (single red and green signals, respectively). The aims of the study were: a) to establish the incidence of MLL rearrangements in a series of unselected dnAML, b) to study the sensitivity of FISH in a series of AML cases carrying 11q23 rearrangements by conventional cytogenetics and/or molecular methods and, c) to ascertain the frequency of deletions possibly surrounding the translocation breakpoints. Interphase FISH was used to screen a variable number of nuclei (100-252) in a total of 73 unselected dnAML cases and in 22 additional cases bearing 11q23 rearrangements at karyotype. Cytogenetic analysis was carried on in all cases. RT-PCR for the detection of MLL-AF9 fusion transcript was used to confirm the molecular cytogenetic data. **Results.** We analyzed 73 unselected cases of adult dnAML with a set of two PACs, 217a21 and 167k13, mapping respectively 5' and 3' to the breakpoint cluster region: 66 cases (90,4%) resulted in a germline FISH pattern (96,9-100% interphase with two fusions), confirming the results of conventional cytogenetic analysis. In 7 cases (6,8%) segregation of green and red signals suggestive of MLL gene rearrangement was observed in 30% to 96,5% of the cells (FISH-positive cases): in 5 patients an 11q23 break was detected at karyotype, whereas in 2 cases an 11q- chromosome was reinterpreted as an 11q23/MLL translocation after FISH and molecular genetic studies. We then applied the dual colour dual fusion probe to an enlarged series of 27 AML cases with 11q23 rearrangements and 7 cases without involvement of band 11q23 at G-banding. In all cases studied FISH correctly detected MLL-AF9 fusion when compared with G-banding analysis and RT-PCR studies, except in one case where an atypical t(9;11)(p23;q23) involving a 9p region distal to AF9 was detected. 3' MLL deletion was found in 1/27 cases, whereas no cases with t(9;11) and deletion flanking the 9p breakpoint was found in this series. We arrived at the following conclusions a) This dual-colour dual-fusion system is sensitive and specific for the detection of MLL/AF9 fusion in AML b) It may be of value for the identification of MLL involvement in cases with an 11q- chromosome at karyotypic analysis c). The incidence of deletions surrounding MLL and AF9 breakpoints may be lower than previously reported in the literature.

0547**THE 5'RUNX1/3'CBFA2T1 FUSION GENE MAY BE GENERATED BY CRYPTIC INSERTION AND INVERSION EVENTS IN ACUTE MYELOID LEUKEMIA**F. Albano¹, F. Albano¹, G. Specchia², L. Anelli², A. Zagaria², G. Saglio³, A. Cuneo⁴, R. La Starza⁵, M. Mancini⁶, L. Vicari⁷, F. Lo Coco⁸, V. Liso¹⁶, M. Rocchi¹⁰¹University of Bari, BARI, Italy; ²Hematology - Univ. of Foggia, FOGGIA, Italy; ³Hematology - University of Torino, TORINO, Italy; ⁴Hematology - Univ. of Ferrara, FERRARA, Italy; ⁵Hematology- Univ. of Perugia, PERUGIA, Italy; ⁶University 'La Sapienza', ROMA, Italy; ⁷A.O.R.N. - Cardarelli Hospital, NAPOLI, Italy; ⁸Hematology-Univ. of Tor Vergata, ROMA, Italy; ⁹Hematology - University of Bari, BARI, Italy; ¹⁰DI.GE.MI. University of Bari, BARI, Italy

Background. Translocation t(8;21) (q22;q22) is a common karyotypic abnormality detected in about 15% of Acute Myeloid Leukemia (AML) cases. The rearrangement results in fusion of the RUNX1 (also known as AML1) and CBFA2T1 (also known as ETO) genes generating a 5'RUNX1/3'CBFA2T1 transcriptionally active fusion gene on derivative chromosome 8. In 1 to 8.5% of AML cases insertions events generating a 5'RUNX1/3'CBFA2T1 fusion gene have been reported, whereas the occurrence of inversions accompanying the t(8;21) has never been observed. **Aims.** We performed a molecular cytogenetic analysis by FISH to verify the frequency of chromosomal insertion and inversion events in AML cases bearing the 5'RUNX1/3'CBFA2T1 rearrangement. A detailed breakpoints characterization has been performed in all cases with insertions and inversions. **Methods.** We report a screening of 82 AML cases bearing the RUNX1/CBFA2T1 rearrangement detected by RT-PCR; all cases were tested by Fluorescence In Situ Hybridization (FISH) with BAC and PAC clones specific for CBFA2T1 and RUNX1 genes. **Results.** Our results revealed that 8 (9.8%) AML cases showed cryptic chromosomal insertions or inversions. FISH co-hybridization experiments with CBFA2T1 and RUNX1 probes revealed the presence of a functional fusion gene on the der (21) instead of the der (8) chromosome in five cases with ins (21;8); a single fusion signal on the der (8) chromosome was detected in the case with ins (8;21). The use of the same clones in FISH studies showed the presence of a single unexpected fusion signal on the 8p derivative chromosome in addition to faint CBFA2T1 and RUNX1 signals on the long arm of der (8) and der (21) chromosomes, respectively. These results suggested that in two cases a pericentric chromosome 8 inversion involving CBFA2T1 gene occurred and that the chromosome 21 was rearranged with the 8p derivative chromosome. Appropriate chromosome 21 and 8 BAC clones were employed to precisely define the size of inserted regions in cases with insertions and the breakpoint on the 8p derivative chromosome in cases showing pericentric chromosome 8 inversion. The insertion size turned out to be very heterogeneous, ranging from a minimum of 2.4 Mb to a maximum of 44 Mb. In both cases with chromosome 8 inversion, the CBFA2T1 gene represents the breakpoint at the chromosome 8 long arm whereas the 8p breakpoint showed different mapping positions in 8p21.3 and 8p21.1, respectively. **Conclusions.** Our study allowed us to reveal five cases with ins (21;8), one with ins (8;21), and two with a pericentric chromosome 8 inversion followed by a t(8;21) translocation. Our results illustrate that (1) heterogeneous mechanisms can lead to the generation of the 5'RUNX1/3'CBFA2T1 chimeric gene; (2) molecular cytogenetic techniques may identify cryptic chromosomal changes, not detected by conventional cytogenetic analysis (3) the crucial role of the 5'RUNX1/3'CBFA2T1 fusion gene in leukemogenesis does not depend on its location.

0548**IMPACT OF BONE MARROW SAMPLING ON MINIMAL RESIDUAL DISEASE MONITORING IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA**

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Detection of minimal residual disease (MRD) is of prognostic relevance in childhood acute lymphoblastic leukemia (ALL). In several current ALL treatment protocols, patients are stratified according to MRD levels in bone marrow samples obtained during or after induction therapy. Although it has been suggested that leukemic cells may not be distributed homogeneously over the body, the impact of bone marrow sampling on MRD levels is not yet known. We compared MRD levels in paired bone marrow samples (two independent punctures at different locations) obtained during the first year of treatment. From 26 childhood ALL patients (20 precursor-B-ALL, 6 T-ALL), all treated according to the DCOG ALL9 protocol. The paired bone marrow samples were obtained at day 15 (n=20), day 28 (n=23), day 42 (n=19), 3 months (n=24), 6 months (n=22), 8 months (n=7), 9 months (n=12), and/or 12 months (n=15) after start of therapy. MRD levels were determined by real-time quantitative PCR (RQ-PCR) analysis of rearranged immunoglobulin and/or T-cell receptor gene rearrangements. Data were analyzed according to the guidelines of the European Study Group on MRD detection in ALL. A quantitative range of at least 10e-4 for at least one MRD-PCR target was obtained in 19 patients; in all patients at least one MRD-PCR target had a quantitative range of 5x10e-4. Twenty-three patients were monitored by two MRD-PCR targets; in these cases the highest MRD level of the two MRD-PCR targets was used for analysis. Generally, MRD levels in the two paired BM samples were very comparable and differed less than 3-fold (Pearson's correlation coefficient: 0.94 for quantitative data). In only two pairs, both obtained at day 15, MRD levels differed more than 3-fold. This difference is probably related to the hypocellularity of the BM during the early induction therapy, resulting in higher chance of obtaining BM samples 'contaminated' with peripheral blood. In six samples, MRD levels were around the quantitative range of the assay (5x10-4/10-4) and could be quantified in one sample only, the other sample assigned 'positive, but no accurate quantification possible'. Finally, in 20 paired samples low MRD levels, outside the quantitative range of the assay, could be detected in one BM sample only. If patients would have been stratified according to MRD levels at day 42 and 3 months, analysis of the two paired samples resulted in different MRD-based risk-groups in five out of 24 patients that could be evaluated. These differences concerned four intermediate-risk versus low-risk and one intermediate-risk versus high-risk stratification. Our data demonstrate that MRD levels are very comparable between BM samples obtained from different sites and that most differences occur with MRD levels at the lower border or below the quantitative range (=reproducible part) of the RQ-PCR assay. v.h.j.vandervelden@erasmusmc.nl

0549**LIGHT CHAIN REARRANGEMENTS IN PRECURSOR-B-ALL: IMMUNOBIOLOGICAL CHARACTERISTICS AND APPLICABILITY FOR THE DETECTION OF MINIMAL RESIDUAL DISEASE**

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We analyzed Vk-Jk and VJ-IJ rearrangements in 100 precursor-B-ALL patients using the BIOMED-2 multiplex primer sets in order to evaluate their frequency, characteristics, and applicability as targets for detection of minimal residual disease (MRD). Vk-Jk rearrangements were detected in 31% of patients; they were significantly more frequent in common-ALL (40%) than in pre-B-ALL (13%) and showed a significant relation with age at diagnosis (highest frequency (55%) between 6 and 10 years) and the presence of TEL-AML1 transcripts (55%). VJ-IJ rearrangements were detected in 17% of patients and showed no significant relation with immunophenotype, age or the presence of TEL-AML1 transcripts. Patients with Vk-Jk rearrangements showed a significantly higher frequency of intron-Kde, TCRG, Vd2-Ja, and TCRB rearrangements than Vk-Jk-negative patients. In contrast, incomplete IGH rearrangements were virtually absent. Patients with VJ-IJ rearrangements showed a significantly higher frequency of Vd2-Ja and complete TCRB rearrangements than VJ-IJ-negative patients, whereas incomplete IGH rearrangements were completely absent. Overall, the mean number of PCR-detectable Ig/TCR gene rearrangements in Vk-Jk-positive, VJ-IJ-positive, and Vk-Jk/VJ-IJ-negative patients was 6.7, 6.8, and 4.4, respectively. Of note, in 4 out of 17 patients with VJ-IJ rearrangements, no IGK-Kde rearrangements were detected, suggesting that the hierarchy of IGK and IGL rearrangements is less strict than in normal B-cell development. Vk-Jk and VJ-IJ rearrangements showed a good stability between diagnosis and relapse (88% and 77% stable, respectively) and reached good sensitivities in RQ-PCR analysis (10^{-4} in 82% and 80%, respectively). Our data indicate that Vk-Jk and VJ-IJ rearrangements are differently regulated in precursor-B-ALL and that both type of rearrangements can successfully be used for the detection of MRD.

0550**A NOVEL APPROACH TO MRD MONITORING IN AML, REAL-TIME QUANTIFICATION OF LEUKAEMIA ASSOCIATED ANTIGENS (WT1, PRAME AND G250)**

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Monitoring MRD in AML patients is important in assessing the effectiveness of treatment and identifying patients at high risk of relapse, to enable intervention to prevent its onset. Several chromosomal translocations have been identified in AML, but are only detected in approximately 30% of patients. This demonstrates the need for alternative approach to MRD monitoring in AML utilizing alternative target genes which are either specifically expressed or significantly upregulated in leukaemic cells. The Wilm's tumour (WT1) gene, which is overexpressed at levels exceeding 10^4 (4) copies in approximately 60% of acute leukaemia patients is an example of such markers. We have examined a number of leukaemia-associated antigens (LAAs) and identified another two genes, PRAME and G250 (CA9), which could provide suitable markers for molecular monitoring of MRD in all AML patients. We have developed real-time RT-PCR (RQ-PCR) protocols for the quantification of these three transcripts, and have examined their levels in 32 AML patients at presentation, and 12 normal subjects. 12/32 patients were also examined at remission, and 5/32 patients at relapse. Our data show that these three markers were able to offer accurate MRD monitoring for all 32 patients. WT1 was identified as suitable for monitoring MRD in 20/32 patients examined (levels of up to 89.25 WT1/ABL), while PRAME was suitable for 11/32 (levels up to 10 PRAME/ABL) and G250 for 8/32 patients (lev-

els up to 11.27 G250/ABL). In 5 patients more than one marker was identified as suitable for MRD monitoring. The levels of all 3 transcripts decreased significantly in remission samples. Relapse samples showed levels corresponding to those detected at diagnosis. 3 patients examined 2-3 months before the onset of relapse showed a significant increase in the levels of the 3 transcripts compared to those detected at remission. These data show the value of this alternative approach to MRD monitoring in AML. The three genes, taken together, were able to offer accurate MRD monitoring for all 32 patients examined and were able to distinguish patients at high risk of relapse up to 3 months before its onset. This approach could simplify MRD monitoring with a choice of few markers only.

0551**IN UTERO ORIGINS OF CHILDHOOD LEUKEMIA**T. Burjanivova¹, J. Madzo², K. Muzikova², R. Marschalek³, S. Pekova⁴, J. Stary², J. Trka², J. Zuna²

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While there is enough convincing evidence in childhood ALL, the data on the pre-natal origin in childhood AML are less conclusive. Our study aimed to screen Guthrie cards (neonatal blood spots) of childhood acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) patients for the presence of their respective leukaemic markers. For the analysis we used PML/RARa, AML1/ETO and CBFb/MYH11 fusion genes, translocations of MLL gene and internal tandem duplication of Flt3 gene (Flt3/ITD) in patients with AML and TEL/AML1 fusion gene and immunoglobulin (Ig) and/or T-cell receptor (TCR) gene rearrangements in patients with ALL. These molecular markers represent suitable candidates for backtracking of leukaemic cells in newborn material, because they are clonotypic and altogether their incidence is ~40% and >90% in AML and ALL, respectively. We screened Guthrie cards from 12 AML patients (4x PML/RARa, 2x CBFb/MYH11 2x AML1/ETO, 2xFlt3/ITD, 3x MLL rearrangement) and from 14 ALL patients (14x Ig/TCR rearrangements, 1x TEL/AML1). One AML patient from our group had PML/RARa fusion gene together with Flt3/ITD, in 1 ALL patient we screened the Guthrie card for both Ig/TCR and TEL/AML1. We designed patient-specific PCR primers and we established nested PCR assay for each clonotypic sequence prior to the analysis of the corresponding Guthrie card. Assay specificity was determined using serial dilutions of patient DNA into the DNA of a healthy donor. The sensitivity of PCR was $\geq 10^{-4}$ in 12 systems in AML patients and in all ALL patients. In one patient with CBFb/MYH11 fusion gene presenting with 22% blasts at diagnosis the sensitivity of PCR was 10^{-3} . In two patients with ALL we reproducibly detected identical rearrangements both in the presentation sample and in the neonatal blood spot on Guthrie card. The first patient harboured two independent rearrangements: TCR-delta Vd2/Dd3 and IgH VH3/JH5 and both were detectable in Guthrie card. In the second patient two PCR systems were optimised with adequate identical sensitivity: Ig \square Vk1/Kde and TCR \square VgI/Jg1.3-2.3. However, only the latter detected leukaemic cells in Guthrie card. We did not find patient-specific molecular markers in any patient with AML. In the present study we confirmed the prenatal origin in 15% of ALL patients. The negative results in the AML group can not disprove the theory that some childhood AML cases are also initiated in-utero. The negative results might be caused by lower size of pre-leukaemic clone or by the higher age at presentation in our AML group compared to ALL patients (median 7 and 3 years, respectively). As for the Flt3/ITD cases the latest data indicate that this aberration is probably of a post-natal origin. However, our present data suggest that the prenatal origin of childhood ALL is a more frequent phenomenon than the prenatal origin of childhood AML. Supported by grants #7436 from the Czech Ministry of Health and #0021620813 from the Czech Ministry of Education.

0552**IMMUNOMAGNETIC SEPARATION OF BONE MARROW CELLS WITH THE PATIENTS OF MM-COMPARISON OF PROGNOSTIC FACTORS WITH THE DELETION OF 13Q14 DETECTED BY I-FISH ON SEPARATED AND UNSEPARATED BONE MARROW CELLS**J. Smejkalová¹, P. Kuglík², H. Filková¹, V. Vranová², A. Oltová¹, J. Stražilová², M. Krejčí¹, L. Pour¹, Z. Adam¹, M. Penka¹, R. Hájek¹¹University Hospital Brno, BRNO, Czech Republic; ²Faculty of Science, Masaryk U, BRNO, Czech Republic

Background. Cytogenetics abnormalities of chromosome 13 are emerging as important prognostic factors in multiple myeloma and have been associated with poor prognosis. **Aims.** and methods. The occurrence of 13q14 deletion and other standard laboratory parameters were determined in 40 patients with multiple myeloma. Interphase fluorescence in situ hybridization was used for detection 13q14 deletion. **Results.** We found that interphase fluorescence in situ hybridization using a locus specific probe for RB1 gene on immunomagnetically selected myeloma cells was more sensitive than non selected cells. The 13q14 deletion was found in 10 of 40 (25,0%) of bone marrow samples without cell selection and in 25 of 40 (62.5%) of samples with CD138+ enriched myeloma cells. Negative correlation was found between albumin and the 13q14 deletion in separated ($p=0,003$) as well as in cells without selection ($p=0,010$). No significant correlation was found between overall survival on selected and non-selected cells ($p=0,830$; $p=0,260$) and a similar result was obtained for treatment response after transplantation on selected cells ($p=0,520$) on non-selected cells ($p=0,190$). **Conclusions.** Our results confirm that immunomagnetic selection of CD138+ cells increases the sensitivity of detection of the 13q14 deletion in bone marrow samples. The correlation was found between albumin and the 13q14 deletion in both of type of cells.

Supported by grant IGA MZÁR 7475-3.

0553**SPLIT FISH ASSAYS DETECT THE CALM/AF10 REARRANGEMENT AT DIAGNOSIS AND SERVE TO MONITOR ACUTE LEUKEMIA**R. La Starza¹, B. Crescenzi¹, C. Matteucci¹, V. Pierini², A. Cuneo³, A. Bardi³, G. Specchia³, C. Ariola⁴, M. Sborgia², D. Magro², R. Foà⁴, A. Krause⁵, M.F. Martelli¹, C. Mecucci¹¹Hematology and Bone Marrow Unit, PERUGIA, Italy; ²Ematologia, FERRARA, Italy; ³Department of Ematologia, BARI, Italy; ⁴Università 'La Sapienza', Rome, Italy; ⁵Klinikum Grobhadern, Munchen, Germany

Background. The t(10;11) (p13;q14)-CALM/AF10 was first identified in the monocytic U937 cell line and subsequently found in undifferentiated leukemia, acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), and non-Hodgkin's lymphoma. Although the different CALM/AF10 fusion transcripts have no evident correlation with the types of leukaemia, they are usually considered a marker of poor prognosis in terms of relapse and event-free survival. **Aims.** To set up double color FISH assays for diagnosis and disease monitoring of the CALM/AF10 fusions. **Methods.** We selected three AML and two T-ALL with a balanced t(10;11) (p13;q14) translocation as the only cytogenetic abnormality in 2/3 AML and in both T-ALL. In the other AML the karyotype also showed an add (19) (p13). At diagnosis, metaphase and/or interphase FISH was performed on bone marrow in all 5 cases, and on mediastinal biopsy embedded in paraffin in one AML. Double color experiments were done with clones RP11-418C1 (red) and RP11-249M6 (green) for the 5'AF10 and 3'AF10 respectively, and with clones RP11-12D16 (red) and RP11-90K17 (green) mapping at the 5'CALM and 3'CALM sides, respectively. The LSI MLL probe (Vysis) assessed the status of the MLL/11q23 gene in two patients. RT-PCR, done as already described (Boh-

lander et al. Leukemia 2000; 14: 93-99), was used to confirm the presence of the CALM/AF10 transcripts. Both interphase FISH and RT-PCR were used for disease monitoring in two patients. **Results.** Clones for AF10 gave co-localization of red/green signals on normal chromosome 10 and separate signals, with green on der (10) and red on der (11), in four cases. In the fifth case (AML with an add (19) (p13), a three way t(11;10;19) (q14;p13;p13) translocation was demonstrated. In all patients clones for CALM gave co-localization of red/green signals on normal chromosome 11, and separate signals, with green on der (11) and red on der (10). FISH also demonstrated a CALM/AF10 rearrangement on mediastinal biopsy in one AML. RT-PCR studies detected the CALM/AF10 transcript in all patients. Interphase FISH and RT-PCR results overlapped during remission and relapse. In one patient, interphase FISH also confirmed an extramedullary leukemic relapse in the mammary gland. **Summary/conclusions:** Using the split signal principle, we developed and validated a set of CALM/AF10 FISH probes which reliably detects the CALM/AF10 rearrangement in bone marrow samples and in the extramedullary localization. It is also a powerful assay for investigating CALM/AF10 fusion in complex chromosomal changes. Disease monitoring by interphase FISH may complement RT-PCR analysis. Supported by: CNR-MIUR, FIRB, and Fondazione Cassa di Risparmio, Perugia, Italy Acknowledgements: BAC clones were kindly provided by Dr. M Rocchi, University of Bari, Italy and TIGEM, Ospedale San Raffaele, Milan, Italy

0554**MYELOID SARCOMAS HAVE AN HETEROGENEOUS PATTERN OF CYTOGENETIC ABERRATIONS: FISH ANALYSIS OF 58 PATIENTS**M.C. Cox¹, S. Bacci², R. Gasbarra³, S. Ascani⁴, E. Sabbatini⁵, F. Lo Coco⁶, S.A. Pileri⁵¹AO S'Andrea, ROME, Italy; ²Institute of Hematology 'Seragnoli', BOLOGNA, Italy; ³Istologia, Osp. Forlanini, ROME, Italy; ⁴Dept. of Hematology, Seragnoli Institute, BOLOGNA, Italy; ⁵Hematology, Seragnoli Institute, BOLOGNA, Italy; ⁶Hematology, Tor Vergata, ROME, Italy

Background. Myeloid sarcoma or granulocytic sarcoma is a rare and ill defined neoplasm presenting as a solid mass composed of haematopoietic precursors in an extramedullary region. Cytogenetic and molecular characterisation of this neoplasm is poor, nonetheless sporadic case reports described the detection of AML1/ETO gene fusion in myeloid sarcomas diagnosed in patients suffering from AML1/ETO+ acute myeloid leukaemia (AML). These observations, lend credence to the notion, that most myeloid sarcomas bear the AML1/ETO gene fusion. **Aims.** To analyse a large series of myeloid sarcomas with a panel of molecular probes, in order to better define the cytogenetic profile of this rare neoplasm. **Methods.** Fifty-eight samples from myeloid sarcomas were analysed by FISH using the following probes: AML1/ETO; CBF-Beta; MLL; EGR1/D5S23, D5S721; D7S486/CEP7; CEP4; CEP8; CEP11; CEP16. Locus specific probes (LSP), were routinely used also to detect aneuploidies: AML1/ETO probe for chromosome 8 and 21, the MLL probe for chromosome 11, the CBF-Beta probe for chromosome 16. If aneuploidy was suspected a confirmatory hybridisation test was carried out with centromeric probes (CEP 8, CEP 11, CEP 16). After pre-treatments slides were co-denatured and hybridised using the Hy-Brite machine (Abbott Laboratories). The slides were analysed with an Olympus BX50 microscope (Olympus, Japan): in all evaluable cases 100-200 cells were counted for each probe. A normal value study was performed in 25 control samples. **Results.** Eight samples out of 58 (13,8%) were not evaluable by FISH analysis, because of lack of hybridisation signals with all the probe sets. Thirty-three out of 58 sarcoma samples could be fully analysed (56,9%), while in the remaining 17 (24,1%) the success rate of FISH analysis varied within the different probe analysed. In the control samples only 2 out of 25 analysed cases were not evaluable (8%), while in the remaining 23 the full

probe set could be tested. FISH showed clonal abnormalities in 22 out of 50 (44%) sarcomas fully or partially analysed, while the rate of individual aberrations was respectively as follows: 10,9% for monosomy 7; 8,3% for MLL rearrangement; 8,2% for trisomy 8; 5% for monosomy 5q (1 case of monosomy and 1 of 5q deletion); 4,4% for inversion 16; 4,4% for trisomy 4; 4,4% for del CBF-Beta (incidentally observed while scoring INV-16), 2,4% for 20q-; 2,2% for AML1/ETO fusion; 2% for trisomy 11. In 2 cases two different aberrations were observed: 20q- and CBF-Beta deletion; trisomy 4 and 5q- Summary/Conclusions. This is, as far as we know, the largest series of Myeloid sarcomas studied by FISH. Unexpectedly the pattern of cytogenetic aberrations observed was very heterogeneous and the incidence of AML1/ETO+ cases was low. The survival of the overall population was poor regardless of cytogenetic results.

0555

THE FOUR AND A HALF LIM DOMAIN PROTEIN 2 (FHL2) INTERACTS WITH CALM

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Background. The balanced t(10;11) (p13;q14) chromosomal translocation results in the CALM/AF10 fusion gene. This translocation is found in acute myeloid leukemia (AML), T-cell acute lymphoblastic leukemia (T-ALL) and malignant lymphoma. CALM (Clathrin Assembly Lymphoid Myeloid Leukemia Protein) is a clathrin assembly protein which plays a role in clathrin mediated endocytosis and trans Golgi network trafficking. CALM is a member of the growing family of endocytic proteins which are found altered in tumors. AF10 is a putative transcription factor that is probably involved in processes related to chromatin organization and appears to have polycomb group gene like properties. FHL2 (Four and a half LIM domain protein 2) was shown to be an antagonist to PLZF (promyelocytic leukaemia zinc finger protein). FHL2 it interacts with the proto-oncogene SKI1 and with β -catenin which both have important roles in the Wnt signalling pathway. FHL2 transmits Rho signals from the cell membrane to the nucleus and is a coactivator for the CREB transcription factors (Fimia et al, 1999). **Aims.** The CALM/AF10 translocation is associated with a variety of acute lymphoid and myeloid leukemias. To learn more about the CALM/AF10 translocation we searched for protein interaction partners of CALM. **Methods.** A yeast two hybrid screen was performed to identify interacting partners. Interactions were confirmed by co transformation assays in yeast. Further confirmations using co-immunoprecipitation is being performed. Deletion mutants of both CALM and FHL2 are used to map the protein interaction domains. We are currently performing co-localization studies of CALM and FHL2 and are testing whether FHL2 might be a transcriptional co-activator for CALM. **Results.** Seven putative protein interaction partners of CALM were detected. One of these interactors was the four and a half LIM domain protein FHL2. The FHL2 interaction domain of CALM was mapped to amino acids 294 to 335 of CALM. Summary Vecchi et al (2001) demonstrated that inhibition of CREM mediated nuclear export leads to the accumulation of CALM in the nucleus and that CALM has transcriptional activator properties, suggesting participation of CALM in nuclear events. This together with the observation that FHL2 has been shown to modify the transcriptional properties of other proteins suggests that FHL2 might regulate the nuclear function of CALM.

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MOLECULAR AND IMMUNOPHENOTYPIC DETECTION OF MINIMAL RESIDUAL DISEASE (MRD) IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)

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Minimal residual disease (MRD) detection is becoming an integral part of contemporary therapeutic protocols for childhood acute lymphoblastic leukaemia (ALL). In addition to monitoring possible relapse following treatment, detection of MRD during treatment provides information regarding the response of the patient to a given protocol. As a risk factor, independent of other known factors (age, sex, white blood count) these results can help stratify patients into higher or lower risk categories and treatment suitably adjusted. A nationwide prospective study of MRD in 63 Irish children with ALL was undertaken to compare both immunophenotypic (flow cytometry) and molecular (PCR) based assays. Molecular analysis involved tracking of fusion-transcript levels in patients with leukaemic-specific chromosomal translocation by real-time quantitative PCR (RQ) PCR. In the absence of a known translocation, patient samples were screened for Immunoglobulin heavy chain (IgH) or T-Cell receptor (TCR) gene rearrangements and allele specific oligonucleotides (ASOs) designed as markers of clonal disease. To date, 35 patients have been successfully tracked at all time points. Patients are tracked at regular intervals, Days 8, 15, and 28, Weeks 7, 16, 24, 33, 42, 52, 82, and 127. 27 have been tracked, by fusion gene transcript levels; TEL-AML (n=8), MLL-AF4 (n=2), E2A-PBX (n=1), BCR-ABLp190 (n=1). The remaining 23 patients have been tracked by RQ-PCR using clonal specific markers. Sensitivity levels of between 10⁻⁴ and 10⁻⁵ were achieved for all patients. 7/35 patients had a rapid clearance of disease with undetectable levels of MRD at end of the induction period. 14/35 has a slower reduction in the leukaemic burden but had undetectable levels of MRD by 3-6 months. 6/35 patients had persistent levels of MRD up to final time-point, while 8/35 patients had re-emerging levels of MRD. For the flow cytometry assay, a panel of 20 antibodies were used to characterize patient-specific aberrant leukaemia-associated phenotypes (LAPs). LAPs were identified in 48 patients, with 43/48 patients having at least two LAPs. 44 patients had B-lineage ALL and 4 had T-lineage ALL. In patients with B-lineage ALL, the most common LAPs were CD58/CD10 and CD66/CD10 co-expression, identified in 39 and 29 cases respectively. In serial analysis of 34 patients, 15 were MRD negative after treatment induction phase at day 28 and remained negative at week 50. These patients showed rapid and sustained response to chemotherapy. High level (> 0.5% total cell) and intermediate level (0.5% < >0.05%) of MRD were detected at day 28 in 5 and 8 patients respectively. These patients were again MRD positive at week 50. 3 patients showed slow MRD level decrease as leukaemic cells were present at day 28 but eventually disappeared by week 50. Finally, despite being negative at day 28, 3 patients were found positive by week 50. Our results show that MRD analysis using both molecular and immunophenotypic approaches is highly sensitive in evaluating response to therapy in childhood ALL and the kinetics of MRD using these approaches may identify subgroups of patients within certain prognostic groups.

Cytokine signaling and transcriptional II

0557

CHEMOKINES SYNERGIZE IN THE RECRUITMENT OF CIRCULATING NEUTROPHILS INTO INFLAMED TISSUE

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Background. The innate immune response against microorganisms is mediated by phagocytes, attracted by chemokines and other G protein-coupled receptor (GPCR) ligands. Originally, we observed increased neutrophil migration by the interaction of inflammatory CXC chemokines such as IL-8/CXCL8 and granulocyte chemotactic protein (GCP)-2/CXCL6 with regakine-1, a CC chemokine constitutively present in plasma. **Aims.** To better characterize regakine-1 and to demonstrate that chemokines synergize in an *in vivo* setting. **Results.** We observed statistically significant synergy between regakine-1 and the neutrophil attractants C5a or IL-8/CXCL8 in neutrophil shape change and migration under agarose. In addition, regakine-1 attracted human bone marrow granulocytes and enhanced their chemotactic response to IL-8/CXCL8 in a dose-dependent manner. *In vivo*, regakine-1 provoked a mild neutrophilia in rabbits upon intravenous injection. Importantly, we also demonstrated that the CC chemokines regakine-1 and monocyte chemoattractant protein (MCP)-3/CCL7 as well as the CXC chemokine stromal cell-derived factor (SDF)-1a/CXCL12 co-operated with murine GCP-2 after intraperitoneal co-administration in mice to increase neutrophil influx. **Conclusions.** Plasma chemokines may regulate the number of circulating leukocytes under homeostatic conditions and may facilitate extra recruitment of bone marrow neutrophils during inflammation. These data also demonstrate that inducible and constitutive GPCR ligands synergize to enhance inflammation and facilitate a more effective immune response.

0558

PLATELETS RELEASE CXCL4L1, A NON-ALLELIC VARIANT OF THE CHEMOKINE PLATELET FACTOR-4 (PF-4)/CXCL4 AND POTENT INHIBITOR OF ANGIOGENESIS

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Background. The chemokine family consists of pro-inflammatory cytokines, primarily involved in chemoattraction and activation of specific leukocytes in various immuno-inflammatory responses. Several chemokines influence tumor growth by regulating angiogenesis. The first chemokine described as a regulator of angiogenesis is platelet factor-4 (PF-4)/CXCL4. Although this angiostatic platelet-derived chemokine has been the subject of extensive research as a candidate anti-cancer drug, its non-allelic human gene variant PF-4^{alt}/PF-4^{var1}/SCYB4V1 has not been previously investigated. **Results.** The product of this non-allelic variant gene of CXCL4, designated CXCL4L1, was isolated for the first time from thrombin-stimulated human platelets and purified to homogeneity. Although secreted CXCL4 and CXCL4L1 differ in only 3 amino acids, CXCL4L1 bound heparin with lower affinity than CXCL4 but was more potent in inhibiting chemotaxis of human microvascular endothelial cells (HMVEC) toward interleukin-8 (IL-8/CXCL8) or basic fibroblast growth factor (bFGF). *In vivo*, CXCL4L1 was also more effective than CXCL4 in inhibiting bFGF-induced angiogenesis in rat corneas. **Conclusions.** Activated platelets release CXCL4L1, a potent regulator of endothelial cell biology, which affects angiogenesis and vascular diseases.

0559

MOLECULAR CLONING OF DOCK10, A NOVEL IL-4-INDUCIBLE FACTOR IN HUMAN B LYMPHOCYTES

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Background. The cytokine interleukin-4 (IL4), produced by Th2 lymphocytes, binds the IL4 receptor (IL4R) present in the surface of hematopoietic progenitors, macrophages, mast cells, and T and B lymphocytes. IL4R activation initiates signalling pathways that lead to STAT6 activation and, subsequently, the development of specific gene expression programmes. IL4 drives the differentiation of CD4⁺ T cells to Th2 cells, which play roles in allergic responses and infections by extracellular parasites. In B lymphocytes, IL4 induces differentiation, proliferative and survival responses. Thus, IL4 prolongs the survival of *in vitro* cultured B-CLL cells. In this pathological entity, characterized by defective apoptosis, recent reports based on microarray analysis suggest that IL4 pathways are up-regulated. Following IL4 stimulation of B-CLL cells, we detected an unexpected RT-PCR amplification product. The fragment was cloned, sequenced, and identified as transcribed sequences from a novel gene, named DOCK10, of which only partial sequences were known. **Aims.** To clone the entire coding sequence of DOCK10. To study the function of this novel IL4-inducible gene. **Methods.** RACE-PCR was used to clone the 5' sequence of DOCK10 mRNA. Sequences deposited in the human genome resources served to complete the coding sequence of DOCK10. Search tools available in the web were used to identify protein domains and homologous genes. Tissue distribution of DOCK10 was studied by RT-PCR and Northern Blotting. A rabbit polyclonal antibody raised against DOCK10 was used for analysis of cytoplasmic, nuclear and whole cell extracts in Western Blotting. Plasmids for inducible expression of DOCK10 were constructed (tet-off system) and stable clones of the K562 cell line were isolated which express DOCK10 following doxycycline removal. **Results.** An oligonucleotide anchored at the 5' end of the cloned RT-PCR fragment was used to prime a 1960-bp RACE-PCR fragment. The most 3' end sequences of the mRNA were known, and additional intermediate sequences were obtained by RT-PCR designed to cover the gap. The DOCK10 gene was found to span a region of 170 kb in chromosome 2q36. It contains 56 exons, and encodes a protein composed of 2180 amino acids. DOCK10 is homologous in structure to DOCK9 (Zizimin1), a Cdc42 activator involved in the regulation of actin cytoskeleton. DOCK10 was found to be most expressed in T and B lymphocytes, and induced by IL4 in B but not in T lymphocytes. In all of 9 B-CLL cases, IL4 strongly up-regulated expression of DOCK10. Up-regulation of DOCK10 was also observed following IL13 stimulation in 1 out of 4 cases. DOCK10 was found to localize both in cytosol and nuclear fractions, with an apparent molecular mass of 250 K, which was also the size of DOCK10 in 4 transfectant K562 clones. **Summary/Conclusions.** DOCK10 is a novel factor whose expression is: a) restricted to lymphocytes, and b) IL4-inducible specifically in B cells. The study of DOCK10 function could provide insights into the understanding of the biological activities of IL4, which may be relevant to B cell malignancy.

0560

STUDY OF DIFFERENT EXPRESSION OF THE CHEMOKINE RECEPTORS CXCR3, CXCR4 AND CXCR5 BY HEMATOLOGICAL MALIGNANT CELLS

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Abstract Background. and Objectives: There is accumulating evidence that apart from their physiologic functions, chemokine receptors may also be involved in migration and dissemination of malignant hematopoietic cells. BM microenvironment plays

an important role in regulation of growth, survival, and differentiation of normal and leukemic hematopoietic progenitors. Direct contact with stromal cells is particularly important for survival of normal and leukemic progenitors. This process is likely to be directed by chemokines secreted by BM stroma and by their corresponding receptors on leukemic cells. Chemokine receptors expressed in variable amounts on leukemic cells are functionally active and may be involved in trafficking and *in vivo* motility of malignant hematopoietic cells, and identified chemokine receptor neutralization may act as a potential treatment for hematological malignancies. **Design and Methods.** This study investigated the expression of three chemokine receptors CXCR3, CXCR4, and CXCR5 on malignant cells from 90 patients, 30 ALL, 30 AML, and 30 NHL patients using flow cytometry. **Results.** CXCR3, CXCR4, and CXCR5, were expressed in 97%, 93%, and 83% of ALL patients ($p < 0.05$), and in 50%, 53%, and 63% of NHL patients ($p > 0.05$) respectively, while in AML patients, the three chemokine receptors were significantly higher ($p < 0.05$) in M3, M4, and M5, interestingly, the three AML subtypes with organomegaly. Similarly, the three chemokine receptors expression was significantly higher ($p < 0.05$) with organomegaly and massive lymphadenopathy in ALL and NHL patients respectively. **Conclusions.** BM microenvironment plays a crucial role in regulating survival, proliferation, and differentiation of both normal hematopoietic and leukemic cells. Localization of hemopoietic progenitors and leukemic cells involves interaction of leucocyte adhesion molecules with counterligands present on BM stromal cells and extracellular matrix. Migration of leukemic cells might be dependent on the expression of chemokine receptors which is expressed by a number of malignant neoplasms and may have a role in tumour metastasis. Different patterns of chemokine receptors identify different malignant cells and may play a role in malignant cell circulation. Since a potential mechanism in trafficking of hematological malignant cells is the interaction of chemokine receptors, which is expressed on malignant cells, and since hematological malignancies have the potential to infiltrate the liver, spleen, lymph nodes and brain, such extramedullary presentation is important to understand the biology of hematological malignancies and also for developing new prognostic parameters and potential therapeutic approaches. **Key Items:** Chemokine Receptors, CXCR, Hematological Malignancies.

0561

TPO TREATMENT RESTORES GATA-1 EXPRESSION AND MEGAKARYOCYTIC DIFFERENTIATION IN GATA-1LOW MICE

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Background. The role of GATA-1 in megakaryocytopoiesis has been firmly assessed by the phenotype of mice carrying deletion of the sequences of the gene required for its expression in Mk (i.e. deletion of the first DNase hypersensitive site, Gata1tm2Sho or GATA-1low mutation). The GATA-1low mice experience severe thrombocytopenia because of defective thrombocytopoiesis. The GATA-1 gene is required at the beginning of the maturation process because the mutation blocks Mk maturation between stage I and II, resulting in accumulation of defective Mk in the tissues of the mutants. The block in maturation includes failure to properly organize α -granules, since von Willebrand factor is barely detectable in mutant Mk and P-selectin, although normally expressed, is found frequently associated with the demarcation membrane system instead than within granules. Conversely, both von Willebrand factor and P-selectin are barely detectable in GATA-1low platelets (Centurione et al., Blood 104:3573-3580, 2004). **Aims.** To clarify the relationship between thrombopoietin (TPO) and GATA-1 in the regulation of Mk differentiation by investigating whether treatment with TPO would affect GATA-1 expression and thrombocytopoiesis in GATA-1low mutants. **Methods.** GATA-1low

mice were injected i.p. with 100 microg/Kg/day of TPO per 5 consecutive days. Mice were sacrificed at day 7 and 14 after beginning of the treatment to evaluate blood platelet counts, levels of GATA-1 expressed in purified Mk (mRNA by quantitative RT-PCR and protein by immunohistochemistry) and Mk morphology (by electron and immuno-electron microscopy). **Results.** TPO treatment increased (by 5-fold) the number of platelets in the circulation of the GATA-1low mice at 14-18 days after treatment. At this time points, the blood contained not only defective megathrombocytes characteristic of the mutation, but also many normal platelets that formed clusters virtually undetected on smears from untreated animals. The blood platelet increases were preceded by increases in Mk (2-5-fold) in the spleen. In contrast with the Mk from untreated animals, the newly formed Mk were strongly positive for GATA-1 by immuno-histochemistry and expressed increased levels of GATA-1 mRNA (Delta Ct=9.7±0.4 vs 7.7±0.4 with mRNA from Mk purified from untreated and TPO-treated animals, respectively). Furthermore, mature Mk with fully mature platelets territories, virtually absent in the tissues from untreated mice, represented 28.5% of all of the Mk present in the spleen of the TPO-treated animals. These Mk expressed normal levels of Von Willebrand Factor and P-selectin and the two proteins were appropriately assembled in the α -granules. **Conclusions.** These results indicate that TPO-treatment restores GATA-1 expression, Mk differentiation and platelet formation unpaired by the GATA-1low mutation and suggest the presence of TPO-responsive elements among the regulatory sequences of the gene not deleted by the GATA-1low mutation. It is possible that these sequences might play an important function in lineage decision.

0562

HUMAN MASK HAS A UBIQUITOUS EXPRESSION IN HUMAN TISSUES AND LEUKAEMIAS AND PARTICIPATES IN A FAK, SRC AND SHP2 MULTIPROTEIN SIGNALLING COMPLEX IN K562 CELLS

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The Multiple Ankyrin Repeat Single KH domain (MASK) protein was first identified in *Drosophila* through a genetic screen designed to identify proteins that interact with the protein tyrosine phosphatase, Corskrew (CSW), a homologue of the tyrosine phosphatase, Shp2, in humans. The phenotypic characterisation of MASK in *Drosophila* suggested that MASK is a novel protein involved in receptor tyrosine kinase signalling (RTKs) and its activity is required for cell differentiation, cell survival and cell proliferation in *Drosophila* eyes. Human MASK (hMASK) is a protein that has been recently described in humans, in the prostate cancer cell line LNCaP. Through Western Blot analysis, we have detected a broad expression of hMASK in normal tissues; skeletal muscle, kidney, lung, small intestine, stomach, liver, spleen, lymph node, peripheral blood leukocyte of normal donors, and the human leukaemia cell lines; K562, HL-60, MOLT4, KG1 and Jurkat. Confocal microscopy, using the K562 and Jurkat cell lines, identified hMASK in the nucleus and perinuclear region. Moreover, computational analysis (PSORT II and NetNES) detected a Nuclear Localisation Signal and a Nuclear Export Signal in the hMASK sequence. Real Time RT-PCR detected mRNA expression of hMASK in normal bone marrow and a varied higher expression (2-12 fold increase) of hMASK mRNA was observed in 8 bone marrow from patients with diagnosis of acute myeloid leukaemia; FAB classification: 1 M1, 3 M2, 2 M4, 1 M5, 1 secondary AML. In K562 cells, the role of hMASK in RTK signalling pathway was evaluated. Western Blot analysis revealed that, in K562 cells, hMASK is associated with the focal adhesion kinase (FAK), the non-receptor tyrosine kinase Src, and the tyrosine phosphatase Shp2. When associated with hMASK, FAK displayed phosphorylation on Tyrosine, an important step for its activation. An association between BCR-ABL and hMASK was not observed.

Focal adhesion kinase (FAK) is a nonreceptor tyrosine kinase that plays an important role in cancer cell motility, invasiveness and proliferation. FAK associates with several different signalling proteins such as Src-family protein tyrosine kinase, Shc, Grb2, PI 3-kinase, tyrosine phosphatase Shp2 and paxillin. The presence of multiple ankyrin repeats suggests a role for hMASK as a scaffolding protein, bringing together many signalling molecules. Our findings are the first to describe the expression of hMASK in different human tissues and to characterise the role of hMASK in the RTK signalling pathway. The identification of proteins that interact with hMASK and that might direct hMASK to various signal transduction pathways will be essential to clarify the role of hMASK in the signalling pathways in general. The ongoing search for proteins and pathways associated with the abnormal phenotype of the leukaemia cell will surely uncover new molecular targets for a rational therapy of leukaemia in the near future.

0563

MODIFIERS OF MITOCHONDRIAL ION CHANNELS ALTER PROLIFERATION IN CD34+ CELL LINES

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Background. Haematopoietic stem cells (HSC) with a low mitochondrial membrane potential (ψ_{mem}) show a higher engraftment-potential than HSCs with a high ψ_{mem} (Spangrude et al 1990 PNAS). Therefore the mitochondrial probe rhodamine123 has been used to determine very early haematopoietic progenitors. To date the mechanism connecting a low ψ_{mem} with high haematopoietic engraftment potential is unknown. Beside the role of mitochondria in apoptosis and ATP-generation they have been shown to be important key regulators of intracellular Ca²⁺. Ca²⁺ itself is also strongly connected to the expansion of HSCs. **Aims.** In this study we investigate the molecular mechanisms of the higher clonogenicity of HSCs with a low ψ_{mem} using CD34+ cell lines. A deeper insight into these mechanisms might help to better define early human progenitor cells and might allow to establish new ways of ex-vivo manipulation of HSCs. **Methods.** We investigated the openers (NS1619, diazoxide, As2O3) and blockers (Cyclosporine A (CsA), 5-Hydroxydecanoate (5-HD)) of the following mitochondrial ion channels: permeability transition pore (PTP: As2O3, Cyclosporine A), mitochondrial ATP-dependent K⁺-channel (mitoKATP: diazoxide, 5-HD), and mitochondrial big K⁺ channel (mitoBK: NS1619). Proliferation was tested using the CD34+ cell line KG1a and the novel c-kit+ and CD34+ cell line CS-1 which can be growth-stimulated by stem cell factor (SCF). Cell proliferation assays included cell counting with electronic control for dead cells (Casy, Schaefer Systems) and flow cytometry measuring dilution of the cell membrane linker PKH67 or CS-1 and KG1a cells, respectively. Cellular Ca²⁺ was determined by flow cytometry using Fluo-3-AM. The colony assays were performed using SCF, erythropoietin and interleukin-6 and were analysed after 12 days. **Results.** The mitochondria depolarising agents NS1619 (25 and 50 μM), diazoxide (100 and 200 μM) and As2O3 (1 μM) substantially inhibited the growth of CS-1 and KG1a cells. The most pronounced growth inhibition was induced by 200 μM diazoxide (1.46 population doublings after 3 days versus 1.96 for the control and 1.77 for 25 μM NS1619 in KG1 cells). The blocker of the mitoKATP channel 5-HD (250 and 500 μM) stimulated the growth of both cell lines. CsA (500 $\mu\text{g/L}$) did not alter the proliferation of KG1a cells. Addition of 100 ng/mL SCF stimulated the growth of native CS-1 cells. Ion channel modifier-induced stimulation or inhibition of the proliferation was not modified by co-incubation with SCF. NS1619, diazoxide and 5-HD showed no effect on the differentiation of peripheral blood CD34+ progenitors in the CFU-assay. It has previously been shown that diazoxide inhibits the proliferation of Jurkat cells by an inhibition of store operated Ca²⁺-entry. Consistent with that data we found a

decreased Ca²⁺ entry into KG1a cells after incubation with 200 μM diazoxide. **Conclusions.** The mitochondrial Ca²⁺-regulation strongly influences the growth of CD34+ cells. We have previously shown that 200 μM diazoxide and 25 μM NS1619 induced comparable mitochondrial depolarisations but they differ substantially in their induction of growth inhibition. This suggests a differential role of mitoKATP and mitoBK channels in the growth control of myeloid cells. These intracellular ion channels are interesting targets for the growth-modulation of CD34+ cells.

0564

THE PRESENCE OF CCR5DELTA32 DELETION MUTATION ASSOCIATES WITH A LOWER INCIDENCE OF AGVHD WHILE ENHANCED CCR5 EXPRESSION INCREASES THE RISK OF EBV INFECTION

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Background. CCR5 is a receptor for beta-chemokines: RANTES (CCL5) and macrophage-inflammatory protein (MIP)-1 α and MIP-1beta. The recent reports have suggested a potential role of CCR5 in perpetuation of some viral infections. Among the others, CCR5 acts as a co-receptor for HIV entry. It has been also documented that the CCR5 deletion mutation (CCR5Delta32) results in a nonfunctional chemokine receptor. The recent literature data have suggested a potential role of chemokine receptor CCR5 in a mouse model of graft-versus-host disease (GVHD). In this model, the aGVHD was prevented when recruitment of donor T cells into gut Peyer's patches was interrupted by disrupting the gene encoding CCR5. **Aims.** The present study aimed to analyse if there is any association between the polymorphism of CCR5 encoding gene and aGVHD in the recipients of allogeneic haematopoietic stem cell transplants (HSCT). In addition, the level of CCR5 gene expression was related with EBV load. **Methods.** The 32-nucleotide deletion within the CCR5 encoding gene (CCR5Delta32 polymorphism) was analysed in 73 recipients of allogeneic sibling HSCT. In addition, at the same time points employing real-time PCR technique, the viral load and the numbers of mRNA CCR5 copies were assessed in 23 patients suffering from haematological disorders. **Results.** Patients carrying the CCR5Delta32 allele (associated with defective CCR5 expression) had less frequently aGVHD as compared to patients lacking this complication (2/11 vs 30/62, p=0.06). The presence of the CCR5D32 allele was found only in one out of 23 patients for whom EBV copies were studied. This patient lacked EBV infection although he presented with over 298 000 copies of CCR5 mRNA/100000 cells of peripheral blood. The higher numbers of EBV copies were detected in patients with enhanced CCR5 gene expression. Among 15 patients with EBV infection, 14 had more than 50 000 copies of CCR5 mRNA/100 000 cells as compared to 3 out of 8 patients lacking EBV infection (having less than 10 of EBV DNA copies/100 000 cells) (0.93 vs 0.38, p=0.009). No association was observed between the polymorphism and expression of the CCR5 gene. **Summary / Conclusions.** The presence of a defective CCR5 lowers susceptibility to aGVHD while increased CCR5 gene expression associates with elevation of EBV copies. These results might suggest that the expression of functional CCR5 plays a role in initiation/perpetuation of aGVHD by promotion of viral reactivation. This work was supported by ALLOSTEM grant.

0565**RGS16 IS A NEGATIVE REGULATOR OF SDF1-CXCR4 SIGNALING IN MEGAKARYOCYTES**

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Regulators of G-protein signaling (RGS) constitute a family of proteins involved in the negative regulation of signaling through heterotrimeric G protein-coupled receptors (GPCR). Several RGS proteins have been implicated in the down-regulation of chemokine signaling in hematopoietic cells. The chemokine SDF-1 activates migration of hematopoietic progenitor cells but fails to activate mature megakaryocytes in spite of high levels of CXCR4 receptor expression in these cells. This prompted us to analyze RGS expression and function during megakaryocyte differentiation. We found that RGS16 and RGS18 mRNA expression was upregulated during this process. Overexpressing RGS16 mRNA in the megakaryocytic MO7e cell line inhibited SDF-1-induced migration and MAPK activation, whereas RGS18 overexpression had no effect on CXCR4 signaling. Knocking-down RGS16 mRNA via lentiviral-mediated RNA interference increased CXCR4 signalling in MO7e cells and in primary megakaryocytes. Thus, our data reveal that RGS16 is a negative regulator of CXCR4 signaling in megakaryocytes. We postulate that RGS16 regulation is a mechanism that controls megakaryocyte maturation by regulating signals from the microenvironment.

0566**G-CSF STIMULATION INCREASES PROTEASOME ACTIVITY IN MYELOID CELLS**

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Background. The proteasome is a multi-catalytic enzyme complex responsible for the degradation of approximately 80% of intracellular proteins. Proteasome activity is known to be increased in malignant cells. We have devised a method for the extraction and measurement of proteasome activity in cell lines and primary human cells and found high activity in individuals undergoing peripheral blood stem cell harvesting (PBSC) for autologous transplantation. **AIM:** The aim of this study was to determine whether G-CSF stimulation was responsible for the increased proteasome activity in mobilised stem cells. **Methods.** Primary cells were obtained from allogeneic bone marrow donors, rib samples excised at thoracotomy and PBSC from autologous donors. The murine FDCP-MIX myeloid cell line was used as a model of normal myelopoiesis. Proteasome was extracted from primary cells and cell lines in an ATP/DTT lysis buffer. The individual proteolytic activities of the proteasome were measured on a cytofluor by rate of turnover of specific fluorogenic substrates: Succ-Leu-Leu-Val-Tyr-AMC for chymotrypsin-like activity (CT-L); Z-Ala-Ala-Arg-AMC for trypsin-like (T-L) and Z-Leu-Leu-Glu-AMC for postglutamyl peptide hydrolase (PGPH). Reaction rates were expressed as arbitrary fluorescence units per minute (AFU/min). To examine the effect of G-CSF on proteasome activity *in vitro* two allogeneic bone marrow samples and FDCP-MIX cells (1x10⁵/mL) were incubated with 100 ng/mL Filgrastim (Amgen Ltd, Cambridge, UK) before extracting and measuring proteasome activity as before. **Results.** Proteasome activity was similar in both rib bone marrow (CT-L median 87 AFU/min, range 58-171 AFU/min; T-L 289, 114-365; PGPH 354, 130-641; n=7) and marrow from allogeneic donors (CT-L median 57 AFU/min, range 25-192 AFU/min, T-L 209, 89-884; PGPH 218, 65-875; n=4). In contrast all three proteolytic activities were higher in G-CSF mobilised cells (CT-L median 188 AFU/min, range 100-711 AFU/min; T-L

796, 290-1634, PGPH 558, 252-2482; n=7). FDCP-MIX cells showed a significant increase in proteasome activity after 24h stimulation with G-CSF *in vitro* (CT-L 138±40%, T-L 198±74% and PGPH 167±51%, n=3). In allogeneic donor 1, cells were incubated with G-CSF for 24 hours and no increase was seen compared to control cells grown without G-CSF. In donor 2, cells were incubated for 72 hours with G-CSF to mimic the conditions used to mobilise stem cells; proteasome activity was significantly increased following G-CSF treatment (CT-L 124%, T-L 150% and PGPH 184%). **Conclusions.** G-CSF stimulation increases proteasome activity in myeloid cells. Proteasome inhibitors have already entered clinical use and further studies will be required to investigate the implications of these findings on such treatment.

0567**ANTI-CD44 MONOCLONAL ANTIBODY INDUCES DIFFERENTIATION OF THP-1 LEUKEMIA CELLS THROUGH ACTIVATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE AND PHOSPHATIDYLINOSITOL 3-KINASE/AKT/PKB SIGNALING PATHWAY**

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Ligation of CD44 with anti-CD44 monoclonal antibody A3D8 induces terminal differentiation of human leukemia cells. However, the underlying molecular mechanisms remain largely unknown. In this study, we examined the importance of Raf-1, MEK/ERK, p38 MAPK, and phosphatidylinositol 3-kinase (PI3-K)/Akt/PKB pathway in A3D8-induced monocytic differentiation of THP-1 leukemia cells. THP-1 cells displayed cytologic changes typical of mature monocytes and an increased expression of monocyte-specific antigen CD14 (49%±4%) and of myeloid-specific antigen CD11b (68%±6%) after 3 days of A3D8 treatment. The level of CD15 was also increased. The increase in the expression of these differentiation antigens was dose and time-dependent. We found that phospho-Raf-1 (Ser338), phospho-MEK^{1/2} (Ser217/221), and phospho-ERK^{1/2} (Thr202/Tyr204) were activated dramatically shortly after the cells were treated with A3D8 and lasted for 72h. Akt/PKB (Ser473) phosphorylation was also observed shortly after the cells were exposed to A3D8 and sustained thereafter. In contrast, the phosphorylation of p38 (Thr180/Tyr182) MAPK and JNK were not increased by CD44 ligation. Preincubation of leukemia cells with 1-10mM of MEK1 inhibitor PD98059 or U0126 for 30 min completely blocked the A3D8-induced activation of MEK^{1/2} and ERK^{1/2} and potently inhibited the THP-1 differentiation. A p38 MAPK inhibitor SB203580 induced synergic effect of the CD44-induced terminal differentiation. Treatment of leukemia cells with PI3-K inhibitor LY294002 or a specific Akt/PKB inhibitor resulted in a near complete inhibition of A3D8-induced terminal differentiation and A3D8-induced activation of Raf-1, MEK^{1/2} and ERK^{1/2}. By contrast, pretreatment of cells with PD98059 and U0126 did not inhibit the A3D8-induced Akt/PKB or Raf-1 activation, suggesting that PI3-K/Akt/PKB is the upstream of Raf/MEK/ERK pathway in A3D8-induced terminal differentiation of THP-1 leukemia cells. Taken together, our findings demonstrated that the activation and cross-talk of PI3-K/Akt/PKB and Raf-1/MEK/ERK signaling pathway play critical roles during the CD44 ligation-induced differentiation of THP-1 leukemia cells.

Dendritic cells and immunotherapy

0568

PHASE I CLINICAL TRIAL OF CT-011, A HUMAN MONOCLONAL ANTIBODY DIRECTED AGAINST A B7 FAMILY-ASSOCIATED PROTEIN, IN PATIENTS WITH ADVANCED HEMATOLOGICAL MALIGNANCIES

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Background. CT-011, a humanized monoclonal antibody that is directed against a B7 family-associated protein, was previously shown to efficiently elicit anti-cancer immune response against a wide range of murine and human tumors (Hardy et al PNAS 94:5756-5760, 1997). Its interaction with both NK cells and CD4+CD45+RO T cells culminates in NK- and T- cell dependent immune responses. CT-011 target-antigen operates through the PI3K pathway to extend the survival of effector/memory T cells and to promote the generation of tumor-specific memory T cells. **Aims.** The purpose of this first in human clinical study was to evaluate the safety and determine the maximal tolerated dose (MTD) of CT-011 single intravenous administration in pts with advanced stage hematological malignancies. **Methods.** We studied the safety profile of CT-011 in 15 pts with advanced hematological malignancies. All pts failed several lines of conventional chemotherapy and radiotherapy as well as allo (n=6) or auto SCT (n=3). Ten of the pts were females and five were males with a median age of 55 (20-77, range) years. Seven had AML, four NHL, three CLL and one HD. CT-011 was given in a single 5h IV infusion in escalating doses starting at 0.2 mg/kg up to 6.0 mg/kg (3 pts at each dose level). One pt at the lowest dose level was re-enrolled five months after the first administration at a higher dose level for a total of 16 administered treatments. **Results.** CT-011 was safe and well tolerated with no treatment-related toxicities. Common adverse events included minimal allergic reactions and low grade fever. No single dose MTD was found in this study. One AML pt with resistant leukemia that was platelet-dependent with $plt < 10 \times 10^9/L$ is currently 7 months post first CT-011 infusion in partial response and is platelet transfusion-independent. Two additional pts (NHL-1, HD-1) remain with stable disease with no disease progression for more than 6 months. Five other pts are alive with active disease with a median follow up of 3 (1-6) months, while seven pts died from their advanced resistant disease. Accrual to this study as well as pts follow up continues. **Conclusions.** A single administration of CT-011 is safe and well tolerated in pts with advanced hematological malignancies. The observed anti-tumor activity may be related to CT-011 interaction with the B7 receptor family-associated protein resulting in enhancement of tumor-specific immune response. Future studies will evaluate the combination of donor lymphocyte infusion and CT-011 for pts with hematological malignancies having minimal residual disease after stem cell transplantation.

0569

INTRACELLULAR EXPRESSION OF ANGIOTENSIN-CONVERTING ENZYME (ACE, CD143) IN LEUKEMIC DENDRITIC CELLS (DC) IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background. DC play a key role in the induction of the adoptive immune response because they are efficient antigen presentation and costimulation of naïve lymphocytes. Leukemic DC (LDC) and DC from healthy donors are characterized by high levels of costimulatory molecules expression. The differ-

ence between LDC and DC from healthy donors existed in the expression of surface ACE, as it is reported to be a high on the monocyte-derived DC in contrast to DC derived from leukemic blasts. **Aim.** We supposed that the absence of surface ACE expression reflects the alteration in the differentiation of LDC resulting in the block of transportation of ACE on the cell's membrane. In order to confirm this proposal we investigated intracellular production of ACE. **Methods.** Blood samples were collected from 8 AML patients at diagnosis before induction chemotherapy. Mononuclear cells were isolated using gradient centrifugation with Ficoll-Paque, leukemic cells has been cultured in the presence of 180 ng/mL calcium ionophore for 4 days. Two healthy donors constituted the control group (non-adherent cells were removed before culturing). DC were stained for surface and intracellular ACE and detected by flow cytometry. Were used two clones of antibodies (1D8 for nonactivated and 9B9 for activated forms of ACE). Statistical data were computed by program 'Statistics for Windows 5.5'. **Results.** DC derived from AML blasts have shown large amount of intracellular ACE: $70 \pm 12\%$ (clone 1D8), $79 \pm 12\%$ (clone 9B9). Surface ACE was detected in $2 \pm 2\%$ (clone 9B9) and $0.8 \pm 0.5\%$ (clone 1D8) cells. In contrast, DC derived from normal monocytes had intracellular ACE in $0.8 \pm 0.1\%$ (clone 1D8), $2.3 \pm 0.6\%$ (clone 9B9) cells, surface ACE positive cells were $60.6 \pm 9.2\%$ (clone 9B9) and $5.6 \pm 0.8\%$ (clone 1D8). The percentages of intracellular ACE producing LDC significantly differed from normal DC ($p=0,0001$ and $p<0,0001$ for clones 1D8 and 9B9 respectively). The percentages of surface ACE positive normal DC cells exceeded such counts in AML patients ($p<0,0001$ for both clones) **Conclusions.** The data confirm the block transport of ACE on the membrane of LDC and alter of their differentiation.

0570

EXPRESSION OF RHAMM/CD168 AND OTHER TUMOR ASSOCIATED ANTIGENS IN PATIENTS WITH B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA

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Purpose. Antigen targeted immunotherapy might be a novel option for the treatment of B-cell chronic lymphocytic leukemia (B-CLL). **Methods.** To define potential target antigens for immunotherapies in B-CLL, we screened the mRNA expression of eleven tumor associated antigens (TAAs) from the literature (fibromodulin, survivin, OFA-iLRP, BAGE, G250, MAGE1, PRAME, proteinase, Syntaxin, hTERT and WT-1), as well as six TAAs defined earlier by SEREX analysis of patients with myeloid leukemias by our group (PINCH, HSJ2, MAZ, MPP11, RHAMM/CD168) or by others (NY-Ren60). Peripheral blood mononuclear cells (PBMCs) from 30 B-CLL patients and 20 healthy volunteers (HV) were examined by conventional and quantitative RT-PCR. The ZAP-70 status was determined by FACS analysis. Mixed lymphocyte peptide cultures and ELISPOT assays were performed for RHAMM/CD168 R3 peptide. **Results.** mRNA of RHAMM/CD168, fibromodulin, syntaxin and NY-Ren60 was expressed in 55-90%, mRNA of HSJ2, MAZ and OFA-iLRP in 90-100% of the patients. No expression of WT-1, h-TERT, BAGE, G250, MAGE1 and survivin was observed. Low (2-20%) expression frequencies of MPP11, PINCH, PRAME and proteinase were detected. Only RHAMM/CD168, fibromodulin, PRAME and MPP11 showed expression in B-CLL patients but not in HV, all others antigens showed expression frequencies of 28-100% in HV. RHAMM mRNA expression was significantly higher in B-CLL patients than in HV. No significant difference of RHAMM expression between ZAP-70 positive and negative patients was observed. Specific CD8+ T cell responses against RHAMM/CD168 in B-CLL patients could be detected *in vitro*. **Conclusions.** RHAMM/CD168 might be a possible target for future immunotherapies in both ZAP-70 (+) and ZAP-70 (-) B-CLL patients.

0571**KIR LIGAND MISMATCHED, ALLOREACTIVE NATURAL KILLER CELLS LYSE PRIMARY SOLID TUMOR CELLS**R.F. Re¹, V. Vecchio¹, A. Velardi², L.R. Loredana Ruggieri², C. Staudacher¹, C. Dogliani¹, M. Bregni¹¹Scientific Institute San Raffaele, MILAN, Italy; ²University School of Medicine, PERUGIA, Italy

Alloreactive NK cells recognize and kill leukemia cells from patients who undergo haploidentical bone marrow transplantation. In this study we addressed the question of whether solid tumors are susceptible to NK cell-mediated lysis. We first analyzed the ability of alloreactive NK cells to specifically kill fresh tumor cells obtained from surgical resections, thus likely miming the in-vivo environment. Tumor cells obtained from 9 biopsies of different histotypes (5 gastric and colon cancers and 4 renal cancers) were tested for susceptibility to NK cell-mediated cytotoxic effect. Tumor cells expressing one KIR ligand allele, thus allowing the recognition of the other two alleles by NK cells, showed >70% lysis, while lysis >50% was observed with tumor cells expressing two KIR- ligand alleles resulting in only one KIR- ligand mismatched pair, at 40:1 effector-target ratio. More interestingly NK cells who recognize specific HLA-C allotypes were able to kill both tumor cells and EBV-LCL target cells mismatched for HLA-C KIR ligand (>35% lysis at 20:1 effector- target ratio), whereas no lysis of target cells with KIR ligand-matched alleles (<12% lysis at 20:1 effector- target ratio). We conclude that the NK-cell mediated anti-tumor effects might provide useful insights for designing new cell therapy approaches in chemotherapy-refractory tumors.

0572**IMMUNIZATION OF NON-HODGKIN LYMPHOMA PATIENTS WITH A NOVEL RECOMBINANT IDIOTYPE VACCINE: RESULTS FROM A PHASE I CLINICAL TRIAL**

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Immunization of patients with B-cell non-Hodgkin's lymphoma (NHL) against the individual immunoglobulin (idiotype) expressed by the malignant clone may induce tumor-specific immune responses. In an effort to ease manufacturing of individual idiotype vaccines, a production strategy based on anchored RT-PCR cloning of the variable segments of the heavy and light diotype genes from lymphoma biopsies was developed. Identified clonal VH and VL segments are inserted into an inducible dicistronic expression vector. Recombinant idiotype Fab fragment is expressed in *E. coli* and purified from the bacterial periplasma by affinity chromatography. 18 B-NHL patients who had relapsed after previous anthracyclin- or fludarabine-based chemotherapy received repeated intradermal vaccinations with 0.5 - 1.65 mg Fab fragment mixed with a lipid-based adjuvant in increasing intervals of 2 - 4 weeks. The entire production process fulfills GMP criteria. 150 µg GM-CSF was injected subcutaneously at the vaccine location immediately after each immunization. Only 7/18 patients had normal CD4+ T cell counts prior to vaccination, and all 10 patients who received 2 concomitant immunizations with a recombinant Hepatitis B vaccine during the course of the idiotype vaccination failed to develop anti-HbS antibodies. As assessed by ELISA for IgM, IgG, and opposite light chain antibodies, 7/17 evaluable patients developed anti-Fab antibodies. In 10/17 evaluated patients, anti-Fab T cell responses were induced by the vaccinations as detected by ELISPOT quantitation of IFN γ -secreting cells upon *in vitro* stimulation with Fab-presenting autologous dendritic cells. Complete regression of pre-vaccination lymphadenopathy occurred in 1 patient (diffuse large cell lymphoma). 10/17 evaluable patients and 10/14 non-secreting B-NHL had progression-free survival (PFS) of at least 4 months after the start of immunizations. 3 of these patients have ongoing

PFS at a median follow-up of 2,2 years. These results demonstrate the feasibility, tolerability, and potent immunogenicity of the tested idiotype vaccine formulation in this group of mostly immunosuppressed NHL patients.

0573**A NOVEL VACCINE FOR HEMATOLOGICAL MALIGNANCIES USING DENDRITIC CELLS**R. Eshel, O. Vainas, M. Shpringer, R. Ben-Yosef, E. Naparstek
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Allogeneic stem cell transplantation has a significant contribution to the improved outcome of patients with hematological malignancies. Nevertheless, a significant proportion of patients suffer from recurrence of the disease, which ultimately leads to death. Therefore, novel therapeutic strategies have been identified to improve the outcome of these patients, such as cellular-based immunotherapies. Therapeutic approaches based on immunological responses are incorporated within routine clinical protocols of hematological malignancies. However, in most of the hematological malignancies, the tumor specific antigens are unknown, and the cellular or non-antigen specific immunotherapy is based on allogeneic bone marrow transplantation. In the occurrence of relapses patients are administered with allogeneic, donor derived T lymphocytes (DLI), aiming to induce a clinically significant graft-versus-tumor responses. The aim of our study was to induce a potent anti-leukemia cytotoxic T lymphocyte (CTL) response, utilizing dendritic-leukemia cell hybrids, to treat leukemic relapse in patients after allogeneic stem cell transplantation. Such fusion cell vaccine has the advantage of presenting known and unidentified leukemic antigens in the context of co-stimulatory signals. For this purpose, the human myeloid leukemia cells K562, were fused with human dendritic cells (DC), as an alternative approach to induce immune responses directed against known and undefined leukemic antigens. Fusion of the leukemic cells with the DCs resulted in the generation of heterokaryons that dually expressed the putative leukemia-associated antigens and MHC class II. The fusion cells stimulated autologous T cell proliferation that demonstrated a significant CTL response, resulting in 40% specific lysis of the target K562 cells, as measured by the AlamarBlue cytotoxicity assay. Our preliminary results clearly demonstrate that the hybrid vaccination approach in AML is technically feasible. Such specific anti-leukemic donor CTLs may be utilized to maximize the anti-tumor effects of DLI in patients relapsing after allogeneic transplantation.

0574**PHENOTYPE AND FUNCTIONALITY OF MATURE DENDRITIC CELLS PRODUCED FROM PBMC OF HEALTHY DONORS**I. Bolonaki¹, I. Nikoloudi², E. Lydaki², H. Koutala³, K. Foundouli², D. Mavroudis⁴, K. Kosmatopoulos⁵¹University Hospital of Heraklion Crete, HERAKLION, CRETE, Greece; ²Transfusion Med, University Hospital, HERAKLION, Greece; ³Flow Cytometry, Medical School, HERAKLION, CRETE, Greece; ⁴Medical Oncology, University Hospital, HERAKLION, CRETE, Greece; ⁵Vaxon Biotech, Genopole, EVRY, France

Introduction. Dendritic Cells (DC) are antigen-presenting cells that are involved in the induction of primary immune responses. The aim of the study was to define the phenotype and evaluate the functionality of mature human Dendritic cells (DC). Materials and methods. Immature DC (iDC) were produced by stimulating adherent peripheral blood lymphocytes (PBMC) from 30 healthy donors with GM-CSF and IL4 for 7 days. The yield of iDC production was similar for all donors and ranged from 15 to 100 X 10⁶ cells. Maturation of these iDC was induced by overnight stimulation with CD40-L. Results. iDC expressed high levels of CD86, HLA-I but low or undetectable levels of

HLA-II, CD40 and CD80. In contrast, m DC further up-regulated HLA-I and CD86 and expressed high amounts of HLA-II, CD40 and CD80. m DC were tested for their capacity to stimulate an allogeneic T-cell response. m DC strongly stimulated proliferation of allogeneic T-cells in a dose-dependent manner. T-cell proliferation was observed even at a T/DC ratio of 1/1000. Moreover, m DC activated naïve antigen specific T-cells. In fact, m DC from HLA-A0201 donors loaded with the HLA-A0201 bound MELAN/A peptide (ELEGILTV) induced specific cytotoxic T lymphocytes after two rounds of *in vitro* stimulation as assessed by tetramer staining, intracellular IFN staining and cytotoxic assay. **Conclusions.** The m DC may be used in the induction of specific cytotoxic T lymphocytes with different cancer peptides and could proceed, in this way, in anti-cancer therapy.

0575

KL-03: A NOVEL EBV+ B-CELL LINE WITH DENDRITIC FEATURES

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Cell lines are valuable research tools for the biology of normal and leukemic dendritic cells. We describe KL-03, a novel Epstein Barr (EBV) positive B-cell line with dendritic features, which grew in liquid culture from the bone marrow cells of a patient suffering from acute myelomonocytic leukemia (AML-M4 FAB subtype) with an hybrid B-immunophenotype by flow cytometry (MPO+, CD 34+, 13+, 33+, 68+, 14+, 123+, Tdt+, 79a+). The patient had a normal karyotype and he was EBV IgG positive. KL-03 cells arose spontaneously in liquid culture of Ficoll-separated blasts (95% purity) without any added cytokines. The cell line has by now reached 22 months in continuous culture with a doubling time of 36 hours. The cells are dendritic by morphology, with long hair-like projections. By immunophenotype (both by flow cytometry and immunochemistry) they share B-cell markers (19+, 20+, Tdt+, 79a+ with a k/L ratio 10/1), and dendritic ones (CD80+, 83+, 86+, 123+, DR++, 40+, Fascin+). No T-, NK- or other lineage-specific markers were found. The KL-03 cell line was found EBV positive by RT-PCR. Cytogenetic evaluation showed a normal karyotype on two occasions at six months intervals. Assays for cytokine secretion at the supernatants by ELISA showed increased amounts of TNF- α . KL-03 cells also stimulated allogeneic T-cells at the mixed lymphocyte (MLR) reaction at a dilution 1:100. Finally, KL-03 cells form large colonies in semisolid cultures in methylcellulose, independently of the cytokines tested (GM-CSF, SCF, IL-3, Epo). In conclusion, we describe a novel EBV+ B-cell line with morphology and function of dendritic cells. To our knowledge, only a few cell lines with similar characteristics have been reported (HBM-Noda, KM-H2, 2001 and BDCM, 2002). Although the leukemic origin of this cell line, due to the lack of a genetic marker, may be questioned, it nevertheless provides an immortal cellular population with overlapping B-lymphoid and dendritic features, similar but not identical to the plasmacytoid dendritic cells.

0576

IMPACT OF PLASMACYTOID DENDRITIC CELL (PDC) RECOVERY ON OUTCOME AFTER REDUCED-INTENSITY CONDITIONING (RIC) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT)

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Among DC subsets, the reconstitution of the natural type I interferon-producing PDCs has been proposed to play a major

role in establishing immune competence. Therefore, we investigated the impact of circulating PDCs measured at the 3rd month after RIC-allo-SCT, in 54 patients with hematological and non-hematological malignancies who received a RIC-allo-SCT from an HLA-identical sibling, in order to determine whether this could provide an indicator for long term outcome. The median absolute count of PDCs measured at 3 months was 0.725/ μ L (range, 0-23.2). In a multiple logistic regression analysis including all relevant parameters (demographic and graft characteristics, RIC regimens, CMV infections, and acute GVHD), only the absence of grade II-IV acute GVHD was associated with an improved PDC recovery at 3 months ($p=0.003$; OR=6.4; 95%CI, 1.9-22). Being the major type I IFN-secreting cells, we also investigated whether PDCs recovered after allo-SCT are functional in response to viral stimulation. Patients experiencing grade 0-I aGVHD could secrete significantly higher amounts of IFN- α as compared to patients with grade II-IV aGVHD (mean, 91 vs. 0 pg/mL respectively; $p=0.002$), likely highlighting the deleterious impact of corticosteroids therapy on PDC function. The CD34+ stem cell dose and other lymphoid subsets infused with the allograft did not affect PDC recovery. Though PDC count could not predict death from progression or relapse, patients with a 'high' PDC recovery profile had an improved overall survival (OS; $p=0.03$), in contrast to patients with a 'low' PDC recovery profile who had an increased incidence of late transplant-related mortality (GVHD, infections) ($p=0.03$). In addition, the overall incidence of late infections (viral, fungal and bacterial) was significantly higher in the 'low' PDC recovery group as compared to the 'high' PDC recovery group (59% vs. 19%; $p=0.002$), illustrating the importance of PDCs in anti-infectious immune responses. In a multivariate analysis, only a 'high' PDC count was significantly predictive of a decreased risk of death ($p=0.04$; RR=0.34; 95%CI, 0.12-0.96). The role of rare immune effector cells would tend to be more evident in truly RIC and less toxic regimens. In this study, we could show that monitoring of PDCs may be useful for patients' management (closer surveillance, infection prophylaxis...), and may have a significant impact on the probability of a favorable outcome in the context of RIC-allo-SCT.

0577

TRANSIENT IMMUNE RESPONSES TO AN hCD40L/hIL-2 AUTOLOGOUS CELL VACCINE IN PATIENTS WITH B-CHRONIC LYMPHOBLASTIC LEUKEMIA ARE ASSOCIATED WITH THE PRESENCE OF REGULATORY T CELLS

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Purpose. Human CD40 ligand (hCD40L) activates the transformed B cells in chronic lymphocytic leukemia (B-CLL) and enhances their capacity to present tumor antigens. Human Interleukin-2 (hIL-2) further potentiates the immunogenicity of hCD40L in pre-clinical murine models. **Experimental design.** We prepared autologous B-CLL cells that expressed both hCD40L and hIL-2. Nine patients were enrolled in a Phase 1 dose-escalating study and received three to eight subcutaneous vaccinations. **Results.** Ninety percent of autologous manipulated B-CLL cells were CD40L-positive compared with <1% before treatment. The mean secretion IL-2 was 1822 (range: 174-3604) pg/mL per 1×10^6 cells. Vaccinations were administered without evidence of significant local or systemic toxicity. A B-CLL-specific T-cell response was detected in seven patients. The mean frequencies of IFN- γ , granzyme-B and IL-5 spot-forming cells among 1×10^5 T cells were 1/1230, 1/1450, 1/4500, respectively, representing a 43 to 164-fold rise over the frequency before vaccine administration ($p < 0.005$ for all molecules). Three patients produced leukemia-specific immunoglobulins. Nonetheless, the anti-tumor immune responses *in vivo* were transient only and no clinical benefit was observed. High levels of circulating CD4+/CD25+/LAG-3+/FoxP-3+ immunoregulatory T cells (T-reg) were present before, during and after treatment and *in vitro* removal of these cells increased

the antileukemic T-cell reactivity. **Conclusions.** These results suggest that immune responses to B-CLL can be obtained with hCD40L/hIL-2-expressing vaccines, but that removal of co-existing T-reg cells *in vivo* may be necessary if clinically effective responses are to be induced and sustained.

0578

DIRECT ISOLATION OF HA-1 SPECIFIC CD8+ T CELLS FROM PERIPHERAL BLOOD FROM HA-1 NEGATIVE DONORS FOR ADOPTIVE IMMUNOTHERAPY

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Graft versus leukemia (GVL) reactivity after allogeneic stem cell transplantation can be mediated by donor T-cells recognizing minor histocompatibility antigens (mHags) on the recipient hematopoietic cells, as shown by the *in vivo* kinetics of mHag-specific donor T-cells in patients responding to donor lymphocyte infusion (DLI). Patients not responding to DLI may lack the *in vivo* environment for an efficient induction of mHag-specific T-cell responses. Adoptive transfer of mHag specific donor T-cells to patients with relapsed leukemia may induce GVL reactivity without graft versus host disease. However, the generation of primary HA-1-specific T cell responses from unprimed donor cells under good manufacturing practice (GMP) conditions has been successful in only a minority of cases. Furthermore, HA-1 specific T cells were only obtained after a long *in vitro* culture period and repeated restimulation, since frequencies of HA-1 specific T cells were very low (<0,02%) in normal donors. The aim of this study was to isolate and enrich HA-1 specific T cells from HA-1 negative donors at earlier time points using HA-1 specific tetramers to shorten the *in vitro* culture period of the T cells. First, peripheral blood lymphocytes (PBL) were depleted of CD4, CD14 and CD19. Then, HA-1 specific T cells were isolated following staining with PE-labeled HA-1 tetramer using FACS sorting of the PE-positive cells. Isolated cells were cultured at concentrations of 50 cells/well and expanded in the presence of allogeneic feeders, EBV-LCL loaded with HA-1 peptide, PHA and 100 IU/mL IL-2. Cells were isolated directly at day 0 without preceding stimulation, or after one week of specific stimulation with dendritic cells loaded with HA-1 peptide, or following non-specific stimulation with anti-CD3/anti-CD28 beads. After a culture period of 1–2 weeks after isolation T cells populations were analyzed for HA-1 specificity by tetramer staining. HA-1 specific T cells could be determined in 1/4 donors after specific stimulation with HA-1 peptide loaded DC. After stimulation with anti-CD3/anti-CD28 HA-1 specific T cells could be determined in 4/4 donors and 27-50% of the wells were positive. When cells were directly isolated at day 0, in 2/3 donors HA-1 specific T cells could be detected in 20 and 100% of the wells. No HA-1 specific T cells could be determined in the PE-negative fractions. These studies shows that despite very low frequencies it is possible to strongly enrich for HA-1 specific CD8+ T cells from PBL from HA-1 negative donors irrespective of prior *in vitro* stimulation. Enrichment for HA-1 specific T cells without prior *in vitro* stimulation will shorten the *in vitro* culture period of the T cells and enhance their *in vivo* potential.

0579

EX-VIVO GENERATION OF ANTI-LEUKEMIA CTL LINES: IL-15 ENHANCES THE EXPANSION OF CTLs AND INCREASES THE NUMBER OF T CENTRAL MEMORY CELLS

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The success of adoptive transfer of ex vivo generated anti-leukemia CTL lines for prevention/treatment of leukemia relapse mainly depends on the number of infused cells and on

their long-term survival and proliferating capacity, as well as on maintenance of leukemia-specific immune response *in vivo*. Previously, we demonstrated that a large number of donor-derived anti-leukemia CTL lines directed against different types of blast cells can be generated by means of 3-4 rounds of leukemia-specific stimulations using tumour cells and 1 round of antigen-independent expansion, supported by IL-2 (25 U/mL) during stimulations. Recently, it has been described that IL-15 enhances the proliferation capacity of memory T cell subsets. In this study, we evaluated i) the distribution of memory T cells subsets in anti-leukemia CTL lines generated with IL-2; ii) the effect of IL-15 (10 ng/mL), used in place of IL-2, on generation of memory T-cells and on the capacity of anti-leukemia CTL lines to proliferate *in vitro* and to maintain leukemia-directed cytotoxic activity. So far, we have studied 3 anti-leukemia CTL lines, one derived from an HLA-identical sibling and 2 from an HLA-partially matched relative. Leukemia-stimulation and antigen-independent expansion resulted in the generation of CTLs, with a predominant phenotypic CD3+CD8+ profile, able to display anti-leukemia cytotoxic activity, using either IL-2 or IL-15. However, cell recovery was 5 to 10 fold increased in IL-15-treated cultures. Also the cytotoxic activity was 15-20% higher in IL-15-treated cultures, without being associated with an increase in alloreactivity. Analysing the co-expression of CD45RA and CCR7, we found a higher number of T central memory (TCM) cells, defined as CD45RA-/CCR7+, in IL-15 stimulated CTLs (3,7%±1,5 vs. 0,4%±0,4 CD8+ cells, p<0,05). The number of T effector memory (TEM) cells displaying effector function was predominant in CTLs and no difference was observed between IL-15- or IL-2-treated cultures (CD45RA-/CCR7-: 30,7%±28,1 vs. 42,5%±14,5; CD45RA+/CCR7-: 42,1%±13,4 vs. 52,3%±9,2, respectively). Our results suggest that use of IL-15 in the expansion phases of anti-leukemia CTL lines enhances the proliferative rate of CTLs, without loss of specificity. Moreover, IL-15 increases the subset of TCM cells, which are potentially able to display long-term survival and capacity to proliferate and differentiate *in vivo* in the presence of limited amount of leukemia blasts.

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EX VIVO GENERATION AND CHARACTERISATION OF CYTOKINE INDUCED KILLER CELLS IN GMP CONDITIONS

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Background. Cytokine induced killer cells (CIK) are CD3+/CD56+ cells that can be expanded *in vitro* following stimulation of peripheral blood mononuclear cells with interferon- γ (IFN- γ) on day 0, anti-CD3 antibody (OKT3) on day 1, followed by expansion in interleukin-2 (rhIL-2) for 18-21 days. These cells have been shown to be cytotoxic against tumour cells with little *in vivo* toxicity. We are planning a phase I study with CIK cells in acute myelogenous leukaemia patients relapsed after allogeneic bone marrow transplantation. CIK cells from the peripheral blood of the donors will be generated *in vitro* in order to administer 1x10⁷ CD3+/CD56+ cells/kg/infusion for up to 3 infusions. **Aims.** In order to fulfill the cell therapy requirements by the Italian authorities, we have set up clinically feasible conditions to generate CIK cells *in vitro* under GMP rules. **Results.** CIK cells were expanded *in vitro* from a mean of 160 x10⁶ PBMC (range 20-525 x 10⁶) from 22 consecutive donors. CD3+/CD56+ cells were 4% of the total mononuclear cells on day 0 (range 1-9%) and reached a mean of 56% after 18-21 days of culture (range 13-82%). An average 328 fold expansion of CD3+/CD56+ cells was obtained (range 29-1300). Thus a mean of 980 x 10⁶ CIK cells could be obtained (range 150-2600 x 10⁶) after 18-21 days culture. In 18/22 donors, expanded CIK preparations satisfied the established criteria for lot release (at least 40% CD3+/CD56+ cells). They showed an average of 46% cytotoxic activity against the K562 erythroleukemia cell line at a 30:1 E:T ratio (range 20-100%), and 42% cytotoxicity against freshly isolated patients' leukemic cells (range 11-40%).

CIK cells could also be expanded from autologous PBMC in patients having still up to 80% of blasts cells in the periphery at day 0. These CIK cell populations at the end of culture had the same phenotype as CIK cells derived from normal donors and were free of leukemic cells, as determined by phenotypic analysis. Finally quality controls assays were performed on 6 consecutive CIK preparations with validated tests (viability, colony assays, measurement of contamination by aerobic and anaerobic bacteria and fungi, endotoxin and mycoplasma), confirming that the preparations were adequate (6/6) for *in vivo* use. **Conclusions.** An adequate number of CIK cells can be expanded *in vitro* in GMP conditions, starting from 200-300x10⁶ leukocytes obtained by leukapheresis. These cells satisfy all criteria of viability, functionality, phenotypic characteristics and sterility required for *in vivo* use in more than 80% of cases.

We thank the AIRC, the AIL-sezione Paolo Belli-Bergamo and MIUR (Project FIRB) for their financial support

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ID PROTEIN-KLH VACCINATION IN PATIENTS WITH MULTIPLE MYELOMA - PHASE II CLINICAL STUDY

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Objectives: Malignant cells in multiple myeloma (MM) produce a monoclonal immunoglobulin which is tumor-specific and can be used for the induction of specific T cells. The idiotype (Id) is expressed at the cell surface of malignant plasma cells and allow the recognition and targeting of these cells by Id-specific T lymphocytes. A phase II clinical study has started in our center, investigating the efficacy and toxicity of Id conjugated with keyhole limpet hemocyanin (KLH) given as a vaccine to patients with MM. The aim of this therapy was to induce specific immune response directed against the tumor cells. 12 patients with stable disease or with slow progression not requiring systemic therapy were immunized six times without interleukin-2 (IL-2) or with IL-2 for the last 3 vaccinations in the case of no response. **Methods.** The specific immune response was monitored by proliferation test of mononuclear cells in presence of antigen. The production of interferon γ from activated T lymphocytes was evaluated by elispot reader. Non-specific effect of Id vaccination was controlled by flow cytometry. The expression of following antigens in peripheral blood was evaluated: T lymphocytes (CD3, CD4, CD5, CD8), B lymphocytes (CD19, CD20, CD45), NK cells (CD16, CD56, CD3), dendritic cells (CD11c, CD123, HLA-DR, CD83), monocytes (CD14), activation markers (CD25, CD69) and others (CD28, CD45RA, CD45RO). **Results.** No significant toxicities were seen during vaccination. Three patients relapsed during study, one during vaccination (after 4th Id vaccination) and two during first year from the start (6th month and 9 month). One patient died without connection with vaccination due to underlying cardiac failure. Id-specific delayed type hypersensitivity skin tests were positive in 7 of 7 tested patients. The generation of Id-specific T-cells proliferative responses was documented in 2 patients. The production of interferon γ was positive in one patient from 12 patients before vaccination and another patient from 8 patients has a positive response after three months of vaccination. We did not find any changes in expression of activation markers. Relative number of lymphocytes, NK cells and memory B cells increased, number of T lymphocytes and dendritic cells decreased. No significant clinical response was observed. **Conclusions.** Id-protein is not strong immunogen therefore Id-KLH vaccine boosted by IL-2 can evoke weak immune response and can not evoke significant clinical response.

Supported by grant IGA CR 7475-3

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GENERATION OF B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (BCP-ALL) SPECIFIC CYTOTOXIC CYTOKINE INDUCED KILLER CELLS (CIK)

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Background. CIK cells (CD3/CD56 double positive cells) are a novel population of immune effector cells characterized by a non MHC-restricted cytotoxicity against a variety of tumoral targets including both solid and haematopoietic tumors. Nevertheless, it has been demonstrated that these cells are unable to kill BCP-ALL blasts. Recent findings have shown that the specificity of T lymphocytes can be redirected by inducing the expression of artificial 'chimeric' receptors, characterized by an extracellular domain specific for a target molecule highly expressed on the surface of cancer cells and by an intracellular signalling domain, which initiates a signalling cascade upon ligation of the receptor. To be effective *in vivo*, redirected effector cells should be also able to migrate into tumor site where they get in close contact and kill tumor cells. **Aims.** to generate and expand CIK cells with specific cytotoxic activity against BCP-ALL cells by transduction with an anti-CD19 chimeric receptor and to study their capability to migrate *in vitro* in response to CXCL12, a chemokine active in the recruitment of cells in the bone marrow. **Methods.** CIK cells were transduced by a spinoculation method. Four different vectors were tested with the aim of evaluating their functionality and specificity: the anti-CD19- \square the \square anti-CD19- \square -truncated, the anti-CD19-4-1BB (a crucial molecule for T cell activation)- \square and a control vector to evaluate GFP expression. Transduced CIK cells were then characterized with regard to their phenotypical characteristics (CD3/CD56 and chimeric receptor expression) and cytotoxic activity against primary BCP-ALL cells in a 51Cr-release assay. Moreover, cells were analyzed for CXCR4 expression by flow cytometry and for *in vitro* chemotactic response to its ligand (CXCL12). **Results.** percentage of CD3+/CD56+ was not altered by transduction. CIK cells were efficiently transduced with the retroviral vectors (average expression of GFP/chimeric receptor above 15% for all vectors tested; n=8). Furthermore, in 5/5 experiments, only CIK cells expressing anti-CD19- \square and anti-CD19-BB- \square receptors showed a strong cytotoxic activity against primary BCP-ALL blasts (average lysis=60%, range=40-73% and 63%, range=43-80% respectively). Moreover, both anti-CD19- \square and anti-CD19-BB- \square -transduced CIK cells, from 4/4 donors, expressed high levels of CXCR4 (mean value=83%, range=65-96% and mean value=76%, range=46-99%, respectively), and cells were also able to migrate in response to CXCL12 (migration index=2.24, range=1.3-2.7 and 2.80, range=2.3-3.3, respectively). **Conclusions.** these results show that anti-CD19 chimeric receptor expression on CIK cells confers them a powerful cytotoxic activity against BCP-ALL cells and that transduced cells are able to migrate in response to CXCL12, suggesting their potential capacity to specifically reach the bone marrow. Based on these preliminary findings we suggest that Chimeric Receptor-modified CIK cells may be an attractive strategy for BCP-ALL immunotherapy.

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IDENTIFICATION OF HLA-A*0201-PRESENTED T-CELL EPITOPES DERIVED FROM THE ONCOFETAL ANTIGEN-IMMATURE LAMININ RECEPTOR PROTEIN FOR BROADLY APPLICABLE VACCINE THERAPIES

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Background. The oncofetal antigen immature laminin receptor

(OFA/iLR) is a potential target molecule for immunotherapeutic studies in several tumor entities including hematologic malignancies. *Aims.* We sought to identify and characterize HLA-A*0201-presented T cell epitopes derived from the OFA/iLR protein. *Methods.* Utilizing the 'reverse immunology' approach, OFA/iLR peptide-specific cytotoxic T lymphocytes (CTL) obtained from leukemia patients (chronic lymphocytic leukemia, acute myeloid leukemia, multiple myeloma, n=60) and healthy individuals (n=10) were analysed in ELISPOT assay, FACS (tetramer staining) and cytotoxicity assays. *Results.* We demonstrate that both allogeneic HLA-A*0201-matched and autologous CTLs recognized and killed endogenously OFA/iLR-expressing tumor cell lines and primary malignant cells from patients with hematopoietic malignancies in an MHC-restricted fashion but spared non-malignant hematopoietic cells. Spontaneous OFA-iLR peptide-specific T cell reactivity was detectable in a significant proportion of leukemia patients. *Summary.* The identification of OFA-iLR-derived peptide epitopes provides a basis for vaccination strategies in patients with OFA/iLR-expressing malignancies.

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NF-KB ACTIVATION BLOCKADE SELECTIVELY INDUCES APOPTOSIS OF ACTIVATED / ALLOREACTIVE T LYMPHOCYTES

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Background. Nuclear Factor kB (NF-kB) plays a major role in activation and induction of immunogenic response of T lymphocytes. While its expression is significantly increased in activated T lymphocytes, resting T cells express low levels of NF-kB. This property may allow to selectively target alloreactive T cells after a mixed lymphocyte culture by the proteasome inhibitor bortezomib, which inhibits IκB degradation and, therefore, prevents NF-kB activation. This property may be important in the allogeneic transplant setting in the prophylaxis and treatment of GVHD. *Aims.* We studied the ability of the NF-kB inhibitor Bortezomib to block T cell activation, proliferation and survival and analyzed the specificity of these effects on alloreactive T cells as compared to resting T cells. *Methods.* T cells, obtained from healthy donors' buffy coats, were cultured either unstimulated (control group) or stimulated with PHA, aCD3/aCD28, allogeneic dendritic cells (APC) or in mixed lymphocyte culture (MLR). Bortezomib at 1nM to 1uM (1 log increments) was added to the cultures. Proliferation and survival of T cells were studied by the MTT assay. Apoptosis and cell death were analyzed by annexinV/7-AAD staining and the effect of the drug on the activation of T cells was assessed by evaluating CD25, CD69 and intracellular IFN expression by flow cytometry. NF-kB and I-κB expression was assessed by Western-Blot. *Results.* NF-kB expression was increased in culture conditions which induced a higher proliferation in T lymphocytes as evaluated by MTT assay. Interestingly, the proliferative blockade induced by Bortezomib correlated with the expression of NF-kB since it was significantly higher in culture conditions where the NF-kB expression was increased (PHA > APC > controls). When assessed by flow cytometry, CD25+ CD69+ cells were specifically targeted by the effect of the drug while it hardly affected proliferation/survival of resting T cells. Thus, the effect on proliferation was due to a selective induction of apoptosis among activated T cells. To further confirm this results we performed MLRs and after three days of culture we separated CD25+ and CD25- cells by Auto-Macs and incubated them with different concentrations of Bortezomib. After 48h we calculated the number of annexin/7-AAD negative events (non-apoptotic cells) after acquisition by FACScan: interestingly, the number of annexin/7-AAD negative events among CD25- lymphocytes only decreased at 1uM of Bortezomib while decreases > 50% were observed at 10-100 nM Bortezomib among CD25+ lymphocytes. The addition of Bortezomib not

only decreased the proliferation and viability of activated T lymphocytes but also the expression of activation markers and the levels of intracellular IFN which were significantly decreased among activated T cells cultured with Bortezomib at doses ranging from 10 to 100 nM. *Conclusions.* At concentrations reached in the clinical setting, Bortezomib inhibits proliferation and induces selective apoptosis of activated T lymphocytes while it barely affects unstimulated T cells. NF-kB blockade also hampers T cell activation and decreases Th1 response as assessed by IFN production in activated T lymphocytes. Thus NF-kB inhibitors could play a role in the management of GVHD.

Genomics and molecular targeting II

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ROLE OF CYP2D6, CYP1A1, CYP2E1, GSTT1 AND GSTM1 GENES IN THE SUSCEPTIBILITY TO ACUTE LEUKEMIAS

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Background. Acute leukemias (ALs) are heterogeneous diseases. Functional polymorphisms in the genes encoding detoxification enzymes cause inter-individual differences, which contribute to leukemia susceptibility. *Aims.* and *Methods.* We aimed to determine whether any association exists between xenobiotic-metabolizing enzymes and pediatric and adult acute leukemia patients (ALL and AML). Acute leukemia patients (ALL, n=155 and AML, n=94) and healthy controls (n=140) were examined by using PCR/PCR-RFLP method for their allele and genotype frequencies. This study presents analysis of loci encoding both phase I (CYP1A1, CYP2D6, CYP2E1) and phase II (GSTT1, GSTM1) xenobiotic-metabolizing enzymes in ALL and AML patients. *Results.* No association was observed between the GSTT1 gene deletion and patients (OR=0.8 95%CI=0.4-1.7 for AMLs and OR=0.9 95%CI=0.5-1.6 for ALLs). Patients with ALL and AML had a higher prevalence of the GSTM1 deletions compared to controls but only the difference among adult AML patients (OR=2.1 95%CI=1.0-4.2) was statistically significant. The CYP2D6*3 variant allele frequency was lower in the overall acute leukemia patients (0.6%) compared to controls (p=0.03). CYP2D6*1/*3 genotype frequency also showed a protective association in AML patients (OR=0.09, 95%CI=0.01-1.7; p=0.04). We also found a risk association for CYP2E1*5 in ALL and AML (OR=3.6, 95%CI=1.4-9.4 and OR=3.9, 95%CI=1.4-10.5, respectively). No association was found for the studied CYP2D6*4, CYP1A1*2A, and GSTT1>null variants and the risk of acute leukemia (ALL or AML). *Conclusions.* This case-control study suggests a contribution of CYP2E1, CYP2D6 and GSTM1>null variants to the development of acute leukemias. As in any case-control study, despite the statistical significance of associations found, chance findings and results due to unknown cofounders cannot be ruled out unless a prospective, truly population-based and larger study is carried out. The study, however, still contributes to the overall understanding of the involvement of these xenobiotic enzyme loci in the development of acute leukemias by providing an insight to their association in a different population.

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SPECIFIC IMMUNOPHENOTYPE FOR SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES

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Background. Splenic lymphoma with villous lymphocytes

(SLVL) has been described more than 10 years ago using morphological findings. Since specific membrane markers such as CD25, CD103 and more recently CD123 have been used for Hairy cell leukemias (HCL) diagnosis, no characteristic immunophenotype is available for SLVL recognition. *Aims / Patients and Methods.* In order to obtain SLVL specific markers, we retrospectively studied our immunophenotype database, concerning 832 B lymphoproliferative disorders (LPD) characterized by CD19 and CD20 expression. The most frequent studied markers were as follow: CD10, CD22, CD23, CD24, CD27, CD77, CD79b, FMC7, integrin b7, CD5, κ/λ Ig light chain, IgA, M, D, G Ig heavy chain. For villous LPD, other monoclonal antibodies (Mo-Ab) were added: CD11c, CD25, CD103. Immunophenotype was performed using multi-color flow-cytometry on a FC500 flow-cytometer (Beckman-Coulter). A marker is considered positive when expressed in more than 30% B-cells, defined by positivity of CD19. *Results.* Loss of CD24 is observed in different B malignancies including SLVL (13/14). CD24 intensity is dim in HCL (n=5/5) or variant HCL (n=1/1). Expression of integrin b7 is detected in many mantle cell lymphomas (n=14/36), rare cases of diffuse large B cell lymphomas and follicular lymphomas (respectively 2/77 and 3/121), all cases of HCL (n=4/4) and SLVL (13/14). For sensitivity, 13 out of the 14 SLVL defined by morphology, demonstrate a B CD19 (+), CD24 (-)ve, integrin b7 (+) phenotype. Except in a dissociated case, the phenotype is CD19+, CD20+, CD22+, CD24 (-), CD25 (-), CD27+, FMC7+, CD11c+, integrin b7 +. Only one case express CD5 and another case is CD79b (-). The dissociated case is CD24+ and integrin b7 (-). It is characterized by other differences: loss of expression of CD11c, the younger age (68 years old compared to a median age of 77), the highest number of circulating villous lymphocytes (32.5 G/L for a median at 6.1). For specificity, only SLVL and HCL are integrin b7 + and CD24 'dim/neg.'. In fact only the 13 cases of SLVL and one case of HCL are CD24 (-), integrin b7 +. *Summary / conclusion:* Among a large panel of mature B LPD (CD45 bright, CD19+), decrease of CD24 associated with integrin b7 expression characterizes villous lymphocytes whereas CD25 bright expression is specific of HCL. The number of HCL variant form is too low to conclude that it is not possible to distinguish SLVL from HCL variant form, however usual markers such as CD11c and CD25 are clearly not relevant for this purpose, in our experience. Role of integrin b7 expression which is able to oligomerize with integrin a4 or integrin aE, ligand of VE-Cadherin, is to be determined in stromal cell interactions.

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EMERGING ANTISENSE CHEMISTRIES AND THEIR POTENTIAL TO REDUCE ONCOGENIC GENE EXPRESSION IN CHRONIC MYELOGENOUS LEUKAEMIA

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Background. Chronic Myeloid Leukaemia (CML) is a stem cell malignancy characterised by a chromosomal translocation involving the bcr and abl genes which results in the production of a leukemia specific fusion protein (p210) with constitutive tyrosine kinase activity. The bcr-abl mRNA represents a suitable target for antisense oligodeoxynucleotide (ODN) based approaches. *Aims.* Three different antisense approaches were employed (i) phosphorothioate ODNs, (ii) Morpholino ODNs (iii) siRNA that mediate their effects either by active destruction of the mRNA or by blocking translation through steric hindrance of ribosomal assembly. In addition optimisation of delivery of antisense ODNs to their target was investigated, as this is a critical factor in the success of an antisense based approach. *Methods.* For design of all antisense molecules in the study, a bioinformatic approach was employed for conformational derivation of mRNA secondary structure, in order to select the most optimum region of the bcr-abl mRNA for mRNA binding. A mod-

el of intramolecular folding and mRNA secondary structure for both b2a2 and b3a2 variants allowed calculation of optimum minimum free energy values. Optimisation of antisense delivery was performed using a number of cell permeabilisation agents - streptolysin-O being the most effective in achieving specific delivery with minimal toxicity to normal hemopoietic cells. All antisense approaches were tested in 4 CML cell lines and in primary material from CML patients at diagnosis or in normal haemopoietic cells. *Results.* Phosphorothioate antisense ODNs were initially employed to target the b2a2 & b3a2 variants in CML. Downregulation of gene expression was achieved but effects were variable and transient. Use of the RNase H independent Morpholino backbone led to a significant antisense effect with prolonged reduction in P210 protein levels as judged by western blotting and this was specific when compared to a sense control sequence and a 5 base pair mis-match control. Testing of morpholino ODNs on normal haemopoietic cells (n = 10) using the CFU-GM assay indicated minimal toxicity. An siRNA approach was developed using four targets on the bcr portion of the bcr-abl RNA - mRNA and protein levels were reduced significantly and activity could be specifically correlated with secondary structure surrounding the target binding site as predicted using the mfold bioinformatics software. Similarly toxicity was minimal in normal cells (n=10). Although both morpholino and siRNA approaches were successful in down-regulating bcr-abl expression, in neither case did this lead directly to apoptosis induction in CML cells. A second target was chosen for mRNA downregulation, the cyclin D2 gene which is implicated in BCR-ABL induced proliferation. Morpholino and siRNA approaches both significantly reduced the proliferative capacity of CML cells relative to controls. *Conclusions.* Morpholino ODN and siRNA approaches proved superior to standard phosphorothioate approaches in downregulating bcr-abl mRNA expression in CML cells while showing minimal toxicity to normal hemopoietic cells. Use of a second target such as cyclin D2 may improve the efficacy of these newer generation antisense approach in chemotherapy resistant CML cells

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NOVEL GENOMIC IMBALANCES IDENTIFIED IN ACUTE MYELOID LEUKEMIA WITH COMPLEX KARYOTYPES USING MATRIX-BASED COMPARATIVE GENOMIC HYBRIDIZATION

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Approximately 10 to 15% of acute myeloid leukemia (AML) cases exhibit complex karyotypes, i.e., three or more chromosome abnormalities without presence of a specific fusion transcript. Using chromosome banding analysis, the majority of such cases cannot be completely described due to the low resolution of this method. To identify novel genomic regions of interest in AML with complex karyotypes we applied comparative genomic hybridization to microarrays (matrix-CGH), a novel technique allowing high-resolution genome-wide screening of genomic imbalances and thus may allow the identification of novel regions harboring potential disease-related genes. We designed a microarray consisting of 2799 different human genomic DNA fragments cloned in bacterial artificial chromosome (BAC) or P1-derived artificial chromosome (PAC) vectors. A set of 1500 of these clones covers the whole human genome with a physical distance of approximately 2 Mb. The remaining 1299 clones either contiguously span genomic regions known to be frequently involved in hematologic malignancies (e.g., 1p, 2p, 3q, 7q, 9p, 11q, 12q, 13q, 17p, 18q) (n=600) or contain oncogenes or tumor suppressor genes (n=699). Using this microarray platform, 53 AML cases with complex karyotypes were analyzed. Genomic losses were found more frequently

than gains; the most frequent losses were deletions of 5q (81%), 17p (62%), 7q (51%); followed by deletions of 16q and 18q (34% each), 3p and 12q (21% each), 20q (19%), 12p (17%), and 13q (11%). The most frequent genomic gains were trisomies of 11q (43%) and 8q (38%); followed by trisomies of 21q (28%), 1p (26%), 9p (21%), 13q (19%), 22q (17%), 6p (13%). In part, some critical segments were delineated to genomic fragments of 0.8 to a few megabase pairs in size. Furthermore, 36 high-level DNA amplifications in 19 different regions were identified; amplifications occurring in at least two cases mapped to (candidate genes in the amplicon) 11q23.3-q24.1 (n=6; ETS, FLI1); 21q22 (n=5; ERG, ETS2); 13q12 (n=3; CDX2); 11q23.3 (n=4; MLL, DDX6); 8q24 (n=2; MYC); 9p24 (n=2; JAK2); 12p13 (n=2; FGF6, CCND2); and 20q11 (n=2; ID1, BCL2L1). In conclusion, using high-resolution genome-wide screening tools such as matrix-CGH, allows unravel the enormous genetic diversity of AML cases with complex karyotypes. A larger number of cases needs to be analyzed to substantiate the findings of this study. Correlation of high-resolution genomic profiling with global gene expression studies may help to identify disease-related genes located in the critical regions.

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ZHX2, CHC1L AND RAN GENE EXPRESSION LEVELS DETERMINE DIFFERENT PROGNOSIS GROUPS IN MM PATIENTS

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INTRODUCTION: Gene Expression Profiling through RNA arrays has provided new clues on Multiple Myeloma (MM) pathogenesis and patient prognostic evaluation. Recently, ZHX2, CHC1L & RAN expression has been painted out as very important keys in MM. In the present paper, we have evaluated these genes by RT-PCR in purified plasma cells from 76 patient with plasma cell dyscrasias. **MATERIAL AND Methods.** Purified Bone Marrow cells were obtained from the following patients: 6 MGUS, 7 smouldering MM, 59 symptomatic MM patients and 2 Plasma cell leukemia (PCL). After RNA extraction, RQ-PCR of CHC1L, RAN and ZHX2 genes was carried out using the standard protocol from TaqMan® gene expression assays in an ABI-PRISM 7700 Sequence Detection System (Applied Biosystems, CA). Expression levels were normalized with ABL gene and expressed in n-fold times compared to the expression in a pool of RNA from mononuclear cells from healthy donors. The expression level of the different genes were evaluated for correlation with the diagnosis, clinical characteristic and prognosis of the patients. **RESULT AND Conclusions.** None of these genes displayed a clear relationship with the different stages of the disease pathogenesis, although ZHX2 gene was slightly more expressed in the indolent forms of these proliferative disorders (MGUS and SMM). Within newly diagnosed symptomatic MM patients, several interesting associations were seen. Thus, decreased expressions of CHC1L were observed in hyperdiploid MM cases than in those with a normal DNA index, confirming the participation of this gene product on chromosomal condensation during the mitosis. No other important associations were seen for this gene, although patients with the lowest expression displayed a very good prognosis, but without reaching statistically significant differences. As expected, RAN expression was related to S-Phase PC, since patients with high S phase values (>1.8%) displayed higher levels of RAN transcripts. This however, only resulted in a marginal impact in the survival. ZHX2 provided the most interesting results, thus decreased levels of ZHX2 were related with unfavourable prognostic indicators such as B2 microglobulin >4 mg/L and Haemoglobin levels <10.5 g/dL. This was significantly associated with a shorter survival. So, patients with a level of ZHX2 expression

lower than two times the expression of control were associated with a very low survival (median of 6 months vs not reached, p=0.019). If we take into account the predicting survival value of these three genes, the following prognosis groups were defined: very good prognosis (Z+ C-), poor prognosis (Z- C+) and intermediate (the remaining patients). **SUMMARY:** In this study we confirm that RAN, CHC1L and especially ZHX2 genes play a role in the pathogenesis and behaviour of MM. This could be helpful in predicting survival of patients.

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MOLECULAR INVESTIGATION OF THE RELATIONSHIP BETWEEN BCR-ABL AND SURVIVIN IN BLOCKING APOPTOSIS IN BCR-ABL+ CELLS USING DNAZYMES TARGETING BOTH GENES

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Suppression of apoptosis plays an important role in the initiation and progression of leukaemia. Survivin is a member of the inhibitor-of-apoptosis (IAP) family of caspase inhibitors. While Survivin is not expressed in most adult tissues, it is upregulated in most tumours. Targeting the Survivin gene with 2 specific DNazyme reduced cell proliferation and induced apoptosis in the BCR-ABL+ cell line K562, using XTT and Annexin-V assays respectively. Real-time RT-PCR and Western-blot analyses showed that these DNazymes have caused a significant drop in the level of Survivin transcripts and protein and as a consequence a significant cleavage of the BCR-ABL protein. The BCR-ABL fusion detected in most CML patients, is a cytoplasmic protein with constitutive tyrosine kinase, and antiapoptotic activity. The mechanism by which BCR-ABL inhibit apoptosis is not well understood. To further examine the relationship between Survivin and BCR-ABL fusion gene, we targeted the BCR-ABL transcript with DNazymes. BCR-ABL-targeting DNazyme caused the cleavage of the BCR-ABL transcript and protein in transfected K562 cells. This DNazyme also produced a significant drop in cell proliferation and induced apoptosis. It is interesting to note that the level of Survivin showed a significant drop after transfection with the BCR-ABL-targeting DNazyme. These data show that BCR-ABL antiapoptotic activity is produced via the upregulation of the Survivin gene. Our data also show that cleavage of the Survivin gene or the inhibition of its activity blocks the antiapoptotic activity of the BCR-ABL oncoprotein by causing the cleavage of the BCR-ABL protein. This effect could be a potential therapeutic model for targeting BCR-ABL+ leukaemia, especially those with resistance to Imatinib. Further data will be presented on the effect of disrupting survivin on Imatinib-resistant cells.

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GENETIC POLYMORPHISM OF HMSH3 IS ASSOCIATED WITH INCREASED RISK OF DE NOVO ACUTE MYELOID LEUKEMIA IN ADULTS

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Background. The DNA mismatch repair (MMR) process is crucial for avoiding recombination error between similar but not identical sequences. Defective MMR often lead to the accumulation of gross chromosomal rearrangements (GCR) which are frequent in hematologic malignancies. hMSH3 is one of the human homologs of the bacterial DNA mismatch repair protein MutS located at 5q11.2-q13.2. Mutations of this gene are known to be associated with variable hematologic and non-hematologic malignancies. **Aims.** We selected all three non-synonymous single nucleotide polymorphisms (SNP) of hMSH3 gene to test whether they are associated with increased susceptibility in

patients with *de novo* acute myeloid leukemia (AML). *Methods.* To investigate this possibility, we conducted a case-control study on 241 patients with *de novo* AML and age- and sex-matched 241 healthy controls. DNA was isolated from peripheral blood or bone marrow samples. Three selected SNPs are rs26279 (A/G transition with Ala1036 Thr change at exon 24) along with two rare SNPs of rs1805354 (T/C with Phe to Leu change) and rs184967 (G/A with Gln to Arg change) which were so rare, we excluded them in this study. Genotyping assays were performed by automated Pyrosequencing™ PSQ HS96A platform. Odds ratios (OR) and linear trends were calculated by conditional logistic regression models using SPSS 12.0. *Results.* Among 241 AML patients, wild-type (AA) individuals were 60 (24.9%), heterozygous (AG) were 163 (67.6%) and homozygous rare genotype (GG) were 18 (7.5%). On the other hand, among the controls the wild-type AA were 121 (50.2%), heterozygous were 106 (44.0%) and the GG were 14 (5.8%). Tests for Hardy-Weinberg equilibrium were conducted by comparing observed and expected genotype frequencies using a Chi-square statistic revealing there was no sampling or genotyping error. Higher occurrence of G allele in AML patients than in controls (OR = 1.43, 95% CI 1.17-1.75; $p=0.001$) was observed. With respect to genotype frequencies patients with *de novo* AML were significantly more likely to be carrying heterozygous (AG) (OR = 2.92, 95% CI 1.95-4.39; $p=0.000$) or homozygous (GG) (OR = 2.73, 95% CI 1.20-6.20; $p=0.017$) than homozygous wild-type (AA). More interestingly as shown by univariate analysis, 127 patients achieving complete remission (CR) who received intensive chemotherapy were also significantly more likely to have higher relapse rate when they were carrying wild-type homozygous AA than AG or GG genotypes (41.2% vs. 30.5% vs. 0%, $p<0.05$). However there was no statistically significant difference among different FAB classifications, risk groups according to the cytogenetics or groups with different molecular abnormalities. There was also no significant difference in CR rate, median duration of overall survival (OS), or median duration of leukemic-free survival (LFS) among the patients with three different genotypes. *Conclusions.* This study demonstrates G allele of rs26279 of hMSH3 could be associated with increased risk of *de novo* AML in adults and patients in CR with GG or AG genotypes have significantly lower relapse rate than with wild-type AA suggesting further analysis of A to G transition-induced structural change (Ala to Thr) in the hMSH3 protein might be necessary for its effect on functional activity as a MMR protein.

0592

BICLONALITY IN B- CHRONIC LYMPHOPROLIFERATIVE DISORDERS

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INTRODUCTION: B- Chronic Lymphoproliferative Disorders (B-CLPD) are a heterogeneous group of diseases typically characterized by monoclonal expansions of a single B-cell clone. Such heterogeneity has been attributed to intraclonal variation. However cases have been described in the literature, with the coexistence of two or more different B-cell clones, with an overall incidence in all B-CLPD being around 5%. **AIM:** To evaluate the incidence, immunophenotypic features and outcome of biclonal B-CLPD. *Methods.* Between January 2003 and July 2004, we studied 115 patients (pts) with B-CLPD, 76 B-Chronic Lymphocytic Leukemia (B-CLL) and 39 B-Non-Hodgkin Lymphoma (B-NHL). Diagnosis was made by clinical, morphologic, immunophenotypic and histological criteria, according to WHO classification. The assessment of monoclonality/biclonality was based on immunophenotypic analysis by flow cytometry. *Results.* From 115 pts with B-CLPD, 14 (12%) showed coexistence of two B-cell clones phenotypically aberrant. The incidence of biclonality was 6,5% (5/76) in B-CLL and 20,5% (8/39)

in B-NHL. Five pts presented B-CLL as the main clone (3 with a concomitant B-NHL and 2 with another B-CLL aberrant clone). In 8 cases, B-NHL was the most representative clone (6 with a minor population of B-CLL and 2 with another B-NHL subset). Eight pts showed identical light chains and five cases different light chains in the two clones. Pts presenting with biclonal B-CLPD are older (median: 75 years) than pts with only one aberrant B-cell population (median: 66 years). Besides the short follow up, we analysed the outcome of pts presenting with 2 B-CLPD clones and no statistically significant differences were observed in the time to begin chemotherapy (CT), responses to CT, disease free survival or overall survival, comparing with monoclonal B-CLPD. *Conclusions.* This study confirms the relatively high incidence of two clones in B-CLPD, supporting the highly heterogeneous behaviour of these pathologies and the importance of immunophenotyping in the assessment of biclonality. In our study, biclonality did not influence the outcome of pts with B-CLPD. However, more studies with larger series of pts are needed to confirm these results.

0593

FLOW CYTOMETRIC CHARACTERIZATION OF B LYMPHOMAS FROM GERMINAL CENTER ORIGIN, USING CD44 AND BCL2 MONOCLONAL ANTIBODIES

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Introduction: Characterization of Non-hodgkin-lymphomas (NHL) using flow-cytometry has been less standardized than leukemias, whereas NHL is a frequent pathology. This work studied the interest of CD44 expression for determination of the germinal center (GC) origin of B cells in different tissues. Since GC and pre- and pro-B cells are CD44^{dim}, CD45 has been used to separate them. *Patients and Methods.* Cells obtained from 3 normal tonsils and 232 lymphomatous lymph-nodes are studied after dissociation (Medimachine). 82 of these cases were Follicular Lymphomas (FL) diagnosed by histology. The majority of them (83%) were characterized by a t(14;18) translocation. Two combinations of Mo-Abs were tested in a multicolor strategy on a FC 500 flow-cytometer (Beckman-Coulter): CD44 (FITC), CD38 (PE), CD45 (ECD), CD34 (PE-Cy5), CD19 (PE-Cy7) (Beckman-Coulter) and Bcl2 (FITC), CD45 (ECD), CD44 (APC), CD19 (PE-Cy7). Only 20 selected FL were permeabilized (Intrastain, DAKO) for the second combination. *Results.* CD44 expression was low in normal as well as tumoral germinal center derived B cells. Thus, using normal B lymphoid tissue, centrocytic cells were detected in the CD44^{dim}, CD45^{bright} fraction (cell sorting). Since CD10 expression was detected in only 69% of the FL cases (57 out of 82), 95% (78/82 cases) demonstrated a CD44^{dim}, CD45^{bright} phenotype. Except a case without t(14;18) translocation, all the B CD44^{dim} cells were bcl2 positive (19/20) compared to the bcl2 staining of reactive B or T cells. GC cells from normal tonsils were bcl2^{weak}. For diffuse large B cell lymphoma (DLBCL) 18 out of 50 cases (36%) demonstrated a CD44^{dim} phenotype. The other cases, representative of the major NHL subtypes, were CD44^{bright}. The combination of 'CD19, CD44, CD45' Mo Abs was able to identify GC B cells in all tissues. In a second step, a high bcl2 expression demonstrated malignancy, similarly to bcl2 immunohistochemistry of germinal center. Rare cases without CD44 loss or without bcl2 expression should be more precisely characterized to differentiate them from reactive GC. Further studies using DNA microarrays are needed to demonstrate a similar link between loss of CD44 and GC origin in DLBCL. The role of this phenotype in order to detect minimal tissue involvement, during staging or minimal residual disease determination, should be evaluated.

0594

IMMUNOCHEMOCONJUGATE OF ANTIDNMT1/HDAC2 BISPECIFIC F (AB)2-BSAB LINKED WITH CLEAVABLE DISULFIDE TO VINORELBINE INDUCES ADCC AND APOPTOSIS IN CHEMORESISTANT DIFFUSE LARGE B-CELL LYMPHOMAJ. Giannios¹, P. Lambrinos², N. Alexandropoulos²¹General Hospital of Athens, ATHENS, Greece; ²Ippokraton Hospital, ATHENS, Greece

Introduction: Non-Hodgkin's Lymphoma (NHL) has been rising and it is the leading cause of cancer mortality in males aged between 15-54 years old. Diffuse Large B-Cell Lymphoma (DLBCL) is the most frequent subtype of (NHL) and it is characterised by potent chemoresistance to standard chemotherapy (CHOP) posing an unresolved clinical problem where most patients die within months. Failure to trigger apoptotic cell death due to epigenomic modifications including changes in DNA methylation and histone acetylation which cause gene silencing is a contributing factor in chemoresistance of human lymphoid malignancies. DNMT1 interacts with HDAC2 to repress transcription of tumour suppressor genes interfering with therapeutic and diagnostic procedures. Another potent factor of chemoresistance consists of overexpression of antiapoptotic oncogenes bcl-xL and bcl-2. *Methods.* Our cohort consists of 46 samples of DLBCL chemoresistant patients. Samples were analysed by DMH microarrays for simultaneous analysis of many CpG island loci on one sample at a time, MS-PCR, ChIP and RT-PCR. *Results.* We detected promoter CpG island hypermethylation of the following genes: ATM, p14, Bid, Apaf-1, p27, p16/p19, bcl11b and ARF-Mdm2-p53. There was overexpression of DNMT1 and HDAC2 suggesting a link between histone deacetylation, cytosine methylation, local chromatin condensation with subsequent transcriptional repression of all the above mentioned tumour suppressor genes. Concurrent methylation at the 5' end of the regulatory region of multiple genes and other loci was seen in the DLBCL samples defining them as CIMP+. We treated DLBCL cells with immunochemoconjugate of anti-DNMT1/HDAC2 bispecific F (ab)2-bsAb linked with cleavable disulfide to vinorelbine-tartrate termed as immunovinorelbine (I-VRL). Post-treatment, there was inhibition of HDAC2 and DNMT1 blocking the 5' CpG island methylation of the silenced genes resulting to transcriptional activation by upregulation of their mRNA. There was histone hyperacetylation which opens chromatin structure in which the DNA is more loosely wrapped around the histones making it more receptive to interaction with transcription factors. Overexpression of the previously silenced genes ATM, bcl11b, p14, Bid, Apaf-1, p27, p16/p19 and ARF-Mdm2-p53 combined with the microtubule depolymerizing action of vinorelbine inhibited metabolic activity and DNA synthesis of tumour cells according to MTT and BrdU assays, respectively. We observed expression alterations of the following genes in response to vinorelbine: downregulation of spindle checkpoint genes BUB3 and BUB2-like protein 1, upregulation of antiproliferative genes PTGF-b, BTG2 and G2 checkpoint gene GADD45, phosphorylation of bcl-xL/bcl-2 leading to their downregulation, activation of caspase-9, 6, 7 and upregulation of p21, bax and PARP cleavage products p24 and p85. Immunological analysis exhibited antibody-directed cytotoxicity (ADCC). Flow cytometry exhibited cell cycle arrest at G2/M phase. There was induction of apoptosis in DLBCL cells confirmed by cleavage of poly (ADP-ribose) polymerase (PARP) and gelsolin, enhancement in annexin V binding, activation of CPP-32 and formation of apoptotic bodies (D2 apoptotic stage) which were phagocytosed by adjacent tumour cells leading to a bystander killing effect. *Conclusions.* This novel therapeutic approach with immunochemoconjugate anti-DNMT1/HDAC2 bispecific F (ab)2-bsAb linked onto vinorelbine-tartrate (I-VRL) may revolutionize DLBCL treatment adding significantly to the current clinical armamentarium due to potential advantages offered by I-VRL over conventional therapy such as well defined mode of action, selectivity and mainly circumvention of chemoresistance by causing DNA demethylation and histone hyperacetylation reactivating transcriptionally silenced tumour suppressor/apoptotic genes and phosphorylation of antiapoptotic bcl-xL/bcl-2.

0595

CLINIC MICROBIOLOGIC PROFILE OF OVERINFECTIONS IN HIGH RISK FEBRILE NEUTROPENIAE.M. Donato¹, R. García Boyero¹, M.C. Mas¹, A. Escolá¹, M. Guinot¹, J. Marco¹, A. López², E. Herrera¹, I. García¹, T. Gozalbo¹, J. Amela¹, G. Cañigral¹¹General Hospital of Castellon, CASTELLON, Spain; ²Arnau de Vilanova Hospital, VALENCIA, Spain

Neutropenic patients with haematological diseases are at high risk of contracting infections, although sometimes are overinfections, and not the initial infection, the final cause of fatal evolution of these patients. The aim of this study was to identify the risk factors for overinfections in high risk febrile neutropenia and to describe its clinic microbiologic profile. The study presents a retrospective analysis of 172 episodes of high risk febrile neutropenia along a 7-year period (from January 1997 to December 2003). The neutropenic episodes were considered as high risk if the duration of neutropenia was >14 days or >7 days with severe neutropenia (neutrophil count <100 cells/mm³). Intensive chemotherapy was applied in all episodes. The median age of the patients was 59 (range 16-76), with 104 males (61%). Most episodes occurred in patients with acute leukemia (n=142, 80%), 25 of them corresponding to autologous transplantation. Acute leukemia was refractory to chemotherapy in 54 cases (31%). A total of 127 (73%) patients received antibiotic prophylaxis with quinolones, and 142 (83%) with azoles. A 83% of the patients had Hickman's catheter (n=98). Overinfection was defined as febrile relapse after afebrile 4 days with microbiological documentation or clinical focus different from that initially detected. The statistical analysis was performed with chi square test and t-test using the statistical program SPSS v10.0. *Results.* Overinfection was observed in 47 episodes (27%) with a median of 12 days after the first fever. A total of 11 episodes of bacteremia were found (3 Gram-positive bacilli, 2 Gram-negative bacilli, and 7 fungal infections, all by Candida species). Microbiological documentation without bacteremia was identified in 7 episodes: 2 Gram-positive bacilli, 3 Gram-negative bacilli, 3 fungal infections and 2 viral infections (HVS and CMV). The most frequent clinical focus was in lung, digestive and vascular catheter with 20, 10 and 9 episodes, respectively. There were 16 deaths, 8 of them directly related with overinfection. Higher values of global mortality ($p=0.002$) and related-infection mortality ($p=0.006$), and longer periods of neutropenia, fever and antibiotic treatment were found in patients with overinfection. The variables with statistical significance ($p<0.05$) associated with overinfection were: refractory disease ($p=0.05$), longer duration of neutropenia ($p=0.002$), previous infection by Gram-negative bacilli ($p=0.005$) and parenteral nutrition ($p<0.0001$). The study of our series confirms the negative influence played by overinfection in the evolution of high risk febrile neutropenia. Overinfection is more frequent during long-term neutropenia and refractory disease. Fungi are the most common pathogen isolated. Special attention must be paid to previous infection by Gram-negative bacilli. The clinical parameters described above may help to identify neutropenic patients at risk of developing overinfection.

0596

IMMUNOPROPHYLAXIS OF THE HEPATITIS B IN CHILDREN WITH MALIGNANT DISEASES AT THE TIME OF THE CHEMOTHERAPYO. Geludkova¹, M. Rusanova¹, N. Predeina², E. Zhukovskaya², M. Getmanova³, I. Borodina¹, V. Pastushenko⁴, N. Golubeva³, E. Kuptcova¹¹Research Institute For Pediatric Hematol, Moscow, Russian Federation; ²Regional Children Clinical Hos, Chelyabinsk, Russian Federation; ³Russian Children Clinical Hosp, Moscow, Russian Federation; ⁴Morozovsky Children Clinical H, Moscow, Russian Federation

Virus hepatitis take a special place among the infections

accompanying malignant diseases. General contamination of virus hepatitis in children with malignant diseases reaches 75%. It is established, that 80-90% of patients with malignant diseases in a case of HBV-contamination develop chronic forms of disease. In the future the patients with a chronic hepatitis have the risk of development of cirrhosis of a liver in 20-40% of cases and a hepatocellular carcinoma - 5-10% of cases. Thus, the successes achieved in treatment malignant diseases, can be levelled in the future by heavy defeats of the liver, essential deterioration of the quality of life in this patients. Till now was considered, that vaccination patients with malignancy is inexpedient in connection with impossibility of formation antibodies (anti-HBs) at the background of polychemotherapy. *Aims.* To estimate efficacy of the hepatitis B immunoprophylaxis in children with malignant diseases. *Materials and Methods.* 250 children with different malignancy at the age from 0 to 16 years (median 6 years) which received the chemotherapy were included in the study. 124 patients received an active immunization by the recombinant vaccines «Engerix B» or «HB-Vax II» by scheme: 0-1-2-6 months, 10 mkg - 21 patients, 20 mkg - 92 patients. 13 patients received a combined immunoprophylaxis-specific antibody «Hepatekt» 20 MU/kg by scheme: 0-1-2-3-4-5 months along with «HB-Vax II», 0-1-2-6 months, 10 mkg. Vaccination was conducted in the children without serologic marker of the hepatitis C at the first 3-7 day after the diagnosis of malignancy. 113 children with malignancy not received the specific immunoprophylaxis, and formed a control group. *Results.* After the 6 months the level of the protective antibodies (anti-HBs) was exceeded 10 mMU/mL (the titre median 15,3 mMU/mL) in 66% of children, which received 20 mkg vaccines, and in 25% of children, which received 10 mkg vaccines ($p < 0,05$). The titre of protective antibodies in 13 patients, which received a combined immunoprophylaxis, was revealed earlier ($p < 0,05$). The titre median of anti-HBs after 3, 6 and 12 months were accordingly 193,5 mMU/mL, 126,2 mMU/mL and 47,4 mMU/mL. The contamination of the hepatitis B in group with specific immunoprophylaxis was 13,7%, in control group - 41%, ($p < 0,05$). *Conclusions.* Vaccination in children with malignancy at the time of chemotherapy in the redoubled dose or combined immunoprophylaxis were effective.

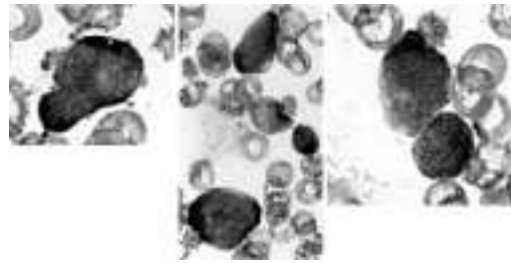
0597

CONGENITAL CYTOMEGALOVIRUS (CMV) INFECTION WITH LEUKEMOID EXPANSION OF HAEMATOGONES WITH PRO-B CELL CHARACTERISTICS

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Hematogones are benign immature B-cells that more often populate the pediatric bone marrow. These cells are similar to acute lymphoblastic leukemia (ALL) cells, raising diagnostic problems. As an example, we present the case of a newborn with congenital CMV infection inducing an expansion of haematogones very similar to pro-B ALL cells. *Methods.* Bone marrow samples were obtained from the iliac spine for MGG-staining, flow-cytometry immunophenotyping, study of IgH gene rearrangements by means of PCR technology, and FISH for MLL rearrangements at 11q23. A 1 month-old preterminal (34 weeks) female was admitted with liver failure and pancytopenia. Her mother had not had any problem during pregnancy and the parents' history was unremarkable. Laboratory investigations revealed isolated thrombocytopenia ($44 \times 10^9/L$) with emoglobin 15.8 g/dL, leucocytes $8.6 \times 10^9/L$ with 36% neutrophils, 51% lymphocytes, 12% monocytes, and hyperbilirubinemia (4.3 mg/dL). CMV serology was negative for IgM antibodies and positive for IgG. In the next few days progressive pancytopenia, increased bilirubin (20.7 mg/dL), and hepatosplenomegaly were noted. Bone marrow aspirate was normo-hypocellular and repleted with atypical cells resembling lymphoblasts (Figure).



Flow cytometry demonstrated a homogeneous expansion of CD34⁺ (26%) CD19⁺/TdT⁺ (20%) CD10- S1g- B-cells, but no T-cell anomaly. The molecular biology/FISH studies ruled out a clonal lymphoid proliferation by showing germ-line configuration of IgH DNA (VH1, 2, 3, 4, 5-JHc and DH1, 2, 3, 4, 5, 6, 7-JHc) and no abnormal signal for MLL at 11q23. Thus, these cells were nonclonal pro-B haematogones. Eventually CMV-PCR analysis was positive confirming congenital CMV disease. Ganciclovir was started leading to clinical improvement and normalization of pancytopenia and marrow morphology, with reduction in B-cell content (CD20⁺ 7%, CD19⁺ 9%). Because of persisting liver damage, liver transplantation had to be performed. Expanded hematogones may have morphological and immunophenotypic features of lymphoblasts. A nonclonal pro-B cell expansion can occur in association with congenital CMV infection at birth, and must be differentiated from infant ALL.

0598

CELL MEDIATED IMMUNE RESPONSES TO INFLUENZA VACCINATION IN HEALTHY VOLUNTEERS AND ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

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Influenza is one of the most common respiratory diseases in humans. The response to vaccination is frequently poor in immunosuppressed individuals. *Aims.* The aim of the present study was to develop an ELISPOT assay for measuring of the specific T-cell response to influenza vaccination. *Methods.* 18 healthy subjects and 6 SCT patients tested before and 4 weeks after influenza vaccination were included in the present study. Peripheral blood lymphocytes were stimulated with four influenza peptides; three based on sequences from the hemagglutinin and one from the M1 protein. The ELISPOT assay and the measurement of intracellular IFN- γ production were used to determine the cell-mediated responses after stimulation with the peptides. *Results.* Influenza vaccination elicited strong cell-mediated immune responses in the healthy controls to all four peptides with 3.2–6.1 fold increases in the number of IFN- γ producing spots/ 10^6 cells. By intracellular staining, it was suggested that CD4⁺ cells mediated the responses to the hemagglutinin peptides. In contrast, there was no increase in the number of IFN- γ producing cells response after vaccination in the 6 SCT patients. *Conclusions.* Our results suggest that the ELISPOT assay might be used as a complement to serology for monitoring of influenza vaccine studies in severely immunocompromised patients.

0599

THE IMPORTANCE OF MANNOSE BINDING LECTIN GENE POLYMORPHISMS IN ACUTE LEUKEMIA'S AND THEIR ROLE IN FEBRILE NEUTROPENIC EPISODESM. Pehlivan¹, H. Onay², A. Ozcan², L. Aslan¹, S. Pehlivan²¹Ataturk Education and Research Hospital, Izmir, Turkey;²Ege University, Izmir, Turkey

Mannose binding lectin (MBL) is a calcium dependent lectin that plays an important role in innate immunity by activating the complement pathway and phagocytosis. The single nucleotide polymorphisms [codon 52 (Allele D), codon 54 (Allele B), codon 57 (allele C), normal (Allele A)] in exon 1 of the MBL gene disrupt the assembly of MBL trimers or accelerate the degradation of the protein. As a consequence functional MBL decreases in the circulation and this causes predisposition to infections and autoimmune diseases. Febrile neutropenia (FN) resulting from chemotherapy is an important cause of mortality and morbidity. There are some articles which support the role of MBL gene polymorphisms in FN, although some articles do not support that correlation. *Aims.* The aim of this study is to investigate the MBL gene polymorphisms in different haematological malignancies and to evaluate their role in FN resulting from chemotherapy. *Methods.* Codon 54 and codon 57 polymorphisms in the exon 1 of the MBL gene were investigated with PCR-RFLP in patients who are hospitalized consecutively in last seven months (27 patients diagnosed as acute leukemia (7 ALL and 20 AML) and 20 patients diagnosed as multiple myeloma-non-Hodgkin lymphoma) and 50 healthy controls. Frequencies of allele A, B and C were compared between patient and control group. The presence of MBL gene polymorphism was evaluated in the lights of distribution of infections, pathogens, duration of neutropenia and duration of febrile episodes in acute leukemia's. *Results.* AB/BB genotype (63%) and B allele frequency (31%) which are important in susceptibility to infections were found significantly high in acute leukemia group compared to multiple myeloma-non-Hodgkin lymphoma and control groups ($p=0.01$ and 0.001 respectively). Median age of the acute leukemia patients was 46 years (16-67), 9 of 27 patients were female and 18 were male. Median duration of neutropenic (neutrophil count $<500/\mu\text{l}$) episodes was 17 days (6-72) and median duration of febrile periods was 8 (1-28) days. There was no relation between presence of MBL gene polymorphism and infectious pathogens, duration of neutropenic episodes, duration of febrile episodes, presence of fungal pneumonia and mortality due to FN. Sepsis was more common in AB/BB group compared to AA group ($p=0.041$). In conclusion, frequencies of AB/BB alleles resulting from polymorphisms in the exon 1 of the MBL gene were significantly high in acute leukemia group and the patients who carry this polymorphism had more sepsis in FN episodes resulting from chemotherapy. These results suggest that the genes which are important for the susceptibility to infections, especially MBL (NRAMP1, Toll-like 2-R, CTLA4-A, IL-1R, IL-R4, Vit D-R), must be investigated in FN.

0600

NOROVIRUS OUTBREAK IN IMMUNOCOMPROMISED PATIENTS ON A HEMATOLOGICAL UNIT-LESSONS TO LEARNJ. Hahn¹, P. Kaiser², S. Boehm³, S. Krause⁴, A. Beck⁵, C. Beyer⁵, E. Holler⁴, R. Andreesen⁴¹University Clinics of Regensburg, Regensburg, Germany; ²Dept. of Microbiol. & Virology, Regensburg, Germany; ³Department of Microbiology & Virology, Regensburg, Germany; ⁴Dept. of Hematology/Oncology, Regensburg, Germany; ⁵Department of Hematology/Oncology, Regensburg, Germany

Noroviruses are known as important pathogens in outbreaks of gastroenteritis in humans, especially in community institutions like day-care facilities for children, aged-care facilities or hospitals. However, there are only few reports about norovirus

infections in immunosuppressed patients. Therefore we here report our experiences with an epidemic outbreak of norovirus infection on our hematological unit. During November 2004 we observed an outbreak of epidemic diarrhea and vomiting on our hematological unit. Outbreak showed a two-peak pattern with a time interval of one week. The first peak occurred on a weekend, making immediate testing impossible. Barrier nursing and standard hygiene measures were instituted for outbreak containment. As these measures didn't prevent a second peak, measures were intensified: change of propanol 75% hand disinfectants to ethanol 95% disinfectants, no allowance of symptomatic staff to the ward unless symptoms stopped at least for two days, stop of admissions of new patients until new cases weren't observed for at least three days and careful follow-up and strict isolation of patients with ongoing fecal virus secretion. By strictly imposing these measures it was possible to contain outbreak within two further weeks. The index patient was a 36 year old woman with chronic Graft-versus-Host Disease 4 years after BMT due to ALL, who was admitted to the ward with symptoms of vomiting and diarrhea one week before the outbreak. She was retrospectively tested positive for norovirus genogroup II by RT-PCR. Thirty patients developed symptoms of diarrhea and/or vomiting during this time frame, 11 of these tested positive for the virus. In 8 patients we could demonstrate fecal viral excretion for more than one week. In four of these 8 patients we were unable to show viral clearance over a period of up to two months. In 4 other patients with prolonged excretion, viral clearance was finally observed after two weeks to two months. Despite delayed virus clearance, symptoms subsided much earlier in most patients. However, one patient got severely symptomatic again, when treated with chemotherapy. Three of the patients with persisting infection died, but none of their deaths was directly related to norovirus infection. 34 members of the staff also developed symptoms of putative norovirus infection, however, the staff was not exhaustively evaluated by PCR. *Conclusions.* In hematological patients under chemotherapy or immunosuppression, norovirus infection was accompanied by prolonged virus secretion as a possible source for new infections. In some patients, infection may lead to clinically uncontrolled disease. Therefore, we recommend immediate testing of norovirus, whenever there are clusters of vomiting and diarrhea on a hematological unit, especially when staff is also involved. Hygiene measures have to be more strict than usual to prevent spreading of these viruses in a population of immunosuppressed patients.

Infectious complications II

0601

LYMPHOCYTE RECOVERY AND VIRAL INFECTIONS IN HLA-HAPLOIDENTICAL TRANSPLANTATION USING UNMODIFIED MARROW AND CD-6 DEPLETED BLOOD STEM CELLS-A COMPARISON WITH HLA-IDENTICAL TRANSPLANTATION

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Background. Allogeneic stem cell transplantation may be curative even in advanced hematologic disorders. In some cases missing an HLA-identical family member, the disease progress does not allow the search of an unrelated donor. Most studies of HLA-haploidentical transplantation use T-cell depletion for prevention of graft-versus-host(GVH) disease and mega-doses of stem cells. We have used unmodified marrow on day 0 and CD6-depleted mobilized blood cells (MBC) on day 6 for haploidentical transplantation. CD6-depleted MBC are devoid of CD4-positive cells, they contain CD34-positive cells, NK-cells and a minority of CD8-positive cells. These cells suppress host-versus-graft and GVH reactions. A factor which confounded interpretation of the haploidentical transplant data was the advanced disease among recipients of this procedure. To avoid

this factor, we decided to include only high-risk adult patients who underwent transplantation HLA-identical related donors during the same years as a control group. **Aims.** To compare (1) lymphocyte recovery, (2) virus reactivation, and (3) clinical outcomes in the first year post transplantation of HLA-haploidentical (haplo) and HLA-identical (id) grafts. **Methods.** We reviewed 90 high-risk adult patients transplanted between November 1996 and May 2004; 47 of them underwent transplantation from CD6-depleted haploidentical related donor and 43 from HLA-ID related donor. Virus reactivation was regularly monitored by polymerase chain reaction including herpes viruses (herpes simplex virus (HSV), varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpes virus 6 (HHV6), human herpes virus 7 (HHV7)) and polyoma viruses (JC and BK). Leukocyte (WBC), absolute neutrophil (ANC) and absolute lymphocyte counts (ALC) were corrected at day 30, 60, 120, 180, 365 after transplantation. Comparison between groups of data were performed with the Mann-Whitney test.

Table. Rate of Viral reactivation

Virus	Haploid ^a	HLA-ID ^a	p-value
HSV	4/42 (10)	0/42 (0)	0.04*
VZV	0/42 (0)	1/42 (2)	NS
CMV	14/47 (30)	8/42 (19)	NS
EBV	31/47 (66)	27/42 (52)	NS
HHV6	19/38 (50)	19/42 (44)	0.01*
HHV7	0/22 (0)	7/33 (6)	NS
JC	8/34 (24)	8/42 (14)	NS
BK	21/34 (62)	19/41 (46)	0.01*

^aValues are given as positive/total number (%)

Results. Neutrophil engraftment (ANC >0.5x10⁹/L) was later in haplo (median 22 days) than in id (median 18 days) (p = 0.01). No differences were observed in the recovery of platelets median days of platelet > 20x10⁹/L were 18 (haplo) and 15 (id) days respectively. Surprisingly recovery of lymphocyte counts did not differ between the groups and the overall survival was not different in both groups; 2 year survival was 22.7 (haplo) and 25.5 (id) (ns). Moreover the rate of viral reactivation was not different for CMV, EBV, VZV and JC, but differences were found for the reactivation of HSV, HHV-6 and BK (Table). **Conclusions.** High-risk patients treated by haploidentical transplantation have a similar overall survival as patients given HLA-identical transplants. Moreover recovery of lymphocyte counts does not differ in both groups. However haploidentical transplants have a higher incidence of HSV, HHV6 and BK virus reactivation.

0602

LETHAL MULTIFOCAL LEUKOENCEPHALOPATHY IN A PATIENT AFTER ALLOGENIC STEM CELL TRANSPLANTATION DESPITE HIGH FREQUENCIES OF JC VIRUS REACTIVE T CELLS

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The reactivation of JC virus in patients with cellular immunodeficiency leads to a demyelinating disease called progressive multifocal leukoencephalopathy (PML). Previous reports demonstrated an association between JC virus-specific cytotoxic T lymphocytes and the early control of PML in patients with HIV. Here we report a 44-year-old woman diagnosed with chronic lymphocytic leukemia (CLL), who underwent allogeneic unrelated stem cell transplantation (SCT) 9 years after diagnosis. Conditioning regimen consisted of treosulfan and fludarabine.

Four months after transplantation the patient developed a thrombocytopenia and received alemtuzumab and rituximab. Nine months after transplant the patient showed up with macrohematuria. BK virus was detected in urine, in serum as well as in the cerebrospinal fluid (CSF) at day +299 and day +325. The presence of BK virus in the CSF was probably due to a proved disorder in the blood-brain barrier. Moreover, she experienced progressive and rapid neurological deterioration with right hemiparesis, impaired consciousness and recurrent seizures. A cerebral MRI scan showed two focal areas of white matter abnormality in the left and right frontal areas. Because of the thrombocytopenia no histological examination could be performed. CSF examination on day +335 disclosed normal values for white cells, protein and glucose but a positive JC virus PCR result (JC/BK consensus, Argene Biosoft), which was confirmed in a further sample one week later. As a consequence of the antibody therapy, immunoglobuline values were very low (IgG 5,92 g/L) at that time. When leukocytes subpopulations were measured, immunophenotyping showed high levels of CD4+ cells (1630/μL) and CD8+ cells (2280/μL), though the CD4/CD8 ratio was decreased (0,71). Moreover, very low values of CD19+ B cells (40/μL) and elevated counts of CD16+ NK cells (720/μL) were found. We then examined the peripheral blood of the patient for the presence of JC virus specific T cells. After overnight incubation with a pool of peptides derived from the JC virus protein VP1, increased frequencies of interferon gamma producing CD8+ T cells were observed in the patient but not in the stem cell donor. The frequency of CD8+ cells against the JC-VP-1 antigen and the JC-large-T antigen was 0,08% and 0,30%, respectively, compared to 0,03% of the negative controls. To our knowledge this is the first report of PML caused by a JC-virus reactivation after allogeneic SCT. Our results show that JC virus diagnosis should always be performed in case of unclear cerebral findings after SCT. Despite recovery of virus reactive T cells, the disease can take a fatal course, as showed in our patient, who died two months after onset of the neurological symptoms.

0603

HIGH INCIDENCE OF SINONASAL MUCORMYCOSIS IN PATIENTS WITH HEMATOLOGIC MALIGNANCY

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Background. Mucormycosis is an uncommon fungal infection. sinonasal mucormycosis is recognized as a potentially aggressive and commonly fatal fungal infection. **Aims.** Determine the incidence, clinical presentation and outcome of paranasal sinus mucormycosis in patients with hematologic malignancy in south of Iran. **Methods.** 550 hospitalized patients with hematologic malignancy in hematology-oncology ward were prospectively studied; the diagnosis of sinonasal mucormycosis infection was made by formalin fixed paraffin embedded tissue derived from biopsies taken from suspicious lesions in patients suspected clinically and radiologically to have infection. **Results.** During 27 months period, we diagnosed and treated 29 patients as having sinonasal mucormycosis. All 29 patients had an underlying hematologic malignancy that caused them to be neutropenic. Relative frequency of mucormycosis in 550 patients with hematologic disorder was 5.2% 22 (75.9%) of patients had AML, with relative frequency of 9.4%. Overall survival in our study among 29 patients was 44.8% and mortality rate directly due to mucormycosis was 55.2%. **Conclusions.** It seems that sinonasal mucormycosis has high incidence among patients with hematologic malignancy in south of Iran. AML patients with chemotherapy induced neutropenia are especially prone to mucormycosis and it is a major cause of their mortality and morbidity. It was postulated that this high incidence might be due to heavy exposure to molded bread.

0604

INFECTIOUS COMPLICATIONS IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH ATRA AND ANTHRACYCLINE MONOCHEMOTHERAPY FOLLOWING A RISK-ADAPTED STRATEGY

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Background: Infectious complications in patients with acute promyelocytic leukemia (APL) have a different profile and relatively low impact on mortality compared to patients with other subtypes of acute myeloid leukemia. As has been reported in APL, therapeutic regimens that use anthracycline monotherapy in combination with all-trans retinoic acid (ATRA) for induction and consolidation are less toxic than those using combinations, particularly with high-dose ara-C. **Aims:** To analyze the incidence, type of infection, and infectious-associated mortality in a large series of patients with APL treated with ATRA and anthracycline alone for induction and consolidation therapy. **Methods:** From November 1996 to December 2004, 666 patients with APL (M/F, 337/329) were included in two consecutive PETHEMA studies (LPA96 and LPA99). Median age was 40 years (range, 2-83). Induction therapy consisted of ATRA and idarubicin (AIDA). Consolidation therapy consisted of three courses of anthracycline monotherapy (idarubicin in courses #1 and #3; mitoxantrone in course #2) that was reinforced with ATRA and increased doses of idarubicin for patients with intermediate- and high-risk after November 1999 (LPA99 study). **Results:** Median duration of neutropenia during induction therapy was 23 days (range, 6-60 days). Six-hundred and six patients (91%) developed febrile episodes (bacteremia 11%, microbiologically documented without bacteremia 27%, clinically documented 28%, and FUO 35%). Eighteen patients (2.7%) died because of infection during induction with a median age of 66 yr (range 35-81 yr) and 13 patients (72%) being older than 60 yr. Median duration of neutropenia was 8, 22, and 9 days for course #1, #2, and #3 of consolidation therapy, respectively. In parallel with longer duration of neutropenia in course #2, a higher rate of febrile episodes (68%) was also observed. Episodes of severe neutropenia (> 15 days) and febrile episodes were significantly higher in patients with reinforced consolidation course #1 (59% and 23%, respectively) and course #3 (63% and 23%, respectively) compared with those receiving non reinforced course #1 (15% and 12%, respectively) and course #3 (10% and 6%, respectively). Distribution of type of infections in each consolidation course was similar and comparable to that observed during induction therapy. Four elderly patients (0.7%) died due to infection during consolidation. **Conclusions:** While infectious complications were frequent during induction and course #2 of consolidation therapy (91% and 68%, respectively), they were uncommon during courses #1 and #3. However, infectious-related mortality was low and mainly affecting elderly patients. It should be note a relatively low proportion of microbiologically documented infections with bacteremia in this series.

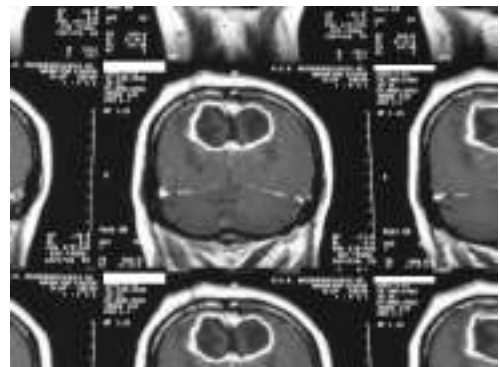
0605

SUCCESSFUL CONTROL OF PULMONARY AND CEREBRAL ASPERGILLOSIS WITH VORICONAZOLE IN A PATIENT AFFECTED BY ACUTE PROMYELOCYTIC LEUKEMIA

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A 52 yo patient, diagnosed with acute promyelocytic leukemia in January 2002, developed pulmonary and cerebral aspergillosis during neutropenia following induction treatment according to GIMEMA AIDA 2000 protocol (Idarubicine, ATRA and metilprednisolone). Pulmonary localisation was characterised by mul-

iple nodules in both lungs, of diameter between 3 and 5 cm. A bronchoalveolar lavage was performed, with the growth of *Aspergillus fumigatus*. Central nervous system localisation was characterised by two bilateral and symmetrical lesions, each about 3 cm of diameter, located in the parietal lobes. Diagnosis was confirmed through a brain biopsy which showed aspergillus hyphae. Culture gave growth to aspergillus fumigatus. Patient symptoms were characterized by fever, visual disturbances, slow ideation, ataxia, headache. Infection worsened after two weeks of treatment with liposomal amphotericine. At this point, amphotericine was stopped, and voriconazole was started. At the same time the patient started prophylactic treatment with diphenilidantoino. During the first 15 days of treatment with voriconazole, patient conditions improved, and he was discharged with persistence of only mild neurological symptoms. Over the following months the treatment slowly resolved the pulmonary lesions, and improved the two cerebral lesions. After 3 years of treatment with voriconazole at 400 mg po qd, patient is in very good clinical conditions. He has no pulmonary symptoms. CT scan of the chest does not show any persistence of active fungal disease. MRI of the brain shows a reduction in size of both parietal lesions. The patient does not present any neurological symptoms and he is continuing prophylaxis with dintoine. Biochemistry shows a persistent increase in colestatic indexes (GGT and ALP) without any elevation of bilirubin and with only mild elevation of AST and ALT. After the first cycle of chemotherapy, the patient still showed persistence of leukemia, with evidence of molecular disease. Due to the fungal infection, he did not underwent any other chemotherapy cycle, but continued only maintenance treatment with ATRA, 1 week every 3 weeks. This treatment obtained the disappearance of pml-rar-alpha. After 34 months of treatment, the patients still maintains a continuous complete molecular remission of leukemia. This case shows that long term treatment with voriconazole can successfully control a cerebral localisation of aspergillus infection. Again, it shows that long term treatment with voriconazole can be very well tolerated, and can be administered without causing severe clinical adverse effects. Cotreatment with antiepileptic drugs is feasible. At the same time, it is worthy to note, as already shown by others, that successful control of Promyelocytic leukemia can be obtained on the middle and long term by only one cycle of induction therapy, and subsequent maintenance with ATRA every three weeks.



0606

DIARRHEA DUE TO CRYPTOSPORIDIUM IN CHILDREN WITH ACUTE LEUKEMIA

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Background. *Cryptosporidium parvum* is an enteric parasitic infection, which causes life-threatening diarrhea in immunocompromised hosts such as those with leukemia. In these patients, Cryptosporidiosis is associated with significant morbidity and mortality, especially among infants and children.

Aims. To access cases of severe diarrhea caused by *Cryptosporidium* in pediatric patients with acute leukemia. Important aspects of disease caused by *Cryptosporidium* are also reviewed. **Methods.** All leukemic children with diarrhea (525 cases) referred to Pediatric Infectious Disease Department were investigated for *Cryptosporidiosis* during approximately 5 years (from 20 March 2001 to 19 January 2005). Stool specimens were taken from them and were investigated for *Cryptosporidium parvum*, using the modified acid-fast stain methods. **Results.** *Cryptosporidium* was detected in 65 (12.38%) individual stool samples collected from 525 pediatric patients with acute leukemia hospitalized in our center. Age range of leukemic patients with *Cryptosporidiosis* (35 boys and 30 girls) was 2.5-12 years old. Ages of 46.2% (30 cases) of them were less than 5 years. Infection with *Cryptosporidium* sp occurred most commonly (in 45 cases) during chemotherapy. In 10 patients, *Cryptosporidiosis* was diagnosed while being followed up for their leukemia. The other patients (10 cases) were infected during the remission. In stool specimens of all patients, mucous and blood were not seen and WBC count was $\leq 2-3$ and RBC ≤ 4 . In the most patients, stool culture was negative and only in 3 cases (4.6%) was positive for *Salmonella* sp. Paromomycin or azithromycin therapy was administered for them and seemed to be beneficial against *Cryptosporidiosis*. **Summary/conclusions:** *Cryptosporidium parvum* should be considered in all leukemic patients with severe or prolonged diarrhea, especially if there is no blood or leukocytes in the stool and infected patients have negative stool culture. Because *Cryptosporidium* can follow a severe course in children with leukemia, which might in some cases result in death; therefore, early diagnosis and treatment of *Cryptosporidiosis* is very important.

0607**FATAL CEREBRAL BLASTOMYCOSIS INFECTION IN A BONE MARROW TRANSPLANTED CHILD**

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Blastomycosis, including paracoccidioidomycosis, has in recent years increasingly been recognized in immunocompromised hosts, particularly patients with acquired immune deficiency syndrome, although most cases occur as sporadic fungal infections in healthy hosts living within endemic areas. We report a 10½-yr-old boy who never travelled outside Europe and was diagnosed with classical X-linked childhood cerebral adrenoleukodystrophy (X-ALD). The patient underwent successful unrelated donor bone marrow haematopoietic stem cell transplantation (HSCT). Biochemical analyses subsequently showed a normalized profile of long fatty acids. Donor transmitted EBV-infection occurring at engraftment was successfully controlled with a single dose of MabThera. Immunosuppression was abolished but immunological workup showed permanently depressed cellular immune response. Three weeks after the HSCT, signs of neurological disturbances rapidly progressed, the patient had increased muscle tone, aphasia and impaired coordination, which left the boy bedridden. EBV-reactivation reoccurred nine months after the HSCT and an additional single dose MabThera was given. Four weeks hereafter, the patient acutely developed severe rigidity and hyperpyrexia disease of uncertain origin. No retinochoroiditis was present, the chest radiograph was normal and CNS morphology by MRI was consistent with advanced ALD. The boy died within 36 hours. Neuropathology confirmed the advanced white matter destruction of ALD-type, but also showed scattered groups of blasted fungi in the central periventricular white matter. The blastomycetes were readily seen in routine staining, strongly basophilic and of variable size around 10 µm in diameter. They showed a marked capsule-like wall and signs of wide-base budding; they were judged to represent blastomyces, probably paracoccidioides. Some single cysts of toxoplasma trophozoites were present as well. While no granulomatous reaction was seen,

there was a mild lymphocytic infiltrate around the vessels. This case report is to the best of our knowledge the first European description of the disease, which is common in Latin America, but rare in organ and bone marrow transplant recipients. Our patient did never reach immunological reconstitution, something we believe plays a critical role in aborting fungal growth.

Transfusion, Apheresis, Granulocytes II**0608****INACTIVATION OF BLOOD-BORNE PATHOGENS IN APHERESIS PLASMA AND PLATELETS USING THE INTERCEPT BLOOD SYSTEM**

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Background. The INTERCEPT Blood Systems for plasma and platelets utilize amotosalen HCl and UVA light to inactivate a wide variety of blood-borne pathogens in plasma and platelets intended for transfusion. Previous studies using the INTERCEPT Blood System for platelets have shown inactivation of a wide range of viruses, bacteria and protozoan parasites in platelet concentrates. In the studies presented here the inactivation of a variety of organisms in jumbo apheresis plasma units is evaluated. These are part of an ongoing series of studies designed to demonstrate the inactivation characteristics of the INTERCEPT Blood System for plasma using treatment components and conditions representative of those intended for commercial use. Previously reported inactivation of these organisms in platelet concentrates is included for reference. **Methods.** For most studies ABO-matched, single-donor jumbo apheresis plasma units were pooled as necessary to make units of 585 mL, inoculated to a final concentration of ~ 6 logs of organisms/mL where possible, and treated with 150 µM amotosalen and 3.0 Joules centimeter squared UVA treatment. A few studies were performed using apheresis (HBV and HCV) or whole blood-derived (T. cruzi) plasma units of 285 mL volume, with amotosalen and UVA treatment adjusted to yield the same treatment conditions used in the jumbo plasma studies. Samples were taken before illumination to determine input titer and after illumination to detect any residual viable organism. N=4 except as noted. **Results.** See Table below. **Conclusions.** High titers of several viruses, two representative bacteria and two protozoan parasites in apheresis plasma units were inactivated to or below the limit of detection by treatment with the INTERCEPT Blood System for plasma. These results are similar to those previously reported for inactivation in platelet units.

Inactivation of Representative Pathogens in Plasma and Platelets

Organism	Mean Log Reduction	
	Platelets	Plasma
West Nile Virus	>6.0	≥6.8
SARS	>6.3 ¹	≥6.5
HSV-1	>6.6	>6.4
HCV	>4.5	>4.5
HBV	>6.5	>4.5
BV/DV	>6.5	>6.7 ²
HTLV-I	4.7	≥4.5
HTLV-II	5.1	>5.7
<i>K. pneumoniae</i>	>6.6	>7.4
<i>S. epidermidis</i>	>6.6	>7.3
<i>T. cruzi</i>	>5.3	>6.0
<i>P. falciparum</i>	≥6.0	≥6.9

¹ Platelet SARS data is from a feasibility study (n=2)

² Plasma BV/DV studies are in progress and results are preliminary (n=1)

0609

GRANULOCYTE TRANSFUSION THERAPY: EFFECTIVENESS OF AN ALTERNATIVE COLLECTION STRATEGY REQUIRING NO SEDIMENTING AGENTSP. Bergamaschi¹, C. Perotti², G. Viarengo², C. Del Fante², D. Sabbioni², T. Santini², L. Salvaneschi²¹IRCCS Policlinico 'San Matteo', PAVIA, Italy; ²IRCCS Policlinico 'San Matteo', PAVIA, Italy

Background. and **Aims.** severe bacterial and fungal infections still represent life-threatening complications in patients with functional neutrophils disorders or profound neutropenia after high dose chemotherapy regimens. The transfusion of granulocytes (PMN) has long been limited because of the inability to obtain adequate quantities of cells. Recently, a renewed interest has been emerging thanks to the availability of granulocyte colony-stimulating factor (G-CSF) for donors mobilization and, as a consequence, enhanced recoveries of PMN in the collections. Granulocytes are currently collected by a third generation cell separator employing sedimenting agents, such as hydroxyethylstarch (HES). These substances proved to be very effective in obtaining red cells depleted products with a high PMN content. However, because of their potential side effects (e.g. severe itching and allergy), the use in healthy donors should be carefully considered especially in the context of unrelated volunteers. Furthermore, there is some evidence that PMN collected after several days versus only one day of stimulation (+1) are qualitatively different, e.g. separation characteristics and cellular function. We report our experience of performing granulocyte apheresis by an alternative collection program based on PMN separation characteristics in day +1 from G-CSF administration, avoiding the exposure of donors to HES. **Methods.** donors were either patient's siblings or enrolled from the community pool of blood donors. They were selected in accordance to general recommendations for blood donors recruitment, in particular concerning infectious disease testing; all signed an informed consent to both G-CSF administration and leucapheresis. A summary of donors characteristics is given in Table 1.

Table 1. Characteristics of the donors (n=10).

Unrelated/related	2/8
Male/female	7/3
Age (years)	41 (27-85)
Mobilization regimen:	
G-CSF alone	8 (80%)
G-CSF plus dexamethazone	2 (20%)
Peripheral blood values:	
WBC ($\times 10^3/\text{mL}$)	37.5 (26.5-58.2)
PMN (%)	88.5 (83-93.5)
PMN ($\times 10^3/\text{mL}$)	33.0 (22.9-53.6)
Ht (%)	40.8 (26.2-49.2)
PLT ($\times 10^3/\text{mL}$)	200 (112-319)

Table 2. Characteristics of PMN leukapheresis (n=19)

No. of procedures per donor	2 (1-4)
Processed blood volume (T)	8.14 (5.0-10.0)
Processed blood volume/donor blood volume ratio	1.9 (1.5-2.5)
Centrifugation speed (rpm)	1021 (613-1551)
Anticoagulant volume infused to the donor (ml)	559 (306-697)
Time of procedure (min)	129 (102-169)
Volume of PMN leukapheresis (mt)	433 (348-699)
WBC content($\times 10^{10}$)	3.4 (0.8-8.3)
PMN content(%)	54.5 (23.7-92.9)
PMN content($\times 10^{10}$)	2.0 (0.35-5.8)
Volume of RBC (ml)	67 (13-196)

Note: mean values and range in brackets.

PMN mobilization consisted in G-CSF alone or combined with dexamethasone, approximately 12h prior to collection. PMN were collected by a continuous-flow centrifugation leucapheresis (COBE Spectra, Lakewood, CO, USA) adding a citrate dextrose solution A (ACD-A) as anticoagulant. The collection program for mononuclear cells (MNC) was used, appropriately modifying the separation parameters of the device. Donors were carefully monitored as respect to vital signs and occurrence of adverse reactions during the donation; calcium gluconate was infused i.v. continuously to prevent or minimize citrate toxicity. The collected PMN bag was maintained at room temperature, irradiated with 2500 cGy and infused within 6h after collection; AB0 typing and cross-match were performed before transfusion to ensure donor-recipient compatibility. **Results.** from 2002 we have performed 19 granulocyte apheresis, whose characteristics are detailed in table 2. We always obtained a PMN dose higher than 1×10^{10} except of 6 cases (31.5%), processing 2 fold the donor's blood volume in 2h on average. Only mild adverse effects (bone pain, headache, weakness) were observed. **Conclusions.** in our hands, the alternative strategy based on the MNC program adapted for PMN collection showed to be effective as well as highly tolerated and safe; this variant was able to preserve the donor from the risks related to sedimenting agents and provides adequate therapeutic doses of granulocytes.

0610

DOUBLE DOSE PLATELETPHERESIS INCREASES NEUTROPHIL ACTIVATION AND PLATELET-NEUTROPHIL COMPLEX FORMATION IN VOLUNTEER DONORS

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Background. and **Aims.** Platelet-neutrophil complexes (PNC) might play an important role in thrombotic and inflammatory diseases. It has been shown that during extracorporeal circulation, such as haemodialysis and cardiopulmonary bypass, platelets may form heterotypic aggregates with leukocytes via platelet CD62P and leukocyte $\beta 2$ integrins. There were conflicting results and limited data on the impact of plateletpheresis procedures on PNC formation and neutrophil activation on donors. In recent years, it has been possible to collect double dose platelets by new generation devices and there were no studies concerning the neutrophil activation and PNC formation on donors during double plateletpheresis (DP). **Methods.** In this study, we investigated the effects of DP with two different devices (Fresenius AS 204 n= 10 and MCS Plus n=22) on *in vivo* neutrophil activation and PNC formation in 22 volunteer donors. Peripheral blood samples were taken immediately before and after apheresis and on the 1th and 7th days. Changes in PNC formation and neutrophils activation was determined by quantitating the CD42b+ neutrophil counts and the amount of mean fleurosans intensity (MFI) of CD62L, CD54, CD50, CD11b/18, CD42b expressions by using a whole blood method on flow cytometry. **Results.** Statistically significant increases were found on 42b+ neutrophil (PNC formation) percentage and counts after apheresis with both machines. CD11b/18, CD50 and CD54 expressions (MFI) on neutrophils did not show any changes after apheresis with both devices. As a marker of neutrophil activation, CD62L expression (MFI) decreased significantly after apheresis with Fresenius machine on the first and seventh days but this was not seen with MCS+ Plus. **Conclusions.** Our results show that DP with Fresenius AS.TEC 204 and Haemonetics MCS+ devices results an increase on PNC formation which may be a risk factor for thrombosis and inflammation. However, clinical significance of these findings during apheresis procedures was still not known exactly.

0611

THE EFFECT OF DOUBLE DOSE PLATELET APHERESIS ON PLATELET ACTIVATION IN HEALTHY DONORS

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Background. and **Aims.** The wide diffusion of multicomponent collection in donor apheresis has led to the yielding of different components, such as double dose plateletpheresis (DP). **Methods.** In this study, we investigated the effects of DP with two different devices (Fresenius AS.TEC 204 n= 15 and MCS Plus n=15) on *in vivo* platelet activation in 30 male volunteer donors. Peripheral blood samples were taken immediately before and after apheresis and on the 1th, 7 th days. Activation of platelets was determined by quantitating the amount of platelet P-selectin (CD62) expression using a whole blood method on flow cytometry. **Results.** We concluded that the plateletpheresis procedure did not cause an increase in platelet activation in donors. On the contrary, circulating activated platelet counts were decreased significantly immediately and first day after apheresis that may be due to the selective collection of activated platelets in the collection bag or removal of activated platelets from circulation by adhesion to leukocytes or reticuloendothelial system. This decrease was not observed in donors who were not smokers. **Conclusions.** Although clinical significance of these findings are not known, further studies are needed to elucidate whether DP is with an increase risk for donors with frequent apheresis, history of thromboembolism or other factors such as smoking.

0612

DRY PLATELETS: QUALITY ASSESSMENT OF A NEW BLOOD COMPONENT

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Background. and **Aims.** transfusion of platelet concentrates obtained by apheresis is highly effective in thrombocytopenic patients affected with oncohaematological diseases. The advantages are essentially due to the reduced risk of multiple allogeneic exposure and transmissible infectious diseases together with the excellent WBC depletion and diminished transfusion reactions. The multicomponent collection technique is quickly growing up, permitting to obtain high quality blood components in one apheresis session and at the same time reducing the costs. Moreover, the availability of several synthetic solutions for platelets storage provides the possibility of preparing plasma-reduced (dry) apheresis platelets offering the advantage of minimising febrile nonhemolytic transfusion reactions, citrate toxicity and allowing ABO-incompatible platelets transfusion. The aim of this study was to test the quality of dry platelets (DryP) in comparison to standard plateletpheresis (SdP) concentrates. **Methods.** the multicomponent apheresis procedures were performed by the single-needle Cobe Trima collection device (Gambro BCT, Lakewood, CO, USA) collecting either DryP or SdP. Within 1h after collection, the bag containing DryP was added with appropriate amount of solution for platelets storage (SSP, MacoPharma) so that platelets were suspended in 70% synthetic medium and 30% autologous plasma. Both DryP and SdP were stored at room temperature with gentle agitation for 4 days. For both concentrates, platelets yield was calculated and *in vitro* studies of membrane glycoproteins expression and aggregation were carried out. Finally, the corrected count increment(CCI) was evaluated 24 hours after transfusion. **Results.** DryP versus SdP yield is shown in graphic 1, as mean value as respect to the time of storage. A comparison between DryP and

SdP in terms of ability to aggregate *in vitro* and membrane glycoproteins expression is reported in table A and B. CCI was found higher than 6x10⁹/L on average and no adverse reactions were registered for both types of plateletpheresis transfused. **Conclusions.** although the results of *in vitro* testing documented a major activation of dry platelets, this new blood component revealed to be as effective as standard plateletpheresis *in vivo*. Furthermore, the availability of dry platelets leads to an improved transfusion safety and to a great flexibility in blood products supply by the transfusion service.

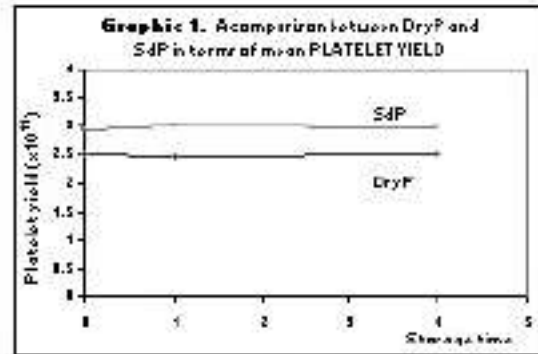


Table A. In vitro aggregation versus different stimuli (mean/percentages values).

	At collocation		Day + 1		Day + 4	
	SdP	Dry P	Sd	Dry P	PSd	Dry P
			Collagen 4 µg/mL			
93	88		97	82	80	53
			ADP 100 µM			
32	11		24	16	16	5
			Ristocetin 1.5 µg/mL			
91	97		77	81	71	52
			Collagen 10 µg/mL + Adrenaline 10 µM			
98	95		93	94	93	80
			ADP 10µM + Adrenaline 10 µM			
87	72		76	61	57	30
			Trap 25 µg/M			
94	94		88	83	87	70

Table B. Expression of membrane glycoproteins (mean/percentages values)

	At collocation		Day + 1		Day + 4	
	SdP	Dry P	Sd	Dry P	PSd	Dry P
			GPI b IIb (MFI)			
5.06	5.75		6.31	6.13	5.26	4.54
			GPIIb - IIIa (MFI)			
35.47	36.71		34.61	37.7	37.76	40.9
			GP IV (MFI)			
11.49	11.4		11.67	10.28	11.65	11.38
			GP 53			
24.23	27.11		21.74	25.91	21.46	31.84
			GMP 140			
21.79	29.29		22.65	30.38	20.58	34.89

0613**MULTIPLE RED CELL APHERESIS IN THERAPY OF HEREDITARY HEMOCHROMATOSIS**

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Background. Hereditary hemochromatosis is one of the most common inherited disorders in which an excessive amount of iron is absorbed from the diet and then deposited in organs. The effective treatment is the regular whole blood removal which causes erythropoiesis activation and leads to decrease of iron stores. Red cell apheresis is an optional method for removing of higher amount of erythrocytes in one session. **Methods.** We performed 262 red cell apheresis in 15 patients with diagnosis of hereditary hemochromatosis (14 x C282Y homozygotes, 1x C282Y + H63D heterozygote) using Haemonetics MCS 3p cell separator (protocol TAE) in which red cells are removed from patients in 2 - 5 cycles; plasma and buffy-coat are reinfused. Collection time, donor convenience, side effects and red cell yield were recorded and analysed. Samples for hematology and iron studies in patients were drawn, analyzed and compared to baseline levels. **Results.** 262 (6 - 70) red cell apheresis in 15 patients (12 male, 3 female), age 47,8 (32 - 62), height 177,4 cm (160 - 190), weight 81,9kg (55 - 110), TBV 5230ml (3627 - 6501). Procedure time was 32 - 87 min. Mean Hb level decreased from 144,7 g/L (129 - 155) before the procedure to 121,6 g/L (107 - 130). Ferritin values decreased from 1227 ug/L (268 - 3998) to less than 20 ug/L (7 - 18) in each of patients. **Conclusions.** Procedures were well tolerated by patients, no serious side effects were seen, 19 mild citrate reactions (7,3%) were noted. Red cell apheresis is an effective procedure for lowering of iron stores in patients with the hereditary hemochromatosis. Decrease of iron stores in patients is individual and depends on many factors.

Supported by research project MZO 00179906

0614**RETENTION OF RBC VIABILITY AFTER PATHOGEN INACTIVATION WITH S-303: USE OF A HYPERIMMUNE (ANTI-S-303) RABBIT TRANSFUSION MODEL**

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Introduction. S-303 treatment of RBC inactivates bacteria, viruses, parasites and contaminating leukocytes. Pre-clinical animal experiments showed no evidence of antibodies (Ab) against S-303 RBC (original process; O-SRBC). In human clinical trials, transfused O-SRBC exhibited normal viability and supported acute surgical anemia comparably to control AS-1 RBC (CRBC). However, repeated transfusions of O-SRBC into 2 patients with chronic anemia resulted in positive cross match tests indicating the presence of Ab to O-SRBC. The S-303 process was modified (M-SRBC) to reduce S-303 RBC membrane binding and therefore immunoreactivity, with retention of pathogen inactivation and *in vitro* RBC function. Aim In contrast to the experience with human O-SRBC in the two patients supported with chronic RBC transfusions, repeated transfusion of rabbit O-SRBC into naïve rabbits failed to induce formation of Ab to O-SRBC. A hyper-immune, rabbit transfusion model was developed to assess the viability of O-SRBC and M-SRBC in the presence of circulating anti-S-303 Ab. **Methods.** High titer ($\geq 1:1000$) Ab to S-303 were induced in New Zealand white rabbits after repeated immunization (days 1, 8, 15, 36, 64) with KLH-S-303 hapten (KLH-hapten) in complete Freund's adjuvant. Ab titers against SRBC were determined by gel card agglutination, or by FACScan with FITC-Goat_anti-rabbit_IgG. Survival of infused biotinylated RBC (4 mL/kg; SRBC or CRBC) mismatched to a major antigen (HgD), was measured. Blood samples were taken 1,3,7,15,21 and 28 days after transfusion and analyzed by FACScan using Streptavidin_PE to determine the proportion of circulating biotinylated RBC. O-SRBC were pre-

pared by the simultaneous addition of 200 μ M S-303 and 2 mM unbuffered GSH. To reduce non-specific protein interactions, M-SRBC were prepared by the sequential addition of 20 mM neutralized GSH, followed by 200 μ M S-303. **Results.** High titer anti-S-303 Abs were induced by KLH-hapten immunization in 2 of the 4 rabbits in group 1 (G1) and all rabbits in group 2 (n=6; G2). Rabbits treated with KLH-hapten developed comparably high Ab titers. The two groups of rabbits were transfused with biotin-labeled rabbit CRBC (G1), or O-SRBC (G2). All rabbits (G1 & G2) exhibited no change in hematocrit or body weight and maintained normal vital signs. KLH-hapten immunization, as expected, did not affect the viability of CRBC in G1 rabbits with or without high titer S-303 Ab. Transfusion of biotin-labeled O-SRBC demonstrated reduced survival in hyper-immune G2 rabbits vs. biotin-labeled CRBC survival in G1 rabbits. After clearance of biotin-labeled O-SRBC, G2 rabbits were transfused with biotin-labeled M-SRBC. Survival of M-SRBC in all G2 rabbits was equivalent to CRBC survival in G1 rabbits. **Conclusions.** In contrast to the naïve rabbit model, the hyper-immune model was capable of detecting accelerated clearance of O-SRBC. In contrast, M-SRBC retained normal viability even in the presence of high titer anti S-303 Ab. The modified S-303 RBC process offers the potential for pathogen inactivation with elimination of immunoreactivity and retention of RBC viability.

0615**INTERCEPT PLASMA RETAINS A BROAD SPECTRUM OF HAEMOSTATIC PROTEINS WITHOUT ACTIVATION OF THROMBIN OR COMPLEMENT**

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Background. INTERCEPT Plasma (I-FFP) is prepared with the INTERCEPT Blood System for Plasma using amotosalen HCl and long-wavelength ultraviolet light to photochemically inactivate a broad spectrum of blood-borne pathogens in plasma intended for transfusion. Recently completed Phase 3 clinical trials, using a prototype plasma processing set, have shown that I-FFP retains proteins necessary for hemostasis in the treatment of acquired and inherited coagulopathies, and in support of therapeutic plasma exchange for TTP. A new processing set intended for commercialization has been developed to improve productivity while maintaining pathogen inactivation efficacy. The prototype set was used to treat approximately 250 mL of plasma, whereas the improved set accommodates up to 635 mL and results in up to three I-FFP doses per photochemical treatment. **Aims.** This study was designed to characterize hemostatic proteins in I-FFP prepared using the improved processing set. Proteins of interest included the vonWillebrand complex, endogenous inhibitors of coagulation, and markers of thrombin generation and complement activation in addition to components of the intrinsic, extrinsic, fibrinolytic, and contact factor pathways. **Method:** Six fresh jumbo (600 mL) apheresis plasma units, collected in sodium citrate using the Haemonetics PCS device, were photochemically treated. Plasma samples, collected before and after photochemical treatment, were frozen below -60°C until batch analysis. Standardized clinical assays were used for all analytes. **Results.** are expressed as the proportional activity (%) remaining in I FFP relative to the activity in plasma prior to treatment(mean \pm SD). Retention of procoagulant factors in I-FFP plasma ranged from 77% to 92%. Factor VIII and vonWillebrand factor activity, antigen, cleaving protease activity (vWF:CP, ADAMTS-13), and multimeric composition remained within normal ranges after treatment. Endogenous inhibitors of coagulation were retained 93% to 100%. Plasminogen and alpha 2-antiplasmin were retained 94% and 78%, respectively. Retention of contact factors was variable; some factors were below the reference range prior to PCT. With the exception of TAT, all markers of coagulation activation were well within normal ranges. The TAT level in one I-FFP unit was slightly above the normal range; all other units had TAT levels that were within the normal range. **Conclusions.** Extensive *in vitro* analysis of coagulation proteins demonstrates that INTER-

CEPT Plasma is functionally similar to untreated plasma. The improved processing set, intended for commercialization, allows up to 3 doses of I-FFP to be produced from a single photochemical treatment.

Table. Retention of hemostasis-related proteins.

Analyte	% Retention	Analyte	% Retention	Analyte	%
Fibrinogen	77 ±3	Factor XI	81 ±8	α1-Antitrypsin	
Factor II	90 ±3	Factor XII	92 ±3	C1-esterase inh	
Factor V	92 ±2	Wf activity	93 ±6	C3a	
Factor VII	78 ±3	Wf antigen	98 ±4	C5a	
Factor VIII	78 ±5	WfCP	89 ±7	α2-Antiplasmin	
Factor IX	83 ±2	Antithrombin	97 ±4	Plasminogen	
Factor X	88 ±2	Protein C	93 ±4	HMWK	
Factor XI	87 ±2	Protein S	100 ±6	Prekallikrein	
D-dimers, F1.2, TAT, FVIIa, FXIa: See text. Wf multimers: Norm					

0616

INTERCEPT PLASMA: PROCESS VALIDATION STUDIES IN THREE EUROPEAN BLOOD CENTERS

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Introduction: INTERCEPT Plasma (I-FFP) is prepared as FFP for transfusion using a photochemical treatment(PCT) system with amotosalen (S-59) and long-wavelength UVA light to inactivate a broad spectrum of blood-borne pathogens. Recently completed Phase 3 clinical trials, supported with an inventory of approximately 10,000 I-FFP units processed at 6 U.S. blood centers in 250 mL units using a prototype processing set, demonstrated retention of coagulation factor activity and hemostatic function for support of patients with congenital and acquired coagulopathies or TTP. The clinical prototype I-FFP system has been modified to treat up to 635 mL of plasma in a single PCT process, yielding up to three 200 mL doses while maintaining pathogen inactivation efficacy. The effects of PCT on coagulation activity and yield using the modified process, intended for commercialization, were evaluated in three European blood centers under routine operating conditions. *Methods.* A total of 90 apheresis plasma units, each approximately 600 mL, were collected at Haukeland University Hospital (Bergen, Norway), the Etablissement Français du Sang (Strasbourg, France), and the Institute of Immunology and Transfusion Medicine, University of Lübeck, (Lübeck, Germany). Plasma was collected using Autopheresis C (Baxter Transfusion Therapies) or Haemonetics MCS devices. I-FFP units were prepared at each center by blood bank personnel using the modified PCT set. Baseline and I-FFP samples were collected, frozen below -60°C, and sent to Cerus for assay of factors I (fibrinogen), II, V, VII, VIII, IX, X, XI, and XIII, proteins C (PC) and S (PS), and antithrombin (AT). Alpha-2 antiplasmin (AP) was assayed by a reference laboratory. Comparative yield data for I FFP prepared using the prototype set for the Phase 3 studies were obtained by testing samples collected during PCT processing from approximately 325 randomly selected plasma units. Results (Table): I-FFP prepared with the modified system yielded 75-76% of baseline fibrinogen and FVIII activity, and 81-97% of baseline factors II, V, VII, IX, X, XI, XIII, PC, PS, AT, and AP. Coagulation factor activity is expressed in IU/dL for all factors

except fibrinogen (mg/dL). Yield of coagulation factor activity is expressed as a proportion (%) of pre-treatment(baseline) coagulation factor activity remaining after PCT. *Conclusions.* Using the improved processing set, coagulation factor activity and yield were similar to that of I-FFP used in clinical trials. The clinical trials demonstrated that I-FFP provided sufficient levels of coagulation factor activity for treatment of congenital and acquired coagulopathies and for therapeutic plasma exchange of TTP. The improved set, intended for commercialization, provides multiple I-FFP doses with a single PCT process.

	Activity, Modified Set		Yield	
	Baseline	I-FFP	Prototype*	Modified
Ff	296 ±90	228 ±52	78 ±7	78 ±7
FII	107 ±17	95 ±15	90 ±5	89 ±5
FV	130 ±25	128 ±24	95 ±4	97 ±4
FVII	113 ±25	92 ±21	82 ±5	81 ±4
FVIII	141 ±41	107 ±35	77 ±7	75 ±6
FIX	95 ±15	80 ±13	85 ±5	85 ±4
FX	112 ±18	100 ±17	90 ±5	89 ±4
FX	102 ±19	90 ±15	90 ±5	88 ±7
F XII	119 ±21	113 ±21	99 ±3	96 ±5
AT	100 ±12	95 ±12	92 ±2	96 ±3
PC	121 ±22	105 ±21	96 ±7	85 ±5
PS	103 ±22	95 ±22	99 ±5	97 ±7
AP	102 ±12	83 ±8	90 ±5	82 ±7

* FVII, AT n=34, PC, PS n=33, AP n=14; all others n=325

0617

ALLOIMMUNIZATION IN MULTITRANSFUSED PATIENTS

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Background. Pretransfusion testing is essential in order to investigate red blood cell (RBC) alloimmunization and to prevent alloimmune hemolytic anemia. *AIM:* A retrospective study was performed to estimate the frequency of alloimmunization against RBC antigens in multitransfused patients. *Methods.* A total of 372 patients (231 males and 141 females), mainly with blood disorders, admitted to our hospital within a 16-month period (from November 2003 to February 2005) were studied. All of them matched for ABO and Rh (D) antigens only and were considered as polytransfused, so long as they received more than 5 units of RBC with an average of 19.5 units (range 6-140). The mean follow-up testing consisted of 4.1 specimens per patient. RBC antibodies were screened with indirect anti-human globulin (IAT) and enzyme tests by a gel centrifugation method (ID Micro Typing System, DiaMed). In case of a positive serum the same test system was used for further antibody identification. Direct antiglobulin test(DAT) was performed only in specimens with positive screening tests by using ID cards with polyspecific and monospecific Coombs reagents. Patients who were transfused with up to 19 RBC units and those who received over 19 were correlated with antibody formation. For the evaluation of the significance of the results, we used the statistical method of chi-square test. *Results.* We identified red cell alloantibodies in 49 out of the 372 patients included in this study during the pretransfusion testing. The incidence of alloimmunization was 13,2%. Seventy-nine antibodies were observed in 49 patients. Anti-E was most frequently found (27.8%) followed by anti-D (13.9%), anti-Kell (11,4%), anti-C (10.1%), anti-Jk (a) (7.6%), anti-c (5.1%), anti-C (w) (5.1%) and further specificities in lower frequency. Twenty-eight out of the 79 antibodies (35%) became undetectable over time. The overall immunization risk was 1.02% per RBC unit transfused. Four patients formed Rh system antibodies after incompatible platelet transfusions. We divided the patients into five groups

with different potential for antibody production. The characteristics of patients, the number of transfusions and the occurrence of RBC antibodies among the 49 polytransfused patients are shown in the table below. No correlation with the alloantibody formation and the number of units transfused was observed ($p=0.167$). *Conclusions.* The frequency of immune red cell alloantibodies in our study population does not differ significantly from this observed in other selected groups of multi-transfused patients. Although the immunization rate is relatively high, the absence of any hemolytical reactions, due to the repeated pretransfusion testing make this procedure a safe and effective method in treating these patients.

Patient group	Incidence n (%)	Age (mean)	Sex (M/F)	pPCR patient	Antibodies		
					1	2	3
Hematology/Oncology	29 (518)	66.4	19/7	28.9	12	7	4
Transplantation	6 (161)	37.6	5/1	39.2	6	0	0
Nephrology	4 (108)	74.3	2/2	10.3	4	0	0
Surgery	10 (268)	51.6	8/2	22.7	5	2	3
Other	6 (161)	68.5	5/1	11.3	4	2	0
Total	49 (1317)	59.6	36/13	24.3	31	11	7

Myeloma - Biology II

0618

THE DISTRIBUTION OF THE D VARIANTS AND WEAK D PHENOTYPES OF RHESUS BLOOD GROUP SYSTEM IN THE GREEK POPULATION

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The Rhesus (Rh) blood group system is involved in the newborn's hemolytic disease, transfusion reactions, and autoimmune hemolytic anemia. The D antigen of the Rh system is now considered to be a mosaic of epitopes. Among Europeans, 1% carries RHD alleles as weak D and partial D. These different phenotypes have distinct immunohematologic characteristics. From January 2003 to December 2004, 3908 blood donors were studied in regard to their ABO groups and Rhesus type, in our laboratory. Data were collected for their age and ancestry. From the 3908 individuals, nine with Rh phenotypes Cce, ce, Cce, Cce, Cce Cce Cce Cce Cce, were characterized as weak D [ID-Card Anti-D (human), DiaMed]. There were further investigated using ID-Partial RhD-Typing Set and genotyped with the use of PCR-SSP technique (weak D-SSP, INNO-train). Genomic DNA was extracted from whole blood collected with EDTA and six RhD-specific primer sets, were used. The PCR products were detected on the gel stained with ethidium bromide, under UV visualization. Five of them were genotyped as weak D type 1, two as weak D type 3 and two of them couldn't be typed with those primers and need further investigation. Molecular classification of weak D offers a more reliable basis than serotyping and is relevant for optimal D transfusion strategies.

0619

MEASUREMENT OF PLASMA CELL PROPIDIUM IODIDE (PC-PI/CD 138) AND APOPTOTIC (PC-AI/CD 138) INDICES AND THEIR RELATION TO THE ACTIVITY OF MULTIPLE MYELOMA

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Objectives. Propidium iodide (proliferative) and annexin V/CD138 (apoptotic) indices vary during different phases of multiple myeloma. The aim of this study is to distinguish specific trends of this variation in course of the disease and to analyze the outcome of longitudinal monitoring for prognosis. *Methods.* Analysed group consists of 120 patients - at the time of diagnosis and different phases of multiple myeloma, some of them reaching remission (at least 50% decrease of monoclonal immunoglobulin, MIG, and less than 5% myeloma plasmocytes in the bone marrow), some being non-responders (less than 50% decrease of MIG or persisting bone marrow infiltration), or experiencing a new progression/relapse of the disease (increase of MIG more than 25%, or more than 25% increase of bone marrow plasmocytes). The patients were treated by standard chemotherapeutic schemes (regimen VBMCP, VAD, MP, CyVAD and CIDEX) or by high-dose chemotherapy supported by autologous peripheral stem cells transplantation. Plasma cell proliferative activity was measured using propidium iodide index (PC-PI/CD138), rate of apoptosis using the annexin-V index (PC-AI/CD138), followed by the method of flow-cytometry (DNA-Prep Reagents Kit, Coulter, software Multicycle fy. Phoenix). *Results.* In the group of patients at the diagnosis ($n=49$), that have reached remission (30 out of 49 patients), a significant decrease of PC-PI has been found in 87% (M: 2,9-2,1%, $p<0,0001$) and at the same time in 83% of them the values of apoptosis have increased (M: 4,7-8,6%, $p<0,0001$). In the non-responders (19 out of 49) PC-PI remained relatively high (M: 2,5-2,6%, $p=0,310$) while the average apoptosis showed even a significant decrease (M: 6,2-3,9%, $p=0,004$). In the group of patients with progression of the disease ($n=36$), the results have been much similar. Those, who have afterwards reached a new remission (17 out of 36), have also had in 94% a significant decrease in the values of PC-PI (M: 2,9-2,2%, $p<0,0001$) with the rise of apoptosis (M: 4,6-8,8%, $p=0,019$) in 76%. Those who had either minimum or no response to the therapy (19 out of 36) showed approximately stable values of both PC-PI (M: 3,0-2,8, $p=1,00$) and PC-AI (M: 5,0-4,7, $p=0,728$). Finally, the group of patients with remission of the disease, followed by progression/relapse ($n=35$), experienced a reverse trend-the rate of proliferation increased in 34 patients -97%, (M: 2,3-2,7%, $p<0,0001$) while the rate of apoptosis showed a significant decrease in 72% (M: 8,3-4,2%, $p<0,0001$). *Conclusions.* Measurement of cytokinetic parameters (proliferative and apoptotic index) of plasmocyte population during different phases of multiple myeloma takes a broad view of the disease, providing valuable information about the character of different populations of plasma cells. It can help us to predict the individual follow up of the disease course and to choose the optimum treatment option.

Founded by grant IGA CR MHCR NC 7503-3/2003

0620**BORTEZOMIB APPEARS TO OVERCOME THE POOR PROGNOSTIC IMPACT OF CHROMOSOME 13 DELETION IN PHASE 2 AND 3 CLINICAL STUDIES**

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Background. In patients with multiple myeloma, deletion of chromosome 13 (del[13]) by conventional cytogenetics is a poor prognostic factor associated with reduced survival. This adverse prognostic impact has been independent of the treatment approach (chemotherapy alone or followed by autologous or mini-allogeneic stem cell transplantation). However, del (13) did not predict decreased survival or response rates in the SUMMIT phase 2 trial with bortezomib (VELCADE®). The APEX phase 3 trial compared bortezomib with high-dose dexamethasone in patients with relapsed multiple myeloma. The patients receiving bortezomib achieved an improvement in survival over those on dexamethasone. Of 168 assessable patients, 24 (14%) showed del (13) by metaphase cytogenetic analysis. **Aims.** Our objective was to evaluate the effect of del (13) on response and survival following treatment with either bortezomib or dexamethasone in the APEX trial. **Methods.** A total of 669 patients with relapsed multiple myeloma were randomized to receive bortezomib 1.3 mg/m² twice weekly for 2 weeks of eight 3-week cycles followed by once weekly for 4 weeks of three 5-week cycles (n = 333) or high-dose dexamethasone 40 mg on days 1-4, 9-12, and 17-20 for four 5-week cycles followed by days 1-4 of up to five 4-week cycles (n = 336). To evaluate the impact of del (13) on survival, a matched-pair analysis comparing 21 of the 24 assessable del (13) patients with 41 patients with wild-type chromosome 13 was conducted. This matched-pair analysis was balanced for adverse prognostic variables, including type of treatment, patient age, and number of lines of prior therapy, as well as β_2 -microglobulin and albumin levels. **Results.** Among 62 evaluable patients in this matched-pair analysis, there was a significant decrease in survival among patients with del (13) (n = 21) compared with those without the deletion (n = 41), regardless of treatment (HR [95% CI] = 3.24 [1.27, 8.23], P = 0.0090). In patients receiving dexamethasone, detection of del (13) by metaphase cytogenetic analysis was associated with markedly decreased survival (HR [95% CI] = 5.72 [1.65, 19.81], P = .0020). In patients treated with bortezomib, however, del (13) was not associated with a difference in survival (HR [95% CI] = 1.23 [0.26, 5.74], P = NS) or response rate (25% del[13] vs 35%, P = NS).

0621**THE THALIDOMIDE ANALOGUE CC-4047 INCREASES THE EXPRESSION OF FCGR1 (CD64) ON NEUTROPHILS IN PATIENTS WITH MULTIPLE MYELOMA**

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A major limitation to the treatment of multiple myeloma by the thalidomide analogue CC-4047 (Actimid®, Celgene, Warren, NJ, USA) is the development of a grade 3/4 neutropenia. Decreases in neutrophil numbers might result from an increase

in apoptosis, enhanced removal from the circulation due to an abnormal expression of adhesion molecules, or to the binding of platelets. We, therefore, examined the phenotype of neutrophils from nineteen patients with multiple myeloma receiving 1-10 mg CC-4047, every other day (e.o.d.), for 28 days. CC-4047 induced substantial dose-dependent decreases in the neutrophil count and increases in the percentage of neutrophils that expressed CD64, but did not alter the expression of the adhesion molecules, CD11b, CD62L or CD162, or increase the binding of platelets. At 5-10 mg there were also significant reductions in the lymphocyte, monocyte and platelet counts. Eight patients receiving 1-5 mg CC-4047 e.o.d. 4-13 months later, had neutrophil counts similar to those on day 1 and dose-related increases in the percentage of CD64-positive neutrophils. We propose that CC-4047 affects leucocyte and platelet kinetics and expands a neutrophil subset that is primed possibly by Interferon-gamma.

0622**TUMOR-SPECIFIC SOMATIC MUTATIONS TARGETING BCL-6 IN MULTIPLE MYELOMA**

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Background. Somatic mutations target multiple loci in normal B-cells, and generally, but not invariably, localise to the germinal centre (GC). Mutations in Ig variable (V) region genes ensure affinity maturation, but the consequences of mutations impacting on the 5'- untranslated region (UTR) of the BCL-6 gene are less well understood. BCL-6 is a transcriptional repressor, which functions to regulate GC formation, and GC-B-cell survival and maturation. Mutations in BCL-6 occur in ~30% of normal GC B-cells and in a variable fraction of lymphoid malignancies. There are indicators however, from malignant B-cells that mutations in specific motifs in the 5'UTR of BCL-6 overtly regulate levels of gene expression, with relevance for tumor origins. The mutational mechanism may also, in part give rise to aberrant chromosomal translocations mapping to this locus. **Aims.** In order to probe true mutational events in multiple myeloma (MM), and their relevance for understanding the tumor cell of origin, we analyzed mutations in the 5'UTR of BCL-6 in malignant cells and compared them to germline polymorphisms in matched T-cells. **Methods.** DNA was isolated after MACS sorted CD138+ bone marrow cells from 4 MM and 1 MGUS patient. A 750 bp fragment of the BCL-6 5'UTR was amplified using a nested PCR strategy. Taq error rate was assessed in a comparable-sized fragment of the beta-globin gene amplified using identical PCR conditions. PCR products were cloned and individual colonies (5 or more for each case) sequenced. To identify polymorphisms of the BCL-6 gene, we amplified the 5'UTR using DNA isolated from sorted, circulating T-cells from the same patient. **Results.** Two mutations, delT520 and G397C were present in 4/5 and 2/5 cases, respectively, and have previously been reported in other B-cell tumors. These alterations were unequivocally identified as polymorphisms, as they could be found in clones obtained from myeloma cells and T-cells in each case. True mutations were identified in 3/5 cases. To distinguish these from Taq error, each mutation was required to be present in at least 2 individual myeloma clones and confirmed in an independent PCR reaction. Interestingly, biallelic identical mutations (T115A, G147A) could be identified in one case, with one allele identified by polymorphism, suggesting specific molecular constraints. A known recurrent mutation (C423G) was identified in another case. Whether recurrent mutations may be specific to MM will require a study of a larger number of cases, using the approach described here. We have preliminary evidence in lymphoma that proteins can bind individual mutational motifs in the 5'UTR of BCL-6 in lymphoma cells (Jardin F et al, unpublished data). Their functional outcome is currently under study. **Conclusions.** The level and pattern of somatic mutations in BCL-6 in MM confirm derivation from a GC B-cell,

which has undergone a comparable level of targeting at this locus as normal circulating memory B-cells harbouring BCL-6 mutations. Such mutations in myeloma clearly do not perturb BCL-6 function to prevent further maturation, as may be occurring in GC-lymphoma.

0623

FLUORESCENCE ACTIVATED CELL SORTING OF PLASMA CELLS FOR FURTHER CYTOGENETIC ANALYSIS

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Background. Multiple Myeloma (MM) is a malignant clonal neoplasia of plasma cells commonly resulting in overproduction of monoclonal immunoglobulins. The plasma cells are phenotypically characterised by a strong expression of CD38 and CD138 but can display an aberrant phenotype compared to normal plasma cells. Besides other markers, asynchronous expression of CD56 is reported in the majority of MM patients. Cytogenetic abnormalities, mostly evaluated by karyotyping and fluorescence in situ hybridisation (FISH), are considered to be the most important prognostic markers in MM. However the detection and characterization of genetic aberrations involved in MM can be hampered by the low proliferative index of plasma cells, the limited extent of bone marrow involvement or the limited proportion of cells bearing the abnormality. Preceding purification of malignant plasma cells may offer some important advantages like higher sensitivity and specificity. **Aims.** In order to overcome most difficulties associated with the detection and characterization of chromosomal abnormalities in MM patients, we developed a protocol for fluorescence activated cell sorting (FACS) of clonal plasma cells from the bone marrow. The protocol was optimised for subsequent cytogenetic and molecular analyses like FISH and comparative genomic hybridisation (CGH). **Methods.** Thirty-five bone marrow samples of MM patients at various stages of treatment and disease were processed. Plasma cells were purified by flow sorting using the FACS Aria (BD). Following red blood cell lysis, immunophenotyping of cells was performed by a five colour staining procedure, using the following mouse anti-human monoclonal antibodies: Igk-FITC, Igλ-PE, CD138-PerCP, CD56-PeCY7, CD38-APC (BD). Intracellular staining of light chains was performed using the Fix and Perm intracellular staining kit according to the manufacturers' instructions (Kaumberg, Austria). For CGH the protocol described by Franke et al. (Blood 2001) was followed and for interphase FISH studies commercial probes were used (Vysis). **Results.** Plasma cells were purified based on the clonal expression of Ig light chains within the CD138+/CD38++ plasma cell gate. The purity of the sorted cell population was determined by reanalysing a small aliquot of the sample by flow cytometry. A purity between 90-95% of the desired population could be obtained. In addition, a May-Grünwald-Giemsa staining of cells sorted directly on microscopy slides showed undoubtedly the typical plasma cell morphology. Subsequent two colour FISH analyses demonstrated clearly the presence of translocations (eg. IgH) and deletions (eg. 13q-) while these abnormalities were often undetectable or at the cut-off level on the corresponding smears. Furthermore, high molecular weight DNA suitable for DOP-PCR could be isolated and successfully used for CGH. **Conclusions.** By optimising the FACS protocol we were able to obtain an almost pure clonal population of plasma cells as confirmed by cell morphology and flow cytometry. In these sorted cell populations, chromosomal aberrations could be detected using FISH and CGH. These data clearly illustrate that the quality of sorted cells is suitable for further cytogenetic and molecular analyses in general. This could be particularly important for more sensitive techniques like micro-array analysis in which a pure homogenous sample is highly desirable.

0624

LACK OF MCL-1 EXPRESSION MAY ENHANCE THE ANTIMYELOMA EFFECT OF BORTEZOMIB WHICH IS INDEPENDENT OF BCL-2 EXPRESSION

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Background. The transcription nuclear factor-kappa B (NFkB) offers a significant survival potential in multiple myeloma (MM) cells. Bortezomib is a proteasome inhibitor, which has been found to be effective in MM in phase II and III studies. It inhibits degradation of I kappa B and hence sequesters cellular NFkB, blocking NFkB transcriptional activity. Bortezomib also enhances apoptosis in MM cells by directly decreasing anti-apoptotic proteins. Expression of antiapoptotic members of the Bcl-2 family (Bcl-2, Mcl-1, Bcl-XL) appears to be responsible for the resistance of multiple myeloma cells to different chemotherapeutic agents. Mcl-1 has been shown to be a major anti-apoptotic protein in MM that appears to regulate cell survival through the JAK/STAT pathway. Bax is a proapoptotic protein and its activation is regulated by bcl-2. **Aims.** The aim of this study was to evaluate the impact of the expression of anti-apoptotic (Mcl-1, and Bcl-2) and pro-apoptotic (Bax) proteins on response and survival of relapsed MM patients, who received bortezomib. **Patients/Methods.** We studied 9 patients with MM (6M/3F; median age: 58 years, range: 34-72 years) who had received more than 4 lines of treatment previously, including autologous stem cell transplantation and were given bortezomib. Six patients had IgG MM, while 1 patient had IgA, 1 non-secretory and one light-chain MM. Bortezomib was given at a dose of 1.3 mg/m², iv, in 3-week cycles, on days 1, 4, 8, and 11 of each cycle. Bone marrow trephine biopsies were assessed before treatment and after 4 and 8 cycles of treatment. The antibodies used for immunohistochemistry in the trephine biopsies were: Bcl-2 and Mcl-1 (Dako, Carpinteria, CA, USA), Bax (Immunotech, Fullerton, CA, USA) along with the standard antibodies to define myeloma cells, such as CD138, CD79a. **Results.** Four out of nine patients had achieved a partial response, while two patients had a minimal response, and one achieved a complete response according to EBMT criteria. Bcl-2 was strongly positive in all pre-treatment biopsy specimens. Two out of 9 patients were positive for Bax but the lack of Bax expression does not seem to influence response. All but two patients were positive for Mcl-1. The median time of follow-up was 25 months (range: 13-30 months). Five out of 9 patients are still alive. The two patients, who were Mcl-1 negative, are still alive after 26 months, while the average time of survival for the mcl-1 positive group was 12 months. **Conclusions.** The anti-myeloma effect of bortezomib is independent of Bcl-2 expression. Moreover, lack of Mcl-1 expression may render the cells more sensitive to bortezomib, something that is translated to a longer survival of the patients. The results have to be confirmed in a larger sample of patients.

0625

OSTEOPONTIN IS AN ANGIOGENIC FACTOR IN MULTIPLE MYELOMA PATIENTS

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Osteopontin (OPN) is a multifunctional bone matrix glycoprotein that it is involved in angiogenesis, cell survival and tumor progression. OPN gene expression by human cells is mainly regulated by the bone specific transcription factor Runx2 namely also CBFA1 (Runx2/Cbfa1). In this study we have investigated the potential production of OPN by human myeloma cells, its regulation and role in myeloma-induced angiogenesis. We show that human myeloma cells directly produce OPN and express its major regulating gene Runx2/Cbfa1. The activity of

Runx2/Cbfa1 protein in human myeloma cells has been also demonstrated. Moreover, we found that interleukin-6 up-regulates OPN production by myeloma cells and in turn OPN increases myeloma cell proliferation. In an 'in vitro' angiogenesis system we showed that OPN production by myeloma cells is critical for the pro-angiogenic effect of myeloma cells. rhOPN treatment stimulated vessel formation as compared to control and the conditioned medium (CM) of myeloma cell lines significantly increased vessel formation in comparison either with control or with VEGF treatment. On the contrary OPN-immunodepleted CM of myeloma cells had not a stimulatory effect on vessel formation and the presence of anti OPN Ab inhibited vessel formation induced by myeloma cells. The expression of OPN by purified bone marrow (BM) CD138+ cells has been also investigated in 60 newly diagnosed multiple myeloma (MM) patients, finding that 40% of MM patients tested expressed OPN. Higher OPN levels have been detected in the BM plasma of MM patients positive for OPN as compared to control subjects. A significant higher MVD was observed in the group of patients positive for OPN, (mean±SE: 29.1±0.7 vs. 17.55±0.37; p<0.01) and similarly, the number of microvessels per field was higher in OPN positive patients in comparison with OPN negative ones (mean±SE: 6.7±0.15 vs. 4.28±0.04; p=0.05). In conclusion our data highlight the direct ectopic production of OPN by human myeloma cells with a Runx2/CBFA1 mediated mechanism and the capacity of myeloma-derived OPN to stimulate angiogenesis *in vitro*. In addition OPN has been detected in a subset of MM patients with higher BM angiogenesis suggesting its potential involvement in pathophysiology of MM-induced angiogenesis.

0626

PIGMENT EPITHELIUM-DERIVED FACTOR (PEDF) INHIBITS MULTIPLE MYELOMA THROUGH ANTIOXIDANT CAPACITY

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Interaction of multiple myeloma (MM) cells and bone marrow stromal cells (BMSC) plays a crucial role in the pathogenesis of multiple myeloma (MM) cells. IL-6 secreted by BMSCs promotes proliferation and survival of MM cells. IL-6 also enhances the vascular endothelial growth factor (VEGF) production of MM cells, which in turn promotes secretion of IL-6 in BMSCs. Thus VEGF is both an autocrine growth factor and IL-6 mediated paracrine growth factor of MM cells. VEGF is also angiogenic factor as increasing microvessel density in the BM of MM patients. VEGF stimulates Rac1 dependent NADPH oxidase to produce reactive oxygen species (ROS), which is an important factor for angiogenic response. Pigment epithelial-derived factor (PEDF), one of serine protease inhibitor superfamily, is the most potent inhibitor of angiogenesis in the mammalian eyes. Recently, we reported that PEDF effectively inhibits growth factor-induced ROS generation by suppressing NADPH oxidase. Accordingly, we hypothesized that PEDF exhibits anti-MM effects by reducing ROS generation. We first examined the role of VEGF- and IL-6-induced ROS generation in MM cells proliferation by using the fluorescent probe CM-H2DCFDA and measuring H3-thymidine incorporation. We found that VEGF promotes ROS generation more effectively than IL-6 stimulation in both RPMI 8226 and U-266 MM cell lines. VEGF- and IL-6-induced MM cells proliferation and ROS generation is completely abrogated by treatment of antioxidant, glutathione peroxidase mimetic ebselen. Then, we examined whether ROS generation is associated with Myeloid cell leukemia 1 (Mcl-1), an antiapoptotic member of the Bcl-2 family, which was shown to be regulated by VEGF as well as IL-6, and necessary for MM cell proliferation. VEGF- and IL-6-induced Mcl-1 up-regulation is also inhibited by antioxidant ebselen in consistent with ROS generation. Next, we examined whether PEDF affects on MM cells proliferation and ROS generation to probe the effect of PEDF on MM. We found that PEDF

alone prevented MM cell proliferation and decreased ROS generation. These PEDF inhibitory effects occurred more prominently on VEGF stimulation, whereas lesser on IL-6 stimulation. IL-6 activates Janus kinase (JAK)/signal transducer and activator of transcription3 (STAT3) pathway following to up-regulation of Mcl-1. Phosphorylation of STAT3 by IL-6 was scarcely decreased with PEDF treatment. These data suggest that PEDF exhibits anti-MM effects through suppressing NADPH oxidase, because NADPH oxidase is activated by VEGF, not but by IL-6. Furthermore to investigate the therapeutic role of PEDF in human MM, we are analyzing the effects of PEDF on patient MM cells for VEGF and IL-6 dependent proliferation and survival. In conclusion, our data provide that oxidative stress derived from ROS generation plays principal role in pathogenesis of MM. Moreover PEDF may be putative endogenous inhibitory factor against human MM, and administration of PEDF could be a novel therapeutic way for MM.

0627

CLINICAL SIGNIFICANCE OF HEPATOCYTE GROWTH FACTOR, PLATELET DERIVED GROWTH FACTOR-AB AND TRANSFORMING GROWTH FACTOR-ALPHA IN BONE MARROW AND PERIPHERAL BLOOD OF MULTIPLE MYELOMA PATIENTS

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Angiogenesis is a process that plays an important role in growth and progression of cancer and there is growing evidence that neovascularization is important in hematological malignancies. Since an increased angiogenic potential has been identified in multiple myeloma, we simultaneously measured the levels of HGF, PDGF-AB and TGF- α by ELISA in both bone marrow and peripheral blood of 30 multiple myeloma patients and 10 healthy controls. The median age of patients was 58 (21 man, 9 woman) and 45 in healthy control group (5 man, 5 woman). According to Durie-Salmon classification 25 were in stage IIIa, 3 were in stage IIIb and 2 were evaluated as stage II myeloma. Three patients had extramedullary plasmacytoma. Median values of HGF were 4183.1 pg/mL and 1088.8 pg/mL, median values of PDGF-AB were 367.7 pg/mL and 330.6 pg/mL, and median values of TGF- α were 44.7 pg/mL and 1.4 pg/mL, in bone marrow and peripheral blood serum of myeloma patients, respectively. Median values of HGF in healthy controls were 1897.2 pg/mL and 1452.6 pg/mL, PDGF-AB values were 224.4 pg/mL and 224.9 pg/mL and TGF- α values were 28.9 pg/mL and 5.4 pg/mL in both bone marrow and peripheral blood serum, respectively. There was significant difference between patients' and control groups' HGF-BM (HGF-Bone Marrow) values (p = 0.001). Detailed analysis of HGF, PDGF-AB and TGF- α according to disease stages, revealed that there was a significant correlation between stages and HGF-BM (p = 0.047), TGF- α -BM (p = 0.028) and PDGF-AB-PB (PDGF-AB-Peripheral blood) (p = 0.006), respectively. When the correlations between all of the parameters were analyzed, we found significant difference between TGF- α -PB and LDH (p = 0.02), TGF- α -PB and HGF-PB (p = 0.002), TGF- α -BM and CD38 (p = 0.046), TGF- α -BM and HGF-BM (p < 0.0001), TGF- α -BM and PDGF-AB-BM (p = 0.048), HGF-BM and HGF-PB (p = 0.044), PDGF-AB-BM and PDGF-AB-PB (p < 0.0001), respectively. Bone marrow HGF levels had a significant effect on survival times, with disease severity in terms of Stages (p = 0.0018, log-rank test). These data show that in myeloma patients, high values of HGF-BM, TGF- α -BM and PDGF-AB-PB were associated with advanced disease stages and especially HGF was playing more significant role in disease processing and related with disease severity. These findings also have led to the concept that there is a symbiotic relationship between the growth of myeloma cells and HGF, TGF- α and PDGF-AB in bone marrow.

0628

HETEROGENEITY OF CD45 EXPRESSION ON MYELOMA PLASMA CELLS IS RELATED TO VEGF EXPRESSION AND BONE MARROW ANGIOGENESIS

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Background. Multiple myeloma (MM) is mainly characterized by the accumulation of malignant plasma cells in the bone marrow. Despite their clonal origin, myeloma plasma cells may consist of subpopulations with discrete morphological and biological features. The heterogeneous CD45 expression on plasma cells is thought to illustrate this intraclonal diversity. CD45 negative phenotype corresponds to plasma cells with increased resistance to apoptosis, less sensitivity to interleukin-6, increased response to insulin growth factor-1 (IGF-1) signaling, a greater potential to circulate in blood and characterize cases with progressive or resistant disease. Recently, in the experimental mouse model 5T2MM, the increase in percentage of CD45 negative myeloma cells in the bone marrow was related to angiogenic switch. **Aims.** We evaluated the CD45 expression on bone marrow plasma cells in patients with MM and investigated its relation to the angiogenesis, the expression of vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFR) on plasma cells and the levels of VEGF in plasma. **Methods.** 28 patients with MM aged from 58 to 91 years old (median 68.8) were studied. Sixteen patients were at the first diagnosis, five patients were at remission and six patients presented relapsed or resistant disease. CD45 and intracellular VEGF expression was studied using three-color flow cytometry in bone marrow aspiration specimens. The expression of VEGFR on plasma cells was also analyzed using cytometry, after incubation of cells with biotinylated recombinant human VEGF and avidin-FICT. Bone marrow microvessels were identified using immunohistochemical staining for CD34 antigen. The microvessel density (MVD) was estimated from the average number of vessels in the three areas with the maximum number of vessels. The result was presented as number of vessels per high-power field (x400). The levels of VEGF in plasma were measured using ELISA. **Results.** The percentage of CD45- plasma cells was ranged widely from 0.5% to 98.5% (median 73.7%). Intracellular VEGF expression was identified in 26 out of 27 cases (96.3%). CD45- plasma cells were exhibited stronger VEGF expression compared to CD45+ plasma cells. VEGFR expression on plasma cells was found in all cases, regardless of the presence of CD45 antigen. Bone marrow MVD in patients was ranged from 6/HPF to 88.3/HPF (median 29/HPF) and was significantly higher compared to controls (median 10.5/HPF, $p=0.005$). A strong positive correlation was found between MVD and the percentage of CD45- plasma cells ($r=0.669$, $p<0.001$). VEGF plasma levels in patients ranged from 8pg/mL to 234pg/mL (median 47.2pg/mL) and were comparable to controls (range from 12pg/mL to 89pg/mL, median 64pg/mL, $p=0.39$). VEGF plasma levels did not relate to the percentage of CD45- plasma cells ($r=0.013$, $p=0.9$) or MVD ($r=-0.71$, $p=0.78$). **Conclusions.** Intracellular expression of VEGF in plasma cell was found in nearly all cases. CD45- plasma cells presented stronger VEGF expression, suggesting an intraclonal heterogeneity of VEGF production depended on the presence of CD45. The percentage of CD45- plasma cells in the bone marrow was also related to the extent of neovascularization. VEGFR expression on plasma cells was found in all cases, in spite of CD45 expression.

0629

FISH ANALYSIS OF SORTED PLASMA CELLS OF MULTIPLE MYELOMA SHOWS HIGH SENSITIVITY IN DETECTING RECURRENT CHROMOSOMAL ABERRATIONS

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Background. Multiple myeloma (MM) is characterized cytogenetically by 14q32 rearrangements, -13/13q-, and various trisomies. These chromosomal changes are known to be of prognostic significance. Conventional cytogenetic analysis is often hampered by the low mitotic index of plasma cells. Fluorescence in situ hybridization (FISH) may be limited by the low percentage of plasma cells in bone marrow of MM patients. The separation of plasma cells may improve the sensitivity of subsequent FISH analysis and might allow identifying additional chromosomal aberrations. **Aim.** The aim was to identify structural and numerical chromosomal aberrations of specific cell populations selected from bone marrow aspirates of MM patients. The selection was performed based upon phenotypical characteristics of cells. **Methods.** Plasma cells of bone marrow samples from 15 MM patients at various stages of disease were identified and sorted on a fluorescence-activated cell sorting (FACS) Aria (BD, US) using expression of CD138+/CD38+/CD56+ or -/cyIgL+. The high purity of the cells (mean 95%) was demonstrated by reanalyzing part of the cells by flowcytometry and microscopy. The high purity plasma cell suspensions were analyzed by dual-color interphase FISH using probes (Vysis) for: IgH, c-myc, t(11;14), t(4;14), bcl6, RB1 (del13), chromosome 8, 1q, 1p, 18q, 19q, 19p. **Results.** Different chromosomal aberrations could be demonstrated in the sorted cell suspensions. The most recurrent aberrations were 13q deletion, IgH rearrangement, gain of 1q, trisomy 19, monosomy 14, gain of 19p, loss of 19q. At least one genetic change could be identified in the sorted suspensions of each MM patient. Despite the high purity of investigated cells the percentage of cells bearing the abnormality varied from 10-80%. **Summary and conclusions** Recurrent chromosomal aberrations could be detected in sorted cells of all investigated MM patients indicating the higher prevalence than hitherto demonstrated by classical methods as well as the high sensitivity of the used approach. The low and different percentages of cells carrying an aberration showed that more than one clone could be identified within phenotypically pure MM cell populations. These findings demonstrate the importance of cell sorting prior to chromosomal analysis.

Myeloma - Clinical II

0630

MELPHALAN, PREDNISONE, THALIDOMIDE AND VELCADE (MPTV) COMBINATION THERAPY FOR RELAPSED MULTIPLE MYELOMAA. Palumbo¹, M.T. Ambrosini¹, C. Cangialosi², N. Pescosta², P. Pregno², P. Omedè¹, I. Avonto¹, G. Aitoro¹, F. Cavallo¹, P. Falco¹, S. Mirto², P. Coser², E. Gallo², M. Boccadoro¹¹A.S.O. S.Giovanni Battista-Osp.Molinette, TORINO, Italy; ²Italian MM Study Group, Italy

Background. Bortezomib (VELCADE™) is a new drug that is effective for the treatment of refractory multiple myeloma (MM). In vitro studies showed that VELCADE can restore sensitivity to Melphalan-resistant MM cell lines. [Ma, Clin Cancer Res. 2003]. In newly diagnosed patients, the addition of Thalidomide to the standard oral Melphalan/Prednisone combination significantly increased response rate and event free survival [Palumbo ASH 2004]. In heavily pretreated patients, the combination Melphalan plus Bortezomib induced a 67% of overall response rate (>25% M protein reduction) [Berenson

EHA 2004]; the combination VELCADE, Thalidomide and Dexamethasone 60% overall response rate [Zangari ASH 2004]. AIM: This study evaluates the efficacy and the toxicity of the association of Melphalan/ Prednisone/Thalidomide/VELCADE (MPTV) as salvage treatment in advanced relapsed/refractory myeloma patients. *Methods.* After 1 or 2 lines of treatment relapsed/refractory MM patients were enrolled in the trial. The MPTV regimen included oral Melphalan 6 mg/sqm administered on days 1-5, oral Prednisone 60 mg/sqm on days 1-5, Thalidomide 100 mg continuously, and VELCADE administered on days 1, 4, 15, 22 of each course, at 1 mg/sqm in the first 10 patients cohort; 1,3 mg/sqm in the second 10 patients cohort and 1,6 mg/sqm in the third 10 patients cohort. Each course was repeated every 35 days for a total of 6 courses. *Results.* Sixteen patients have been enrolled in this study, median age 63 years, 75% IgG 7% IgA 12% Bence Jones. Four patients were treated with MPTV as second line therapy, 12 patients as third line. Eleven patients received prior autologous transplant, 5 thalidomide based regimen and 7 conventional chemotherapy. Patients who received at least 1 course of MPTV were evaluated for toxicity and response. At time of evaluation, 3 patients received 1 entire course of MPTV, 4 patients 2 courses, 1 patient 3 courses, 1 patient 5 courses, 1 patient 6 courses. All this patients received VELCADE at dose of 1 mg/sqm. In evaluable patients, response rate (according to the EBMT criteria) was: 20% near complete response (1 patient after 2 courses, 1 patient after 6 courses), 30% partial response, 20% minimal response, 30% stable disease. Toxicities were evaluated according to Common Terminology Criteria (CTCAE ver.3.0). Grade 4 adverse events were 1 neutropenia and 1 thrombocytopenia. Grade 3 events were: neutropenia (6 patients), thrombocytopenia (3 patients), anaemia (1 patients), febrile neutropenia (1 patient), sepsis (1 patient), pneumonia (1 patient). The most common grade 2 non hematologic toxicities were: constipation (4 patients), sedation (1 patient), paroxysmal atrial tachycardia (1 patient). Among the 5 patients with baseline peripheral neuropathy, 4 patients remained stable, and 1 patient worsened (grade 2). Treatment-related neuropathy grade 1 developed *de novo* in 1 patient. *Conclusions.* Initial results showed that MPTV combination is a promising regimen for relapsed/refractory myeloma. Recruitment is ongoing, an update of the trial will be presented.

0631

THALIDOMIDE MAINTENANCE FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION PROLONGS PROGRESSION-FREE AND OVERALL SURVIVAL IN MULTIPLE MYELOMA - RETROSPECTIVE ANALYSIS OF 111 PATIENTS AT A SINGLE CENTRE

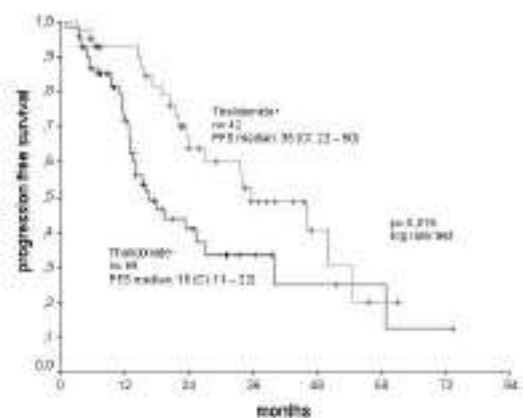
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Background. Autologous stem cell transplantation (ASCT) is standard of care of multiple myeloma (MM) patients up to age of 65 years, however, all patients relapse. Aim of the study: To test the hypothesis that thalidomide maintenance treatment improves survival after ASCT in MM. *Methods.* We retrospectively analysed data of all 111 MM patients transplanted in our unit between Jan.1996 and Dec.2004. During this period, our policy was to offer interferon (IFN) (3x3MU/week) maintenance to each patient; after January 2000 thalidomide (thal) + INF (200 mg/day) were offered. Finally, whether patients received IFN/thal or not depended on two factors: drug tolerance, and thalidomide availability. This 'randomisation' resulted in two groups of patients: one receiving, and the other not receiving thalidomide (thal+/thal-) according to the availability of the drug. We considered a patient thal+ if at least 3 months of 200mg/day thal was given following ASCT before progression

of MM. IFN+ was defined as at least 6 months interferon 3x3MU/weeks. There was no difference between the thal+ (42 pts) and thal- (69 pts) groups regarding prognostic factors. The median and (confidence interval) of thal+/- groups were as follows: age 53,8 years (43,4-65)/52 years (42-60,2), time-to-transplant from diagnosis 13,3 months (5,8-71)/13,7 months (7,5-45), LDH 350 IU/L (181-965)/402 IU/L (209-1001), albumin at diagnosis 46 g/L (37-56)/45 g/L (34-54) and beta-2 microglobulin at the time of transplant 1,54 ug/L (0,50-5,51)/1,66 ug/L (0,50-7,83), respectively. The two groups did not differ in the proportion of patients receiving IFN, or the median IFN dose. We compared the overall and progression free survivals of the two groups using the SPSS statistical program. *Results.* Patients receiving thalidomide maintenance following ASCT had a significantly better progression free (p=0,015) and overall (p= 0,0025) survival with a median follow-up period of 22 months (see Figure). *Conclusions.* 200mg/day thalidomide is tolerable and effective following ASCT. Post-transplant thalidomide maintenance seems to be advantageous for MM patients. Testing this hypothesis in a prospective randomised trial is warranted.

Progression free survival of autologous patients



0632

KOS-953 (A HEAT SHOCK PROTEIN 90 INHIBITOR) AS SINGLE AGENT OR IN COMBINATION WITH BORTEZOMIB IN PATIENTS WITH RELAPSED REFRACTORY MULTIPLE MYELOMA

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Background. KOS-953 is a novel Cremophor-based formulation of 17-AAG, a HSP90 inhibitor. It causes protein degradation and apoptosis of MM cells, including cells from patients resistant to BZ or immunomodulatory agents. Stress from BZ treatment can significantly up-regulate HSP90; by blocking HSP90 function, KOS-953 enhances MM cell sensitivity to BZ. In MM, two dose escalating trials of KOS-953 are in progress: single-agent and in combination with BZ. *Aims.* Define the toxicity and the recommended dose of KOS-953 when given as single-agent or in combination with BZ. Determine the PK of 17AAG and its active metabolite (alone and in combination with BZ). Evaluate biological activity of the combination. *Methods.* All

patients receive a one-hour IV infusion of KOS-953 twice weekly for 2 out of 3 weeks; in the combination trial, this is preceded by BZ. In both trials, PK and PD are performed following the 1st and 4th infusion. PD is assessed in bone marrow aspirates (myeloma cells are examined for apoptosis, proliferation, pAKT, and change in HSP, IL-6 and IGF-R1 expression) and PBLs (change in HSP70/90 levels). In the combination trial, PBLs are examined for changes in proteasome 20S function. **Results.** Enrollment and dose groups are presented below. Single-agent trial: dose limiting toxicity was observed in one patient at 220 mg/m²: a pt with liver infiltration of plasma cells had a Grade 3 elevated ALT. No DLT was observed at 275 mg/m². DLT has not been observed to date in the combination trial. Drug-related toxicities are similar for the two trials, consisting of Grade 1-2 elevated transaminases, nausea, fatigue, diarrhea, anemia, myalgias and rash (thrombocytopenia was also observed in the combination trial). Elimination half-life (parent) equals 2.4±1.0 hours (5.4±1.4 hours for the metabolite). Clearance (parent) equals 50.2±12.2 L/hr; V_{ss} 147±40 L. At 220 mg/m²: AUC_{tot} and C_{max} were 9553 ng/mL*h and 4623 ng/mL (parent); 4852 ng/mL*h and 789 ng/mL (metabolite). BMAs at 220 mg/m² showed increased apoptosis (determined by mitochondrial potential) comparing screening and Day 11 (p=0.01). PBLs showed reactive induction of HSP70 following KOS-953 treatment. Evaluation of 20S proteasome function in the combination trial showed a 40% decrease at 0.7 mg/m² BZ at the end-of-infusion. In the single-agent trial: 1 patient had a MR at 150 mg/m² and 1 patient had an MR at 220 mg/m² (93% reduction in kappa light chain). Combination trial: 1 BZ-refractory patient had stable disease (continuing in cycle 6) at the first dose level; one patient with a minor response (100% reduction of urine M-protein; 31% reduction in serum IgA) at the second dose level. **Conclusions.** Dose escalation in both trials continues. KOS-953 demonstrates signs of activity as single-agent in heavily pre-treated patients, and in combination with BZ in patients in whom BZ had previously failed. PK parameters for KOS-953 at these doses are comparable to the 17-AAG formulation utilized by the NCI.

Monotherapy Trial			Combination Trial			
47/12 M; median age 62 yrs; HPS 90; prior regimens 4, range 2-19			37/36; median age 71 yrs; HPS 90; prior regimens 4, range 2-6			
Dose Level	KOS-953 mg/m ²	# pts	Dose Level	BZ mg/m ²	KOS-953 mg/m ²	# pts
1	150	4	1	0.7	100	3
2	220	9	2	1.0	100	3
3	275	3	3	1.0	150	In progress
4	340	In progress				

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BORTEZOMIB (VELCADE®-V) IN COMBINATION WITH PEGYLATED LIPOSOMAL DOXORUBICIN (D) AND THALIDOMIDE (T) YIELD HIGH RESPONSE RATE IN PATIENTS (PTS) WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA (MM)

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Background. The proteasome is an established target for anti-cancer therapy. Modulation of the proteasome activity can sensitize as well as overcome resistance to chemotherapy. Orłowski et al recently reported enhanced clinical activity of pegylated liposomal doxorubicin when combined with bortezomib, a proteasome inhibitor, in pts with hematologic malignancies. T alters the cytokine milieu and acts as an antiangiogenic as well as a non-specific immunomodulating agent with clinical activ-

ity in MM. We and others have shown increased antitumor activity of T when combined with V or an anthracycline based regimen. In a phase II clinical trial we investigated a new dosing schedule of V with D together with continuous dose of T as a salvage regimen for pts with relapsed or refractory (rel/ref) MM. **Aims.** of this study was to assess the efficacy of the VDT combination in pts with rel/ref MM. **Methods.** All pts with rel/ref MM were eligible for this study. V was given at 1.3mg/m² (D1, 4, 15, 18), D at 20mg/m² (D1, 15) every 4 weeks with daily T (200 mg) for 4-6 cycles. SWOG criterion was used for response evaluation. Low-dose coumadin (1-2 mg) was used for prevention of venous thromboembolism (VTE). **Results.** All pts are evaluable for toxicity and 14 of 21 pts are evaluable for response. Three pts did not complete therapy and are deemed not evaluable (1 pt died of sepsis prior to completing 1 cyc and 2 pt with PR after 1 cyc were taken off study for non-compliance) while 4 pts are too early in the course of treatment for response assessment. Objective clinical response (CR+PR+SD) was noted in 11 (78%) pts, with 2 CR (14%), 6 PR (42.8%) and 3 SD (21.4%). Toxicity: Most commonly reported adverse event was Grade I/II fatigue (n=8) and constipation (n=5). Grade III/IV thrombocytopenia and neuropathy was noted in 5 (23.8%) and 2 (9.5%) pts respectively. Two pts developed Gr. II plantar-palmar erythrodyesthesia (PPE), 1 had Gr. III cellulites and 4 had Grade I/II sensory neuropathy. No VTE was noted. **Conclusions.** VDT is a new non-steroid regimen that is well tolerated and has high response rates in pts with rel/refr MM. Responses were noted despite prior failure to steroids, thalidomide, bortezomib and D. VTE or neuropathy are not limiting.

No. of patients	21
Median age (range)	56 (44-80)
Sex	9M, 12F
Median B2M (range)	3.8 (1.7-81.1)
Median prior therapies (range)	3 (1-7)
Immunoglobulin type	
IgG	13
IgA	3
LC	3
Non-secretory	2
Median % of Bone marrow involvement (range)	70 (5-90)
Refractory disease	15 (71%)
Prior thalidomide	12 (57%)
Prior Denamethasone	19 (90.4%)
Prior Adriamycin	14 (66%)
Prior Transplant	9 (28.5%)
Prior velcade	4 (19%)

0634

PREDICTIVE VALUE OF MINIMAL RESIDUAL DISEASE (MRD) QUANTITATIVE DETECTION IN MULTIPLE MYELOMA (MM) PATIENTS IN COMPLETE REMISSION AFTER STEM CELL TRANSPLANTATION

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Background. Molecular evaluation of minimal residual disease (MRD) following high-dose therapy and stem cell transplanta-

tion (SCT) for Multiple Myeloma (MM) can be performed by means of patient-specific PCR for IgH rearrangement. Attainment of sustained molecular complete remission (MCR) was previously reported to offer an advantage in terms of prolonged event-free survival and reduced risk of relapse in comparison with persistence of molecularly detectable MRD. Within this latter group the use of quantitative methods of molecular analysis could be of value in identifying patients with different residual tumor burden and, consequently, different risk of relapse. **AIM** In the present study we developed a simple and reliable assay to quantify MRD in bone marrow samples obtained from patients who received either autologous or allogeneic SCT for symptomatic MM. **Methods.** By using Real-time PCR, we used SYBR Green chromophore as 'universal probe' and we adopted the comparative Ct method to determine the amount of MRD. More specifically, the number of IgH copies detected in the diagnostic sample was set to 100% and the value of the post-transplant samples was given as a percentage of the sample taken at baseline (IgH ratio). **Results.** We performed a retrospective quantitative analysis of 71 BM samples from 12 patients; all of whom were persistently PCR positive by qualitative monitoring of MRD. With a median follow-up of 24 months, 4 patients out of 12 showed signs of relapse after 20 to 52 months (1 after an allogeneic SCT and 3 following an autologous transplantation), while the remaining 8 patients were still in CR (1 after an allogeneic SCT and 7 following an autologous transplantation) after 35 to 69 (median 57 months). **Results.** of our analysis showed a statistically significant difference in IgH ratio values between patients who relapsed and those who remained stable (mean ln IgH value = -0.81 vs. 0.86, respectively; $p = 0.018$). In order to obtain a cut-off value that could predict for relapse, we subsequently evaluated our results by applying a Bayesian approach. The objective of this analysis was to obtain a Positive Predictive Value (PPV), which is the likelihood of relapse for an individual patient with a positive test. A cut-off value of 2.1 was identified. The sensitivity of our test was 100%; in fact all relapsed patients overcame the cut-off IgH value. Nevertheless, the specificity of the test was 75%, as two patients in CR overcame at least once the cut-off value (resulting false positive): therefore, the PPV was 0.67, which actually means that the probability of relapse will be 67%, when the IgH cut-off value has been overcome during follow-up. **CONCLUSION** These results need to be validated by the analysis of a larger series of patients. If confirmed, IgH ratio could provide a simple and reliable method for predicting early relapse after SCT for MM.

Supported by Università di Bologna, Progetti di Ricerca ex-60% (M.C.); Ministero dell'Università e Ricerca Scientifica (MIUR), progetto FIRB, RBAU012E9A_001 (M.C.); and Fondazione Carisbo.

0635

RESPONSE TO PRIMARY THERAPY FOR MM WITH THALIDOMIDE-DEXAMETHASONE IS NOT ADVERSELY AFFECTED BY T (4,14) AND DEL (13)

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Background. Multiple Myeloma (MM) is a plasma cell (PC) neoplasm characterized by a profound genomic instability. In spite of this karyotypic complexity, several recurrent genomic abnormalities have been identified. These abnormalities define specific subgroups of MM patients and are supposed to be involved in the pathogenesis of the disease. In this context, it has been shown that one of these subgroups, involving at least one-half of MM patients, carries at diagnosis translocations involving the immunoglobulin heavy-chain (IgH) locus and/or deletion of chromosome 13 (del (13)). Among IgH translocations, t(4;14)

(p16;q32) is one of the most commonly reported. Both del (13) and t(4;14) have a poor prognostic relevance and are often associated at diagnosis. Translocation t(4;14) results in the production of a chimeric fusion transcript between MMSET and IgH, and, in about 70% of t(4;14) positive cases, it leads to the activation of the fibroblast growth factor receptor 3 (FGFR3). **AIM:** We investigated the frequency of t(4;14) and del (13) in a series of 52 previously untreated patients with symptomatic MM who received first-line remission induction therapy with thalidomide and dexamethasone in preparation for subsequent autologous transplantation. The relationship between these chromosomal abnormalities and response to treatment was also analysed. **Methods.** For this purpose we isolated the CD138+ plasma cell fraction from the bone marrow taken at diagnosis from MM patients. We analysed 1) the presence of t(4;14) by RT-PCR of the hybrid transcript MMSET/IgH, 2) the over-expression of FGFR3 by Real-time RT-PCR, and 3) the presence of del (13) by FISH analysis. The relationship between these two chromosomal abnormalities and response to thalidomide-dexamethasone was also investigated. **Results.** Translocation t(4;14) was detected in 15/52 patients (29%). Among these patients, 10/15 (67%) displayed both MMSET/IgH fusion gene and FGFR3 over-expression, thus supporting the discrepancy between MMSET/IgH positivity and FGFR3 over-expression. Del (13) was detected in 19/47 patients who could be evaluated (40%). Patients with translocation t(4;14) were more likely to carry also del (13) than t(4;14) negative patients (64% vs.31%, respectively; $p=0.05$). Patients with translocation t(4; 14) and/or del (13) had the same probability to respond to thalidomide-dexamethasone remission induction therapy (69%) than patients who lacked these unfavourable karyotypic abnormalities (51%). **Conclusions.** The frequency of t(4;14) in our series of newly diagnosed MM patients was 29%, a value higher than that found in other series reported so far. At the opposite, the presence of del (13) was consistent with data from the literature. Translocation t(4; 14) and/or del (13) had no adverse influence on response to primary therapy with thalidomide-dexamethasone which can thus be considered as a valid treatment option in preparation for autologous transplantation in preparation for autologous transplantation even for patients with adverse chromosomal abnormalities.

Supported by Università di Bologna, Progetti di Ricerca ex-60% (M.C.); Ministero dell'Università e Ricerca Scientifica (MIUR), progetto FIRB, RBAU012E9A_001 (M.C.); and Fondazione Carisbo.

0636

ARSENIC TRIOXIDE WITH ASCORBIC ACID AND HIGH-DOSE MELPHALAN: A NEW PREPARATIVE REGIMEN FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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Background. High-dose (HD) melphalan followed by an autograft produces a CR rate of 40% and a median event-free survival of 24 to 36 months in patients with multiple myeloma. Arsenic trioxide (ATO) + ascorbic acid (AA) enhance the anti-myeloma activity of melphalan. Based on these preliminary data, we initiated a phase I/II study of ATO + AA + melphalan as conditioning regimen for patients with multiple myeloma undergoing an autologous transplant. **Aims.** To evaluate the safety and efficacy of ATO in combination with AA and HD melphalan in multiple myeloma. **Methods.** Twenty patients with secretory myeloma (10 males, 10 females; age 49-68 years) were treated between 4/04 and 11/05. All patient received melphalan 200 mg/m² IV over 2 days and ascorbic acid 1000 mg/day IV x 7 days. They were randomized to no ATO (arm 1), ATO 0.15 mg/m² IV x 7 days (arm 2) and ATO 0.25 mg/m² IV x 7 days (arm 3). Six patients had a prior autograft. **Results.** Patients were randomized between 3 arms (7 in arm 1, 6 in arm 2 and 7 in arm 3). With a median follow up of 108 days post-autograft, no dose-limiting toxicity or non-relapse mortality was seen. Median time

to neutrophil engraftment (ANC >500/dL) was 9 days. No graft failures or delays in engraftment were seen in the ATO arms. Sixteen of the 20 patients (80%) achieved a response (CR + PR), with no significant difference in responses between the three arms. The impact of ATO on melphalan pharmacokinetics is being analyzed. *Conclusions.* Arsenic trioxide, in combination with ascorbic acid and high-dose melphalan, is safe as conditioning regimen for multiple myeloma. The impact of this combination on the outcome and melphalan pharmacokinetics is being evaluated.

0637

MARKED ACTIVITY OF VTD REGIMEN COMPRISING VELCADE (V) PLUS THALIDOMIDE (T) AND ADDED DEXAMETHASONE FOR NON-RESPONDERS IN ADVANCED AND REFRACTORY MULTIPLE MYELOMA (MM)

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Background. Velcade and thalidomide have been shown to be effective as single agents in patients with relapsing and/or refractory MM. *Aims.* A Phase I/II trial was initiated to determine the activity of the V+T combination in patients with advanced and refractory MM and to investigate the cumulative toxicities, especially neurotoxicity. *Methods.* V was given in group 1 at a dose of 1.0 mg/m² on days 1, 4, 8 and 11 every 21 days and, in the absence of > grade 2 neurotoxicity, T was added with the second cycle at dose increments of 50, 100, 150 and 200 mg daily in cohorts of at least 10 patients. A second group was then started at V 1.3 mg/m² with the addition of T in the same incremental dosing schedule. Dexamethasone was added as early as with cycle #4 if partial response (PR) was not achieved. PR was defined as ≥50% reduction in serum M protein or ≥75% reduction of urine M protein. *Results.* A total of 85 patients have been enrolled (V 1.0 mg/m² + T 50 mg, n=12; T 100 mg, n=10; T 150 mg, n=10; T 200 mg, n=14; V 1.3 mg/m² + T 50mg, n=11; T 100 mg, n=10; T 150 mg, n=12; T 200 mg, n=6). Patient characteristics included prior autotransplant in 81 patients, tandem transplants in 56; prior thalidomide exposure and resistance in 62; more than 5 year of prior therapy in 31; abnormal cytogenetics (CA) in 63 patients including deletion of chromosome 13 in 40 patients. Cumulative incidences of response according to different levels of M protein reduction are depicted in Table 1. 13% of patients obtained ≥PR after the first cycle with V alone, including 5% achieving ≥n-CR; maximum response was noted after completion of 3 additional cycles with combined V + T: 52% achieved ≥PR, 12% ≥ n-CR, and 68% had ≥25% M protein reduction. EFS at 12 months for all 85 patients was 37%; 40% for those achieving ≥PR versus 10% for the others (p = 0.0006); 51% for patients receiving V 1.3 mg/m² versus 26% for those on 1 mg/m² (p = 0.004). The quality of response and a higher dose of V (1.3 mg/m²), but not of T, were associated with better EFS (p=0.004), whereas cytogenetic abnormalities negatively affected EFS (p=0.04). On univariate analysis of 14 potentially relevant prognostic markers, prior therapy > 5 yrs was associated with improved EFS whereas prior T and cytogenetic abnormalities were associated with inferior EFS. On multivariate analysis, duration of prior therapy and prior thalidomide were confirmed to significantly affect EFS. The most common toxicity was myelosuppression (especially thrombocytopenia and neutropenia); grade ≥3 neurotoxicity occurred in 11% of patients. *Conclusions.* Collectively our data indicate good tolerance and activity of the VTD regimen in a very high risk patient population.

0638

COMBINED TC-99M SESTAMIBI (MIBI) AND TC-99M V-DMSA (V-DMSA) SCINTIGRAPHY IS CORRELATED WITH THE INTERNATIONAL STAGING SYSTEM (ISS) IN MULTIPLE MYELOMA (MM)

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Background. MIBI has been proposed as a safe and effective tracer in detecting active disease in patients with MM. V-DMSA has been shown to concentrate in some tumours as well as in inflammatory conditions. The combined use of MIBI and V-DMSA scans in patients with MM has been suggested, since the uptake of each radiopharmaceutical is dependant on different factors. According to ISS, serum β2-microglobulin (β2-M) and serum albumin (alb) were the dominant predictive factors and that different cut-offs of these parameters could separate patients into three stages with significantly different outcome. *Aims.* The purpose of this study was to assess the relationship between predictive factors of MM and the combined MIBI and V-DMSA scans. *Methods.* Twenty-three MM patients (12 females, 11 males, aged 63.6±14.2 years [mean± SD]) were included in the study. Eighteen pts were stage I, 2 were stage II, and 3 were stage III. Eighteen mCi MIBI and 20 mCi V-DMSA were injected intravenously and whole body scans were obtained 10 min and 2 hours post injection respectively, at intervals of at least 48 hrs. MIBI scans were scored according to intensity (I) and extension (E) of the radiotracer uptake. A summed score (S) of E and I, ranging from 0-6, was computed for each patient. The total number of MIBI and V-DMSA positive lesions (TPL) was determined in each patient. Lesions that were V-DMSA positive but MIBI negative were considered as non-active (NAL). The ratio of NAL/TPL (R) was calculated for stage I and stages II and III together. The relationship between ISS, β2-M, alb and the R ratio and S score was assessed. *Results.* β2-M and alb levels ranged in stage I between 1.1-3.1 mg/L (2.2±0.7) and 3.5-4.8 mg/L (4.19±0.42) while in stages II and III 3.5-12 (7.36±3.6, p=0.03) and 2.4-4.3 g/dL (3.44±0.74, p=0.08) respectively. The R was 0.59±0.42 (range 0-1) for stage I and 0.05±0.11 (range 0-0.24) for stage II and III (p<0.001). The S was 2.28±2.2 (range 0-6) and 5.2±0.44 (range 5-6) (p<0.001) respectively. R and S were well correlated (r=-0.8, p<0.001). There was an inverse correlation between R and β2-M (r=-0.5, p<0.05) and S and alb (r=-0.44, p<0.05) and a positive correlation between R and alb (r=0.5, p<0.05) and S and β2-M (but not statistical significant, r=0.4, p>0.05). *Conclusions.* The combined use of these two agents could provide useful information's in the detection of more extensive disease activity, well correlated with alb, β2-M and ISS staging system that is highly predictive for overall survival of patients with MM.

0639

DYNAMIC FLUORO-DESOXY-GLUCOSE POSITRON EMISSION TOMOGRAPHY STUDIES FOR THE PREDICTION OF CHEMOTHERAPY RESPONSE IN MULTIPLE MYELOMA

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Background. There is no optimal staging procedure for multiple myeloma. Especially extramedullary myeloma is usually not detected by standard staging. Fluoro-desoxy-glucose positron emission tomography (FDG-PET) is able to provide detailed information on tumour localisation and metabolism. *Aims.* We evaluated the FDG-PET in staging of multiple myeloma in comparison to standard radiological bone scans. Secondly, we examined the biological behaviour of myeloma during the course of chemotherapy, as the metabolic changes of a tumour under

influence of cytotoxic substances appear to have predictive value. **Methods.** We investigated a group of nine patients with plasmacytoma or multiple myeloma. All patients received an anthracycline-based chemotherapy. Three FDG-PET-studies were carried out (1. prior to the chemotherapy, 2. after the first course of chemotherapy, 3. after the third course). The clinical follow-up data and the EBMT-criteria for progressive disease (PD), stable disease (SD), partial remission (PR) and complete remission (CR) served as reference for the PET-data. The following parameters were retrieved from the dynamic PET studies: standard uptake value (SUV), fractal dimension (FD), two compartment model with computation of the kinetic parameters k1, k2, k3, k4 and the vessel density (VB). Furthermore, the FDG-influx according to Patlak was calculated using the rates of the two-compartment model and the formula $(k1 \times k3) / (k2 + k3)$. Discriminant analysis was used for data analysis. Due to the limited number of patients we dichotomised the patients into two groups, namely PD and SD/PR. Furthermore, we evaluated each parameter separately with regard to response. **Results.** 8 patients presented themselves with multiple myeloma and one patient with extramedullary myeloma. Two patients had been previously treated. One patient has just ended the first cycle and has not yet been re-evaluated. Best parameters for the discrimination between SD and PD were the influx and k3 of the first study with an overall correct classification rate (CCR) of 83%, followed by the distribution volume VB and the SUV with an overall CCR of 80%. A high VB, a high SUV and a high FD were associated with PD. **Conclusions.** These data of our ongoing study demonstrate the value of the FDG-PET in the staging of multiple myeloma. Our analysis revealed a combination of parameters to be helpful for the prediction of therapy outcome in these patients. We will analyse the PET data in respect of other outcome parameters such as survival.

0640

INCREASED INCIDENCE OF HERPES ZOSTER VARICELLA (HZV) REACTIVATION IN MULTIPLE MYELOMA (MM) PATIENTS (PTS) TREATED WITH BORTEZOMIB: AN ANALYSIS FROM THE APEX STUDY

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Background. Bortezomib is approved for the treatment of relapsed/refractory MM. Two large clinical trials (APEX and SUMMIT) confirmed the clinical activity of bortezomib in MM. During the Phase 2 clinical trials, there was a suggestion of an increased incidence of HZV among advanced MM pts treated with bortezomib. This prompted us to review the experience from the APEX Phase 3 randomized trial where bortezomib was compared to high dose dexamethasone. **Aims.** To investigate if bortezomib treatment predisposed MM pts to HZV reactivation and to identify possible underlying mechanisms.

Patients and Methods. This is a review of 663 pts treated on the randomized dexamethasone (dex) vs. bortezomib-APEX study. Data were available for 332 and 331 pts treated in the dex vs. the bortezomib arms, respectively. Pts developing HZV at any time point during the course of treatment on study were identified. The time of onset of HZV, concomitant medications, prior antimyeloma therapies, absolute lymphocyte counts

(ALCs) and neutrophil counts (ANCs) were analyzed in addition to the severity and outcome of the HZV. Antibiotic prophylaxis was not recommended in this study and in particular, there was no requirement for prophylactic antivirals. **Results.** Among the 332 pts in the dex arm and 331 in the bortezomib arm, 15 (5%) and 42 (13%) pts developed HZV (p=0.0002), respectively. Baseline demographic and disease characteristics were similar between treatment groups. The proportion of pts ≥ 65 years old with a history of prior SCT was similar in both groups.

The median time to onset of HZV from start of therapy was 51 vs. 31 days for the dex vs. bortezomib pts (p=0.221). The median ALC at the time was significantly different with 1,750/uL in the dex arm vs. 1,050/uL in the bortezomib arm (p=0.028). HZV was reported as Grade 3 or 4 in 5/15 (33%) dex and 6/42 (14%) bortezomib pts and as a serious adverse event in 3/15 (20%) dex and 6/42 (14%) bortezomib pts. There were no deaths due to HZV. **Conclusions.** HZV was reported more frequently in bortezomib treated multiple myeloma patients than in high dose dexamethasone treated patients. Although the time of onset was similar, the severity of lymphopenia was greater in the bortezomib patients. Analysis of the prophylactic use of antiviral therapy in both treatment groups is ongoing. The role prophylactic antiviral therapy in prevention of HZV reactivation in currently investigated in an ongoing single agent bortezomib clinical trial in pts with previously untreated MM.

	Bortezomib N=41	Dexamethasone N=95
Median (range) Age (yr)	63 (50-78)	61 (37-91)
Gender, Male (%)	42	67
Race, White (%)	100	90
Karnofsky ≥70 (%)	85	85
Baseline Median (range) CRP (mg/L)	4 (0-37.5)	4 (0-135.2)
Baseline Median (range) ESR (mg/L)	3.4 (1.3-35.1)	4.3 (2.3-33.3)
Baseline Median (range) Hemoglobin (g/L)	109 (70-140)	102 (67-121)
Baseline Median (range) Platelet Count (10 ⁹ /L)	211.3 (85-387)	308 (15-383)
Baseline Median (range) ALC (10 ⁹ /L)	1.34 (0.34-5.94)	1.11 (0.39-2.18)
Baseline Median (range) ALP (10 ⁹ /L)	2.83 (0.32-4.74)	2.06 (0.30-6.25)
Received steroids prior to treatment (%)	85	81
Received alkylating agents prior to treatment (%)	83	81
Received anthracycline prior to treatment (%)	39	81
Received Thalidomide prior to treatment (%)	45	35
Received Vinorelbine prior to treatment (%)	39	75
Received BMT prior to treatment (%)	71	75

0641

NEW INTERNATIONAL STAGING SYSTEM EVALUATED IN PATIENTS UNDERGOING AUTOLOGOUS TRANSPLANTATION: EXPERIENCE OF CZECH MYELOMA

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Background. Recently new International Staging System (ISS) was presented by Greipp et al. It has shown promise in patients treated by conventional as well as high-dose chemotherapy and is based on a simple combination of serum β 2-microglobulin and albumin values. We compared ISS with Durie-Salmon staging system (DS) in the two large cohorts of patients who underwent autologous transplantation (AT): **Methods.** and

Results. We have retrospectively analyzed group of 133 pts. represented single center experience (BRNO) and 185 pts. generated from multicentric trial 4W of Czech Myeloma Group. All patients with newly diagnosed MM had the same therapy and were transplanted to one year after diagnosis. The aim of our analysis was to evaluate both ISS and DS systems in our set of patients. Clinical stages according to DS were as follows: BRNO/4W trial stage I - 12%/11%, stage II -13%/20%, stage III - 75%/69%. Clinical stages according to ISS were the following: BRNO/4W stage 1 - 38%/43%, stage 2 -45%/36%, stage 3 - 17%/21%. Initial values of κ 2-microglobulin and albumin were not available for 6% pts. from Brno, 8% of pts. from trial 4W. No ISS stage 3 occurred in the clinical stage I and II in both groups except 10% of ISS stage 3 in DS II of the trial 4W. Median OS of pts. was: for single center experience BRNO/4W trial DS stage I - 67.6/76.6 months, DS stage II - 71.0/82.5 months, DS stage III - 71.3/67.3 months. Differences in survival among patients with clinical stages according to DS system were not statistically significant in both groups. Medians of OS were as follows (BRNO/4W): for pts. with ISS stage 3 was 23.6/45.7 months, with ISS stage 2 - 57.5/77.7 months and with ISS stage 1 72.8 months/not reached in 4W trial. Patients with ISS stage 3 had significantly shorter EFS and OS than others and this was statistically significant for single center (BRNO; $p=0007$ for EFS; $p=0005$ for OS) as well as multicentric trial (4W; $p=0.0039$ for EFS; $p=0.07$ for OS). The differences in EFS and OS between ISS stages 1 and 2 were not significant. **Conclusions.** We proved that in the hand of one single center and one single trial group outcome of AT correlated with the stage according to ISS. Especially ISS stage III was very useful to define subgroup with poor prognosis. We also found no significant correlation between DS staging system and survival after transplantation.

1. Greipp PR, Miguel JF, Durie BG et al. New International Staging System (ISS) for multiple myeloma from the International Myeloma Working Group. *Blood* 2003; 102: 190a (abstr. 664).

Myeloproliferative disorders

0642

HEMATOPOIETIC AND ENDOTHELIAL PROGENITOR CELL TRAFFICKING IN PATIENTS WITH MYELOPROLIFERATIVE DISEASES

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Background. The presence of circulating hematopoietic progenitor cells has been described in patients with myeloproliferative diseases (MPD). However, the exact nature of such progenitor cells has not been specified until now. **Aims.** The aim of this work is to show that the endothelial cell lineage is involved in myeloproliferative diseases. **Methods.** We looked for hemangioblast markers (early common precursors to the hematopoietic and endothelial cell lineage) on the circulating cells of 53 patients with MPD. Peripheral blood was analysed for expression of CD34, CD133, KDR and vWF by quantitative PCR. Clonogenic stem cell assays were performed to assess differentiation towards the hematopoietic and endothelial cell lineage. Patient data (essential thrombocythemia (ET, $n=17$), polycythemia vera (PV, $n=21$) and chronic idiopathic myelofibrosis (CIMF, $n=15$) were compared with data from normal controls ($n=16$) and patients with secondary thrombo- or erythrocytosis ($n=17$). **Results.** Trafficking of CD34 positive cells was increased above the physiological level in 3/17 patients with ET, 5/21 patients with PV and 13/15 patients with CIMF. A subset of patients with CIMF co-expressed the hemangioblast markers CD34, CD133 and KDR, suggesting the presence of hemangioblasts among the circulating progenitor cells. Clonogenic stem cell assays confirmed differentiation towards both the hematopoietic and the endothelial cell lineage in 5/10 patients with CIMF. **Conclusions.** Endothelial progenitor cells are present in the blood of a subset of patients with CIMF.

0643

THALIDOMIDE VERSUS PLACEBO IN MYELOID METAPLASIA WITH MYELOFIBROSIS: A RANDOMIZED DOUBLE-BLIND MULTICENTER STUDY BY THE GOELAMS GROUP

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Background. Thalidomide have been used in non randomized studies in patients with Myeloid Metaplasia with Myelofibrosis (MMM) with some benefits on anemia, transfusion, spleen size and platelet count. **Aims.** To compare thalidomide to placebo in increasing the hemoglobin level by 2 g/dL or reducing RBC transfusions by 20% in anemic patients with MMM. **Methods.** Fifty two patients with hemoglobin under 9 g/dL or RBC transfusions were enrolled in a randomized multicentric double-blind trial to receive either thalidomide 400 mg/d or placebo for 6 months. **Results.** According to the Dupriez score, one patient was at low risk, 29 at intermediate risk and 22 at high risk. Only 17 patients received more than 200 mg/d thalidomide for more than 2 months, 11 patients completed the 6 months, and 52% discontinued thalidomide. During the trial, eight patients died in the thalidomide group, and 6 in the placebo group. No difference was observed between thalidomide and placebo neither in the increase of hemoglobin level (1 patient in each group), nor in the reduction of RBC transfusion (3 and 5 patients respectively). The only significant result was the increase in echographic spleen size which was less in the thalidomide group than in placebo (respectively 0.4 cm and 2 cm, $p < 0.05$). No benefit of thalidomide was observed in the Dupriez score, the severity score of Barosi, survival, and any other clinical or biological data. Somnolence, constipation, weight gain and edema were significantly more frequent in the thalidomide group (respectively $p < 0.001$, < 0.001 , < 0.05 , and < 0.01). **Conclusions.** Thalidomide was not beneficial in these patients with advanced MMM.

0644

EFFECTS OF C-KIT D816V ON GROWTH AND DIFFERENTIATION OF BA/F3 PROGENITOR CELLS

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Background. The pathologic hallmark of systemic mastocytosis (SM) is differentiation and cluster formation of mast cells (MC) in hematopoietic tissues. The somatic c-kit mutation D816V is detectable in a majority of all SM patients independent of the proliferation-status of MC or subtype (indolent or aggressive) of disease. **Aims.** The aim of the present study was to elucidate the role of the c-kit mutation D816V in the pathogenesis of SM. **Methods.** For this purpose, a Ba/F3 cell line with doxycycline-inducible expression of c-kit D816V was established. To investigate effects of c-kit D816V on gene expression, affymetrix gene chip technology was applied. **Results.** We found that c-kit D816V provides a strong signal for mast cell differentiation and cluster formation in Ba/F3 hematopoietic progenitor cells without enhancing their growth thereby resembling the clinical presentation of indolent SM. As assessed by gene chip analysis, induction of c-kit D816V resulted in expression of various differentiation antigens including mouse mast cell protease 5, mi transcription factor, histidine decarboxylase (HDC), secretory granule proteoglycan and IL-4 receptor. In

addition, c-kit D816V was found to induce expression of several adhesion molecules in Ba/F3 cells, including ICAM-1 (CD54), and LAMP-3 (CD63). Upregulation of differentiation-associated and adhesion-linked cell antigens in Ba/F3 cells was confirmed by RIA (histamine), real time PCR, and flow cytometry. c-kit D816V did neither induce expression of granulomonocytic antigens such as myeloperoxidase, IL-3 receptor, or GM-CSF receptor, nor expression of 'late stage' mast cell antigens such as FcγRI. In a next step, signal transduction pathways underlying c-kit D816V effects on Ba/F3 cells were examined. In these experiments, the c-kit D816V-induced cluster formation in Ba/F3 cells was counteracted by the MEK inhibitor PD98059, but not by the PI3-kinase inhibitor LY294002 or the mTOR-targeting drug rapamycin. **Conclusions.** In summary, our data establish a role for c-kit D816V in differentiation and cluster formation of neoplastic (mast) cells. Additional genetic hits, apart from c-kit D816V, may be responsible for aggressive growth of MC in advanced MC neoplasms.

0645

TRANSCRIPTION FACTOR EXPRESSION PROFILING OF POLYCYTHEMIA VERA AND NORMAL CD34+ CELLS. PRELIMINARY EVIDENCE OF STEM CELL RESISTANCE TO TRANSFORMING GROWTH FACTOR BETA (TGF-B)

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Polycythemia vera (PV) is a clonal disorder of unknown etiology involving a multipotent hematopoietic progenitor cell and characterized by trilineage hematopoietic cell hyperplasia in the absence of a recognizable stimulus, extramedullary hematopoiesis and, in some patients, transformation to acute leukemia or bone marrow failure. Signal transduction is altered in PV as indicated by growth factor hypersensitivity or independence, resistance to apoptosis and aberrant gene expression. To define the regulation of gene expression in PV, we examined the transcription factor profile of peripheral blood (pb) CD34+ cells from 5 PV patients with immunophenotypically similar mobilized CD34+ cells from 2 controls. The pbCD34+ cells were purified to greater than 95% with a viability of greater than 98% using immunomagnetic bead technology. Both PV and control CD34+ cells were greater than 95% CD38+ and the cell cycle status of each was similar. Nuclear extracts from these cells were probed with a commercial DNA binding array (Panomics) representing the canonical sites for 150 transcription factors. The arrays were analyzed using quantitative densitometry. Transcription factor expression was considered elevated only if it was 1.5 fold greater than background. Transcription factor expression was largely similar between PV and control CD34+ cells for AP-1 and 2, c-Myb, P53, CREB, NF-kb, E2F1, EGR, GRE, NF-E1 and 2 and GATA. However, CDP expression was increased in all 5 PV patients and none of the controls and this was confirmed by EMSA. Gamma interferon/interferon specific response element(GAS/IRE) and HSE expression was also increased in all PV patients and only one control while Nuclear factor I (NF-I) and Transcription factor II D (TFIID) expression was increased in 4/5 PV patients and 1/2 controls. Since CDP and NF-I over-expression confers transforming growth factor B (TGF-B) resistance, we hypothesize that TGF-B resistance may be implicated as a potential mechanism in the phenomenon of myeloaccumulation and apoptosis resistance in PV. To further explore this hypothesis, we cultured CD34+ cells from control and PV subjects in IL-3 and IL-3+TGF-β. Viability, by supravital staining, at 20 hours was higher in PV (86%) compared to controls (60%) cultured in IL-3+TGF-β. Additionally, TGF-β down-regulated the anti-apoptotic protein Bcl-xL in the cytosol of controls, but not PV CD34+ cells as evidenced by Western blotting (see Figure).

We also examined transcription factor profiles in those cultured cells at baseline, 6, and 20 hours using the same commercial array described above. We observed a high expression of GATA and NFκB in PV CD34+ at 20 hours under the influence on IL-3 and was even higher in the presence of TGF-B.

Smad 3/4, the major TGF-B receptor effector TF is increased in both normal and CD34+ cells in response to TGF-B at 6 hours providing internal validity for our results. Interestingly, Smad 3/4 plateaus down in normal cells at 20 hours but continues to rise in PV cells. Our results suggest that resistance to TGF-B in CD34+ may be instrumental in the pathogenesis of PV.

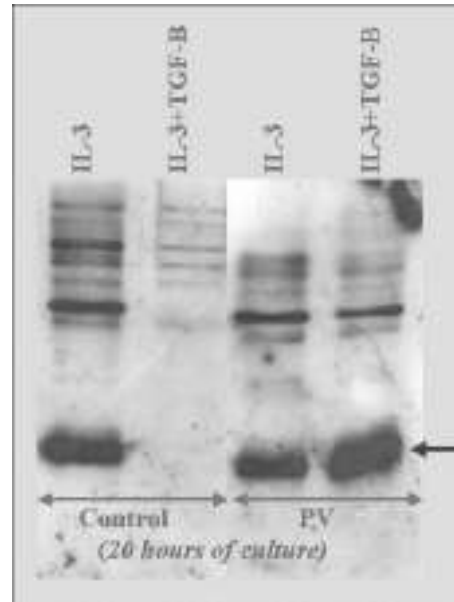


Figure 1. Cytosolic Bcl-xL by Western blot.

0646

IMMUNOHISTOCHEMICAL DETECTION OF HISTIDINE DECARBOXYLASE (HDC) IN NEOPLASTIC MAST CELLS IN PATIENTS WITH SYSTEMIC MASTOCYTOSIS

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Background. Synthesis of histamine in hematopoietic progenitor cells may be one of the earliest events in mastopoiesis. We therefore asked whether the key enzyme involved in histamine production, histidine decarboxylase (HDC), can be employed as an immunohistochemical marker for the detection of immature neoplastic mast cells (MC) in patients with mast cell proliferative disorders. **Aims. and Methods.** To address this question, we examined bone marrow biopsy specimens in a cohort of 102 patients with mastocytosis (cutaneous mastocytosis, n=10; indolent systemic mastocytosis, n=46; smouldering systemic mastocytosis, n=4; systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease, n=25; aggressive systemic mastocytosis, n=13; mast cell leukemia, n=3; mast cell sarcoma, n=1) and 20 control cases (normal bone marrow) using an antibody against HDC and the indirect immunoperoxidase staining technique. In addition, highly purified (>98% pure) CD117/KIT++ sorted immature MC (from 2 patients with mast cell leukemia and 1 with mast cell sarcoma) were analyzed for expression of HDC mRNA by RT-PCR analysis. **Results.** In normal bone marrow and cutaneous mastocytosis, no HDC-positive mast cell infiltrates could be detected. By contrast, HDC-positive infiltrates of MC were detectable in all patients with systemic mastocytosis, independent of the subtype of disease or maturation stage of MC. The anti-HDC antibody produced clear diagnostic staining results not only in patients with indolent systemic mastocytosis but also in those with mast cell leukemia and mast cell sarcoma, in which MC are particularly immature. In these patients, expression of HDC

could also be re-confirmed at the mRNA level by RT-PCR analysis performed with RNA of highly enriched MC. The immature human MC leukemia cell line HMC-1, derived from a patient with mast cell leukemia, was also found to express HDC at the mRNA and protein level. **Conclusions.** In summary, HDC is expressed in neoplastic MC in patients with systemic mastocytosis independent of the maturation stage of cells or the variant of disease. HDC should therefore be considered as a new MC marker for the screen panel of antigens employed to diagnose high grade MC malignancies.

0647

HYDROXYUREA IN MYELOPROLIFERATIVE DISORDERS: A NEW TASK FOR AN OLD DRUG?

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Background. Hydroxyurea (HU) is a chemotherapeutic agent used to treat myeloproliferative disorders (MPD) and other pathologic conditions. A role of HU in reducing the risk of thrombosis associated to MPD was previously suggested. The antithrombotic effect of HU could go beyond its capacity to reduce the platelet number. Actually, epidemiological studies did not find any correlation between incidence of thrombotic events with either platelet number or their *in vivo* or *ex vivo* activation status. So that, the prothrombotic status in MPD could be due to leukocyte activation and/or the presence of platelet-leukocyte aggregates. **Aims.** We analyzed here leukocyte and platelet markers of function in 18 patients with MPD: 12 without chemotherapy treatment and 6 under HU treatment. Both groups of patients were compared to a control group of 15 healthy donors. **Methods.** Platelet, PMN and monocyte markers of activation or degranulation, as CD11b, tissue factor (TF), Fibrinogen (Fg), P-selectin, von Willebrand Factor (VWF) as well as circulating platelet - leukocyte (PMN and monocytes) aggregates were determined using three-colour flow cytometry. The results obtained were expressed as means±SEM of proportion of positive cells. The results (illustrated in the table) showed that, all platelet alterations (increase in P-selectin and tissue factor expression, decrease in platelet content of Fb and VWF) were not different in the HU treated MPD as compared to non treated patients. On the contrary, the leukocyte activation markers (CD11b, TF expression and TF total content) were partially corrected in the group of patients under HU treatment. Finally, the proportion of circulating platelet-PMN as well as platelet-monocyte mixed aggregates in patients treated with HU was not different as compared with to the control group: platelet-PMN: in controls 3±1, in MPD 14±4 and under HU treatment 4±1. For platelet-monocytes in controls 3±1, in MPD 22±9 and under HU treatment 3±1. **Conclusions.** These results are in agreement with previous indications that HU may prevent thrombotic complications in MPD and suggest that reduced leukocyte activation could be involved.

Table 1. Comparative results with and without HU treatment.

	Platelet markers/leukocytes			Thrombocytes		Blood Platelets	
	CD11b expression	TF expression	TF total content	TF oxidized	TF oxidized	Fibrinogen expression	Platelet content
Control	30±11	1.0±7	1.0±5	0.3±0.04	3.3±1.1	7±1	93±1
MPD	30±6	1.6±2	3.2±5	<0.1	5.1±2	3±2	30±3
MPD HU	28±6	0.4±1	2.0±1	0.3±0.1	3.4±1.4	3±2	10±7

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CLINICAL AND HAEMATOLOGICAL CHARACTERISTICS OF POLYCYTHAEMIA WHEN DIAGNOSED ACCORDING TO ORIGINAL PVSG VERSUS WHO RED CELL MASS CUT-OFF VALUES

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Background. The classification of myeloproliferative disorders (MPD) still relies on PVSG criteria. Those criteria were updated by Pearson et al., and in particular a red cell mass (RCM) greater than 25% above the patient's mean normal predicted value based on surface area is now retained to diagnose polycythaemia vera (PV). Recent WHO criteria for PV also retained this cut-off value for RCM. Therefore, in clinical practice most patients with thrombocytosis and diagnosed with Ph-negative MPD will be classified as primary thrombocythaemia (PT) if their RCM is below 125% of the predicted value, and as PV above 125% regardless of their platelet count. **Aims.** As the clinical management and risk of thrombosis and haemorrhage of PT and PV patients are clearly different, we compared the diagnosis of PV patients according to original PVSG criteria (i.e. PV is considered if the RCM is above 32 ml/kg in females and 36 ml/kg in males) and revised PVSG or WHO criteria (i.e. RCM above 125% of predicted value). **Methods.** Between 1994 and 2004, measurement of RCM was performed in 500 patients because of unexplained thrombosis, thrombocytosis or raised haematocrit in a single center. **Results.** were expressed both as absolute values (in ml/kg) and as percentage of predicted value based on surface area. **Results.** 140 patients out of 500 had a RCM above 125% of the predicted value. Among those 140 patients, 66 were females, and 18 (27%) had absolute values below the PVSG cut-off of 32 ml/kg. In these 18 patients, mean measured RCM was 29 ml/kg (±2.3), but was 135% (± 8) of predicted value. Their median age was 62.5 years (range 25-83), and their mean haematocrit at diagnosis was 46% (±6.7). Among 74 males with an RCM above 125% of the predicted value, 27 had measured RCM below 36 ml/kg. In these 27 patients, the mean measured RCM was 33 ml/kg (± 1.7), but was 132% (± 5.7) of predicted value. Their median age was 59 years (range 41-82), and their mean haematocrit at diagnosis was 49% (±5.4). In all, 45 patients out of 140 would have been diagnosed as PT according to initial PVSG criteria, and are now diagnosed as PV according to revised PVSG or WHO criteria. **Conclusions.** In a large cohort of patients evaluated for MPD on clinical grounds, we found that in 32% of cases the diagnosis of PV would not have been retained if RCM was not expressed according to the predicted value. Furthermore, the mean haematocrit of these patients was below 50%, emphasizing the importance of RCM measurement to properly classify MPD. Correlations with history of thrombosis, serum erythropoietin levels, endogenous erythroid colony formation and bone marrow histology findings will be presented.

0649

THE IL-8 INHIBITOR REPARIXIN BLOCKS THE MIGRATION OF CD34+ CELLS OF NORMAL DONORS AND MYELOFIBROSIS PATIENTS

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Background. The chronic myeloproliferative diseases (CMD) are clonal haematological tumours derived from a multipotent haematological stem cell which shows proliferation and differentiation into several lineages in a relatively normal manner. Idiopathic myelofibrosis (IM) is a form of CMD characterised by bone marrow fibrosis, ineffective hematopoiesis and myelo-

proliferation with extramedullary hematopoiesis, particularly in spleen and liver. Increased numbers of CD34+ cells are present in the blood of IM patients. The molecular mechanisms that are responsible for the abnormal extravasation of CD34+ cells from the bone marrow to the blood, and migration into spleen and liver, are still unclear. IL-8 has been shown to play a role in the regulation of normal CD34+ cells mobilisation and proliferation and in the control of megakaryocytic proliferation and differentiation in IM. Reparixin is a small molecule inhibitor of IL-8 (CXCL8) which locks the IL-8 receptor CXCR1/2 in an inactive conformation. AIMS. We have therefore investigated the spontaneous and IL-8 induced chemotaxis of CD34+ cells from normal donors and myelofibrosis patients. *Methods.* CD34+ cells from normal donors and IM patients bone marrow were purified by positive selection. Chemotaxis was performed using Boyden chambers with PVP free 5 micron filters. 50000 CD34+ cells were plated in each well and migration was measured after 6 hours at 37°C. *Results.* CD34+ cells from both normal donors and IM patients showed spontaneous migration (mean 61 and 97, respectively). No significant and reproducible migration was observed in response to 50 ng/mL IL-8 (mean 66 and 83, respectively). In contrast SDF-1 induced a consistent 2-3 fold increase in C34+ cell migration, as expected. Interestingly, preincubation of CD34+ cells for 15 minutes with Reparixin at 10nM significantly inhibited the spontaneous migration of normal and IM CD34+ cells by 41% and 60%, respectively (n=5 in each case). Moreover a dose response experiment showed that the effect was dose-dependent. reparixin also reduced the CD34+ migration in presence of SDF-1. The mechanism of action of the migration block is under investigation, since CD34+ cells from either normal donors or IM patients did not express detectable levels of the CXCR1 and CXCR2 IL-8 receptor. *Conclusions.* Reparixin deserves further *in vitro* studies for its possible application to inhibit the motility of CD34+ stem cells *in vivo*, with particular interest in the context of myelofibrosis.

0650

ENHANCED PLATELET ADHESION IN ESSENTIAL THROMBOCYTHEMIA ASSESSED BY A NOVEL PLATELET FUNCTION ASSAY

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Background. Essential thrombocythemia (ET) is a diagnosis first described in 1934 and subsequently classified as a myeloproliferative disorder in 1951. The median survival in ET exceeds 2 decades. Despite intensive clinical and laboratory research, relatively little has been accomplished concerning the pathogenesis of ET. ET has a paradoxical predisposition to bleeding and thrombotic complications that are major causes of morbidity and mortality. Furthermore, the risk of both thrombosis and bleeding in low-risk patients who are not receiving any cytoreductive therapy may not be significantly different from that of the age- and sex-matched control population. *Aims.* The aim of this study was to compare platelet activity between ET-patients and healthy blood donors (controls). This was accomplished with the use of a newly developed platelet adhesion assay. *Methods.* Microplates were coated overnight with albumin, collagen, fibrinogen or thrombin-activated plasma. Blood was collected from 30 ET-patients (mean age=63) and 14 controls (mean age=48). Platelet rich plasma (PRP) was prepared by centrifugation and PRP together with ADP or ristocetin were added to the microplates. Platelets were allowed to adhere and the amount of platelet adhesion was detected enzymatically. Percentage platelet adhesion was calculated and basal adhesion (adhesion to surface without added activator) was subtracted from all adhesion values. The obtained values, reflecting the ability of platelets to get activated, were used for statistical analysis. *Results.* In general, platelets from ET-patients were more sensitive to *in vitro* stimulation than control-platelets. Distinct differences were seen with ADP as activator. On collagen, fibrinogen and thrombin-activated plasma surfaces the ADP-response were maximal for controls at 1 µM while ET-platelets responded even more at 10 µM. The adhesion after treatment with 10 µM ADP was significantly increased for ET-platelets

(n=30) compared to controls (n=14) on the collagen (p<0.001), fibrinogen (p<0.001) and thrombin-activated plasma (p<0.01) surfaces. Significant differences sustained when the controls were compared with a sub-group of 14 age-matched ET-patients. Furthermore, platelets from ET-patients adhered better than control-platelets when using 1 mg/mL ristocetin as activator. Comparing all ET-patients with controls showed significant differences for albumin (p<0.05), collagen (p<0.01) and fibrinogen (p<0.01). As for ADP, significant differences persisted also when the controls were compared with the age-matched ET-patients. *Conclusions.* Opposite to the majority of other studies we report an increased sensitivity of ET-platelets compared to controls to different platelet activating stimuli *in vitro*. We suggest that this might reflect insufficient control of platelet activation, which could result in the increased propensity for thrombosis commonly observed in the clinical situation. Thus, such platelet activity measurements may be of clinical importance.

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GENE EXPRESSION ANALYSIS BASED ON REAL TIME PCR OF 95 GENES CODING FOR TYROSINE KINASES IN PH NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background. Ph negative Chronic Myeloproliferative Disorders (CMPD) are likely characterized by a deregulated tyrosine kinase (TK) activity which is well identified and characterized only in a small subset of patients. The possibility to identify additional TK involved in the pathogenesis of CMPD possesses a relevant clinical significance since it offers the possibility to design new molecules able to inhibit in a selective manner the specific target, giving the patient the possibility to be treated with a new molecular approach. *Aims.* The aim of the present study was to identify the presence of activated TKs in CMPD through a gene expression analysis based on Real Time PCR of 95 genes coding for Tyrosine Kinases. *Methods.* We analysed the expression level of 95 genes in 16 patients affected by CMPD and 10 BM samples obtained from healthy volunteers. The quantitative analysis was performed using the ABI Prism 7900 (Applied Biosystems) using the micro fluidic card and the assays-on-demand system. The micro fluid card configuration was designed in order to allow the analysis in a single card of the expression level of the selected 95 TKs and three house-keeping genes in duplicate. The series of the patients studied included 3 patients affected by primary eosinophilic syndrome (HES), one of them was characterized by the presence of the hybrid transcript FIP1L1-PDGFRalpha and one by the presence of a cytogenetic marker, 4 patients were affected by chronic myelomonocytic leukaemia (CMML) and 9 patients were affected by Ph negative CML like diseases, four of them characterized by a cytogenetic marker. The final value of expression has been calculated using the software SDS 2.1. *Results.* The first result obtained is represented by an impressive homogeneous expression of the TK within the normal samples. By contrast, in our series of patients we were able to identify 30 genes which resulted to be expressed in a significant different way respect to the normal samples. The majority of them were upregulated and few of them significantly downmodulated. The selected genes have been then clustered based on their biological significance. A further analysis allowed us to select 6 genes which appeared to be of particular interest in different subgroups of patients. These genes have been further studied in an enlarged series of patients. *Conclusions.* This study allowed us to identify a pattern of TK expression in different subgroups of CMPD and particularly to identify 6 genes which probably play a key role in the pathogenesis of CMPD.

0652**IMMUNOHISTOCHEMICAL EVALUATION OF BONE MARROW BASOPHILS IN CML AND OTHER MPDS**

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Background. Basophils are highly specialized granulocytes that express a unique profile of antigens and increase in myeloproliferative disorders (MPDS). In chronic myeloid leukemia (CML), basophilia is an independent prognostic variable. So far, however, no reliable immunohistochemical approach for routine detection and enumeration of bone marrow (bm) basophils has been established. **Aims.** and **Methods.** To overcome this disadvantage, we have applied the anti-basophil antibody 2D7 on formalin-fixed, paraffin-embedded bm sections from patients (pts) with chronic myeloid leukemia (CML; chronic phase, n=37; accelerated phase, n=9), other myeloproliferative disorders (chronic idiopathic myelofibrosis [CIMF], n=10; polycythemia vera [PV], n=11; essential thrombocythemia [ET], n=19; systemic mastocytosis [SM], n=7), and normal/reactive bm (n=34). **Results.** As assessed by serial section-staining of bm specimens, the 2D7 antibody was found to be a basophil-specific immunohistochemical reagent. In these examinations, 2D7+ bm basophils co-expressed myeloperoxidase (MPO), VEGF, histidine decarboxylase, tryptase, CD9, CD15, CD43, CD44, and CD68, but did not express B- or T-cell restricted antigens or mast cell chymase. On the other hand, the 2D7 antibody was found to react with basophils only, but did not react with eosinophils, blast cells, mast cells, lymphocytes, monocytes, or megakaryocytes. Corresponding results were obtained from immunocytochemical staining experiments using highly enriched (sorted) CML basophils, basophil-depleted bm cells as well as the CML-derived basophil cell line KU812. In bm sections, 2D7+ basophils were found to increase significantly in number in pts with CML compared to normal bm (median 2D7+ cells/mm²: normal bm: 7; CML: 28 [p<.05]; CIMF: 7; PV: 5; ET: 2). The highest numbers of bm basophils were recorded in accelerated phase CML (122 2D7+ cells/mm²). **Conclusions.** We have established a useful immunohistochemical staining procedure for basophil detection and quantification in normal bm and pts with myeloid neoplasms. This approach should enable the quantification of basophils in these pts and the monitoring of bm basophils during follow up examinations and anti-leukemic therapies.

0653**PRV-1 EXPRESSION IN THE CLINICAL MANAGEMENT OF PATIENTS WITH POLYCYTHEMIA (RESULTS OF THE PV-NORD GROUP)**

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Background. Clinical management of patients with elevation of the hematocrit is still a major concern. Because these patients fall into diseases of distinct prognosis, it is of an utmost importance to reach a clear diagnosis. In particular, patients with an increased red blood cell mass and no evidence for secondary erythrocytosis are diagnosed with Polycythemia Vera (PV) when PVSG criteria modified by Pearson are met or with Idiopathic Erythrocytosis (IE) in the contrary. **Aims.** PRV-1 gene overexpression in granulocytes has been reported to be a sensitive and specific marker of PV, but studies have partly contradicted its interest. We have evaluated the interest of this marker in new patients addressed for the diagnosis of an elevated hematocrit and compared it with Epo levels and endogenous erythroid colony (EECs) formation. **Methods.** As most of the discrepancies for PRV-1 expression measure-

ment have been reported to be dependent on the methodology used, we employed the method developed in Pr Pahl's laboratory. After having conducted all measurements, 8 blinded samples were sent to Pr Pahl's laboratory and the results verified independently. Analysis of both results obtained for these 8 samples showed a very high correlation ($r^2 = 0.90$) between the two labs. Total RNA purified from circulating granulocytes isolated 24h after sampling were studied by real time quantitative RT-PCR. PRV-1 gene expression was normalized with 18S RNA and the results in each sample expressed as the ratio between PRV-1 and 18S copy numbers. For every patient, red blood cell mass, circulating Epo and EECs formation was measured. **Results.** PRV-1 expression was measured in 34 patients. First, according to the red blood cell mass results, patients were classified as having either false erythrocytosis (n= 4) (median PRV-1 = 0.010; range 0.002 to 0.016) or polycythemia (n= 30). Taking into account PVSG-Pearson criteria these polycythemia patients were then classified as: IE patients (n= 10; median PRV-1 = 0.007; range: 0.001 to 0.037), PV patients (n= 19; median PRV-1 = 0.11; range: 0.007 to 8.7) and one patient had secondary erythrocytosis (PRV-1 = 0.147). PRV-1 expression was found significantly different between IE and PV patients (Wilcoxon test, p=0.0001). The ROC curve analysis (area under the curve = 0.95) demonstrates the ability of PRV-1 expression to serve as a diagnostic test to classify these disorders. Nevertheless, as with most markers, a grey zone where the results are not informative is delineated. In our study 1 IE patient and 3 PV patients fell into this grey zone, and 1 patient with PV presented very low PRV-1 expression. In this study, other conventional markers such as Epo and EEC were not more informative: indeed low Epo levels were observed in 86% of PV and 33% of IE, and positive EEC in 78% of PV but also 37% of IE. **Conclusions.** Our study confirms the potential interest of PRV-1 measurement in the management of patients presenting with polycythemia. If performed following the methodology presented herein, it allowed distinguishing PV and IE patients at first presentation.

0654**CORRELATION OF PRV-1 EXPRESSION WITH ENDOGENOUS GROWTH OF ERYTHROID (EBFU-E) AND MEGAKARYOCYTIC (ECFU-MK) CIRCULATING PROGENITORS IN ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA**

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Background. Essential thrombocythemia (ET) and polycythemia vera (PV) are clonal myeloproliferative disorders lacking specific biological markers. Endogenous BFU-E is a well recognized positive marker for PV. Recently, PRV-1 expression has been suggested to be considered as a new diagnostic marker in ET and PV. **Aim.** The aim of the present study was to analyze the correlation between PRV-1 overexpression and endogenous growth of hematopoietic progenitors, in patients with ET and PV from a single institution. **Patients and methods.** Patients diagnosed with ET (n=88; 22M/66F) and PV (n=64; 35M/29F) according to the PVSG criteria were included in the study. Circulating BFU-E and CFU-MK were available in 64 ET and 42 PV patients, respectively. In vitro cultures were performed at diagnosis. At the time when PRV-1 expression was analyzed, 38/88 ET and 25/64 PV patients were receiving therapy with myelosuppressive/platelet lowering agents as follows: hydroxyurea±ASA (n=45); anagrelide±ASA (n=16) and busulphan (n=2). Thirty-three patients only received ASA and 56 patients did not receive any of the aforementioned treatments. Phlebotomy was performed in PV patients when hematocrit was higher than 0.45L/L and 0.42L/L, in men and women respectively. The expression of PRV-1 and the reference gene beta-glucuronidase (GUS) was quantified by real time RT-PCR (ABIPrism 7900HT). Relative values were standardized against granulocytes from normal healthy donors (n=38) which were assigned an arbitrary relative value=1. PRV-1 quantification in normal healthy donors ranged from 0.1 to 15. PRV-1 was considered overexpressed for values greater than 15. eBFU-E (with-

out EPO) and eCFU-MK (without LCM) were studied. *Results.* An increase in the expression of PRV-1 was observed in 45/88 (51.1%) ET and in 60/64 (93.7%) PV patients. Relative median values of PRV-1 were 17.88 in ET and 400.84 in PV. Among ET patients, 35/64 (54.7%) presented eBFU-E growth, 40/64 (62.5%) showed eCFU-MK growth and 49/64 (76.6%) had either of two. Regarding PV patients, 39/42 (92.8%) had eBFU-E growth, 22/42 (52.4%) showed eCFU-MK growth and 41/42 (97.6%) showed eBFU-E and/or eCFU-MK growth. The correlations between PRV-1 positivity (PRV-1>15) and endogenous growth of BFU-E and/or CFU-MK are shown in the table. A strong correlation between PRV-1 positivity and eBFU-E was found in PV. A good correlation was also observed between eBFU-E and/or eCFU-MK and PRV-1 positivity in ET (85.2%). When considering patients who presented eBFU-E growth, 18/35 (51.4%) ET patients and 38/39 (97.4%) PV patients overexpressed PRV-1. *Conclusions.* The best correlation between PRV-1 overexpression and endogenous eBFU-E growth was found in PV (95%). PRV-1 overexpression might be used, like eBFU-E, as a positive diagnostic criteria for PV.

Acknowledgements. FIS 03/345, SAF-2001-4947, C03/07, C03/10 and Shire Ibérica

	eBFU-E	eCFU-MK	eBFU-E and/or eCFU-MK
ET-PRV-1 positive n=27	18/27 (66.6%)	17/27 (62.9%)	23/27 (85.2%)
PV-PRV-1 positive n=40	38/40 (95%)	21/40 (52.5%)	39/40 (97.5%)

0655

CLINICAL AND HEMATOLOGICAL PRESENTATION OF CHILDREN AND ADOLESCENTS WITH POLYCYTHEMIA VERA

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Background. Polycythemia vera (PV) and congenital erythrocytoses constitute extremely rare diseases in pediatric and juvenile patients. Systematic data on clinical and laboratory evaluations as well as on treatment modalities are sparse. *Aims/Methods.* We developed a protocol (PV-ERY-KA 03) for the systematic collection of clinical, hematological, biological as well as treatment data of children and adolescents with PV or congenital erythrocytoses. The collaborative trial was initiated in March 2004. *Results.* To date, 5 (2 male / 3 female) pediatric patients with PV have been enrolled. The median age at onset was 14 years (7-18.5 years). 4/5 patients are of German, 1/5 of Turkish origin. Only 3 patients presented with PV-related symptoms (Budd-Chiari syndrome, isolated headache, or headache together with tinnitus, dizziness and weakness). At first presentation, the median hemoglobin content was 18 g/dL (15.5-21.1 g/dL), the median hematocrit 0.60 (0.59-0.73). Thrombocytosis was present in 4/5, leukocytosis in 2/5 patients. All patients presented with mild to marked splenomegaly. Granulocytic PRV-1-mRNA expression was assessed in 4/5 patients and significantly elevated in 3 of them, the 4th patient had a borderline PRV-1 expression. Treatment strategies included phlebotomy (4/5), hydroxyurea (1/5), IFN-alpha (1/5), and aspirin (3/5). Stem cell transplantation from a matched unrelated donor was performed in one patient (now in complete remission). Because of progressive liver failure, the patient with Budd-

Chiari syndrome underwent orthotopic liver transplantation. *Conclusions.* The age of onset and clinical presentations in these rare cases of childhood PV are varied. Different treatment strategies are applied. A collaborative assessment of children with PV may help to optimise treatment in these patients, but may in addition contribute to a better general understanding of the disease's pathogenesis.

0656

CIRCULATING ENDOTHELIAL PROGENITOR CELLS ARE CLONAL IN PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA (MMM)

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Background. We have previously shown that patients with MMM have an increased number of circulating CD34+ cells compared with patients with other Ph-negative myeloproliferative disorders (Polycythemia Vera, PV, and Essential Thrombocytemia, ET) and healthy subjects. A deeper analysis of circulating CD34+ cells in MMM patients has indicated that a variable proportion of these cells co-express the VEGFR-2 and the CD133 antigens, suggesting that endothelial progenitor cells (EPCs) could also be increased in the peripheral blood (PB) of MMM patients. *Aims.* We have investigated the hypothesis that circulating EPCs in MMM are increased compared with PV/ET patients and with healthy subjects by growing EPCs colonies *in vitro* and that they belong to the neoplastic clone by studying the X-chromosome inactivation pattern and the loss of heterozygosity in individual endothelial and hematopoietic colonies. *Methods.* Endothelial colonies were obtained by plating 1x10⁶ PB mononuclear cells (MNCs) from 29 patients with MMM, 12 with PV or ET, and 10 healthy subjects, in fibronectin-coated 24 well plates in liquid culture containing 10% FCS and VEGF for 7 days at 37°C, 5% CO₂. The endothelial origin of the colonies was confirmed by *in situ* staining with anti-VE-cadherin, anti-CD31, anti-von Willebrand factor, and anti-CD45 antibodies. X-chromosome inactivation was assessed by determination of DNA methylation at the Humara or PGK loci in 6 informative female patients and 2 healthy female subjects. Briefly, DNA was extracted from at least 8 single endothelial colonies and a pool of 7-8 colonies, digested with the restriction endonuclease HpaII and amplified by PCR. The loss of heterozygosity was studied in 1 male patient who had a 7q34 deletion in 50% metaphases, resulting in a loss of heterozygosity at the polymorphic marker D7S684 that was evaluated by microsatellite analysis in 10 individual endothelial colonies and 10 individual CFU-GM colonies grown in a classic clonogenic assay. *Results.* EPC-derived colonies were higher (median 21 x 10⁶ PBMNCs, range 0-100) in MMM patients than in PV/ET patients (7.5 x 10⁶ PBMNCs, 0-38) and normal subjects (16.5 x 10⁶ PBMNCs, 2-26) (p=0.01 and p=0.05, respectively). Among MMM patients, those with prefibrotic MMM (n=6) had higher endothelial colonies than those with MMM in a fibrotic stage (n=23) (58 x 10⁶ PBMNCs vs 28 x 10⁶ PBMNCs, p=0.05). Moreover, whereas healthy subjects showed a polyclonal pattern of X-chromosome inactivation in the endothelial colonies, all tested MMM patients showed a selective inactivation of the same chromosome X that was also inactivated in the polymorphonuclear cells. Loss of heterozygosity at the polymorphic marker D7S684 was observed in 6 out of 10 and 4 out of 9 hematopoietic and endothelial colonies, respectively. *Summary.* Taken together, our results show that in MMM patients circulating EPCs are increased compared with other myeloproliferative disorders and with healthy subjects and that they are clonal, suggesting that the disease stems from a hemangioblastic progenitor cell. These results provide a novel thinking of the pathogenesis of MMM and pave the way for the development of new theoretical approaches.

0657**IMATINIB MESYLATE FOR IDIOPATHIC HYPEREOSINOPHILIC SYNDROME (HES). A PHASE II MULTICENTRIC ITALIAN CLINICAL TRIAL**

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We studied 141 patients with eosinophilia, 55 (39%) of whom with idiopathic eosinophilic syndrome (HES), by molecular analysis for expression of FIP1L1-PDGFR α , TEL-PDGFR β , FGFR1-BCR and BCR-ABL chimerical transcripts. Sixteen patients (29%) were positive for the FIP1L1-PDGFR α rearrangement and all of them showed previously unreported, abnormal-sized fusion transcripts. We enrolled forty-six out of these fifty-five HES patients (including 16 FIP1L1-PDGFR α positive patients and 30 negative for this rearrangement) in a multicentric Italian phase 2 clinical trial, and treated 45 of them with imatinib mesylate (100 to 400 mg daily). The median follow up was 9 months (range: 1-30). Rapid, haematological complete responses (HCR) were recorded after one month of therapy in all the FIP1L1-PDGFR α and a decrease in eosinophil count was recorded in 8 out of 30 FIP1L1-PDGFR α -patients (100% vs 26.6%). Furthermore, a molecular complete remission (defined as the disappearance of FIP1L1-PDGFR α at qualitative RT-PCR evaluation) was also recorded in all the tested patients after a median period of 2 months of therapy. No significant toxicity was recorded. We conclude that imatinib mesylate is effective and well tolerated in HES patients. FIP1L1-PDGFR α rearrangements may be useful molecular markers for both imatinib responsiveness prediction and minimal residual disease monitoring.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L. grants.

0658**LEUKEMIC TRANSFORMATION OF POLYCYTHEMIA VERA: A SINGLE CENTER STUDY OF 23 PATIENTS**

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Background. Polycythemia vera (PV) belongs to the group of Philadelphia-negative chronic myeloproliferative disorders. Acute leukemia (AL) may occur as late event of PV with an incidence of 5.3 x 1000 person-years. Studies with long-term follow-up indicate that transformation to AL is part of the natural evolution of PV. Myelosuppressive agents as hydroxyurea or pipobroman, when used as the sole treatment, seem not to increase the natural risk of AL. **Aims.** To define the presenting features and outcome of 23 patients who developed AL within a cohort of 414 consecutive PV patients (3208 person-years of follow-up, from 1970 to December 2004). Patients and methods This study includes 23 patients with AL post-PV diagnosed at the Division of Hematology, IRCCS Policlinico San Matteo, Pavia between 1981 and November 2004. Diagnosis of PV was made according to the criteria in use at the time of first observation. Treatment of PV consisted of pipobroman alone (n=14,

61%), sequential use of two or more myelosuppressive agents (n=6, 26%), phlebotomy and pipobroman (n=2, 9%), phlebotomy alone (n=1, 4%). Diagnosis of AL was according to World Health Organization (WHO) criteria, with 20% blast threshold for diagnosis. **Results.** Median age was 68 years, and 18 patients (78%) were >60 years old. At diagnosis of AL, most patients had WBC count >10 x 10⁹/L (n=17, 74%), Hgb <10 g/dL (n=13, 57%), and platelet count >50 x 10⁹/L (n=17, 74%). Leukemia was of myeloid origin in 22 out of 23 patients (96%). The most frequent subtype was M0-M1. Of 14 patients in whom cytogenetic analysis was available at leukemic transformation, 12 showed high-risk abnormalities including complex karyotype (n=10), del (7) (q22) sole (n=1) and del (X) (q26) sole (n=1), while 2 had a normal karyotype. In patients whose karyotype was available at diagnosis of PV, cytogenetic evolution was documented at progression to AL. Treatment consisted of supportive care and/or low-dose chemotherapy (n=15), or induction chemotherapy (n=8) with standard-dose cytarabine plus idarubicin, high-dose cytarabine, or a fludarabine-based regimen. Of 8 patients treated with induction chemotherapy, only one (13%) obtained a complete hematological response. Allogeneic stem cell transplantation was offered to a single patient, who is alive at day +70. The outcome of patients was poor with a median survival of 2.9 months (range 0.6-20.1), with no significant differences between palliation (median 2.5 months, range 0.6-20.1) and intensive treatments (median 5.6 months; range 0.7-8.3). **Conclusions.** This study shows a consistently poor outcome in patients with AL post-PV irrespective of the treatment employed. It may result from distinct clinical and biological features of this type of AL: namely, relatively old age, and unfavorable cytogenetics. Intensive regimens do not translate into a significant increase of survival. Allogeneic stem cell transplantation might be the treatment of choice for the rare patients achieving complete response after induction chemotherapy.

0659**RELATIONSHIP BETWEEN PERIPHERAL BLOOD AND BONE MARROW CD34-POSITIVE CELL COUNTS IN PATIENTS WITH PH-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDER AND ITS POTENTIAL CLINICAL IMPLICATIONS**

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Background. Philadelphia (Ph)-negative chronic myeloproliferative disorders (CMDs) include polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis with myeloid metaplasia (MMM). A peripheral blood (PB) CD34-positive cell count > 15 x 10⁶/L easily allows MMM to be distinguished from the remaining CMDs classified according to PVSG (PV, ET) and Italian criteria (MMM) (Haematologica 2003;88:1123-28). WHO classification recognizes a prefibrotic stage of MMM characterized by bone marrow hypercellularity without fibrosis. A recent paper (Br J Haematol 2005;128:42-8) reports normal circulating CD34-positive cell counts in patients with cellular phase MMM. **Aims.** To study the relationship between PB and bone marrow (BM) CD34-positive cell counts in patients with CMD. Patients and **Methods.** From April 2002 to October 2004, 77 paraffin embedded bone marrow biopsies and concomitant PB CD34 counts were available from 66 patients with CMD. For this analysis, in order to avoid any influence of cytoreductive treatments on parameters in study, we consider only 45 samples of patients who never received cytoreductive treatment. Diagnosis of CMD was according to WHO classification: 11 patients had PV, 5 ET, 6 prefibrotic MMM and 23 fibrotic MMM. PB CD34 counts were assessed by flow cytometry analysis as previously described. In order to identify BM CD34 cells, we examined 10 randomly selected fields at 400 magnification from paraffin sections immunostained for anti-CD34. BM CD34 counts were expressed as percentage of all the hematopoietic nucleated cells in the area. In the same fields, the absolute num-

ber of CD34-positive vascular structures was recorded. Non-parametric Mann-Whitney U test was applied to compare PB and BM CD34 counts between groups of patients. **Results.** Median PB CD34 counts were $3.1 \times 10^6/L$ (range 0.8-7.2) in PV, $3.4 \times 10^6/L$ (range 0.8-6.6) in ET, $5.4 \times 10^6/L$ (range 3.9-10) in prefibrotic MMM and $24.5 \times 10^6/L$ (range 2.3-1102) in fibrotic MMM. Mann-Whitney test showed that PB CD34 count significantly distinguished PV from prefibrotic ($p=0.009$) and fibrotic ($p=0.0001$) MMM, ET from fibrotic MMM ($p=0.007$), and prefibrotic from fibrotic MMM ($p=0.02$). Median BM CD34 counts were 0.9% (range 0.4-1.4) in PV, 0.9% (range 0.9-1.1) in ET, 0.7% (range 0.5-0.9) in prefibrotic MMM, and 0.5% (range 0.2-1) in fibrotic MMM. Mann-Whitney test showed that BM CD34 count significantly distinguished PV from fibrotic MMM ($p=0.0004$), ET from prefibrotic ($p=0.04$) and fibrotic MMM ($p=0.001$), and prefibrotic from fibrotic MMM ($p=0.02$). Finally, there was an inverse relationship between PB CD34 count and BM CD34 count ($r=-0.49$; $p=0.001$). **Conclusions.** This study shows that in CMD the higher the CD34 count in PB, the lower the CD34 count in BM. The two tests are clearly useful to distinguish patients with fibrotic MMM from those with PV and ET. Our findings also indicate that patients with ET have lower PB CD34 count and a significantly higher BM CD34 count than patients with prefibrotic MMM. The combination of PB and BM CD34 cells might be useful in the diagnostic work-up of patients with CMD.

0660

IMATINIB-MESYLATE THERAPY IS NOT EFFECTIVE IN SYSTEMIC MASTOCYTOSIS WITH D816V C-KIT MUTATION

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Human systemic mastocytosis (SM) is a rare disease characterised by an abnormal mast cell accumulation in various tissues. It usually occurs as a sporadic disease that is often persistent or progressive in adults, and it is often associated with eosinophilia. SM has been supposed to be associated with two classes of constitutive activating c-kit somatic mutations: the so-called 'enzymatic site' type (EST) mutations, affecting the structure of the catalytic portion of the kinase (e.g., D816V) and the 'regulatory' type (RT) mutations, affecting the regulation of an otherwise normal catalytic site (e.g., V560G). Recently, imatinib mesylate has proven to be effective in the treatment of chronic myeloproliferative disorders (CMPD) and hypereosinophilic syndromes (HES) associated with rearrangement of the PDGFRs, and in some patients with systemic mastocytosis with eosinophilia (SM-eos), characterised by CHIC2 gene deletion. Kinase inhibitors blocking constitutive c-kit activation, such as imatinib, might be used as therapeutic agents in SM, but there is increasing *in vitro* evidence that imatinib is able to inhibit both wild-type and RT mutant c-kit, but has no effect on EST mutant kit. Since 2002 we studied six patients diagnosed for SM, that were symptomatic. The diagnosis was based on histological and immunohistochemical evidence and met in every case the major classification criteria. We defined two patients as aggressive SM (ASM), and three of them as indolent sporadic mastocytosis (ISM); one of the patients could be considered SM with an associated clonal hematologic non-mast cell lineage disease (AHNMD) classified as SM-NHL. Five of them were male and one female. Trypsase serum levels were elevated in all of them. Two patients had both absolute and relative eosinophilia (45% and 19%, respectively). We treated four of them with imatinib (400 mg/die) for a median period of 3 months (range 1,5-5 months). Before therapy, mastocytes from all of them were found to carry the D816V mutant of c-Kit but not other genomic abnormalities typical for CMPD/HES (FIP1L1-PDGFRalpha, PDGFRbeta-ETV6 and FGR1-BCR fusion transcripts). One patient, with elevated count of eosinophils in peripheral blood, showed an initial response to imatinib, but lost it after 1 month from the beginning of treatment. Another patient

had a transient decrease of the mastocyte bone marrow infiltration. The remaining two patients were definitely non-responsive. Our observations confirm the *in vitro* data showing that imatinib treatment is scarcely effective for SM characterised by EST c-kit mutations, even if associated with hypereosinophilia.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna and A.I.L. grants.

Non-Hodgkin lymphoma - Biology

0661

PVSG AND WHO BONE MARROW EVALUATION IN 90 ESSENTIAL THROMBOCYTHAEMIA PATIENTS TREATED WITH PEG INTERFERON A-2 B. PRELIMINARY RESULTS

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Ninety ET patients diagnosed according to the PVSG criteria were enrolled in a phase II study (sponsored by the Schering Plough Company) designed to evaluate the efficacy, safety and tolerability of a two years PEG Interferon a-2 b (PEG Intron) treatment. The patients, followed in 16 Italian Centres belonging to the GIMEMA Cooperative Group and judged at high risk, had been previously treated with cytoreductive (97%) and antiplatelet(91%) drugs. At the start of the study the patients, 60 F and 30 M, mean age 45 years, showed splenomegaly in 22% of cases. The hematological response (HR: PLT<500 x10⁶/L) was observed in 64/81 (79%) and 48/55 (87%) of the patients on PEG Intron treatment at the end of the first and second year, respectively; the spleen enlargement disappeared in 75% of cases. The Bone Marrow data reported in the study CRFs document that the baseline increase of cellularity, of granulopoiesis, of megakaryocytes (MK) number, size and ploidy significantly decreased during PEG Intron treatment, while the MK displasia and fibrosis rate globally increased. In these patients a revision of the Bone Marrow biopsy slides was blindly performed by an Expert Pathologist Panel by applying the WHO criteria. The evaluation of two-thirds of cases showed the following distribution at the baseline: true ET (23%), IMF-0 (pre-MF17%), IMF-1 (early MF 40%), IMF-2/3 (classical MF 3%), MPD-U (17%). Interestingly, the true ET patients never showed spleen enlargement that, otherwise, was present in IMF-0 (20%) and IMF-1/2/3 (42%) patients. The patients with true ET did not show any bone marrow evolution during the PEG Intron treatment, while the IMF-1/2/3 frequency significantly increased. Moreover, the rate of the HR at the end of the first year was very high in true ET (93%) and lower in IMF-0 (60%) and in IMF-1/2 (71%) patients. All these preliminary data suggest that the patients classified as true ET (WHO Criteria) are not splenomegalic, have no myelofibrotic evolution and show a better response to the IFN treatment.

0662**UTILIZATION OF FLOW CYTOMETRY IN THE DIAGNOSIS OF CUTANEOUS LYMPHOID LESIONS**

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Background. The histologic diagnosis of cutaneous lymphoid lesions remains a challenging area of dermatopathology and is augmented by incorporation of immunophenotypic and genotypic data. A differentiation between cutaneous lymphoid hyperplasia and lymphoma is often hindered by the inability of routine paraffin-tissue immunohistochemistry (IHC) to define surface immunoglobulin (sIg) light chain expression. Flow cytometry (FCM) is a powerful and well established technique in hematopathology. However, it is generally under utilized in dermatopathology. **Aims.** To improve the analysis of surface Ig light chain expression and to increase the yield of immunophenotypic data obtained from skin biopsies, we evaluated the utility of FCM immunophenotyping on skin biopsies. **Methods.** Diagnostic FCM were performed on skin specimens of 32 patients, including 24 routine punch biopsies (entire 4mm, or one half of a 4 or 6 mm punch biopsy). The punch biopsies were transported on ice or RPMI solution. Cells were obtained by mechanical mincing. Fluorescent signals were measured by either a three-color or four-color flow cytometer. **Results.** We found that the specimens yielded sufficient material for at least a panel of 3 antibodies. The yield ranged from 9,000 to 3.0 million cells (average 712,565, median 400,000), with viability ranging from 25 to 99% (mean 76%, median 85%). Specimens transported on RPMI had better viability. Two cases of cutaneous lymphoid hyperplasia showed polyclonal B-cells by FCM, confirmed the histologic impression. Both patients had complete resolution of their clinical lesion either spontaneously or after intralesional steroid injection. Eighteen (18) cases were histologically diagnostic of lymphoma. Demonstration of a clonal B-cell proliferation by sIg light chain restriction (14) or absence of sIg expression (1) confirmed the histologic diagnosis. In many of these cases, this eliminated the need for redundant IHC stains or molecular assays. Another eight (8) cases were histologically suspicious but difficult lesions due either to processing artifact, mixed cellular infiltrate, paucity of neoplastic cells or indeterminate histology. Detection of sIg restriction in these cases enabled us to reach a diagnosis of lymphoma. Overall, evidence of a clonal B-cell proliferation was detected by FCM in 89% (24/27) of cutaneous primary or secondary B-cell lymphomas, compared to 40% (4/10) by IHC. FCM immunophenotyping also provided key diagnostic evidence for an anaplastic plasmacytoma. For subclassification of the lymphomas, additional markers (3 to 23) were analyzed by FCM on 20 specimens. FCM immunophenotypic data also contributed to the diagnosis on three (3) T-cell lymphomas. **Conclusions.** Our experience demonstrates that FCM immunophenotyping can be successfully applied to routine skin biopsies and contributes significantly to the diagnosis and subclassification of skin lymphoid lesions.

0663**EXTRANODAL (EN) INVOLVEMENT OF NON-HODGKIN'S LYMPHOMA (NHL) IN KOREA: FREQUENCY, MANIFESTATIONS AND CLINICAL CONSEQUENCES**

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Background. It has been known that EN involvement of NHL is frequent in East Asia, however, Korean data is not well informed and quoted infrequently. **Aims.** This study was aimed to assess the high frequency of EN involvement in Korean patients with NHL and to show the features of their EN manifestations. **Methods.** Between April 1998 and December 2003,

586 NHL patients diagnosed at Asan Medical Center were available to evaluate the clinical features. **Results.** The most frequent subtypes of total NHL were diffuse large B-cell lymphoma (45.4%), EN marginal zone B-cell lymphoma (23.2%), EN NK/T-cell lymphoma, nasal type (5.8%), peripheral T-cell lymphoma, unspecified (5.1%), and mantle cell lymphoma (3.6%), in decreasing order. Among total NHL, 267 (45.6%) patients were in stage I, 120 (20.5%) patients in stage II, 73 (12.5%) patients in stage III, and 126 (21.5%) patients in stage IV. EN involvement was present in 421 patients (71.8%). One EN site was involved in 363 patients, 2 sites were involved in 45 patients, and 3 EN sites were involved in 13 patients. Total 492 sites of EN involvement comprised stomach, bone marrow, head & neck, intestines, and lung & pleura, in descending order. Three hundred fifty-eight (61.1%) cases were classified as primary EN lymphomas (PENL). The primary sites of PENL were stomach (27.0% of total patients), head & neck (9.7%), intestine (8.5%), lung & pleura (3.2%), orbit (2.7%), and central nervous system (2.6%), in decreasing order. The most frequent subtypes of PENL were EN marginal zone B-cell lymphoma, diffuse large B-cell lymphoma, EN NK/T-cell lymphoma, nasal type, and peripheral T-cell lymphoma, unspecified, in decreasing order. While 81% of primary nodal lymphoma patients had aggressive histologies, PENL comprised significantly less patients with aggressive subtypes (59%, P<0.001). More PENL patients (74%) was in stage I/II than those of nodal lymphoma (56%, P<0.001). International Prognostic Index score was significantly higher in PENL (P<0.001). Patients with PENL had worse scores in LDH, stage, and EN factors. With median follow-up of 29 months, median overall survival of total patients was 49 months. PENL showed median OS of 51 months comparable to 48 months in nodal lymphoma. When grouped by aggressiveness and stage, PENL showed no significant survival difference from those of nodal lymphoma with same histology, aggressiveness, and stage. There was no significant factor for survival by multivariate analysis in the group of indolent PENL. Advanced stage and more than 1 EN site were the 2 significant prognostic indicators for poor survival in aggressive PENL. **Summary/conclusions:** PENL and EN involvement are much more frequent in Korea than in any other countries. In this analysis, PENL itself does not imply the poor prognosis when compared with counterpart nodal lymphoma.

0664**AGGRESSIVE PRIMARY GASTROINTESTINAL NON-HODGKIN'S LYMPHOMA IN KOREA: A CLINICOPATHOLOGIC ANALYSIS OF 100 PATIENTS WITH SPECIAL APPLICATION OF INTERNATIONAL PROGNOSTIC INDEX FOR SURVIVAL**

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Background. Primary extranodal lymphoma is much more prevalent in Korea than western countries. Primary gastrointestinal lymphoma is one of the most frequent extranodal lymphoma, however, their clinical features and prognostic indicators are not well defined in Korea. **Aims.** This study was aimed to obtain the information of clinical features and treatment results of patients with aggressive primary gastrointestinal non-Hodgkin's lymphomas (APGIL) in Korea. **Methods.** Between May 1998 and November 2003, 100 APGIL patients at Asan Medical Center were available to assess the clinical courses. **Results.** Male patients were 60. Median age was 53 years (range, 15-77 years). A total of 47 patients had primary gastric NHL. The small bowel, ileocecal region, colon, and rectum were involved in 26, 3, 12, and 3 patients, respectively. Multiple GI involvements were observed in 9 patients. Twenty-eight patients were in stage IE, 38 in IIE, 14 in IIIE, and 20 in IVE. Ninety patients had B-cell lymphoma (diffuse large B-cell lymphoma (DLBL) 76, mantle cell lymphoma 9, and Burkitt's lymphoma 5). T-cell lymphoma was present in 10 patients (peripheral T-cell lymphoma 6, enteropathy-type T-cell lymphoma 2, and anaplastic large cell lymphoma 2). With a median follow-

up of 29 months, median overall survival (OS) of total patients was 57 months and median event-free survival (EFS) was 41 months. B symptom, T- or B-phenotype, and involved site did not affect the survival. Sixteen patients with primary surgical resection did not show survival difference to those without surgery. Median EFS for DLBL was 57 months compared with 11 months of non-DLBL histology ($P < 0.001$). LDH level ($p = 0.04$), stage ($p = 0.001$), and number of extranodal sites ($P < 0.001$) showed significance for EFS among factors of International Prognostic Index (IPI). Median EFS of each IPI risk group was as follows: 56 months for low risk, not reached for low-intermediate-risk, 11 months for high-intermediate risk, and 4 months for high-risk, $P < 0.001$). Cox multivariate analysis revealed that DLBL histology ($p = 0.004$), younger age ($p = 0.043$), and less number of extranodal sites ($p = 0.005$) were independent prognostic factors for better EFS. Summary/conclusions: We are reporting the unique pattern of clinical manifestation of APGIL from a single institution in Korea. The IPI system has a prognostic value for Korean APGIL. DLBL histology, younger age, and less number of extranodal sites are the independent indicators of prognosis in Korean APGIL.

0665

IMMUNOGLOBULIN KAPPA GENE REPERTOIRE AND MUTATION PATTERNS IN FOLLICULAR LYMPHOMA

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Immunoglobulin kappa (IGK) gene usage and somatic mutation patterns were studied in a series of 42 follicular lymphoma (FL) cases. Forty-eight clonal IGKV-J rearrangements were amplified in 24 kappa-FL cases (3 cases with double rearrangements), 11 lambda-FL cases (one case with double rearrangements) and 7 cases for which information on light chain expression was missing (2 cases with double rearrangements). Thirteen different germline IGKV genes were identified; four genes (IGKV1-39/1D-39, IGKV4-1, IGKV1-5, IGKV2-28/2D-28: 14/9/4/4 rearrangements) accounted for 64,5% of the whole series. Complete analysis of the KCDR3 region was possible in 46/48 IGKV-J rearrangements; IGKJ2 was the most frequent gene (15/46 rearrangements), followed in order by IGKJ4, IGKJ1, IGKJ3 and IGKJ5. Thirty-three rearrangements were rearranged in frame (IF), while 13 were out-of-frame (OF). Twenty-four out of 48 IGKV-J sequences had >98% homology to germline (unmutated); 13/24 were OF rearrangements, mostly (10/13) from lambda-FL cases. The most frequent genes among mutated IF rearrangements in kappa-FL were IGKV1-39/1D-39 and IGKV4-1. The mean CDR3 length of both IF and OF rearrangements was 9 aminoacids. N nucleotides were detected in 11/33 IF rearrangements; evidence for 5' (IGKV) and 3' (IGKJ) exonuclease activity was obtained in 30/33 and 24/33 rearrangements, respectively. Somatic mutation analysis showed that some positions were 'hotspots' (eg. 31 in IMGT-CDR1) or 'universal coldspots' for replacement mutations (both invariant C at positions 23/104 and W/41 in all IF sequences). Average replacement/silent(R/S) ratios in CDRs and FRs were, respectively, 4.27 and 1.78. Analysis after the multinomial distribution model disclosed significant evidence for positive selection by classical T-dependent antigen for 8/24 mutated rearrangements (30%); this percentage is identical to the one previously reported for IGHV genes (30% of mutated IGHV genes were selected in a series of 55 FL cases reported in Br J Haematol. 1999;107: 625). In conclusion, the present series demonstrated biased usage of IGKV genes in both productive (IF) and non-productive FL sequences. The impact of the somatic hypermutation machinery varied significantly among productive rearrangements; nevertheless, the potential contribution of IGKV genes in antigen selection of the clonogenic B-cells in FL is comparable to that of IGHV genes.

0666

SUBSETS OF T-CELLS IN TUMOUR TISSUE MAY AFFECT THE OUTCOME OF FOLLICULAR LYMPHOMA: AN UPDATED ANALYSIS

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Background. Follicular lymphoma (FL) is characterized by a highly variable clinical course, with survival after diagnosis ranging from less than one year, to more than 20 years. The prognosis for the individual patient is difficult to assess. Sporadic reports have suggested that a high number of T-cells in FL lymph nodes may cause spontaneous regression of the lymphoma. **Aims.** We wanted to gain more knowledge of the prognostic importance of the non-malignant T-cells in the tumours. **Methods.** In the pathology archives at Karolinska University Hospital, Huddinge, we identified a total of 231 patients diagnosed with FL between 1 January 1994 and 31 December 2003. For 135 of these patients, flow cytometric data from assays made on diagnostic FL lymph nodes have been found, including markers for CD3, CD4, CD8, CD16/56, CD20, CD19 and CD25. Only cells within the flow cytometric lymphocyte gate were counted. The median age of the patients at diagnosis was 59.6 years (range 33.9-87.6 years), and 43.7% were male. The patients had received a variety of standard treatments. The median follow-up for surviving patients after diagnosis was 5.6 years (range 0.3-10.9 years). In the majority of cases, the histological grading was FL grade 1 and 2. For statistical grouping, the patients were divided by the median values from the flow cytometry results. Comparison has been made, for each CD, between the group with higher-than-median counts and the group with median-and-lower counts. Kaplan-Meier analyses of the preliminary data have been performed.

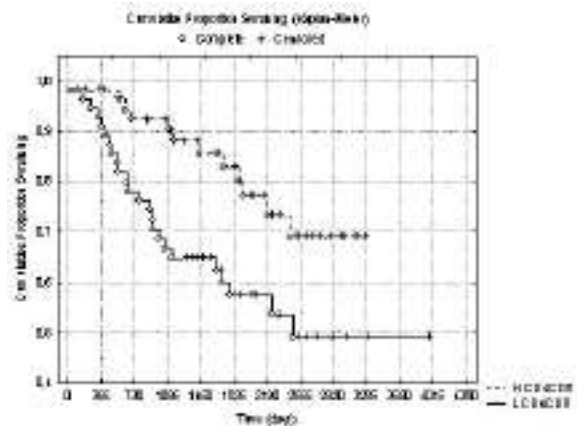


Figure. Overall Survival, High and Low Levels of CD4+CD8+.

Results. Median number of T-cell subsets in the lymph nodes were: CD3+: 30% (range 4-83%), CD4+CD3+: 23% (2-69%), CD8+CD3+: 6% (2-26%), CD25+CD3+: 6% (1-31%) and CD4+CD8+: 0.5% (0.02%-21%). The median number of total and clonal B-cells was 66% (15-94%) and 62% (13-94%), respectively. The level of CD3 positive cells did not have any relation to overall survival time. However, it seems that patients with a high CD4+ count might have a prolonged overall survival, and, likewise, high levels of CD25+ T-cells seem to be related to longer survival. Moreover, the group with high levels of double positive CD4+CD8+ cells shows a significantly better overall survival ($p < 0.014$), compared to the group with low levels. Among the patients with high levels of CD25+ T-cells,

only 29% had bone marrow disease, but among them with low levels of CD25+, 54% had bone marrow disease. A similar difference of bone marrow involvement was observed between the high and low CD4+ groups (32% vs. 44%), and between the high and low CD4+CD8+ groups (31% vs. 52%).

Summary/Conclusions. The study indicates that different T-cell subsets in FL lymph nodes may predict clinical course. Our results are thus in accordance with recent suggestions of an 'immune response signature' being of importance for prognosis in FL.

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TARGETING NF-KB AND INDUCTION OF APOPTOSIS BY DEHYDROXYMETHYLEPOXYQUINOMICIN (DHMEQ) IN BURKITT LYMPHOMA CELLS

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Background. Dehydroxymethylepoxyquinomicin (DHMEQ) is a derivative of the weak antibiotics epoxyquinomicin C, which was isolated from the culture broth of *Amycolaptosis sp.* We previously confirmed that DHMEQ inhibited NF- κ B activities in human myeloma cells. NF- κ B is a critical regulatory factor that activates the transcription of a number of genes, including growth factors, cell adhesion molecules and anti-apoptotic factors. As NF- κ B is activated in Burkitt lymphoma cells, we studied the effects of the new specific NF- κ B inhibitor, DHMEQ, for Burkitt lymphoma cells. **Aims.** In the present study, we evaluated the efficacies of new NF- κ B inhibitor, DHMEQ, for human Burkitt lymphoma cells and clarified its signaling pathways. **Methods.** We analyzed the proliferation of human Burkitt lymphoma cell lines, HS-Sultan and Daudi by MTS assay. The percentage of apoptotic and necrotic cells were analyzed by FACS with FITC-conjugated annexin-V and propidium iodide. The activities of nuclear NF- κ B was measured by TransAM NF- κ B kit (Active motif). Briefly, the binding of nuclear NF- κ B protein to the oligonucleotides including NF- κ B binding sites was measured by ELISA-based assays. Protein expression of several intracellular signaling molecules was analyzed by Western blotting using specific antibodies. **Results.** DHMEQ inhibited the proliferation of human Burkitt lymphoma cell lines, HS-Sultan and Daudi in dose- and time-dependent manners. Apoptosis was detected using FITC-conjugated Annexin-V by FACS. 51.3% of HS-Sultan and 29.9% of Daudi were in apoptosis 24 hours after treatment with 5 microgram/mL DHMEQ. Formation of apoptotic bodies were observed after treatment with DHMEQ by Giemsa staining. The activation of caspase-3 in HS-Sultan and Daudi was confirmed with the specific antibody against the active form of caspase-3 by FACS. When the Burkitt lymphoma cells were pretreated with pan-caspase inhibitor, z-VAD-FMK, DHMEQ-induced apoptosis was inhibited by 93.0% in HS-Sultan and 83.1% in Daudi, indicating DHMEQ-induced apoptosis was caspase-dependent. The activities of nuclear NF- κ B protein were suppressed by 81.4% in HS-Sultan and 76.2% in Daudi, one hour after treatment with DHMEQ compared to vehicle. Inhibitor of apoptosis (IAP) protein is known to regulate the activation of caspase, and NF- κ B regulates the transcription of IAP in various cells. The expression of IAP-1 but not IAP-2 was suppressed by DHMEQ in both HS-Sultan and Daudi cells. These results suggested that DHMEQ activates caspase-3 by suppressing the expression of IAP-1 proteins, followed by inducing apoptosis in Burkitt lymphoma cells. **[Conclusions]** In conclusion, we demonstrated that a new NF- κ B inhibitor, DHMEQ, induces apoptosis through caspase pathways in human Burkitt lymphoma cells *in vitro*. We are currently studying its *in vivo* efficacies using experimental animal models for human Burkitt lymphoma.

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GENE EXPRESSION PROFILING OF MICROARRAY GENE SIGNATURES FOR LYMPHOMA DIAGNOSIS AND MONITORING

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Background. Microarray gene expression profiling has identified certain 'Indicator' genes predictive of outcome in DLBCL and follicular lymphoma (FL). However, such methods rely on relatively large amounts of fresh starting tissue, and, therefore, measurement of Indicator genes in routine practice is difficult. **Aims.** To test the use of Indicator genes as a diagnostic tool for lymphoma, we have developed a simple, clinically practical polyA PCR based method for analysis of Indicator profiles in DLBCL and FL, specifically for use in very small tissue samples. **Methods.** PolyA RT-PCR, an extremely sensitive technique enabling global mRNA amplification from ng amounts of RNA, was applied to RNA extracted from 67 archived human frozen lymph nodes (LN). The resultant cDNA was analysed by TaqMan™ real-time PCR for 36 candidate Indicator genes (from Shipp et al & Alizadeh et al) using specific 3' directed primers. The expression level of each gene was quantified against human DNA, and the data normalised using the mean expression of four housekeeping genes (IF2-b, Gap, Rbs9 and beta actin). **Results.** The results demonstrate ability to distinguish between reactive and neoplastic LNs and between DLBCL and FL. Specifically, ACTA expression was reduced in neoplastic compared to reactive LNs ($p < 0.03$), whilst Urokinase ($p < 0.01$) and KIA0233 ($p < 0.01$) distinguished between DLBCL and FL. Preliminary analysis also indicates correlation with length of survival. Specifically, expression levels of EAR2, 5HT2B, HSF1, HSP27, ID2, KIA0233 & PKCG (all at $p < 0.03$) were correlated with interval to death in FL, and BSP1 with interval to death in DLBCL ($p < 0.04$). **Conclusions.** These results validate a simple, sensitive and robust method for analysing lymphoma Indicator genes. Crucially, initial amplification using polyA PCR makes the technique applicable to very small clinical samples, such as needle core biopsies or fine needle aspirates, facilitating routine clinical application.

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HEPATITIS B VIRUS INFECTION AND B-CELL NON-HODGKIN'S LYMPHOMA: AN ITALIAN MULTICENTER CASE-CONTROL STUDY

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Background. A high prevalence of hepatitis C virus infection in B-cells non-Hodgkin's lymphomas (B-NHL) is now well demonstrated. There are also several demonstrations of B-NHL regression in some patients with HCV infection and indolent lymphoma after antiviral treatment. Few data are instead available on the role of hepatitis B virus (HBV) in B-NHL. The present study is a multicentric case-control study conducted from January 1998 to February 2001 to evaluate the role of HBV infection in B-NHL. **Methods.** All were new diagnoses cases of non

pre-treated B-NHL; the controls were enrolled during the study period from the departments of traumatology, ortopedics, ophthalmology, surgery, gynaecology, otorhinolaryngology, medicine, dermatology and dentistry in the same hospitals where the cases were identified. HBV markers were tested in cases and controls; HBV-DNA detection was performed in HbsAg and Anti-HBc positive cases and controls. Adjusted odds ratios and their 95% confidence intervals were computed using multivariate logistic regression model. **Results.** 400 cases of B-NHL and 392 controls were tested: HbsAg was present in 35 (8.8%) cases and in 11 (2.8%) controls; OR (age and residence adjusted) was 3.68 (95% C.I.=1.50-9.04). Anti-HBs was positive in 28.3% of cases and 37% of controls, anti-HBc in 41.6% of cases and 29.1% of controls. The highest HbsAg+ prevalence was observed among cases between 36 and 55 years of age. A highest HbsAg prevalence was observed among indolent(8.3%), as well as aggressive (9.1%) B-NHL. Anti HBs+ and antiHBc+ cases were also equally distributed among indolent and aggressive lymphomas. **Conclusions.** The present study suggests that the risk of B-NHL is increased in HBV carriers and warrants further investigation of the possible role of hepatitis B virus in the pathogenesis of B-NHL.

0670

ABERRANT SOMATIC HYPERMUTATION IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

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Background. Primary mediastinal large B-cell lymphoma (PMLBCL) is recognised as a subtype of diffuse large B-cell lymphoma (DLBCL) arising in the mediastinum. PMLBCL displays specific clinical, morphological and molecular features suggesting that PMLBCL may represent a distinct clinico-pathologic entity. Aberrant somatic hypermutation of PIM-1, PAX-5, RhoH/TTF and c-MYC has been described as a molecular feature distinctive of DLBCL contributing to malignant transformation of these tumors. **Aims.** To investigate whether aberrant somatic hypermutation is associated with PMLBCL. **Methods.** We performed mutational analysis of PIM-1, PAX-5, RhoH/TTF and c-MYC in a panel of 19 PMLBCL. For comparison, 19 DLBCL were also analysed. A region spanning up to 1.5 Kb from the transcription start site and previously shown to contain >90% of mutations in B-cell lymphoma was analysed by PCR amplification and DNA direct sequencing. **Results.** Mutations targeting at least one of the four genes were found in 14/19 (73.6%) PMLBCL and 13/19 (68.4%) DLBCL, while mutations in more than one gene were found in 7/19 (36.8%) PMLBCL and 9/19 (47.3%) DLBCL. PAX-5 was mutated in 9/19 (47.3%) PMLBCL (mean mutation frequency: $0.20 \times 10^{-2}/\text{bp}$) and in 7/19 (36.8%) DLBCL (mean mutation frequency: $0.18 \times 10^{-2}/\text{bp}$); RhoH/TTF was mutated in 6/19 (31.5%) PMLBCL (mean mutation frequency: $0.08 \times 10^{-2}/\text{bp}$) and in 8/19 (42.1%) DLBCL (mean mutation frequency: $0.27 \times 10^{-2}/\text{bp}$); PIM-1 was mutated in 3/19 (15.7%) PMLBCL (mean mutation frequency: $0.09 \times 10^{-2}/\text{bp}$) and in 7/19 (36.8%) DLBCL (mean mutation frequency: $0.11 \times 10^{-2}/\text{bp}$); c-MYC was mutated in 6/19 (31.5%) PMLBCL (mean mutation frequency: $0.23 \times 10^{-2}/\text{bp}$) and in 5/19 (26.3%) DLBCL (mean mutation frequency: 0.11×10^{-2}). The mutation pattern was overall similar between PMLBCL and DLBCL and was consistent with the physiological somatic hypermutation process. Among PMLBCL, a total of 74 mutational events were detected, the majority of which were represented by single base-pair substitutions (n=66), whereas only 8 deletions of a short DNA stretch were observed. Of the 66 single base-pair substitutions, 41 were transitions and 25 were transversions, with a transition/transversion ratio of 1.64 (expected 0.5;

$p=0.001$) and a G+C/A+T ratio of 3.6. Eleven out of 66 (16.6%) single base-pair substitutions fell within RGYW/WRCY motifs. Among DLBCL, a total of 87 mutational events were detected with a prevalence of single base-pair substitutions (n=81), whereas only 4 deletions and 2 insertions of a short DNA stretch were observed. Of the 81 single base-pair substitutions, 42 were transitions and 39 were transversions, with a transition/transversion ratio of 1.07 (expected 0.5; $p=0.001$) and a G+C/A+T ratio of 1.89. Twenty six out of 81 (32.1%) single base-pair substitutions fell within RGYW/WRCY motifs. **Conclusions.** First, aberrant somatic hypermutation is involved in the pathogenesis of PMLBCL. Second, aberrant somatic hypermutation targets both PMLBCL and DLBCL with similar prevalence, distribution and mutational pattern. Since aberrant somatic hypermutation has been advocated as a molecular marker of DLBCL, our results corroborate the notion that PMLBCL represents a subtype of DLBCL rather than a distinct clinico-pathologic entity.

0671

FAMILIAR WALDENSTROM'S MACROGLOBULINEMIA (WM): REPORT OF A FAMILY WITH 4 FIRST DEGREE RELATIVES WITH WM AND MOLECULAR ANALYSIS OF THE VH GENE USAGE IN THE REST OF THE FAMILY

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Background. Genetic factors may contribute in the pathogenesis of Waldenstrom's macroglobulinemia (WM). This statement is supported by reports of familial clustering of WM. **Aims.** The purpose of the present study is to present a family with 4 members suffering from WM. **Patients and Methods.** Eight years ago, a 77 year old woman presented to our section with mild anemia and a monoclonal IgM compound in the serum. Bone marrow (BM) smears and biopsy revealed 80% infiltration by lymphoplasmacytes and the diagnosis of WM was made. Her sister was also suffering from WM (followed in another Centre). In both sisters, the disease was indolent, they never required chemotherapy and both died of other cause, in advanced age (85y and 83y respectively). Our patient had two sons that at the age of 52 and 46 years presented a serum monoclonal IgM. At presentation, the first one had a monoclonal IgM value of 1680 mg/dL and a 40% BM lymphoplasmacytic infiltration with no other findings, signs or symptoms; he is regularly followed in our section for 24 months and he is stable and in good condition. The second son, presented to us 16 months ago with a serum monoclonal IgM value of 886 mg/dL and a 10% BM infiltration by monoclonal lymphoplasmacytes and plasmacytes. While under follow-up examination only, he developed 9 months ago peripheral neuropathy with positive anti-Mag antibodies and improved with rituximab administration. Nor the mother or the sons had HCV antibodies. The first son has 3 children and the second two. Buccal and blood mononuclear DNA of the mother, both sons and their children, was analysed for the IgH rearrangement by PCR. Primers for the leader region of VH were used. Subsequently PCR products were introduced into a TOPO vector (Invitrogen) and sequenced by an automated Sequencer (MWGi). The nucleotide sequence data were analyzed using the Blastn program of Genbank. **Results.** Blood monoclonality was demonstrated in the three patients (mother and two sons) and in two non-affected members (one daughter of each son). The analysis of VH usage is complete in three members so far. The mother had mutated genes while the two non-affected members had unmutated VH genes using the VH3 family. **Conclusions.** This is an extremely interesting family with four affected members with Waldenstrom's macroglobulinemia in two generations. Moreover the detection of an asymptomatic monoclonal lymphocytic population in two additional members of the third generation and the ongoing study of all the rest of the members may elucidate further the pathogenesis of the disease.

Non-Hodgkin lymphoma - Clinical III

0672

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA-EVALUATION OF SIX YEARS EXPERIENCE

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Background. Primary Central Nervous System Lymphoma (PCNSL) is a rare form of extranodal non-Hodgkin's lymphoma (NHL) that accounts for 4% of all primary brain tumours and less 5% of non-Hodgkin's lymphoma. Despite the large number of non-randomized PCNSL trials, therapeutic issues remain unsolved. **Aims.** To analyse retrospectively the experience of Hospital São João in Primary Central Nervous System Lymphoma's treatment between 1998 and 2004. **Material and Methods.** In this period, 18 patients were diagnosed as having PCNSL. Six of these patients were excluded from the analysis: five were HIV related and one had concurrent systemic NHL. Diagnosis was established in brain tissues by stereotactic biopsy in 50% of the patients and surgical resection in the other 50%. All specimens showed diffuse large B cell lymphoma, involving predominantly the frontal lobes. The primary chemotherapy regimen included HD-MTX and steroids in all patients; this was followed by whole brain radiotherapy and, three weeks later, by two courses of high dose cytarabine in all patients (9/12) that were able to complete treatment. **Results.** The median age was 54 years (37-72) and male/female rate 8/4. The main clinical features at diagnosis were headache, hemiparesis and personality changes. Five of the 12 patients analysed (42%) are alive and disease free with follow-up times varying from 6 to 56 months. These five patients are currently in complete remission with a median survival time of 36 months, without major neurotoxicity. Two of these five patients had relapsed, one ocular and the other in the cranial skin; these patients entered a second complete remission after chemo+radiotherapy in first case and chemotherapy followed by autografting with BEAM conditioning in the second case. Seven patients died: four (33%) were early deaths and did not start specific treatment (chemotherapy and/or radiotherapy). **Conclusions.** Our experience shows that PCNSL is an eminently curable condition when treated adequately. Early deaths are a major limiting factor of the outcome of the disease.

0673

EFFICACY AND SAFETY OF YTTRIUM 90 (90Y) IBRITUMOMAB TIUXETAN THERAPY WITH RITUXIMAB MAINTENANCE IN PATIENTS WITH UNTREATED LOW-GRADE FOLLICULAR LYMPHOMA

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Background. Recent trials have demonstrated the efficacy of rituximab induction therapy, when followed by maintenance rituximab, in patients with untreated low-grade follicular NHL. Earlier studies, employing low-dose total-body irradiation as an initial treatment for follicular lymphoma (FL), have previously shown the inherent radiosensitivity of the disease. **Aims.** Based on these findings, we evaluated the efficacy and safety profile of frontline 90Y ibritumomab tiuxetan (Zevalin®) delivered prior to maintenance rituximab in patients with low-grade FL. **Methods.** Patients were eligible for treatment if they presented with histologically confirmed, previously untreated low-grade FL. Additional eligibility criteria included stage III or IV disease and a need for therapeutic intervention because of constitu-

tional symptoms, evidence of disease progression, or organ dysfunction directly attributable to NHL. Patients were excluded if there was evidence of histologic transformation to aggressive disease. On day 1, patients received an initial infusion of rituximab (250 mg/m²) followed by an imaging dose of indium 111 (111In) ibritumomab tiuxetan (5 mCi). One week later, a second infusion of rituximab (250 mg/m²) was given, followed by an injection of 90Y ibritumomab tiuxetan (0.3 or 0.4 mCi/kg, depending upon platelet count). Rituximab maintenance therapy (375 mg/m² x 4) was scheduled at 6-month intervals over 2 years. Endpoints included clinical response rates using standard International Workshop Response Criteria (IWRC) and toxicity. **Results.** Ten patients, presenting with grade I or II FL, have been treated with 90Y ibritumomab tiuxetan, and 8 are evaluable for treatment response. The median age of patients was 58 years (range, 40-82) and 50% (5/10) of patients had stage IV disease. Delivered doses of 90Y ibritumomab tiuxetan ranged from 21-32 mCi. Toxicities were primarily hematologic with grade 3 cytopenia occurring in 38% (3/8) of patients. Of the 8 patients qualifying for evaluation, 5 (62%) had complete responses and 3 (38%) had partial responses to 90Y ibritumomab tiuxetan induction. **Conclusions.** These data illustrate that frontline 90Y ibritumomab tiuxetan therapy produces high rates of response (100% in this small population) in patients with low-grade FL. Toxicities following administration of radiolabeled antibody were manageable. Further follow-up is needed to determine the long-term efficacy of combining frontline radioimmunotherapy with antibody-based maintenance.

0674

ANAPLASTIC LARGE CELL LYMPHOMA (ALCL): CLINICAL PRESENTATION AND OUTCOME OF 65 PATIENTS FROM A SINGLE INSTITUTION

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Background. Anaplastic CD30+ large cell lymphoma (ALCL) is a relatively rare type of aggressive lymphoma (2% of all NHL) often associated with t(2;5) (p23;q35) involving anaplastic lymphoma kinase (ALK). The lack of ALK expression is considered an adverse prognostic factor. **Aims.** In a retrospective study we evaluated treatment outcome and prognostic factors in 65 patients (pts) with ALCL. **Methods.** Histologic diagnosis was based on routine H&E and immunostaining of CD30. ALK protein expression was examined in 49 pts. All pts were treated with CHOP or CHOP-like chemotherapy. Kaplan-Meier method, log rank test, and Cox's model were used in a statistical analysis. **Results.** Median age was 40 years (range 15-83). 37 pts (57%) were male, 35 (54%) were in clinical stage (CS) III or IV, 35 (54%) had B symptoms, 32 (49%) had bulky disease, 33 (52%) had LDH > N, 30 (47%) had IPI score >1, and 62 (93%) was T or null cell ALCL. ALK was present in 22 of 49 cases tested (45%). IPI distribution by ALK+/- was similar (p>0.05). Median overall (OS) and progression free survival (PFS) was 51.2, 95% C.I.=[28.8; 161.5] and 28.0 months, 95% C.I.=[8.9; 59.9] for all patients. Five year OS and PFS for 22 ALK positive vs 27 ALK negative pts was 56% [95% C.I. (32%, 75%)] and 19% [95% C.I. (1%, 52%)] vs 50% [95% C.I. (27%, 70%)] and 35% [95% C.I. (16%, 55%)] (p=0.82). On multivariate analysis, CS IV (p<0.001), B symptoms (p=0.001), and bulky disease (p=0.024) were independent adverse factors for OS. CS IV (p<0.001), B symptoms (p=0.039), and male sex (p=0.052) were significant factors for PFS. **Conclusions.** ALK positivity was relatively low in this series of CD30+ ALCL pts and had no statistically significant influence on pt outcome. Stage IV, tumor bulk, and B symptoms were adverse factors for OS, and male sex - for PFS. These results appear comparable to expected outcome of patients with aggressive lymphoma as a whole.

0675**MOLECULAR ASSESSMENT BY BCL-2/JH QUANTITATIVE PCR IN 115 PATIENTS WITH FOLLICULAR LYMPHOMA IN ADVANCED STAGE TREATED WITH FLUDARABINE, CYCLOPHOSPHAMIDE AND MITOXANTRONE**

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Background. Patients (pts) with advanced stage FL remain incurable with current treatments. Fludarabine-based combination chemotherapy, including FCM, has shown remarkable activity in pts with FL in progression or relapse. **Aims.** To assess the efficacy of RQ-PCR in predicting clinical and molecular remissions in patients with FL treated with FCM. **Methods.** Between July 1999 and December 2003, 115 pts <65 yrs (53M/62F; median age: 53yrs), with FL (grade I, 57 cases; grade II, 52) in stages III-IV have been included in this study. The FCM regimen consisted of 6 cycles of fludarabine (25 mg/m²/day, days 1-3), cyclophosphamide (200 mg/m²/day, days 1-3) and mitoxantrone (6 mg/m², day 1). In 110 pts DNA material was available for molecular assays. RQ-PCR analysis was performed either at diagnosis (at peripheral blood [pb], bone marrow [bm] and lymph node [ln]), at the end of treatment, and during the follow-up. **Results.** The distribution of pts according to the bcl-2/JH breakpoint was: MBR, 67 (61%), mcr, 8 (7%), no MBR/mcr, 35 (32%). There was a strong correlation between the RQ-PCR values in pb and bm (R: 0.82; p<0.001). Discordant bm and pb cases were as follows: bm+/pb-, 2 pts; bm-/pb+, 5 pts. In 47 of 53 pts (89%) in whom DNA material from pb, bm and ln was available, the results on bcl-2/JH were concordant between pb/bm and ln. Pb RQ-PCR initial values correlated with bulky disease and bm involvement. The clinical outcome and pb molecular assessment at different time-points are detailed in the table. **Conclusions.** In patients with advanced FL, FCM treatment results in a high molecular CR rate as detected by RQ-PCR of the BCL 2/JH rearrangement. **Results.** in peripheral blood and bone marrow are concordant in 89% of the cases.

	Pre-FCM (N=34)	FCMx3 (N=32)	FCMx6 (N=54)	6-12 mo. post FCM (N=38)
CR rate (%)	0	50	87	81
Molecular CR (%)	0	76	88	71
RQ-PCR pb*	3.2 ± 10	0.060 ± 0.062	0.001 ± 0.004	0.006 ± 0.01

*Arbitrary units

0676**RITUXIMAB PLUS CHOP-14 FOR THE TREATMENT OF PATIENTS WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMA**

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Introduction. Standard first line therapy for aggressive Non Hodgkin's Lymphoma (NHL) consists of CHOP chemotherapy. The addition of Rituximab to CHOP has produced a survival benefit. Moreover the dose-dense CHOP has recently shown an improvement in response and survival rate of patients with aggressive NHL. **Methods.** In order to evaluate the efficacy and feasibility of dose-dense CHOP plus Rituximab we planned a schedule with Rituximab 375 mg/m² and CHOP therapy on day +1 given every 2 weeks, with G-CSF starting from day +7

to +11 for 6 cycles. We administered prophylaxis with sulfamethoxazole and trimethoprim and itraconazole. From January 2002, we have enrolled 44 patients with aggressive NHL (FL grade III = 13, DLBCL = 31). The mean age was 62 years (range 29-76). Two patients were stage I with bulky disease, 8 stage II, 7 stage III and 27 stage IV. Eighteen/44 (41%) patients presented B symptoms, 18 (41%) bone marrow involvement, 28 (64%) increased LDH values and 31 (71%) had at least one extranodal station involvement. According to the IPI score, 24 patients were Low Risk (IPI = 0-1) and 24 High Risk (IPI = 2-3). **Results.** All patients completed the planned 6 cycles and were evaluable for response. Thirteen (29%) patients delayed treatment (anemia=1, neutropenia=11, thrombocytopenia=1). No important extra-hematological toxicities were observed, particularly with regard to mucositis and cardiotoxicity. Thirty-six patients (82%) obtained a CR and 8 (18%) a PR (the overall response rate was 100%). After a median follow-up of 20 months (range 3 - 36), 6 patients (14%) have relapsed. The 2-year OS and DFS were 89% and 78% respectively. **Conclusions.** R-CHOP-14 is a feasible and active therapeutic protocol devoid of severe toxicity for the management of patients with aggressive NHL.

0677**IFOSFAMIDE, EPIRUBICIN, ETOPOSIDE (IEV) AND AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELL TRANSPLANT: A FEASIBLE AND EFFECTIVE SALVAGE TREATMENT FOR LYMPHOID MALIGNANCIES**

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Background. The combination of ifosfamide, epirubicin, and etoposide (IEV) has shown good tolerability and marked therapeutic activity against HLs and aggressive NHLs and excellent efficacy in mobilising PBSCs. Patients and methods. The IEV schedule consisted of epirubicin 100 mg/m² over 30' on day 1, etoposide 150 mg/m² over 1 hour on days 1-3, and ifosfamide 2.5 g/m² over 1 hour on days 1-3 together with MES-NA. Patients who proceeded to HDT received conditioning therapy with BCNU, etoposide, Ara-C and melphalan (BEAM, in HLs and NHLs), or melphalan 100 mg/sqm and mitoxantrone (MM patients). The present study currently includes 65 patients with a median age of 53 years: 27 with aggressive NHL, 20 with HL, 7 with indolent NHL, and 11 with MM. Fifty-five patients received IEV for disease that was refractory to conventional induction regimens or in first or second relapse; 4 patients with high risk NHL and 4 with MM were treated with IEV while in CR after chemotherapy in order to mobilise PBSCs. **Results.** Ninety per cent of patients with HL responded to IEV, and 85% of them achieved CR. Both aggressive and indolent NHLs were less responsive (ORR 55% and 33%, respectively; CRR 45% and 16.5%, respectively). MM patients showed intermediate responsiveness (ORR 50%, CRR 30%). Among 16 HLs and NHLs treated for chemorefractory disease, only 2 responded to treatment (1 CR, 1 PR). In our series, more than 90% of patients with HL and NHL who received IEV while in PR following the previous therapy responded to our salvage therapy (CR rate of 82% and 80%, respectively). Similar response rates were observed among patients treated in relapse. IEV was well tolerated in most patients. Haematological toxicity was not negligible, and 37% and 30% of patients received RBC and platelet transfusions, respectively. Twenty-six per cent of patients had fever of unknown origin, but no life threatening infections were recorded. No severe heart complications were observed. PBSC mobilisation was successful in 37 out of 39 patients (95%) and led to the collection of a median of 16, 12, and 13.7x10⁶ CD34+ cells / Kg in patients with HL, NHL, and MM, respectively. All 37 patients actually underwent autologous stem cell transplant, following a 1 to 2 month interval after the end of IEV. Two patients were submitted to allogeneic transplant. Median overall survival in HL, aggressive

NHL and indolent NHL have been 32 (5-60), 16 (2-46), and 14 (4-42) months, respectively. Median EFS have been 31 (5-60), 7 (2-46), and 7,5 (4-42) months, respectively. *Conclusions.* In conclusion, our study confirms that IEV±HDT is a well tolerated and effective salvage treatment for lymphoid malignancies, and that IEV acts as an excellent stem cell mobiliser.

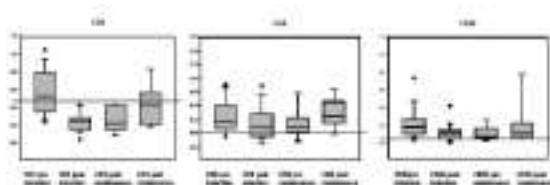
0678

PERIPHERAL T-LYMPHOCYTE SUBSETS IN PATIENTS TREATED WITH RITUXIMAB-CHLORAMBUCIL COMBINATION THERAPY FOR INDOLENT NHL

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Chimeric anti-CD20 antibody rituximab has considerably changed the treatment of follicular lymphoma patients. Combination of rituximab with chemotherapy has significantly improved the response rate in this setting of patients. When used as single agent, a maintenance treatment can significantly prolong EFS without an increased incidence of side effects. Prolonged rituximab treatment is associated with a depletion of circulating B-cells without an increased incidence of infections. So far no data are available on T-cells peripheral subset after a rituximab-chemotherapy combination treatment. We retrospectively investigated the effects of rituximab-chlorambucil combination therapy on peripheral blood lymphocyte subsets in 25 low-grade or follicular B-cell NHL patients (12 newly diagnosed and 13 relapsed/refractory). The regimen consisted of chlorambucil 6mg/sqm daily for 6 consecutive weeks in association with a standard 4-weekly rituximab as induction phase. After reevaluation, the responding patients received 4 additional cycles with chlorambucil (6 mg/sqm daily for 2 weeks monthly) plus rituximab (once/monthly). All the patients completed the planned treatment and were evaluable for response and toxicity. After induction, we observed absolute G2-G3 lymphopenia in 13 and 6 patients respectively; after consolidation 10 pts had G2 and 5 pts G3 absolute lymphopenia; after a median of 9 months (3-30months) from the end of treatment 6 pts showed G2 lymphopenia. As expected, after the first administration of rituximab, all the patients presented a reduction of CD19/20+ positive cells below 0.1 that was present at reevaluation after induction and consolidation therapy as assessed by flow cytometry. All the patients presented a marked CD4+ reduction independently of the levels at baseline (median absolute CD4+ 246 cell/mL) and this trend was maintained during the therapy (median absolute CD4+ 216cell/mL); at median follow up of 9 months, in 50% of the patients absolute CD4+ T cells recovered within normal range as shown in figure 1. On the other hand we observed no significant reduction of CD8+ and CD56+ absolute value. About infections: 2 patients developed cutaneous HZV, 1 perianal abscess, 1 patient was hospitalized for HBV reactivation. Our experience suggests that chlorambucil-rituximab combination therapy induces a selective decrease of absolute CD4+ T cells during treatment. However, no major infections were observed: the lack of correlation between the drop of CD4+ and high rate of infections could be related to the relative short period of absolute CD4+ low count. Trials using Rituximab alone or in combination for longer period of time as maintenance therapy could be useful to confirm our observation.



0679

DOSE-DENSE R-CHOP14 SUPPORTED BY PEGFILGRASTIM IN DIFFUSE LARGE B CELL LYMPHOMA: A STUDY OF FEASIBILITY AND TOXICITY

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Background. CHOP chemotherapy (CT) in a dose-dense setting (CHOP14) or with the addition of rituximab (R-CHOP) may improve response rate and survival of patients with diffuse large B-cell lymphoma (DLBCL). Besides, in dose-dense regimens, G-CSF proved instrumental to deliver on time the planned CT dose. *Aims.* This phase II study was designed to evaluate feasibility, toxicity and efficacy of a dose-dense CHOP regimen, with the addition of rituximab (R-CHOP14), supported by pegfilgrastim as first-line therapy in patients affected with DLBCL. *Methods.* Eligibility included patients with DLBCL in stage II-IV, aged 18-70. The CHOP regimen was delivered every 14 days, preceded on day 1 by rituximab (375 mg/m²) and followed on day 3 by pegfilgrastim (6 mg per cycle). Fifty patients were enrolled; 41 have completed the program and 35 are evaluable for response to therapy, so far. Ten patients received adjuvant RT on sites of prior bulky disease or residual disease after CT. Median age was 55 yrs (range 22-70) and M/F ratio 0.92. Half of patients were in stage IV (16% with bone marrow involvement) and 40% had bulky disease. Age-adjusted IPI 0-1 accounted for 52%, IPI 2 for 38% and IPI 3 for 10% of patients. Toxicity was calculated over a total 253 cycles of therapy; feasibility was calculated over 203 cycles, not considering the cycles 1. *Results.* Cycles were delivered on time in 189/203 instances (93%). Delays occurred in 14 occasions; 3 cycles (1.5%) were postponed for grade 2 neutropenia and 11 for non-hematological toxicity. Average relative dose intensity was 95% for doxorubicin and cyclophosphamide, and 91% for vincristine. R-CHOP14 produced a complete response (CR) in 27 of 35 evaluable patients (77%); four patients (11%) proved resistant to this approach. The ANC nadir occurred on day 10, with a median value of 1.5 x 10⁹/L (range 0.01-23.4). Grade 3 and 4 neutropenia occurred in 33% and 18% of 253 cycles, respectively, with a mean duration of grade 4 neutropenia of 2±1.3 days. Neutropenic fever developed in 8 instances (3% of cycles), with a median duration of 5 days (range 2-10). Febrile episodes occurred in 16 additional cases with ANC above 1 x 10⁹/L. Fourteen severe adverse events (SAE) were registered (4 of them developed off-therapy) and consisted of interstitial pneumonia in 8 cases (in 3 *Pneumocystis carinii* was documented), bacterial pneumonia in 2, septic shock and GI hemorrhage in one case, each. Eight patients died so far; two of them during the program (both over 60 with advanced disease). *Conclusions.* These results indicate that a single dose per cycle of pegfilgrastim successfully supports dose dense R-CHOP14 in DLBCL, allowing on-time delivery of therapy in 93% of cycles, with optimal average dose intensity. The overall program produced CR in 77% of patients, with low incidence of febrile neutropenia. Incidence of febrile episodes unrelated to grade III-IV neutropenia was relevant and mostly due to interstitial pneumonia. The occurrence of *Pneumocystis carinii* pneumonia was unexpectedly high and cotrimoxazole prophylaxis is mandatory.

0680**RITUXIMAB TREATMENT FOR SPLENIC MARGINAL ZONE B-CELL LYMPHOMAS (SMZL)**

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Background. Splenectomy has traditionally been considered as standard first line treatment for SMZL. However it carries significant complications, especially in elderly patients. **Aims.** The purpose of the present study is to evaluate the safety and efficacy of Rituximab for the treatment of SMZL. **Patients and Methods.** Thirteen patients with SMZL, diagnosed in our Department were treated with Rituximab. Diagnosis was established using standard criteria. Eleven of them received Rituximab as first line treatment at a median time of 2 months (1-120) after diagnosis. The remaining two received Rituximab one and 14 months after splenectomy respectively. Four patients were symptomatic. Patients' median age was 68 yrs (range 50-78) and four were male. All non-splenectomized patients had palpable splenomegaly before treatment. The median size of the spleen was 10 cm below left costal margin (range 3-20 cm). 11/13 patients had anemia, 6/13 leukocytosis, 9/13 lymphocytosis, 3/13 leukopenia and 4/13 thrombocytopenia before treatment initiation. Rituximab was administered for six weekly cycles of 375mg/m². 6/13 patients received maintenance treatment, starting at a median time of four months (range 2-7) after the completion of the six cycles. Maintenance was given as one 375mg/m² dose every two months. Complete clinical response was defined as disappearance of palpable splenomegaly. Complete hematologic response was defined as the restoration of all hematologic parameters to normal values and partial hematologic response as an improvement of abnormal values without complete normalization. Molecular remission was defined as PCR negativity for IgH rearrangement in patients with negative bone marrow biopsy after treatment. **Results.** All non-splenectomized patients achieved a complete clinical response with Rituximab treatment. Symptomatic patients had resolution of disease/splenomegaly related symptoms. 8/13 (62%) patients achieved a complete hematologic response, including the two previously splenectomized patients and 5/13 (38%) a partial hematologic response. Anemia was resolved in 8/11 patients, leukocytosis in 6/6, leukopenia in 1/3 and thrombocytopenia in 4/4 patients. Bone marrow biopsy after treatment disclosed persistent but reduced infiltration in 6/10, disappearance of lymphomatous infiltration in 3/10 and remained unchanged in a single patient. 2/3 patients with negative bone marrow biopsy were in molecular remission, while one patient remained PCR positive after treatment. He subsequently received a second course of four weekly cycles and became PCR negative. No patient presented infectious complications after Rituximab administration. Infusion related side effects were easily treated with steroids, antihistamines and paracetamol. Two patients, who did not receive maintenance treatment progressed with reappearance of splenomegaly both at 7 months after completion of treatment and were retreated with six cycles of Rituximab. One of them had a second response and the other remained with stable disease. All patients are alive. Median follow up after treatment initiation is 11 months (range 2-17) and median response duration has not been reached yet. **Conclusions.** Rituximab is a safe and effective treatment for SMZL and can be considered as an alternative to splenectomy as first line therapy. Maintenance may be important for consolidation of response, and deserves further evaluation in subsequent studies.

0681**T-CELL NON-HODGKIN'S LYMPHOMAS IN KOREA: A SINGLE INSTITUTIONAL EXPERIENCE**

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Background. The incidence of T-cell non-Hodgkin's lymphoma (NHL) is known to be more frequent in Far East Asia. Their frequency and clinical course however, have not well been defined in Korea. **Aims.** We aimed to define the relative frequency and the clinical features of T-cell NHL in Korea. **Methods.** Retrospective analysis was performed on 586 NHL patients who were diagnosed at Asan Medical Center between January 1998 and December 2003. **Results.** There were 101 patients of T-cell NHL (17.2% of total NHL). The most frequent subtypes of T-cell NHL were extranodal NK/T cell lymphoma, nasal type (NASAL) (33.7%), peripheral T-cell lymphoma, unspecified type (PTCL-U) (29.7%) and anaplastic large cell lymphoma, T/null cell, primary systemic type (ALCL) (9.9%), in decreasing order. Sixty-four patients (63.4% of T-NHL) had primary extranodal lymphomas with no difference from 60.6% of B-cell NHL cases. Seven pathologic subtypes in this study could be classified into 3 prognostic subgroups: favorable (cutaneous ALCL), 5 year overall survival (OS) 100%; intermediate [PTCL-U, ALCL and precursor T-cell lymphoblastic lymphoma (T-LBL), NASAL], 5 year OS 60-70%; unfavorable [enteropathy-type T cell lymphoma (ETTL), angioimmunoblastic T cell lymphoma (AITL)], 5 year OS 50%. When grouped 3 frequent T-cell NHL (NASAL, PTCL-U, and ALCL) together, their survival was clearly depicted by IPI scores (p=0.0069). Median survival was not reached in low risk group, 41 months in low-intermediate risk group, and 10 months in both high-intermediate and high risk group. In univariate analysis of IPI elements and other clinical features, IPI category of stage (p=0.0133) and extranodal site (p=0.0005) were significant predictors of survival. Cox multivariate analysis revealed the number of extranodal sites was the only independent prognostic indicator for the 3 frequent T-cell NHL in Korea (p=0.042). **Conclusions.** The relative frequency of T-cell NHL seems to be decreasing compared with previous Korean studies, and becoming similar to that of western countries. Still NASAL is frequent in Korea. Korean ALCL does not have a favorable prognosis. New paradigm of diagnostic and therapeutic approach is warranted for Korean T-NHL.

0682**NON-GASTROINTESTINAL EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA (EMZL): A SINGLE CENTER EXPERIENCE**

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Background. Extranodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue (MALT lymphoma) is a distinct entity with specific clinical and pathologic features. The stomach is the most common and best studied site of involvement, but other non-gastrointestinal (non-GI) sites may also be involved. **Aims.** To assess the clinical data of patients with non-GI EMZL and correlate disease localization with clinical features, treatment modalities and patient outcome. **Patients and Methods.** We reviewed the medical files of 40 consecutive patients with an initial diagnosis of EMZL according to the REAL/WHO classification criteria, who presented with a clinically dominant non-gastrointestinal site of involvement. **Results.** The median age of the patients was 50 years (23-87) and 20/40 (50%) were males. The most commonly affected sites were skin (n=13; 33%) and salivary glands (n=11; 28%), followed by lung (n=5; 13%), eye (n=5; 13%), oral cavity non-salivary (n=2; 5%) and upper airway (n=1; 2%), while 3 cases (8%) presented with

more than one extranodal site. Lymph node involvement was present in 5 patients (13%) and bone marrow involvement in 3 (8%). Associated autoimmune disorders were noticed in 6 patients (16%) and monoclonal gammopathies in 5 (13%). Ann Arbor stage was I in 29 patients (72%), II in 3 (8%), and IV in 8 (20%). Two patients (5%) presented B-symptoms. Advanced stage disease was observed in patients with salivary gland involvement and lung involvement (50% and 40% respectively). In patients with salivary gland involvement, autoimmune manifestations were encountered in 25% and dissemination to other extranodal sites in 17%. In patients with primary lung involvement, a long period of time (2 years) was observed between the onset of the disease and diagnosis; 1 of them (20%) presented immunoblastic transformation. The 1st line treatment, for the 13 patients with extranodal skin lymphoma (SALT), was interferon- α (in 54%), Mabthera I.V or intraleisional (in 31%) or resection alone in the rest. Of the other 29 patients, 72% received chlorambucil intermittently (in combination with mabthera or radiotherapy in some of them). With a median follow-up of 53 months (6-216), 5 deaths are recorded so far; the 5- and 10-year overall survival was $82\pm 8\%$ and $65\pm 13\%$. The 5-year overall survival was 90% or higher for each non-lung presentation vs $60\pm 22\%$ for patients with lung involvement ($p=0.01$). The only other feature associated with inferior overall survival was age >60 years ($p=0.003$). **Conclusions.** Our data confirm the previously described indolent behavior of EMZL, with the exception of patients with lung involvement, who appear to have a more aggressive behavior. Although EMZL are frequently localized at the time of diagnosis, they have a tendency to disseminate among various MALT sites and extensive staging procedure is needed at diagnosis and during follow-up. The optimal management of EMZL is not well established and may be site-related.

0683

RITUXIMAB WITH OR WITHOUT CHEMOTHERAPY TO TREAT HEMATOLOGIC MALIGNANCIES IN PATIENTS INFECTED WITH HEPATITIS C VIRUS: HIGH INCIDENCE OF VIRAL LOAD INCREASE BUT LOW RISK OF LIVER DYSFUNCTION

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Background. Reactivation of chronic hepatitis B virus (HBV) infection with the development of fulminant hepatitis has been reported following Rituximab, given alone or associated with chemotherapy. Less is known about the risk of hepatic injury after Rituximab in patients infected with hepatitis C virus (HCV). **Aims.** This study reports the monitoring of viral load and liver function tests in a series of HCV positive patients receiving Rituximab-containing treatments for different haematological malignancies. **PATIENTS & Methods.** Eighteen consecutive HCV-positive patients were studied, thirteen had B-cell lymphoma (subtypes: Diffuse Large Cell [3], Follicular [3], small-lymphocytic/ marginal-zone [5], post-transplant lymphoproliferative disorder [2]), four had cryoglobulinemia and one patient had a diagnosis of Canale-Smith syndrome; thirteen patients were at diagnosis while five were at relapse. HCV infection was identified by anti-HCV antibodies (chemiluminescence) and by HCV RNA detection (reverse-transcription polymerase chain reaction). Seven patients received Rituximab alone, two in combination with involved-field radiotherapy and nine in combination with chemotherapy; six of these latter patients were managed with conventional chemotherapy, while three underwent an intensive program with autograft, including Rituximab given for *in vivo* purging purposes. Liver function tests (LFTs) and viral load were evaluated before and during chemo-immunotherapy, and at follow-up, with post-therapy controls performed up to 49 mos. since treatment completion (median follow-up: 20 mos.). **Results.** Fourteen patients were positive for both anti-HCV and HCV RNA, whereas four patients had only anti-HCV antibodies; four patients were also positive for the

antibody to the core antigen of HBV, in absence of HBsAg and HBV DNA serum detection. All patients had normal pre-treatment LFTs. During treatment, ten patients displayed a variable increase of the viral load, recorded at a median of 5 mos. after treatment start. No abnormalities of LFTs were recorded during and after treatment, with the exception of one patient who received the intensive chemo-immunotherapy program for an aggressive lymphoma at relapse; this patients had a pre-treatment HCV viral load of 1,240,000 IU/mL and a slight increase of liver enzymes; he presented a marked 'flare' of hepatitis C, with a HCV viral load of 4,500,000 IU/mL at 3 mos. after autograft and peak values of alanine-aminotransferase and aspartate-aminotransferase of 3011 U/L and 431 IU/L, respectively, at 6 mos. after autograft. Values of viral load and LFTs spontaneously normalized without any specific therapy within 14 months. No acute or fulminant hepatitis were recorded neither severe liver-related complications. **Conclusions.** Increase of HCV viral load is frequently detectable in HCV infected patients receiving Rituximab +/- chemotherapy for haematological malignancies; viral recurrence, however, does not imply serious abnormalities of liver function, unless intensive programs with autograft are employed; thus, prophylaxis with Ribavirin does not seem to be firmly required in HCV positive patients undergoing Rituximab alone or in combination with conventional chemotherapy.

0684

SINGLE AGENT RITUXIMAB IS ACTIVE AS FIRST LINE THERAPY IN PATIENTS WITH NEWLY DIAGNOSED AIDS-RELATED LYMPHOMA (ARL). A PILOT TRIAL OF THE SWISS GROUP FOR CLINICAL CANCER RESEARCH

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Background. The activity of rituximab in ARL is controversial. While several phase II studies of chemotherapy-rituximab combinations indicated an improved outcome in comparison to historical controls, a randomized trial of the AIDS Malignancies Consortium showed no benefit of adding rituximab to CHOP, which resulted in increased toxicity without improvement in response rates (Blood 102:1488a, 2003). The activity of rituximab monotherapy in ARL has not been established. **Methods.** Based on the high activity of rituximab in post-transplantation lymphoproliferative disorders, we initiated a single agent phase II study in newly diagnosed ARL. Patients with intermediate or high grade lymphoma were treated with rituximab 375mg/m² weekly x4 (treatment phase). Patients with no change, partial or complete response were followed for an additional 8 weeks (observation phase). Patients showing signs of progression at any time point during the treatment or the observation phases went off study and were treated with CHOP. Highly Active Antiretroviral Therapy (HAART) was mandatory. **Results.** 8 patients were treated between 2000 and 2003. Baseline characteristics: median (range) age 41,6 (35,2-75,8) years; Stage III-IV 75%; IPI 3 (1- 4); CD4 141 (5-985) cells/mm³; HIV viral load 71500 (<100-106) RNA copies/mL. 5 pts completed the treatment phase, 1 progressed during the therapy phase and 2 during the observation phase. Responses included 2 CR/Cru and 3 PR, corresponding to an overall response rate of 62% (5/8). Side effects included fever and fatigue, but no infection. Among the 5 responders, 2 progressed, for an overall disease-free survival of 25 months (5-50). Response to chemotherapy included no response in 2 cases of early PD, and CR in one case of PD and two late relapses. **Conclusions.** Although the number of patients is small, the results from this pilot study suggest that rituximab monotherapy in newly diagnosed ARL has significant and sustained activity, with minimal toxicity. The addition of rituximab to chemotherapy regimens for ARL deserves further study.

Platelets

0685

Q-TWIST ANALYSIS OF RITUXIMAB PLUS CVP VERSUS CVP ALONE AS FIRST-LINE TREATMENT FOR ADVANCED FOLLICULAR LYMPHOMA

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Background. In a randomised phase III study (Marcus R, et al, Blood 2005), the addition of rituximab to each of eight cycles of CVP chemotherapy (R-CVP) has been shown to significantly improve clinical outcomes without an increase in clinically significant toxicity in patients with previously untreated follicular lymphoma. **Aims.** We evaluated the impact of R-CVP compared to CVP on patients' quality of life, using quality-adjusted time without disease symptoms or treatment toxicity (Q-TWiST) as a measure of benefit. All patients who received at least one treatment were included in the analysis (n=321). **Methods.** Overall survival was partitioned into three distinct health states: relapse (REL), toxicity (TOX) and time without disease symptoms or toxicity (TWiST). Toxicity in this analysis was defined as any treatment-related adverse event occurring during or within 24 hours following treatment administration. Differences in health states with 95% confidence intervals (CI) between R-CVP and CVP were calculated based on the 30-month median follow up data. **Results.** The addition of rituximab to CVP was not associated with an increased burden of toxicity (p=0.2266, 1.7 and 1.8 months for R-CVP and CVP respectively). The mean time R-CVP patients spent in REL was significantly shorter than observed with CVP (mean±SD: 9.9±1.2 months vs 16.2±2.2 months; p=0.0055). Patients in both treatment arms experienced TWiST, but R-CVP patients achieved a 7.5-month (95% CI: 5.00–9.91 months) additional benefit in mean time (24.2±0.9 months and 16.7±0.8 months, respectively; p<0.0001) spent in TWiST compared to CVP alone. Each health state was assigned a utility ranging from 0 to 1, with 1 being the optimal state of no toxicity and no symptom of disease. Q-TWiST was calculated as (mTOX × TOX) + (mREL × REL) + (mTWiST × TWiST). With utility coefficients of 1.0 for all health states, the unadjusted difference in mean survival time between R-CVP and CVP was 0.97 months (95% CI: -3.2–5.1 months). Using, for example, patient-reported utility coefficients of 0.5 for both TOX and REL and 1.0 for TWiST, the quality adjusted overall difference in mean survival time was 4.2 months with R-CVP compared with CVP alone (30.0 vs 25.8 months). **Conclusions.** By integrating quality of life assessment into a survival analysis, these Q-TWiST data indicate that the addition of rituximab to each of eight cycles of CVP in the first-line treatment of follicular NHL affords a statistically significant TWiST and a substantial increase in Q-TWiST benefit over CVP alone at a median follow-up of 30 months. It is anticipated that this benefit will be further increased with extended follow-up. This quality-adjusted survival benefit further supports the use of R-CVP as an effective first-line regimen for follicular lymphoma.

0686

SEVERE IMMUNE THROMBOCYTOPENIA DUE TO HPA-1A HOST-DERIVED ANTIBODIES AFTER NON-MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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Background. The human platelet(plt) antigen (HPA) system is independent of the HLA system. Therefore, host- or donor

derived alloimmune thrombocytopenia can develop after allogeneic haematopoietic stem cell transplantation (HSCT) even in HLA-matched donor-recipient pairs. We report the first case on a stem cell recipient developing thrombocytopenia due to host-derived HPA-1a antibodies after non-myeloablative allogeneic HSCT. A 52-year-old male patient was diagnosed with multiple myeloma in 6/2003. Treatment consisted of 3 cycles vincristin, adriamycin and dexamethason followed by tandem autologous stem cell transplantation. Because of progressive disease he received 4 cycles of bortezomib, and after complete remission a stem cell allograft(7,99x10⁶/kg BW CD34+ cells) from his histocompatible (HLA A,B,C,DR identical) brother after reduced intensity conditioning regimen with fludarabine (3x30 mg/m²) and alkeran (100 mg/m²). He had received only twice packed red blood cell concentrates and one plt concentrate before allogeneic HSCT. Stable bilinear engraftment occurred around d12 but was accompanied with a continuous decrease of plt counts. Between d10 and d19 the patient received seven plt transfusions, containing a median of 2,96x10¹¹ plt/unit(range 2,7-3,21x10¹¹ plt/unit) from random donors. The corrected plt count increments at 12 to 24 hours after these transfusions were <2500/μL. Therefore, and because of even a further decline of platelet counts to 1000/μL on d19 we investigated the presence of plt antibodies. **Methods.** The patient's serum was tested by antigen capture ELISA assays (PAKPLUS® and PAK1®, GTI) and a solid phase assay (Capture-P®, Immucor). The MAIPA assay was used to confirm the results obtained by the above mentioned assays. In addition, we tested the patient's serum by the MASPAT kit(CLB) against plt from the donor and against homozygous HPA-1a plt obtained from our donor pool. Stored recipient's DNA from the time before HSCT was used for genotyping. Genotyping for HPA -1, -2, -3 and -5 of the donor and the recipient was performed by PCR-SSP (HPA, Protrans). **Results.** The patient's serum obtained on d19 after HSCT reacted strongly with the donor's plt due to anti-HPA-1a antibodies and antibodies against HLA class I antigens. The patient's genotype before transplantation was HPA-1bb, -2aa, -3ab, and -5aa; the donor was HPA-1ab, -2aa, -3aa, and -5aa. Thus, the antibodies were host derived and directed against the donor's plt. Serum samples obtained on d38, d45 and d65 after HSCT contained antibodies against HLA class I antigens but HPA-1a antibodies were not anymore detectable. No HLA antibodies were detectable on d149 after HSCT. **Conclusions.** The severe thrombocytopenia was caused by host-derived HPA -1a antibodies. Fortunately, plt counts started to increase on d20 spontaneously and the patient could be discharged at d27 (plt 51.000/μL) with a complete donor chimerism. The decrease of the serum antibodies parallel to the increase of the plt count strongly suggests a progressive elimination of residual host cells. We conclude that the HPA mismatch between recipient and host affected thrombopoietic engraftment and the success of plt transfusions.

0687

ASSESSMENT OF PFA-100 DEVICE TO DETECTION OF PLATELET HYPER-AGGREGABILITY. RELATIONSHIP WITH THROMBO-HAEMORRHAGIC EVENTS IN POLYCYTHAEMIA VERA AND ESSENTIAL THROMBOCYTHAEMIA PATIENTS

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INTRODUCTION: Chronic myeloproliferative disorders (cMPD) are characterised by thromboembolic complications. Estimation of hyperaggregability of platelets is important for diagnosis and prevention of vascular events. **OBJECTIVES:** 1) To evaluate a method for detection of a hyperaggregable state of platelets by using an PFA-100 (Dade Behring). 2) To correlation the hyperaggregability with thrombo-haemorrhagic events. 3) To compare the turbidimetric aggregation and PFA-100 for monitoring the platelet hyperaggregability. **MATERIALS AND Methods.** Patients: we studied 65 patients (ET in 41 and PV in 24 patients). 39 females, mean age 56 years (range age 25-89). Evo-

lution time from diagnosis was 2-17 years (mean 8). We reported the thrombo-haemorrhagic events and the therapy employed. **Methods.** Platelet activation was measured by 'Platelet Function Analyzer' (PFA-100[®]) (DADE Behring International Inc., Miami, FL.). Citrated total blood samples were collected. Normal reference of closure time was: Col/ADP 71-118 seconds and Col/Epi 94-193 seconds. Platelet hyperaggregability status was defined to Col/ADP and Col/Epi with a closure time lower 71 and 94 seconds, respectively. We reported platelet number. A group of 25 (age/sex matched) healthy subjects were control group. Turbidimetric aggregation: It was measured by aggregometer MCP-40 (Menarini Diagnostics). Determinations were analysed with platelet rich plasma (PRP). **Results.** 1) 16 patients (11 ET, 5 PV) presented platelet hyperaggregability by PFA. In contrast, only in one patient this phenomenon was seen by turbidimetric aggregation. 2) There are not correlation between the platelet number and hyperaggregability. Three patients with a platelet number higher than million had not thrombo-haemorrhagic complication. 3) Platelet hyperaggregability could be an intrinsic phenomenon to MPD, because that not changes with the therapy employed. 4) We reported a higher number of thrombotic events in this subgroup with hyperaggregability. **Conclusions.** 1) PFA-100 is a simple, rapid, and automated method and thus should be suitable for routine clinical use for screening of high thrombo-haemorrhagic risk. 2) PFA-100 presents more sensibility for detection of a hyperaggregable state of platelets than turbidimetric aggregation. 3) Platelets of the MPD patients have an activation status, that not modificate with the therapy.

ID Patient	MPD	Platelet (10 ⁹ /L)	PFK-COL/ADP (17-118 sec)	PFK-COL/Epi (94-193 sec)	Event	Therapy
01	ET	120	82	182	None	HS
05	ET	518	121	87	None	None
08	ET	343	87	182	ITD	HS
02	ET	1,400	70	280	None	HS
09	ET	618	70	280	None	HS
10	ET	873	70	290	Heart failure	HS
16	ET	874	70	280	Stroke	HS
17	ET	874	70	280	Heart failure	HS
20	ET	272	70	280	None	HS
40	ET	1,346	70	280	None	HS
42	ET	713	80	188	Stroke	HS
44	ET	954	80	74	None	HS
47	ET	641	81	112	ITD	HS
50	ET	843	107	76	ITD	HS
54	ET	1,000	134	49	None	None
59	ET	108	147	78	ITD	HS
64	ET	753	70	290	None	HS

MPD: Myeloproliferative Disorders; ET: Essential thrombocythemia; PV: Paroxysmal nocturnal hemoglobinuria; HS: Hydroxyurea; ITD: Idiopathic Thrombocytopenic Purpura; None: No treatment; Stroke: Ischemic stroke

0688

THE EFFECTS OF CHLORIDE CHANNEL BLOCKERS ON PLATELET CYTOPLASMIC FREE CALCIUM CONCENTRATION AND PLATELET AGGREGATION

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Objective. To explore the effects of chloride channels on the regulations of platelet cytoplasmic free calcium concentration ([Ca²⁺]_i) and platelet aggregation (PAG). **Methods.** Platelet were separated freshly and then activated by thrombin; the chloride channel blockers 4,4'-diisothiocyano-2,2'-disulfonic acid stilbene (DIDS) or niflumic acid (NFA), and calcium channel block-

ers 1-[β-[3-(4-methoxyphenyl) propoxy]- 4-methoxyphenethyl]-1H-imidazole hydrochloride (SK&F96365) or Nifedipine were added to react with the activated platelets. The effects of each agent on platelet [Ca²⁺]_i and PAG were detected. The combine effects and the interactions among chloride channel blockers (DIDS, NFA) and calcium channel blockers (SK&F96365 Nifedipine) were also investigated. **Results.** Both DIDS and NFA [the concentration were 12.5, 25, 50, 100 and 200 μmol•L⁻¹ respectively] could inhibit the PAG induced by thrombin (1U/mL) and the effect was dose-dependent. Compared with the control, they had no significant effects on resting [Ca²⁺]_i. Compare with the control group, DIDS (100 μmol•L⁻¹) SK&F96365 (100 μmol•L⁻¹) and Nifedipine (100 μmol•L⁻¹) could significantly reduce the PAG, Ca²⁺ release and Ca²⁺ influx activated by thrombin in platelet (P<0.05). DIDS (100 μmol•L⁻¹) and SK&F96365 (100 μmol•L⁻¹) could enhance each other's effect on reducing the PAG, Ca²⁺ release and Ca²⁺ influx (P<0.05). DIDS (100 μmol•L⁻¹) and Nifedipine (100 μmol•L⁻¹) could enhance each other's effect on reducing Ca²⁺ release (P<0.05). NFA (100 μmol•L⁻¹) and SK&F96365 (100 μmol•L⁻¹) could weaken each other's effect on Ca²⁺ release (P<0.05). NFA (100 μmol•L⁻¹) and Nifedipine (100 μmol•L⁻¹) could weaken each other's effect on PAG, Ca²⁺ release and Ca²⁺ influx activated by thrombin in platelet (P<0.05). **Conclusions.** The chloride channel blockers DIDS and NFA have no effect on the resting [Ca²⁺]_i and the leak calcium influx of platelet. DIDS can inhibit the Ca²⁺ release, Ca²⁺ influx and PAG of platelet induced by thrombin, while NFA can only inhibit the Ca²⁺ release of platelet induced by thrombin. There are interactions between chloride channel blockers and calcium channel blockers in resting [Ca²⁺]_i and PAG of platelet. The opening of chloride channel can influence the cellular calcium movement of platelet.

0689

ASSOCIATION BETWEEN HELICOBACTER PYLORI INFECTION AND IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background. Helicobacter Pylori (HP) is a Gram-negative bacterium that has been implicated in the pathogenesis of some autoimmune diseases such as rheumatoid arthritis, autoimmune thyroid diseases and idiopathic thrombocytopenic purpura (ITP). However, the studies so far published on the relation between ITP and HP infection are controversial. Seroprevalence of HP infection in healthy individuals is greatly variable from country to country; the current HP seroprevalence in Greece is approximately 49.2%. **Aims.** The primary aim of our study was to evaluate the possible role of HP infection in the pathogenesis of ITP by comparing the prevalence of HP infection in patients with ITP with the general population. The secondary aim was to compare the characteristics of HP positive and HP negative patients with ITP. **Methods.** 23 Greek adult patients with ITP [5 males (21.7%) and 18 females (78.3%); mean age 46.3±18.1 years] with a mean duration of ITP of 86±78 months were studied. ITP was diagnosed on the basis of the presence of isolated thrombocytopenia (platelets <100x10⁹/L) and megakaryocytic bone marrow hyperplasia; other causes of thrombocytopenia were excluded. All patients had been previously treated with corticosteroids while at the time of the evaluation five patients (21.7%) were receiving corticosteroids, two patients (8.7%) were receiving cyclosporine and 16 patients (69.6%) were not receiving any treatment. The mean platelet count at ITP onset was 27.9x10⁹/L±22.3x10⁹/L. At the time of the study the mean platelet count was 233x10⁹/L±179x10⁹/L; eight patients (36.4%) had less than 150x10⁹/L platelets and four patients (18.2%) had less than 100x10⁹/L platelets. 14 patients (60.9%) had undergone splenectomy. None of the patients had a history of gastric or duodenal ulcer. Patients were not eligible for the study if they had been treated for HP or if they had been

treated with either antibiotic or proton pump inhibitor within the past 8 weeks. HP infection was assessed by ¹³C urea breath test. In order to see if the status of HP could be predicted by gastrointestinal (GI) symptoms, all patients were asked to complete a short standardized questionnaire at the time of breath test. **Results.** HP was detected in 6 patients (26.1%) but none of them had symptoms from the GI tract. 6 patients (26.1%) had symptoms from the GI tract but none of them was HP positive. There were no differences between HP positive and negative patients in any of the examined parameters (age at diagnosis and at the time of the study, gender, platelet count at the onset of ITP and at the time of evaluation, splenectomy, presence of GI symptoms). Four patients had a platelet count less than $100 \times 10^9/L$ and three of them (75%) were HP positive. **Conclusions.** Considering that: a) the prevalence of HP infection in our ITP patients was significantly lower than in the healthy Greek population and that b) HP positive and negative patients with ITP did not differ in any of the examined parameters, our study does not support the existence of a causal relationship between HP infection and ITP.

0690**HELICOBACTER PYLORI ERADICATION CAN INDUCE PLATELET RECOVERY IN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP CHR)**

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Introduction: Over the last 5 years, several studies has reported improved platelet counts in H. pylori positive ITPchr patients (pts.) following standard triple eradication therapy. Review of published studies indicates an overall response rate of 53% in 193 pts. in whom H. pylori was eradicated. Cohorts from Japan and Italy report higher response rates. **Aims.** to assess the prevalence of H. pylori infection and the efficacy of its eradication on the platelet count in a series of 54 adults with ITPchr. Patients and **Methods.** 54 adults with ITPchr (12 male, 42 female; median disease duration 6 years; median age 51 years; mean platelet count: $70 \times 10^9/L$; 7 refractory; median follow-up 10 months) entered the prospective study between February 2002 -December 2004. All immunosuppressives were withdrawn at least 1 month before examination. Pts. were not eligible if they had been treated for H. pylori within 2 years or if they had been treated with either an antibiotic, proton pum inhibitor or bismuth within the past 4 weeks. H. pylori infection was confirmed in 39 (72%) pts through a ¹⁴C urea breath test, and whenever possible by histological examination. 30 H. pylori positive pts. received triple eradication therapy: 23 pts. pantoprazole (PPZ) 40 mg bid, amoxicillin (AMC) 1000 mg bid and clarithromycin (CAM) 500 mg bid for 7 days; CAM was replaced with metronidazole (MNZ) for 500 mg tid in 7 pts. The pts whose H. pylori infection was not eradicated after the first treatment received the reeradication with PZP 40 mg bid, AMC 1000 mg bid, and MNZ 500 mg tid for 7 days. Platelet counts were monitored monthly and assessed at 3 and 6 months after the end of treatment. Complete response (CR) was defined as platelet count $>150 \times 10^9/L$. Partial response (PR) was defined as platelet count $>50 \times 10^9/L$ or an increase $>30 \times 10^9/L$ with respect to the baseline value. Data were analyzed by t test; percentages were compared by χ^2 test (Fisher exact test for value ≤ 5); a p value <0.05 was considered statistically significant. **Results.** 1) H. pylori infection has been documented in 39/54 (72.2%) pts. which is significantly higher then in general Serbian population (55%). 2) The H. Pylory-positive pts. were significantly older then H. Pylory-negative pts. (54: 42 years) and had a significantly lower platelet count ($66: 84 \times 10^9/L$). 3) Successful eradication was confirmed in 23/30 (77%) pts. 4) Stable platelet recovery was registered in 5/23 treated pts. (21.7%). Namely, CR was achieved in 2 and PR in 3 pts, including 1 highly refractory pt. The response was maintained for a median of 9 months. 4) No platelet recovery was registered in either H.

Pylory-negative pts. (0/15) or H. pylori positive unsuccessfully eradicated pts. (0/7) - control group. 5) A significant platelet recovery is registered after H. Pylory eradication ($p < 0.05$). **Conclusions.** Even though the pathogenetic mechanisms of H. pylori-induced thrombocytopenia remain obscure, the results of this small prospective study support the efficacy of an H. pylori eradication treatment for ITP pts.

0691**DETERMINATION OF RETICULATED PLATELETS IS NOT USEFUL IN DIFFERENTIAL DIAGNOSIS OF PERIPHERAL AND CENTRAL THROMBOCYTOPENIA**

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Background. Reticulated platelets (so-called immature platelet fraction, IPF) have an increased ribonucleic acid amount and are the youngest platelets in circulation. The quantity of reticulated platelets increases with increased platelet production and has been supposed to be high only in peripheral thrombocytopenias. **Aims.** To determine the value of IPF in distinguishing peripheral from central thrombocytopenias, defined as platelets less than $100.000/\mu L$. **Methods.** The percentage of reticulated platelets was determined by analysis of platelet rich plasma incubated with platelet specific CD61 monoclonal antibody and incubation with the fluorescent thiazole orange that specifically colours ribonucleic acid. We analysed 20 samples from healthy donors, 10 from patients with Immune Thrombocytopenic Purpura (ITP), 18 samples from patients during chemotherapy-induced aplasia (CIA), 6 from patients with Myelodysplastic Syndromes (MDS). Diagnoses of ITP and MDS were performed on bone marrow aspirate. Statistical analysis was performed with SPSS 10.1 for Windows and a Student t Test was done in order to compare means. **Results.** The reference range for the IPF in healthy blood donors (mean platelet value 194.500) was established as $1.21 \pm 0.5\%$. All analysed categories had a significantly higher mean of reticulated platelets compared to the healthy controls ($33.54 \pm 27.8\%$ $p < 0.0001$). When comparing immune thrombocytopenias with so-called central thrombocytopenias (MDS, CIA) there was no significant difference in IPF ($p = 0.054$ for ITP versus MDS and 0.669 for ITP versus CIA). **Conclusions.** Although only low numbers of cases have been analysed, determination of IPF seems not to distinguish peripheral from central thrombocytopenias and probably will not preserve thrombocytopenic adult patients from performing bone marrow aspiration. Bone marrow aspirate should remain the gold-standard for the differential-diagnosis of thrombocytopenia.

0692**GENETIC VARIATION IN PLATELET GLYCOPROTEIN RECEPTORS GPIIIA AND GPIA ARE NOT ASSOCIATED WITH DIABETIC RETINOPATHY IN TYPE 2 DIABETES MELLITUS**

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Background. Recent studies show that platelets may play important role in the development of diabetic microangiopathy. The glycoprotein complex (GP) Ia/IIa is a major collagen receptor, while GPIIb/IIIa is a fibrinogen receptor on platelets. Recently, linked polymorphism within the coding region of the GPIa gene (C807T and G873A) were identified that are related to GPIa/IIa surface expression. The T807/A873 allele is associated with higher expression of this receptor. This polymorphism creates restriction site for Bgl II enzyme. Another polymorphism in GPIIIa receptor (PIA^{1/2}) may play important role in a platelet aggregation and adhesion. **Aims.** The aim of the pres-

ent study was to assess the associations of the $PIA^{1/2}$ and Bgl II polymorphism in platelet GP complexes and risk to diabetic retinopathy. *Material and method:* We have examined 75 patients with type 2 diabetes, 30 without diabetic retinopathy (DR) and 45 with it and 123 controls for $PIA^{1/2}$ polymorphism and 117 for Bgl II polymorphism. There were no significant differences between the duration of diabetes ($p=0.103$, 9.8 ± 5.5 years in patients without retinopathy and 11.5 ± 3.95 in patients with retinopathy), age ($p=0.619$, 58.2 vs. 59.4 years), level of HbA1c (8.3% vs. 9.2% , $p=0.103$) and sex distribution ($p=0.39$) in both groups. For genotyping of these two polymorphisms, we used the method of polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using a DNA isolated from peripheral blood. *Results.* Distribution of the PIA genotypes in the group without DR was ($A1/1=26$, $A^{1/2}=4$ and $A2/A2=0$), in the group with DR ($A1/1=39$, $A^{1/2}=5$ and $A2/A2=1$) and in the healthy controls ($A1/1=93$, $A^{1/2}=27$ and $A2/A2=3$). We found no differences in the distribution of the $PIA^{1/2}$ genotypes or allele in both groups of patients ($p=0.69$) and controls ($p=0.17$). Distribution of the Bgl II genotypes in the group without DR was (Bgl II $+/+ =0$, $+/- =18$ and $-/- =12$), in the group with DR (Bgl II $+/+ =2$, $+/- =21$ and $-/- =22$), and in the healthy controls (Bgl II $+/+ =8$, $+/- =61$ and $-/- =48$). There were no significant differences in the distribution of the Bgl II genotypes or allele in both groups of patients ($p=0.32$) and controls ($p=0.42$). *Summary:* These results suggest that this polymorphism does not contribute to the development of diabetic retinopathy, contrary to some other recent studies.

0693

BRUCellosis INDUCED ISOLATED THROMBOCYTOPENIA: A REPORT OF 6 CASES

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Background: Brucellosis constitutes a major public health and economic problem in the many parts of the developing countries including Turkey. *Brucella* infection has been reported to cause hematological abnormalities including anemia, leukopenia, thrombocytopenia and clotting disorders. Thrombocytopenia is less common manifestation with an incidence varying from 1% to 8% in adults. *Aims.* In this study we aimed to investigate the incidence of thrombocytopenia in outpatients who were diagnosed as brucellosis. *Methods.* In the present study we describe 6 isolated thrombocytopenic patients with manifestations of brucellosis observed during the course of active infection. This prospective study was carried out in the department of hematology and infectious disease of Gaziantep University between June 2004 and February 2005. Patients who were known to have any chronic or acute systemic disease other than brucellosis and those younger than 14 years of age were excluded. A diagnosis of brucellosis was established by the isolation of *Brucella* species in the blood, bone marrow culture and by a compatible clinical picture with the detection of specific antibodies at significant titers (Wright's seroagglutination test, over 1/160) or demonstration of at least a fourfold rise in the antibody titer in serum specimens drawn 2-3 weeks apart. *Results.* 6 of the patients had fever over the 38°C , headache, myalgia and artralgia. 2 had hepatomegaly and splenomegaly, 3 had petechia. Serologic tests including EBV, HIV, Rubella, CMV, Parvovirus and HbsAg were negative. The blood cell count revealed isolated thrombocytopenia of 6 patients. Number of platelets were ranged from 19000 to 95.000/ μL . The tube agglutination tests for brucellosis were positive for all patients (1/160-1/2560). 3 patients showed positivity for bone marrow and blood culture. *Brucella mellitensis* was isolated from three patients. Bone marrow aspiration showed normal cell proliferation and maturation, but also revealed an increased number of megakaryocytes. Rifampicin and doxycycline was commenced.

Thrombocytopenia was improved immediately after rifampicin and doxycycline therapy. The platelet count was in normal limits at one month after the therapy was commenced and progressive reduction at the titers of the agglutination test for brucellosis was observed. *Conclusions.* The pathogenesis of thrombocytopenia in brucellosis remains obscure but, several possible mechanisms may account for it including; hemophagocytosis, disseminated intravascular coagulation, severe immune reactions sometimes even with presence of platelet antibodies. Another mechanism that could be implicated in brucella attributed thrombocytopenia is granulomatous infiltration of the bone marrow in the chronic form of the disease. The hematological consequences of brucellosis should always be kept in mind in the differential diagnosis of isolated thrombocytopenia in endemic areas. Patients with isolated thrombocytopenia should be investigated for brucellosis in the presence of fever, family history or a traveling history to countries where the disease is endemic.

0694

EFFICACY OF RITUXIMAB (MONOCLONAL ANTIBODY ANTI CD20) IN CHRONIC SYMPTOMATIC REFRACTORY ITP DURING CHILDHOOD

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Background. Children with chronic ITP usually do not have severe bleedings and therefore do not need therapy; only a minority needs medical treatment because of symptomatic thrombocytopenia. Rituximab has been explored in autoantibody-mediated diseases because of its B cell depleting effect. Its effect on ITP has been reported in adults. We report the efficacy of Rituximab in 19 pediatric patients with chronic refractory symptomatic ITP. *Methods.* 15 females and 4 males (median age at diagnosis 7 years, range 3-16), were treated with Rituximab for two to five weekly doses (375 mg/m²). Patients were previously treated with IVIG (19/19); prednisone (10/19); m-prednisolone (16/19); desametasone (9/19); cyclosporin (2/19); Ig anti-D (2/19); splenectomy (6/19). Response to Rituximab was defined as positive if platelet count did rise above 50000/microL for at least 7 days. The absence of relapse during all follow-up was defined as complete remission (CR). *Results.* As of December 31, 2004, the median follow-up for the 19 patients was 24 months (range 3-37). Overall response rate to Rituximab was 65% and it was achieved at a mean time of 16.5 days (range 2-120) from the first dose. Six patients relapsed after a median time of 4.5 months (range 3 - 8); 7 still maintain a platelet count > 50.000/microL (median follow-up time: 27 months, range 8-37). CR and was achieved in 3/6 splenectomized and 4/14 non-splenectomized patients, respectively ($p=NS$). One patient was treated before and after splenectomy and was considered twice. CR was detected in 6/15 patients who received four or five weekly infusions (47%) and in 1/5 (20%) treated with two or three infusions. Duration of ITP at the time of Rituximab treatment did not influence the response rate. Patients achieving CR showed significantly decreased levels of CD19+ cells after 4 months as compared to non responding or relapsed patients. ($p < 0.05$). The treatment was well tolerated, with only mild transient side effects in 5 children. No major infections were reported during follow-up. *Conclusions.* Our data show the efficacy and tolerability of Rituximab in children with refractory symptomatic ITP. No difference was detected between splenectomized and not splenectomized patients. The total dose of Rituximab positively correlates with response and patients achieving CR showed a more prolonged B cell depletion.

0695

A PROSPECTIVE STUDY OF GESTATIONAL THROMBOCYTOPENIA

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Background. Gestational thrombocytopenia is a benign condition, affecting up to 8% of pregnancies. Thrombocytopenia is relatively mild with platelet counts usually remaining greater than 70.000/mL and is characterized by an absence of history of maternal thrombocytopenia or bleeding. The cause is unknown, but is considered that increased platelet destruction plays a crucial role. Thrombocytopenia is detected incidentally by routine blood testing. Platelet counts return to normal within 2–12 weeks following delivery. We investigated the incidence of this condition in a total of 500 pregnant women. **Methods.** We studied 500 pregnant women in twelve months period (2003) who attended in obstetric clinic of our hospital. In all of them we recorded the platelet counts and the mean platelet volume (MPV) pre-delivery and post-delivery. Blood count determinations are obtain using electronic particles counters. We performed examination of peripheral blood film to verify the low platelets count. In order to exclude other causes of thrombocytopenia we included in our study the above serological markers, anti-HAV, HBsAg, HBeAg, anti-HBs, anti-HBc, anti-HBe, anti-HCV, anti-HIV. Pregnant women with thrombocytopenia were detected for the presence of anti-platelet antibodies. The normal platelet count ranges from 150–400 x10⁹/L, and the normal MPV, for platelet count of 150 x 10⁹/L, is 7.5–11 fl. Screening of hemostasis including PT, INR, APTT, and FIB were detected in all women pre and post delivery using automatic coagulation analyzer. **Results.** We found 35 women, mean age 25,8 years old, out of 500 (7%) with mild thrombocytopenia in the late of pregnancy. Pre-delivery platelet counts ranged from 91 to 139 x10⁹/L mean value 125x10⁹/L, MPV ranged from 8.5 to 14.2 fl mean value 10.3fl. In 20 out of 35 women (57.2%) MPV were above the normal limits, with mean value 12.3fl, and the platelet counts below 120x10⁹/L. Post-delivery, at the end of the first week, the platelet counts ranged from 113 to 206x10⁹/L mean value 151x10⁹/L, MPV ranged from 8.5 to 14.6 fl mean value 10.4fl. In 13 out of 35 women (37%) MPV were above the normal limits, with mean value 12.1fl. None of the women had positive anti-platelet antibodies. 17 women had positive serological markers of HBV infection, 6 of them were chronic carriers of HBsAg and 11 had evidence of HBV past infection. One was anti-HAV IgM positive, none had anti-HCV, and anti-HIV. We didn't found disorders of screening of coagulation in our patients. **Conclusions.** 1. In our group, excluding all others causes, low platelet counts classified as gestational thrombocytopenia. 2. The incidence of benign thrombocytopenia was 7% in the late of pregnancy. 3. Platelets number tends to return to normal in a few days after delivery. 4. Increased MPV in correlation with low platelet counts support the theory that platelet destruction is probably the cause of gestational thrombocytopenia.

0696

ELEVATED CYTOKINE INTERLEUKIN-6 IN UNSTABLE ANGINA AND THE EFFECT OF DUAL ANTIPLATELET THERAPY, ON PLATELET ACTIVATION

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Background. In patients (pts) with unstable angina (UA), an increase risk for adverse outcome has been observed if interleukin-6 (IL-6), a major inducer of inflammatory response by CRP production by the liver, is elevated. Activated platelets as

well as plaque inflammation participate in the pathogenesis of ACS. While aspirin is an effective therapy for UA the addition of clopidogrel an ADP receptor inhibitor of platelets, further improves clinical outcome. However, soluble P-selectin (s-PS), a marker of platelet activation has not been predictably altered. **Aims.** We evaluated the effect of clopidogrel on s-PS in association with IL-6 levels in pts with UA. **Methods.** 120 consecutive pts who were admitted for UA were randomized to receive aspirin (325 mg, daily) and clopidogrel (300 mg loading dose followed by 75 mg daily) (group: Asp+Clop), or aspirin (325 mg, daily) (group: Asp), additionally to usual medical therapy. 37 pts from Asp+Clop group (mean age 70±4 years, 32 males, 5 females) and 38 pts from Asp group (mean age 67±6 years, 34 males, 4 females) who did not develop myocardial infarction were studied. Blood samples were collected at 0, 8, 48 hours and on day 6. IL-6 and s-PS were determined by immunoassay and ELISA accordingly. **Results.** Pts on Asp+Clop compared to pts on Asp alone had at baseline similar clinical characteristics. Overall, s-PS levels were similar among the two groups at 0, 8 and 48 hours and at day 6 (Asp+clop vs Asp: 55.6±23 vs 53.75±22.9 ng/mL p=0,4, 52.41±16 vs 54.14±20 ng/mL p=0.5, 50.17±13.7 vs 60.8±37.9 ng/mL p=0.1, 50.53±17.5 vs 54.64±18.97 ng/mL p=0.4). When the effect of clopidogrel on s-PS was examined by IL-6 quartiles, pts that had IL-6 levels at the highest quartile (IL-6 above 8.4 pg/mL) had a significant increase in s-PS at 8 hours, if they received Asp only (52±12 to 69.41±21 ng/mL, p=0.043), and levels of s-PS were greater than in pts on Clop+Asp (Clop+Asp vs Asp: 46.6±14 vs 68.41±21 ng/mL, p=0.01). This difference was not maintained at 48 hours and 6 days). **Conclusions.** In pts with UA and high IL-6, treatment with Asp+Clop is better than Asp alone in prohibiting early P-selectin elevation. This could be related to the greater intensity of platelet activation in UA in the presence of a more severe inflammation as indicated by higher IL-6 levels.

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PILOT STUDY OF RITUXIMAB THERAPY IN ADULT IMMUNE CYTOPENIAS IN VENEZUELA

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Background. Rituximab is an anti-CD20 monoclonal antibody which can deplete B-cells producing antibodies. The standard treatment for chronic immune thrombocytopenic purpura, autoimmune hemolytic anemia and Evans syndrome due to antibodies includes combination of glucocorticoids, high doses of immunoglobulins, immunosuppressive drugs and splenectomy. Patients who are refractory to these therapies constitute an important therapeutic challenge to Rituximab. **OBJECTIVES.** This study examined the efficacy and safety of Rituximab in 8 patients, four with chronic immune thrombocytopenic purpura, three with recurrent autoimmune hemolytic anemia (AIHA) and one with Evans syndrome with refractoriness to other treatments. **STUDY DESIGN:** Four patients with chronic immune thrombocytopenic purpura for 5 to 21 years (one of the patients also with diagnostic of LED), 25 to 75 years of age, with platelet counts < 30,000 /ul, with platelet antibodies, normal bone marrow cellularity and megakaryocytes count received Rituximab at a dose of 375 mg/m², once weekly for 4 weeks. Platelet response was characterized as complete (CR) if a count >150,000/ul was achieved and partial (PR) if 50,000 to 150,000/ul. Three patients with autoimmune hemolytic anemia with direct Coombs test(IgG) positive and one patient with Evans syndrome also received the therapy with Rituximab. Hemoglobin complete response was characterized for normal Hb and Hto for their sex and age and partial remission if their Hb increased at least two grams. **Results.** Three of four patients with chronic immune thrombocytopenic purpura (75%) achieved a CR before the therapy with Rituximab was com-

pleted and they maintained their complete response 6 months later. The last patient with purpura had only partial remission (platelet count: 117,000-133,000/ul) but he is still in partial remission 7 months after Rituximab therapy. One patient with AIHA for 10 months had complete remission for only one month, the second patient (with recurrent AIHA for one year) has been in CR for one year and the third patient (with AIHA and with antiphospholipid syndrome for one year and 7 months) achieved CR and has been in CR for 1 month up to date. The patient with diagnosis of Evans syndrome for one year had CR for two months up to date. Therapy was well tolerated, and no infectious complications occurred. *Conclusions.* Long lasting chronic immune thrombocytopenic purpura, autoimmune hemolytic anemia and Evans syndrome refractory to corticosteroids, high doses of immunoglobulin and immunosuppressive agents can still respond to four weekly infusions of Rituximab so that the splenectomy can be deferred. Thus, administration of Rituximab may be considered prior to splenectomy in patients with refractory immune cytopenias and high risk of complications following splenectomy.

0698

CONGENITAL THROMBOCYTOPENIA CAUSED BY DELETIONS IN 11Q CAN BE DETECTED BY FLUORESCENCE IN SITU HYBRIDIZATION (FISH) USING A SUB-TELOMERIC 11Q PROBE AS A NEW DIAGNOSTIC TOOL

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Neonatal thrombocytopenia occurs in about 1% of all newborns. Inherited forms like 11q- or Jacobsen syndrome are rare. However, they may remain undetected with karyotyping because the deleted regions in 11q often involve small subtelomeric regions. Here we report on the detection of deletions in 11q in two newborns with normal routine karyotypes who were shown to carry subtelomeric deletions in 11q by means of fluorescence in situ hybridization (FISH) using a subtelomeric 11q probe (Abbott, Diagnostics, Wiesbaden, Germany). Both children showed thrombocytopenia (18.000/ μ L and 26.000/ μ L, respectively) and dysmegakaryopoiesis (absence of normal megakaryocytes and presence of micromegakaryocytes) associated with facial dysmorphism, cardiac defects and psychomotoric retardation. In the second case, the mother and the grandmother also showed mild thrombocytopenia. In both patients, FISH analyses on peripheral blood and bone marrow showed the loss of the telomere-associated region of 11q distal of the MLL gene. In the first patient, the deletion of 11q resulted from an unbalanced complex rearrangement with duplication of 11p. As the source of this chromosomal aberration, a paternal pericentric inversion of chromosome 11 was identified. The partial monosomy 11q and the partial trisomy 11p in the first patient were confirmed by comparative genomic hybridization (CGH) analysis. Array/matrix CGH assisted in determining the breakpoints at 11p15.1 and 11q24.1. No structural aberrations of 11q were found in the mother of the second patient, but further investigations are under way. These findings give further evidence that small subtelomeric deletions of 11q and probably mutations of genes located therein cause thrombocytopenia. Since it can be very difficult to detect these deletions by karyotyping, FISH using a subtelomeric 11q probe seems to be an extremely useful new diagnostic tool. This new method should be applied in children with congenital thrombocytopenia, in particular if they have additional complex dysmorphic features.

0699

PURE THROMBOCYTOPENIA AND ACUTE LYMPHOBLASTIC LEUKEMIA AT DIAGNOSTIC IN ADULTS

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Background. To perform a bone marrow aspiration in patients presenting with a pure thrombocytopenia (i.e. without either leukopenia, anemia or MCV increase) still remains a matter of debate. This procedure is proposed in order to rule out an Acute Lymphoblastic Leukemia (ALL) before prescribing corticosteroids, that could induce a transient remission and further lessen the cure rate. Population and *Methods.* In order to evaluate if ALL may present as a pure thrombocytopenia in adults, we retrospectively studied all ALL diagnosed and treated at Edouard Herriot Hospital of Lyon, France, between 01/01/1980 and 07/31/2004. Sex, age, presence of spleen, liver and lymph node enlargement in patients as well as complete blood count (CBC) at presentation were collected from clinical records. *Results.* 458 ALL were diagnosed during the study period. There were 278 males and 180 females, with ages ranging from 12 to 89 (median = 35 yrs; 1st/3rd quartiles = 22 and 53 yrs). Platelet count (Pt) ranged from 2 G/L to 402 G/L (median = 52 G/L, 1st/3rd quartiles = 28-106 G/L). In addition, 220 (48%) patients (pts) presented with lymph node enlargement, whereas 185 (40%) and 116 (25%) pts presented with spleno and hepatomegaly. Of 354 (77%) pts who had thrombocytopenia i.e. platelet count < 150 G/L, 37 (8%) had no lymphoblast on peripheral smears, and 1 had no additional cytopenia (Hb > 125 and [80 < MCV < 100] and [4.0 < WBC < 10.0]). This patient was a 26 yrs male, presenting without spleen, liver and lymph node enlargement and with Pt = 42 G/L, Hb = 132 g/L, MCV = 88 fl, WBC = 6.2 but an absolute neutrophil count of 1.3 G/L (24%). *Conclusions.* No ALL presented as a pure thrombocytopenia in our experience. However, because some of the additional abnormalities were very mild, we recommend careful examination of the CBC and of the blood smear. If corticosteroid prescription can be delayed, the bone marrow examination can be omitted under the cover of a strict follow up of CBC. If patient cannot be referred to a trained Center, bone marrow analysis is recommended.

Stem cells - Biology and therapy II

0700

HARRIS SYNDROME: A GEOGRAPHIC PERSPECTIVE

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Congenital thrombocytopenias, once considered rare and obscure, are now recognized with increasing frequency, due to the evaluation of platelet number as a part of routine blood testing. Nonmuscle myosin heavy chain II-A mutation is responsible for MYH9-related disease, which is characterized by macrothrombocytopenia, granulocyte inclusions, deafness, cataracts, and renal failure. Harris et al described a new syndrome ACMT (Asymptomatic Constitutional Macro Thrombocytopenia) characterized by mild to severe thrombocytopenia, giant platelets [Mean platelet volume (MPV)>10fL] with normal platelet functions without any bleeding symptoms in blood donors from West Bengal. In view of confusion between ACMT and CAMT (Congenital A Megakaryocytic Thrombocytopenia) we now refer this entity as Harris syndrome. A

prospective study was conducted over 8 months at the Christian Medical College and Hospital, Vellore, to determine the geographic distribution of Harris Syndrome in India and neighboring countries. Of 10,200 blood donors screened over a period of 8 months; 1002 were randomly screened for Harris Syndrome. Nine hundred and thirty one blood donors were residents of India, and remaining 71 were from Nepal, Bhutan and Bangladesh. All donors were questioned about history of abnormal bleeding episodes. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA). Automated platelet counts were performed using a Coulter STKS (Coulter, Hialeah, Florida) within 2 hours of collection. Peripheral smears were examined to confirm the thrombocytopenia and presence of giant platelets. The samples were screened with dHPLC mutation detection system. All exons and flanking intronic regions of the MYH9 gene (May Hegglin gene) were assayed. All abnormal profiles were sequenced. There was no history of any abnormal bleeding episode in any of the donors. A peripheral blood smear confirmed the diagnosis of thrombocytopenia and giant platelets. There were no abnormal inclusion bodies in the leukocytes. Thrombocytopenia was graded as mild ($100-150 \times 10^9/L$), moderate ($50-100 \times 10^9/L$) and severe ($<50 \times 10^9/L$). **Results.** were divided into five groups based on the birthplace of each donor. There was a statistically significant difference in platelet count ($P < 0.000$) and mean platelet volume ($P < 0.000$) between eastern states and all other states. No mutations were found that would cause pathological consequences or expression anomalies. In conclusion, MYH9 is not mutated in these samples and another gene with a probable similar function is the likely causative factor. Further genetic and molecular studies should yield new insights into the pathogenesis this syndrome.

1. Harris VKN, Sukesh CN, Biju G. Asymptomatic constitutional macro thrombocytopenia among West Bengal blood donors. *Am J Med* 2002; 112: 742-3.

Table 1. Severity and distribution of Harris syndrome.

Region n (%)	East 280 (97% Normal 50)	North 133 (17% Normal 25)	West 111 (97% Normal 5)	South 252 (97% Normal 5)	Remaining Countries 71 (97% Normal 5)
Mean Platelet Volume (fL)	10.7±2.1	9.3±2.2	9±1.5	7.6±1.1	9±2
Mean Platelet Count (x 10 ⁹ /L)	213±59	213±61	260±51	260±96	211±88
Severe ($<50 \times 10^9/L$) n (%)	2 (0.5)	0	0	0	0
Moderate ($50-100 \times 10^9/L$) n (%)	31 (8)	0	0	0	0
Mild ($100-150 \times 10^9/L$) n (%)	160 (26)	24 (18)	12 (6.5)	12 (4.5)	6 (8.5)
Normal ($>150 \times 10^9/L$) n (%)	254 (25)	108 (20)	109 (21)	250 (25.5)	65 (21.5)

0701

COMPARISON OF CORD BLOOD AND BONE MARROW MONONUCLEAR CELLS BY SERIAL ANALYSIS OF GENE EXPRESSION (SAGE)

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Background. Stem cells have great plasticity being capable of differentiation into several types of tissues and repairing damage organs. Umbilical cord blood and bone marrow represent efficient sources of hematopoietic stem cells. However, some studies analyzing the sub-population CD34+ have demonstrated that cord blood and bone marrow differ qualitatively and quantitatively. It seems that cord blood stem cells are more primitive having greater self-renewal, proliferation, expansion, multilineage differentiation capacity and due to their lymphocyte immaturity, can be transplanted without total HLA compatibility. Recent studies demonstrate better trafficking and homing, promoting better transplantation recovery. The gene expression of cord blood and bone marrow stem cells, sub-population CD 34+/CD 38/Lin/++ have been recently studied with

micro array and serial gene expression analysis confirming differential expression of some genes related with proliferation differentiation and engraftment capacity in these sources. These studies also demonstrated that serial analysis detected a great number of transcripts than the micro array method. **AIM:** As both cord blood or bone marrow mononuclear cells can be used for bone marrow transplantation, the aim of this study is to analyze the gene expression of cord blood mononuclear cells, using serial analysis of gene expression (SAGE). **Methods.** The results were compared to the ones obtained in the bone marrow mononuclear cell library already deposited in Sagemap database. **Results.** Cord blood revealed a total of 44.924 tags representing 15.519 unique tags (7772 known genes; 3935 sequence predicted or annotated; 3812 no matches). Bone marrow showed a total of 36577 tags representing 13075 unique tags (6711 known genes; 3371 sequence predicted or annotated; 2993 no matches). Genes were annotated by Gene Ontology Consortium and category distribution was similar in both libraries. We found 23.981 common genes. Taking in to account fold change difference, in gene expression ≥ 10 and ≤ 10 ($p \leq 0.05$) 48 genes, which had UniGene number, were detected. Thirty-four were more expressed in cord blood and were related with immune response, cell cycle and homing. SAGE validation was performed by quantitative real time polymerase chain reaction (qPCR) of 10 differentially expressed genes that confirmed results. Alpha, beta and gamma globin results were comparable with hematopoiesis ontogeny. F11 receptor; CDC25B; TGFA; SMARCC2; SKP1A; NKG7; were more expressed in cord blood and S100-A8 was in bone marrow. Fourteen higher expressed genes were chosen for function description. **Summary/Conclusions.** Genes related with homing, cell cycle differentiation, and immune response seem to be higher expressed in cord blood cells. Further studies verifying the role of these genes will probably contribute for better knowledge regarding cord blood cell biology.

0702

DISTINCT (CIRCULATING VS. INCORPORATED IN THE TISSUE) AC133+ PRECURSOR POPULATIONS IN LYMPHOMA PATIENTS

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It has been suggested that circulating AC133+ cells (acting as progenitors) can be used, in cancer patients, as surrogate markers of tumor angiogenesis. Although their role during tumor growth is not completely understood, AC133+ cells have also been detected within tumor biopsies. In the present study, we investigated the systemic (circulation) and the local (tumor-incorporated) presence of AC133+ cells, in Lymphoma patients, and their potential involvement in tumor growth as endothelial progenitor cells (EPC). Circulating AC133+ cells were magnetically isolated (MiniMac System) from peripheral blood (PB) samples of 25 Lymphoma patients while tumor-incorporated AC133+ cells were isolated from Lymphoma biopsies. To determine their endothelial differentiation potential, the isolated cells were characterized (RT-PCR, flow cytometry) for EPC markers (AC133, KDR, c-Kit, CD34), and cultured in EPC differentiation conditions (EGM-2 Clonetics-VEGF, FGF, IGF..., gelatin 1%). After the differentiation assay, the expression of endothelial (EC) markers (VE-cadherin, KDR), was determined by RT-PCR and immunofluorescence. Finally, systemic vascular endothelial growth factor (VEGF) levels were measured in patient's plasma samples, by ELISA. By RT-PCR, 44% (11/25) of the PB Lymphoma samples expressed AC133. The majority (9/11) of them was also c-Kit+, while only 2 were AC133+c-Kit+KDR+. No sample obtained from these patients differentiated into EC; cell survival was low and only hematopoietic markers (RT-PCR) could be detected after the cultures. The presence of circulating AC133+ was not associated with the systemic levels of VEGF. Interestingly, 73% of the AC133+ patients evidenced bone-marrow infiltration with malignant cells. To understand as to whether AC133+ cells mobilization was affect-

ed upon treatment, AC133 expression was determined in PB samples of 7 Lymphoma patients after therapy. AC133+ cells were still detected in 5 of them, regardless of responding or not to the treatment regimen. Again, no association between VEGF levels and the presence of AC133+ cells in the circulation was identified. These results were in contrast to those seen with tumor-incorporated AC133+ cells. Thus far, 83% (5/6) of the Lymphoma biopsies expressed AC133. Here, after cell isolation, EPC differentiation takes place, resulting in VE-cadherin+ KDR+ AC133- EC, in 75% (3/4) of the tested biopsies. These results suggest that circulating and incorporated AC133+ cells may coexist in the same patient during tumor growth. First, circulating AC133+ cells were shown to differ from incorporated ones in the expression of AC133 mRNA isoforms (RT-PCR, DNA sequencing, RACE). In addition, circulating AC133+ cells coexpressed CD34 and CD45 antigens (flow cytometry) while tumor-incorporated AC133+ cells expressed CD19 (lymphoid marker). To study their molecular characteristics further we determined their gene expression profiles by Affymetrix microarrays. These AC133+ cell populations differ mainly in the expression of genes involved in cell adhesion, signaling pathways, cell cycle, metabolism and transcription regulation. These data report for the first time that, during lymphomagenesis, two populations, molecularly and functionally distinct, of AC133+ precursor cells can be found. Their relative contribution towards Lymphoma growth and their use to monitor therapeutic responses and tumor progression is under scrutiny.

0703

CD133 POSITIVE MESENCHYMAL STEM CELLS ISOLATED FROM TERM UMBILICAL CORD BLOOD AND MOBILIZED PERIPHERAL BLOOD

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Background. Umbilical cord blood (UCB) and mobilized peripheral blood (MPB) represent two important hematopoietic sources for stem cell transplantation to replace a defective hematopoietic system or to treat malignant diseases. Their potential use as source of mesenchymal stem cells (MSC) has been a long time debated and remains to be validated. **Methods.** In this study CD133 positive cells from UCB (n=8) and MPB (n=6) were isolated using immunomagnetic microbeads with a purity of 90,4±1,03%. After the second passage, MSC derived from UCB and MPB were compared with bone marrow (BM) MSC and identified by their positive expression of mesenchymal markers (SH2, SH3) and negative expression of hematopoietic markers (CD14, CD34, CD45 and HLA-DR). **Results.** These MSC were also able to differentiate into osteocytes, adipocytes, chondrocytes and neuronal/glial cells after specific induction. Two weeks after mesodermal induction, we assessed lipid vacuoles, calcium deposits and chondrogenic matrix using oil red O, silver nitrate and toluidin blue respectively. Differentiation into neuronal/glial cells required 10 days of induction in presence of NPBm+ cAMP+ IBMX+ NGF+ Insulin. In these conditions, cells displayed typical neuron-like morphology with typical long thin neurites and were positive for Nestin, Tuj-1, MAP-2 and GFAP. After four passages, 6,8.108 and 3,3.107 mesenchymal stem cells were obtained from the CD133 positive fraction respectively for UCB and MPB. In comparison with BM-MSC derived from two normal donors after classical sternal aspiration or from particle fraction removed by nylon filtration prior to transplant, 3,8.106 to 7,8.109 MSC were obtained respectively. These results demonstrate that CD133 positive cell fraction contains MSC with high proliferative potential. We also demonstrated that UCB- and MPB-MSC express Oct4, an octamer binding transcription factor present on undifferentiated cells, as embryonic stem cells with high proliferative capacity. **Conclusions.** The CD133 positive selection could thus be useful not only for hematopoietic stem cell transplantation as previously described but also to isolate MSC from UCB and MPB. UCB and MPB represent thus two new sources of MSC with an easy accessibility.

0704

OUTPATIENT STEM CELL TRANSPLANTATION: THE MEXICAN EXPERIENCE WITH 132 PATIENTS

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INTRODUCTION. Allogeneic stem cell transplantation after non-myeloablative chemotherapeutic conditioning has been shown to reduce systemic toxicity and make possible the procedure on an ambulatory setting. **OBJECTIVE.** To describe the characteristics of patients selected for an ambulatory stem cell transplant based on a non-myeloablative conditioning regimen. **MATERIAL AND Methods.** 132 patients (82 males and 50 females) with the following diagnosis were included: 34 CML, 2 CLL, 27 ALL, 31 AML, 7 NHL, 5 HL, 3 MM, 1 biphenotypic leukemia, 1 Sezary Sd., 2 myelofibrosis, 1 lung adenocarcinoma, and 1 renal cell cancer, as well as 19 with non-malignant disorders. Ages ranged between 1 and 71 years, with a mean of 36 years. In all patients Karnofsky scale values were superior to 70. All patients tolerated oral intake. Hemoglobin values ranged between 5.2 and 18.4 g% with a mean of 11.2 g%. Platelet counts ranged between 8 and 1,650 K/uL with a mean of 222.9K/uL. Serum creatinine ranged between 0.54 and 2.7 mg/dL with a mean of 0.99 mg/dL. The conditioning scheme was: busulfan 4 mg/kg/day (-6, -5) and cyclophosphamide 300 mg/m²/day (-4, -3, -2), fludarabine 30mg/m²/day (-4, -3, -2). **Results.** 103 patients (78%) successfully completed an ambulatory transplant, 95 (72%) had a Karnofsky score >100. Hematologic mean values were 12 g% (hemoglobin), 248K/uL (platelets) and 0.9 g/dL (creatinine). Twenty nine patients (22%) started the transplant on an ambulatory basis but were hospitalized because of fever and/or mucositis (inpatient days: 4 to 24). Karnofsky scale values in these patients ranged between 80 and 100. Mean values from these patients were 11.1 g% (hemoglobin), 191K/uL (platelets) and 0.93 mg/dL (creatinine). **CONCLUSIONS.** Seventy eight percent of the patients admitted to the study completed the stem cell transplant on an ambulatory setting. Factors positively associated with this outcome were normal hematological values and Karnofsky scores >70. Together, these values reliably predict whether an ambulatory stem cell transplant could be successfully completed.

0705

CHEMOKINES REQUIRED FOR MIGRATION OF MESENCHYMAL STEM CELLS INTO MARROW OR INJURED TISSUES

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Background. Intravenous infusion of mesenchymal stem cells (MSC) after chemotherapy are currently used to accelerate the hematopoietic recovery but their potential of migration and homing to bone marrow is poorly understood. **Methods.** In this study, bone marrow MSC (BM-MSC) were isolated by the classical plastic adhesion method and cultured in alpha-MEM medium supplemented with 15% FCS. Cultured MSC were identified through their expression of SH2, SH3, CD44, CD105 but lacked typical hematopoietic antigens: CD14, CD31, CD34, CD45 and CD62-e. Their potential of differentiation was evaluated by their ability to differentiate into adipocytes, osteocytes, chondrocytes and neuronal/glial cells under specific induction. The MSC migration was evaluated using matrigel invasion chamber assay. MSC were used at 5.105 cells/mL in the upper chamber. In the bottom chamber, different cytokines were tested for their chemoattractive properties: SDF-1 (100 ng/mL), PDGF-bb (100 ng/mL), IGF (0,5 µg/mL) and IL-6 (100 ng/mL) in a serum free medium. BM-MSC conditioned medium (CM) at 10% was also used as chemoattractant. After 24 hours, cells that had not migrated were removed from the top

of the insert by scrubbing with a cotton swab and cells that migrated through the membrane were counted after staining with 0,5% crystal violet. **Results.** Two weeks after specific induction, lipid vacuoles, calcium deposit and chondrogenic matrix were stained using oil red O, silver nitrate and toluidin blue respectively. We observed that MSC migration was greater in the presence of PDGF-bb, IL-6 or with 10% CM with respectively an increase of 6,6; 5,6 and 5 fold compared to spontaneous migration. Only a small number of MSC migrated in response to SDF-1, and this observation correlates with the very low expression of CXCR4 receptor (0,67±0,08%). Indeed, we demonstrated that CXCR4, the SDF-1 receptor was mainly intracytoplasmic expressed (74,9±1,3%), and the surface expression of CXCR4 was very low. **Conclusions.** IL-6 and PDGF-bb are important cytokines produced by bone marrow microenvironment or by injured tissues. In this study, we thus demonstrate that these chemokines could be involved in the migration of MSC to bone marrow as well as to damaged tissues.

	Ctrl	SDF-1	IGF	10%CM	IL-6	PDGF-bb
Migrated MSC ⁺	18,7±2,7	74,9±1,3	42,2±2,8	89,9±7,9	77,1±4,1	80,5±4,3

Number of cells observed in 100 fields of view.

0706

ABNORMAL RETENTION OF RADIOPROTECTIVE HEMATOPOIETIC CELLS WITHIN THE BONE MARROW MICROENVIRONMENT IN CXCR4^{-/-} CHIMERIC MICE

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1. Within the BM microenvironment(BMM), self-renewal, proliferation and differentiation of hematopoietic stem cells (HSC) occur through specific interactions with stromal cells and extracellular matrix proteins. 2. The chemokine SDF-1 and its receptor CXCR4 play an important role on HSC biology but the mechanisms are not fully understood. 3. We used 14.5 dpc CXCR4^{-/-} and CXCR4^{+/+} fetal liver cells to reconstitute lethally irradiated recipient mice in order to further investigate the role of CXCR4 receptor on hematopoiesis. 4. We show that radioprotection of lethally irradiated mice injected with less than 2x10⁶ fetal liver (FL) cells from E14.5 CXCR4^{-/-} embryos was markedly impaired when compared to CXCR4^{+/+} counterparts (10% survival in CXCR4^{-/-} engrafted mice versus 65% survival in CXCR4^{+/+}), but this defect was rescued when hosts were engrafted with 5x10⁶ cells. This quantitative defect contrasted with a similar content in hematopoietic colony forming cells (CFCs), CFU-S and Lin-/lowSca-1+c-kit+ cells in E14.5 CXCR4^{-/-} and CXCR4^{+/+} FL. In addition, we show that the homing of CFCs was significantly altered in the spleen but not in the BM as detected with a CFSE-staining assay. Nevertheless, mice engrafted with 5x10⁶ cells from either E14.5 CXCR4^{-/-} and CXCR4^{+/+} FL shown similar levels of chimerism at both 5 and 16 weeks post transplant. Thus, no significant difference was observed either on the total number of circulating leukocytes and erythrocytes in both group of chimeras. Interestingly, CXCR4^{+/+} chimeras exhibited a thrombocytosis before week 12, which was not observed in CXCR4^{-/-} engrafted mice. Later platelet count returned to normal. As reported in other studies, CXCR4^{-/-} chimeras were characterized by a decrease in the absolute number of circulating lymphocytes and an elevated number of circulating granulocytes, from week 2 to week 16 post transplant. Also, using a CFCs assay, we observed a 30-fold increase in CFCs in the circulation of CXCR4^{-/-} chimeras (8544±249 CFCs per ml of blood versus 280±42 CFCs per ml of blood in CXCR4^{+/+} chimeras, p<0.001) and this increment was already observed before hematopoiesis had reached a steady state level at week 2 (4760±480 CFCs per mL of blood in CXCR4^{-/-} mice versus 140±28 CFCs per mL of blood in CXCR4^{+/+} mice, p<0.001). Secondary transplantations were

performed using irradiated Ly5.1 hosts injected with a mixture of either 100 µl CXCR4^{+/+} or 100 µl CXCR4^{-/-} chimeras' PBLs and 1.5x10⁵ BM Ly-5.1+ cells. At week 5 post transplant, all recipients engrafted with PBLs from CXCR4^{-/-} donors exhibited a significant level of chimerism in contrast to those injected with PBLs from CXCR4^{+/+} donors (6.4±3.1% versus 0.7±0.6% of circulating Ly5.2+ cells in control mice). 5. Altogether, our results demonstrate that the defect in hematopoietic reconstitution of CXCR4^{-/-} fetal liver cells is more related to an altered anchorage in the BM than a real defect in the homing of primitive hematopoietic cells. This further underlines the role of CXCR4 and SDF-1 in the circulation and mobilization of hematopoietic precursors, progenitors and stem cells.

0707

IMPLICATION OF HIF-1ALPHA BUT NOT HIF-2ALPHA IN THE HYPOXIC RESPONSE OF HUMAN HEMATOPOIETIC PROGENITORS

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Background and Aims. Maturing hematopoietic cells are exposed to hypoxia as they develop and migrate within the bone marrow microenvironment. Previous studies using non hematopoietic cell lines and monocytes showed that CXCR4 is strongly induced by hypoxia but little is known on the regulation of CXCR4 by hypoxia in the other hematopoietic cells and during hematopoietic development. **Methods. and Results.** We analyzed the expression and regulation of hypoxia-inducible transcription factor-1alpha (HIF1alpha) and 2alpha (HIF2alpha), the master regulators of metabolic adaptation to hypoxia, during hematopoiesis. Real time quantitative RT-PCR showed that HIF-1alpha mRNA was present on all the non hematopoietic and hematopoietic cells lines including HL-60, HEL, TF1, K562, KG1, U937, Jurkat and Mo7e. In contrast, HIF-2alpha mRNA expression was variable among the cell lines and was detected only at very low level in some cells such as KG1, Jurkat and HEL. CXCR4 induction by hypoxia was observed in the cells that exhibit significant expression of HIF-2alpha mRNA and HIF-2alpha protein accumulation. In contrast, VEGF induction by hypoxia was observed in the cells that expressed HIF-1alpha and exhibited HIF-1alpha protein accumulation. Human CD34+ cells expressed high levels of HIF-1alpha mRNA, whereas HIF-2alpha mRNA was barely detected. Interestingly, CXCR4 receptor expression was not induced by hypoxia or hypoxia mimetic reagents such as cobalt chloride and desferrioxamine whereas a strong induction of VEGF expression was observed. **Conclusions.** Altogether these data indicated that the hypoxic responses of human hematopoietic progenitors are independent of HIF-2alpha. Moreover, they establish that CXCR4 regulation by hypoxia is linked to HIF-2alpha protein expression.

0708

IDENTIFICATION OF CXCR4 AS A NEW NITRIC OXIDE REGULATED GENE IN HUMAN CD34+ CELLS

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Background. and Aims. Nitric oxide (NO) is a short-lived free radical which serves as a messenger molecule for diverse pathological and physiological functions. Recent studies have shown that NO enhanced the leukocyte trafficking. Although NO is involved in the regulation of hematopoiesis, its role in the trafficking of hematopoietic progenitor cells is not defined. **Methods and Results.** Here, we show that several NO donors, NOC18, sodium nitroprusside (SNP), Sperm NO and SIN-1 increased 5 to 10 fold CXCR4 membrane expression in a large fraction of CD34+ cells from both mobilized peripheral blood, and cord blood but exhibited a weak effect on BM CD34+ cells, mature cells and most of the hematopoietic cell lines tested (Mo7e, UT-7, CEM, HL-60). Increased surface expression of CXCR4 on both PB and CB CD34+ cells is apparent shortly

upon incubation at 37°C (2 hours) and is sustained during 24h culture in presence of NO donors. This up-regulation of CXCR4 expression by NO can be completely inhibited by cycloheximide suggesting that it is protein synthesis dependant mechanism. Accordingly, NO induction of CXCR4 expression was associated with an increase in CXCR4 transcription, as actinomycin D treatment inhibited NO effect on CXCR4 mRNA expression. Furthermore, NO up-regulates CXCR4 mRNA transcription in presence of puromycin, implying that a pre-existing protein mediates the function of NO. The effects of NO are not mediated through the protein kinase G-mediated signaling pathways, but via redox regulation and thiol modification. Most of CFU-E (colony forming unit-erythroid) progenitor cells exhibited low CXCR4 expression, which is not up regulated by NO. In contrast, CFU-GM exhibited higher CXCR4 expression, which was largely increased by NO. CXCR4 up-regulation by NO was also observed in primitive cells such as LTC-ICs. In *in vitro* chemotactic assays, NO treated cord blood CD34+ cells exhibited increased migration properties to SDF-1. **Conclusions.** This study demonstrates for the first time that NO modulates the transcriptional activity of CXCR4 gene in hematopoietic stem cells. The role of nitric oxide in the *in vivo* trafficking of hematopoietic progenitors is under study.

0709

EVIDENCE THAT BONE MARROW STROMAL CELLS (BMSC) CAN EXHIBIT IMMUNOSTIMULATING EFFECTS

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It has been reported that bone marrow stromal cells (BMSC) exert a strong immunosuppressive effect on mixed lymphocyte reaction (MLR). This effect is thought to be mediated by different factors including transforming growth factor (TGF)-beta, hepatocyte growth factor (HGF), prostaglandin E2 (PGE2) or indoleamine-dioxygenase (IDO). This immunosuppressive effect may both favour engraftment of bone marrow transplantation and avoid the graft versus host reaction. However, the ratio between BMSC and lymphocytes used for *in vitro* experiments is conceivably higher than that obtainable *in vivo* after BMSC administration. Moreover, BMSC are thought to support the growth of CD34+ precursors, thus exerting a stimulating effect. To better understand the functional consequences of BMSC/lymphocytes interaction possibly occurring *in vivo*, we analysed the effect of different BMSC/lymphocytes ratios *in vitro* on cell proliferation and cytokine production. To this aim we obtained BMSC and peripheral blood lymphocytes (PBL) from either healthy donors (n=4) or from patients (n=10) suffering from acute myeloid leukemia (AML) in post-chemotherapy complete remission. BMSC were characterised by the surface expression of SH2, SH3 and SH4 surface markers, besides ICAM1 (the counter receptor of LFA1), CD44, beta-1 integrin and HLA class I antigens. We found that cultured BMSC constitutively produce TGF-beta and strongly inhibit (up to 90% of inhibition) MLR when used as a third party at the BMSC:responder ratio of 1:5, in keeping with findings reported by other authors. The BMSC-mediated inhibition is lost at the BMSC:responder ratio of 1:20. Surprisingly, at this ratio BMSC strongly induce proliferation of the responder, as assessed by ³H-thymidine uptake and labelling of responder lymphocytes with CFSE; this effect is still present at 1:200 ratio. Of note, BMSC do not express counter-receptors, such as CD80 or CD86, for accessory molecules but are able to activate ex-vivo isolated allogenic PBL, in the absence of a stimulator. Indeed, BMSC induce the expression of activation markers including CD25 and CD69 and PBL proliferation at a BMSC:PBL ratio of 1:2000, but not at 1:20. Moreover, BMSC trigger the production of the pro-inflammatory cytokines TNF-alpha and IFN-gamma, by PBL upon 24hrs stimulation. Altogether, these findings indicate that BMSC can also activate, beside inhibit,

lymphocytes *in vitro*. It is possible that this phenomenon occurs *in vivo* as well, since the number of BMSC administered during BM transplantation is very low, compared to the number of lymphocytes that conceivably are still present in the recipient. Thus, prior to design therapeutic trials employing BMSC, it would be useful to get a better knowledge of the molecular consequences of their interaction with lymphocytes, in order to forecast what will happen *in vivo*.

0710

HYPOXIA SELECTS DRUG-INSENSITIVE PROGENITORS WITHIN CLONAL LEUKAEMIA CELL POPULATIONS

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1. We previously showed that normal haematopoietic stem and progenitor cells exhibit different levels of resistance to severe hypoxia, which contributes to maintain stem cell features, while protecting stem cells from differentiation commitment. Leukaemia stem cells also appear to require such a protection, as differentiating agents often suppress leukaemic growth. On this basis, we undertook the characterization of the effects of hypoxia on leukaemia stem and progenitor cells. 2. The question we addressed with this study was whether clonal and apparently homogeneous myeloid leukaemia populations, such as the Friend's murine erythroleukaemia (MEL) and K562 cell lines, comprise in fact cells endowed with different levels of hypoxia-resistance, corresponding to different levels within the progenitor/stem cell hierarchy. 3. Our experimental system, based on cell incubation in normoxia or hypoxia (0.1% oxygen), allowed to discriminate between clonogenic progenitors detectable in semisolid cultures (CFC) and hierarchically higher culture-repopulating progenitors (CRC) which correspond to short-term marrow-repopulating stem cells. 4a. We found that severe hypoxia strongly inhibited the overall growth of MEL cells and almost completely suppressed the CFC subset within the MEL cell population. Such a marked sensitivity to hypoxia of CFC when compared to the overall leukaemia cell population parallels that of normal CFC we previously showed. In hypoxia, on the other hand, a cell subset survived whose expansion power after transfer to normoxia (an indicator of CRC) was unchanged in comparison to that of cells always incubated in normoxia. 5a. Thus, the MEL cell population comprises hypoxia-resistant CRC as well as hypoxia-sensitive CFC. Such a heterogeneity is therefore typical not only of leukaemia populations progressing *in vivo* to a polyclonal state, but also of monoclonal populations. The hypoxia-resistant CRC subset most likely contains leukaemia stem cells which resemble stem cells believed to survive in the severely hypoxic core of fast-growing solid tumors and to be responsible for tumor progression, treatment resistance and long-term relapse of disease. 4b. The use of K562 cells enabled us to match the effects of hypoxia with those of antileukaemic drugs such as the BCR/Abl inhibitor STI-571. Hypoxia strongly inhibited the overall growth of K562 cells, and hypoxia-selected cells, after transfer to normoxia, underwent a lag phase before exponential growth was triggered. The latter phase, however, followed a kinetics very similar to that of equal numbers of cells always incubated in normoxia. CFC, when compared to the overall K562 cell population, exhibited a higher sensitivity to hypoxia and much faster kinetics of decrease in hypoxia and increase after transfer to normoxia. Cell number decrease in day-7 hypoxic K562 cell cultures was unaffected by the presence of STI571. 5b. The kinetics of cell number increase after transfer to normoxia was similar irrespective of cell treatment with STI571 in hypoxia, indicating that hypoxia-resistant CRC are STI571-insensitive. When the cell population generated in normoxia from CRC was tested for sensitivity to STI-571, its growth was markedly inhibited by STI571, indicating that the clonal expansion of hypoxia-resistant CRC results in the generation of an STI571-sensitive progeny.

0711

DIFFERENTIATION OF UMBILICAL CORD BLOOD CD14+ CELLS INTO ENDOTHELIAL LIKE CELLSM. Rabiei¹, M. Soleimani², A.A. Movassaghpour³¹Immunology, Asthma and Allergy Institute, TEHRAN, Iran; ²T.M.U, Hematology dept., TEHRAN, Iran; ³T.M.U, Hematology Department, TEHRAN, Iran

Background. It has been shown that circulating human non-adherent CD34+ cells co-expression vascular endothelial growth factor (VEGF-R2) and AC133 have the capacity to differentiate into adherent mature endothelial cells. However, prior studies have demonstrated that a much bigger subset of primary adherent mononuclear cells can also form endothelial progenitor cells (EPC). **Aims.** So, this study initiated to evaluate the capacity of CD14+ as monocyte-macrophages to differentiate into an endothelial phenotype. **Methods.** CD14+ cells were isolated from umbilical cord blood by adherence separation and magnetic bead selection (Purity>90%) by antibody against CD14+ and culture on fibronectin-Coated plastic dishes in DMEM medium supplemented with VEGF (10 ng/mL), bFGF (2 ng/mL) and 20% FBS. **Results.** After 2 week of culture, using fluorescence activated cell analysis we observed expression of the endothelial markers included Von Willebrand Factor (vWF), PECAM1 (CD31). The proportion of cell expressing these markers further increased after 4 weeks (96 and 92.5% vs. in 65 and 18% of cells, respectively). **Summary/conclusions** The present study data indicate that under angiogenic stimulation, Cord blood macrophages develop to endothelial phenotype with expression of specific surface markers and suggesting that these cells population may be recruited for vasculogenesis and my use for cell therapy in future.

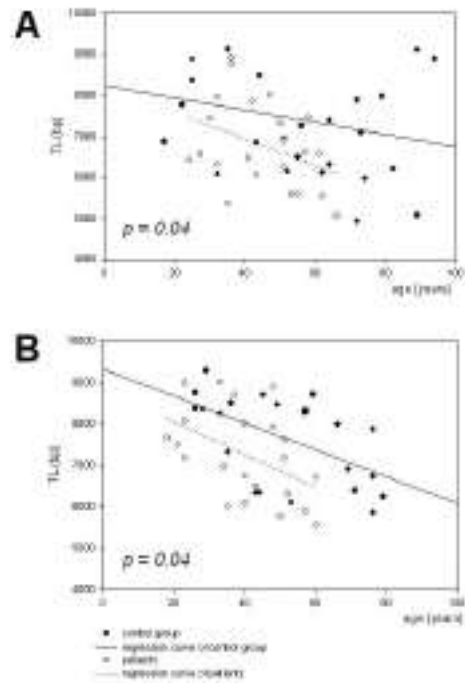


Figure 1. Correlation TL & age in granulocytes (A) and BM (B)

All patients were in continuous Complete Remission since autograft and displayed cell blood counts within the normal range. TL was determined on BM and PB by Southern blot, using a chemiluminescence-based assay; the same samples were also studied for *in vitro* growth characteristics, assessed by short and long-term culture assays. **Results.** TL was found slightly shortened in PB mononuclear cells from post-autograft patients compared to age-related healthy subjects (median TL: 6,895 bp, range 6,022-8,864 vs 6,973 bp, range 4,936-11,470, respectively; $p=ns$). However, autografted patients displayed a significant reduction of TL in granulocytes and in BM samples compared to the age-matched controls (median TL on BM cells: 7,004 bp, range 4,631-9,004 in post-autograft vs 8,113 bp, range 5,856-9,285 in controls; $p<0,05$) (Figure 1A & B). When *in vitro* growth of hematopoietic progenitors was assayed, a significant reduction of BM erythroid (BFU-E), myelo-monocytic (CFU-GM), multipotent (CFU-Mix) and long-term initiating (LTC-IC) progenitor cells was found in autografted patients compared to normal controls. **Conclusions.** accelerated TL erosion of hematopoietic cells and marked reduction of BM immature and committed progenitors are distinct markers of autografted patients; these features persist at long-term following autograft, in spite of the large quantities of PBPC re-infused and the stable haematological recovery, confirmed by the persistence of normal cell blood counts. The defective hematopoiesis might be ascribed either to inability of autografted stem cells to fully reconstitute the hematopoietic system or to an irreversibly damaged BM microenvironment.

Thrombosis and thrombophilia III

0712

EVALUATION OF HEMATOPOIESIS AT LONG TERM AFTER AUTOGRAFT: EVIDENCE OF ACCELERATED STEM/PROGENITOR CELL SENESCENCE ASSOCIATED WITH PERSISTENT REDUCTION OF BOTH COMMITTED AND IMMATURE PROGENITOR CELLSA. Rocci¹, I. Ricca¹, C. Della Casa¹, P. Longoni², M. Battistelli², R. Francese¹, M. Compagno¹, F. De Marco¹, D. Caracciolo¹, M. Boccadoro¹, D. Ferrero¹, M. Ladetto¹, C. Carlo-Stella², C. Tarella¹¹A.O. S. Giovanni Battista, TORINO, Italy; ²Istituto Nazionale Tumori, MILANO, Italy

Background. Telomere length (TL) is considered a valuable marker of cell senescence and a predictor of replicative cell capacity. For instance, a progressive telomere shortening affects hematopoietic cells following *in vitro* expansion. Aim of this study was to investigate the rate of cellular senescence assessed as TL of hematopoietic cells following the replicative stress induced by bone marrow reconstitution following stem cell autograft. The *in vitro* functional characteristics of these cells were evaluated as well. **MATERIALS AND Methods.** Both TL and *in vitro* growth characteristics of bone marrow (BM) and peripheral blood (PB) cells were evaluated at long-term in 32 patients with a previous diagnosis of lymphoma, who had received intensive chemotherapy and peripheral blood progenitor cells (PBPC) autograft. Median time of follow-up since autograft was 6.5 years (range 3.5-10). There were 20 males and 12 females; median age at autograft was of 42 years (range 21-60). In all patients, autograft had been performed with large quantities of PBPC (median autografted CD34+ve cells/kg: 7×10^6 , range 2-24), allowing a rapid and stable hematological reconstitution.

0713**ANTITHROMBIN III SUBSTITUTION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA UNDER TREATMENT WITH L-ASPARAGINASE**

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Background and Aims. L - Asparaginase (L - Ase) is an enzyme whose action is based on protein synthesis blockage. L - Ase treatment, used in patients with acute lymphoblastic leukemia (ALL) causes a reduction in coagulation inhibitors such as antithrombin III (AT III). In our study 30 children treated with L - Ase received AT III concentrates as adjuvant treatment. The aim of the study was to increase the reduced AT III concentration usually found in these patients, during this phase of treatment since AT III deficiency is thought to be associated with an increased risk of thrombosis. **Patients and Methods.** Thirty paediatric patients received L - Ase during induction therapy for ALL, according to the BFM 95 protocol (5000 IU/m² on 8 alternate days). In these patients AT III concentrate was administered at a dosage of 20 - 25 IU/Kg/day for 8 doses starting with the first L - Ase infusion. Twenty patients who received L - Ase without AT III substitution served as controls. **Results.** AT III administration resulted in significantly higher median plasma AT III nadir values than those in the controls (46-76% respectively). In the group receiving AT III, D - Dimer levels, which can be considered as early markers of a state of hypercoagulability were very significantly lowered. **Conclusions.** Our data suggest that in ALL patients receiving L - Ase, according to the BFM 95 protocol, infusions of AT III cause satisfactory plasma AT III levels. Large randomized trials are needed to improve our understanding of the role of AT III. Possibly AT III corrects the state of hypercoagulability due to L-Ase therapy in ALL.

0714**GIESSEN THROMBOPHILIA STUDY: MANIFESTATION AGE OF THROMBOEMBOLISM - AN UPDATE**

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Background. Severity of thromboembolism can be calculated upon manifestation age of thromboembolism. Thus, we searched for differences in manifestation age of thromboembolism in different types of hereditary thrombophilia. We were especially interested, whether combined thrombophilic defects are really more severe and have earlier manifestation age of thromboembolism than single defects. **Patients:** In total, 1.354 patients were examined for thrombophilic risk factors. 936 / 1.354 patients had already experienced thromboembolic complications. **Methods.** In all patients, the following parameters were measured: PT, aPTT, antithrombin, aPC Ratio, protein C and S, factor XII, plasminogen, homocysteine, F. V Leiden mutation, Prothrombin (G20210A)- and MTHFR (677T)-polymorphism and screening for antiphospholipid antibodies. Blood samples for thrombophilia screening were taken 3 months after thromboembolism at the earliest. **Results.** 1. In 480/936 patients with thromboembolism, no coagulation defect was found. 302/936 patients had MTHFR-polymorphism (677T) and/or elevated homocysteine levels, 229 patients combined defects, 201 Factor V Leiden mutation, 44 protein S deficiency, 24 protein C deficiency, 26 prothrombin-polymorphism (G20210A), 5 antithrombin deficiency and 28 patients were identified with antiphospholipid syndrome. 2. Except antithrombin deficiency (19+3 years) and antiphospholipid syndrome (30+11 years), there were no differences concerning manifestation age in different types of thrombophilia: average manifestation age was between 34+14 in patients with Factor V Leiden mutation and

39+14 years in patients with MTHFR-polymorphism and/or elevated homocysteine levels. In patients without thrombophilic defect (n=480) manifestation age of thromboembolism was 37+14 years. **Conclusions.** Except in patients with antithrombin deficiency (19+3 years), no differences concerning manifestation age of thromboembolism was found in different types of hereditary thrombophilia. Even patients with combined defects (double defects 36+15, triple defects 35+11 years) had similar manifestation age as patients with single or even as patients without coagulation defects. Thus, it is unsure, whether patients with combined coagulation defects do really need prolonged anticoagulant treatment.

0715**THROGA, AN INNOVATIVE METHOD FOR QUANTITATIVE DETERMINATION OF THROMBIN GENERATION POTENTIAL IN BLOOD AND PLASMA**

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Background. Thrombin becomes available in soluble form in blood during extrinsic and intrinsic activation of coagulation. Thrombin enzymatically converts fibrinogen to insoluble fibrin and is the strongest platelet aggregation agonist. Thrombin controls the growth of somatic cells via PAR's and binds to proteins in blood. Exact determination of maximal thrombin generation potential offers an important diagnostic tool for a precise assessment of the current state of the coagulation system. For this purpose, a completely new, original method was developed, the THROGA (THROmbin Generation Assay). **Method:** To citrated blood or citrated plasma a defined amount of hirudin is given and furthermore tissue factor and actin are added for activation of coagulation. Following a shaking period (550 rpm, 20 min) the thrombin generating reaction is stopped by addition of EDTA. By measurement of hirudin content in the sample using Ecarin Chromogenic Assay, hirudin consumption is determined. This measured hirudin consumption exactly corresponds to generated thrombin quantity. **Results.** In healthy volunteers the amount of generated thrombin was determined to be 75±11 thrombin units in 1 ml blood, in 1 ml plasma 116±13 thrombin units were measured. First investigations in blood and plasma of thrombophilic patients have shown different thrombin activation patterns. In thrombophilic patients the blood born thrombin amount was significantly higher than the thrombin amount generated in plasma. On the other hand, there are patients showing an increased thrombin generation in plasma at normal generation capacity in blood. The additional measurement of 'blind thrombin', i.e. thrombin bound to proteins and receptors, in a blood or plasma sample was of interest for estimation of a thrombophilic 'background' in patients. THROGA was also shown to be suited for the exact determination of the efficacy of oral anticoagulants. **Conclusions.** THROGA is a new simple test for determination of maximal coagulation potency in blood and plasma. Using THROGA, a fast detection of a thrombophilic state in patients is possible allowing referral to differentiated diagnostics and therapy.

0716**RETROSPECTIVE AND PROSPECTIVE ANALYSIS OF THE INCIDENCE OF VENOUS THROMBOEMBOLISM IN FIRST-DEGREE RELATIVES OF FACTOR V LEIDEN AND FACTOR II G20210A CARRIERS**

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Data on thrombotic risk associated with FVL and FII G20210A derive mainly from casecontrol studies and information on relative risks (RR) and annual incidence of VTE in asymptomatic carriers is scarce and, in the case of South American populations, still lacking. We evaluated retrospectively and prospectively the incidence of VTE in firstdegree relatives of

FVL and FII G20210A carriers. Information on previous thrombotic events and exposition to acquired risk factors for VTE was collected from all relatives using a validated questionnaire. Retrospective analysis was performed by evaluating subjects from 5 yrs-old to the beginning of study (February 2000) or first VTE. For FVL, 85 family members of 21 carriers were included; 36 subjects (35 heterozygotes and 1 homozygote) carried the mutation. 1212 and 1506 years of observation were recorded in the group of FVL carriers and non-carriers, respectively. Annual incidence of VTE was 0.33% in FVL carriers and 0.07% in non-carriers (RR=4.96, CI95 0.55-44.32). For FII G20210A, 101 family members of 23 carriers were included; 52 subjects (48 heterozygotes and 4 homozygotes) carried the mutation. 1574 and 1634 years of observation were recorded in the group of carriers and non-carriers, respectively. Annual incidence of VTE was 0.19% in carriers and 0% in non-carriers (RR=∞). Prospective analysis was performed by evaluating the subjects from the beginning (February 2000) to end of study (May 2004) or first VTE. 92 family members of 21 FVL carriers were included; 44 subjects were FVL heterozygotes. 132 and 161 years of observation were recorded in the group of carriers and non-carriers, respectively. Annual incidence of VTE was 2.27% in FVL carriers and 0.62% in noncarriers (RR=3.6, CI95 0.38 -34.23). In the prospective analysis, 113 family members of 23 FII G20210A carriers were included; 64 subjects (60 heterozygotes and 4 homozygotes) carried FII G20210A. 194 and 151 years of observation were recorded in the group of carriers and non-carriers, respectively. Annual incidence of VTE was 1.55% in carriers and 0% in non-carriers (RR=∞). In conclusion, the incidence of VTE in asymptomatic carriers of the two most common prothrombotic mutations was found to be low in the retrospective and prospective analysis. Since uncertainties still remain regarding the actual benefits of the identification of an inherited prothrombotic state in asymptomatic carriers, the present findings indicate that routine thrombophilia mutation screening in asymptomatic subjects is not justified.

0717

REFRACTORY CHRONIC AUTOIMMUNE TROMBOCITOPENIC PURPURA: TREATMENT WITH RITUXIMAB: REPORT OF A CASES SERIE

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There are many reports about the efficacy of the autoimmune thrombocytopenic purpura treatment with rituximab. This antibody seems to be more effective and safe than others treatments in the refractory and chronic phase of this disease, and, additionally, the response duration is more prolonged. We present the report of 14 patients with refractory and chronic (CR) ITP whom were treated with rituximab. We collected prospectively the data for 14 consecutive patients whom were referred to the hospital because CR ITP. All of them was previously treated with at least one treatment scheme. All of them received 4 doses (one each week) of Rituxmab 375mg/m and were followed each 2 weeks. We evaluated the response 4 and 8 weeks after the end of treatment and the response durations was also registered. The patients were from 17 to 70 years old, with a median basal platelet count of 12,550/mm³. Only one patient didn't respond, 7 patients showed complete response and 4 patients partial response. Seven patients have sustained response. Rituximab seems to be an effective and safe treatment for patients with CR ITP. There is a lack of randomized studies to confirm this observation.

0718

GENETIC MARKERS OF INFLAMMATION AND VENOUS THROMBOEMBOLISM

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The inflammation is a key component of vascular diseases and thrombosis. Therefore, the genes coding for inflammatory cytokines or chemokines could be feasible candidates to risk factors for venous thromboembolism (VTE). The interleukin-1 (IL-1) and interleukin-6 (IL-6) are cytokines involved in endothelial activation and synthesis of acute inflammatory reactants including some coagulation factors such as fibrinogen and factor VIII. The monocyte chemoattractant protein-1 (MCP-1) is a chemokine producing monocyte recruitment to the inflammatory focus and a procoagulant state (increasing the tissue factor expression on endothelial cells and monocytes). High serum levels of IL-6 and MCP-1 were recently associated with recurrent deep vein thrombosis (DVT) (van Aken et al, *Thromb Haemost* 2000; 83: 536). We aim to know any possible influence between their main genetic determinants and the VTE. Population and methods. We studied 270 consecutive patients with an objectively diagnosed VTE and 270 healthy controls of similar gender and age [in overall 62.1 (14.0) y, 49.3% males]. Among the patients, 93 (34%) have suffered a pulmonary embolism (PE) with or without an associated DVT and 59 (22%) a recurrent episode of VTE. We genotyped (by PCR-RFLP) the main functional polymorphisms of genes encoding four of the most important inflammatory cytokines and chemokines: three polymorphisms located in a promoter region [-889C>T of interleukin-1alpha (IL-1A), -174 G>C of interleukin-6 (IL-6) and -2518 A>G of monocyte chemoattractant protein-1 (MCP-1)] and +3954 C>T in the exon 5 of the interleukin-1beta (IL-1B) gene. *Results.* The allelic frequencies were similar for the eight alleles between patients and controls. However, some of the 12 genotypes showed weak associations with the clinical background. The carriers of an homozygous genotype for the variant allele of the MCP-1 (-2518GG) seem protected against the VTE [OR= 0.36 (0.14-0.93), p<0.05] whereas his heterozygosity (-2518AG) appears more frequently in recurrent cases [OR= 2.04 (1.15-3.61), p<0.05]. Furthermore, the subjects with homozygosity for the variant allele of the IL-6 (-174CC) could be protected against the PE [OR= 0.38 (0.14-1.00), p=0.05]. *Conclusions.* Although a strong influence of the studied genetic markers in VTE seems to be excluded, the apparent protective role associated to the -2518GG genotype of the MCP-1 deserves further analyses. Other genotypes of these inflammatory molecules weakly associated with clinical background of the VTE.

This work was supported by the grant FIS #010839

0719

PATIENTS WITH UNUSUAL VENOUS THROMBOSIS (CEREBRAL AND SPLANCNIC VENOUS THROMBOSIS): GENETIC AND ACQUIRED THROMBOPHYLIC FACTORS

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Background. In patients with unusual venous thrombosis i.e. cerebral venous thrombosis and splanchnic venous thrombosis the genetic and acquired risk factors are often present. *Aims.* We wanted to verify the role of thrombophilia and of a possible underlying myeloproliferative disorder or other malignancy. *Methods.* We studied 15 patients with unusual venous thrombosis; we evaluated the frequency of familiar, genetic and acquired thrombophilic risk factors. The diagnosis of cerebral venous thrombosis was made by CT scan, MRI or cerebral arterial angiography. In the patients with splanchnic venous thrombosis the diagnosis was made with Ultrasonography-Doppler

ultrasound and CT scan (1 case was diagnosed during laparoscopy for acute abdomen). We performed a coagulation study including tests for inherited or acquired predisposition to thrombophilia: AT-III, protein C and protein S deficiencies, LAC-ACA antiphospholipid antibodies, hyperhomocysteinemia, factor V Leiden and G20210A prothrombin gene mutations, APC resistance, high plasma levels of coagulation factor VIII. In some cases bone marrow biopsy was made to rule out the possibility of an underlying myeloproliferative disorder. Besides an occult neoplasia was investigated and also the use of oral contraceptive therapy (OCT) was taken into account. **Results.** Among the 9 patients (6 female; median age of 41 years, range 18-56) with splanchnic vein thrombosis only one patient presented no risk factors. Three patients had a combination of genetic and acquired risk factors: G20210A prothrombin gene mutation in association with OCT; factor V Leiden gene mutation in association with OCT; hyperhomocysteinemia with a concomitant diagnosis of essential thrombocytemia (ET). In a woman, who was in OCT, an ET was diagnosed at the time of portal thrombosis. One patient had various genetic polymorphisms of factor XIII, fibrinogen and PAI genes. One patient was affected by ET; one by myelo-monocytic chronic leukaemia, and another patient was affected by idiopathic myelofibrosis and had a positive familiarity. As regarding the six patients (4 female, median age 38 years, range 26-56) with cerebral venous thrombosis a complete thrombolytic screening study was performed in 5/6. Two patients presented a combination of genetic and acquired risk factors: the first had high plasma levels of coagulation factor VIII and APC resistance in association with OCT; the second one had factor V Leiden gene mutation, LAC/ACA antibodies and OCT. The third patient had a combination of two acquired factors: OCT and a breast cancer that was discovered at the time of thrombosis. The fourth patient had only one acquired factor because at the time of thrombosis it was diagnosed essential ET; this patient was the only one presenting also a familiarity for thrombosis. The fifth patient had no risk factors. In 75% of these patients OCT represented an acquired risk factor. **Conclusions.** Despite the limitations of the sample size, our data suggest that other predisposing factors are often associated with the genetic thrombophilia. The OCT and the myeloproliferative disorders seems to be important determinants in the global thrombotic risk. A screening for inherited thrombophilia should be made; it is important to research a possible underlying myeloproliferative disorder or other malignancy.

0720

SUCCESSFUL USE OF DANAPAROID IN TWO PREGNANT WOMEN WITH VALVE PROSTHESIS AND HEPARIN-INDUCED THROMBOCYTOPENIA TYPE II (HIT TYPE II)

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Background. Anticoagulant therapy for the prevention of thromboembolism in pregnant women with prosthetic heart valves is associated with an increased risk to the mother and/or the fetus. A life-threatening complication of the therapy with heparin is HIT type II. Newer non-heparin anticoagulant drugs such as Danaparoid (Orgaran TM) have not yet been reported to be safe and effective for this indication. **Case 1:** A 26-yr old woman with tricuspidal valve prosthesis conceived under oral anticoagulation (phenprocoumon). Phenprocoumon was discontinued at the 6th week of gestation and full dose unfractionated heparin (UFH) therapy was given UFH. On day 12 after initiation of UFH, the platelet count dropped from 202/nl to 143/nl. One day later, the diagnosis of HIT type II was supported by a highly positive heparin/platelet factor 4 antibody titer tested by ELISA. UFH was discontinued and anticoagulation with danaparoid subcutaneous (s.c.) 3000 IU twice a day (target anti-Xa level: 0.7-1.0 IU/mL) was initiated. The patient's platelet count rose progressively. The patient was well until the 37th week of gestation, when she developed preeclampsia.

Danaparoid was discontinued (anti-Xa level: 1.05 IU/mL) and a caesarean section was performed after 43 hours when the anti-Xa level had dropped to 0.3 IU/mL. The patient delivered a healthy boy. Danaparoid was reinitiated 2 hours after delivery (target anti-Xa level: 0.6-1.0 IU/mL) followed by gradual introduction of phenprocoumon. **Case 2:** A 37-yr old woman with a St. Jude Medical mitral-valve prosthesis conceived under phenprocoumon. Because of HIT type II with a thrombosis of the valve prosthesis under UFH in a previous pregnancy, phenprocoumon was discontinued at the 5th week of gestation and anticoagulation with danaparoid s.c. 3750 IU twice a day (target anti-Xa level: 0.9-1.2 IU/mL) was initiated. A valve thrombosis or valve insufficiency was excluded by echocardiography every 4 weeks. Gynecologic examinations showed a regular and uneventful course of pregnancy and sonographic regular development of the fetus until the 32th week of gestation. At this time the woman presented with a placental hematoma (5x1 cm) which did not increase over the following week. Because of increasing cardiac insufficiency a cesarean section was performed when after discontinuation of danaparoid the anti Xa-level had dropped to 0.7 IU/mL. The woman delivered a healthy boy. Danaparoid was reinitiated s.c. after delivery (target anti-Xa level: 0.8-1.0 IU/mL) followed by gradual introduction of phenprocoumon. **Conclusions.** HIT type II in a pregnant woman with a prosthetic heart valve can be successfully managed with danaparoid.

0721

A CASE OF A YOUNG MAN SUFFERING OF AGENESIS OF THE INFERIOR VENA CAVA, DEEP VEIN THROMBOSIS AND MULTIPLE COAGULATION ABNORMALITIES

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Background. It has been noticed previously in very rare reports that congenital malformations of vena cava inferior could be followed by extensive venous thrombosis. **Case Report.** A 19-year old sportsman in previously excellent health condition presented with sudden onset of arterial hypertension (200/110 mm Hg). Among other clinical findings abdominal ultrasound revealed a mass in the right hemiabdomen. Computed tomography (CT) imaging revealed abnormal venous structure surrounding right kidney with initial hydronephrosis of the same kidney. Few days later a patient has been hospitalized urgently for acute abdominal pain, oedema and change of colour of the right leg. Deep vein thrombosis (DVT) was suspected and confirmed by angiography. Despite high dose heparine DVT progressed quickly to the left leg. His general condition was deteriorated also due to Gram negative sepsis. **Methods.** A CT and MRI scan revealed agenesis of inferior vena cava (IVC), an extension of thrombosis affecting the ilio-femoral veins on both sides, the right retroperitoneal veins and collateral system around the right kidney. Multiple clotting perturbations was found: deficit of protein C, factor V Leiden mutation, positive lupus anticoagulant, slightly elevated PAI, high level of D-dimer and fibrinogen, thrombocytosis. **Course and Treatment.** Agenesis of inferior vena cava and DVT of the both legs was complicated by right ureter compression and initial hydronephrosis, hypercoagulable state and Gram negative sepsis. Spontaneous prolongation of PTT makes a heparin treatment difficult to prescribe. The patient has been treated with high heparin dose (heparine 40 000 IJ/24h/i.v.) and adequate antibiotics and his general condition improved. Since the patient is in high risk of thrombosis a long term anticoagulant treatment with coumarin has been prescribed. After two years follow up he is in excellent condition. **Conclusions.** Agenesis of the IVC is a very rare condition. We describe the patient with extensive deep vein thrombosis and multiple coagulation abnormalities.

0722

COAGULANT ACTIVITY IN HAEMOPHILIACS WITH AND WITHOUT THROMBOPHILIC MUTATIONS

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Introduction: There is a considerable variability in bleeding pattern in haemophiliacs. It has been seen that a significant number of haemophiliacs show a co-existence of mutations related with increased risk of thrombosis (Foka et al 2003). It has also been suggested that these thrombophilic genetic defects may actually protect haemophiliacs from recurrent bleeding episodes or may even lead to thrombosis in several cases (Makris et al 2003). In order to study their coagulant activity, F1+2 and TAT were selected as markers of the generation of prothrombinase (PG). We simultaneously applied the Hicks & Pitney test to compare our results, and we used the Thrombin Generation test (TG) as a confirmatory standard test. **Materials- Methods.** 65 subjects were studied (58 patients with moderate-mild haemophilia, 42±15 years and 7 normal subjects (NS) 45±10 years). Subjects were divided in four groups: 1-27 haemophiliacs without mutations (HN), 2-15 with FVLeiden and/or FIIG20210A mutations (HHet), 3-16 MTHFR homozygotes (HTT) and 4-7 NS. The levels of F1+2 and TAT were tested with ELISA. The Hicks & Pitney test was applied in 10 consecutive incubations in 37°C. Initially we added Cephalin and CaCl₂ in equal and sufficient quantity of diluted PPP (1/10). 0,1ml from this mixture is added to a new tube containing 0,1ml substrate normal plasma and 0,1ml CaCl₂. Simultaneously 0,1ml from each incubation is frozen for measurement of F1+2 and TAT. Concerning the TG, we used the Thrombogram-Thrombinoscope™ assay. TG or Endogenous Thrombin Potential (ETP) was studied according to the assay described by Hemker et al (2000). Four major parameters can be assessed in this test: lag time, peak height, time to peak and endogenous thrombin potential. **Results.** It can be seen in the charts that haemophiliacs with no mutations show a gradual prothrombinase generation.

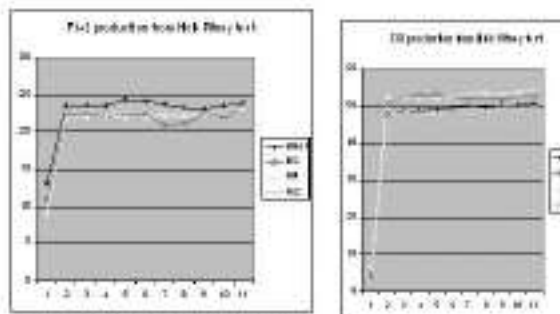


Table 1. Results from the TG

	ETP	Peak
NS	7090.3 ± 217.4	359.5 ± 72.8

HHet show a rapid generation that is also expressed by the concomitant increase in the levels of F1+2 and TAT. A similar response was observed in the group of NS. This can be explained as an adjustment of the coagulation mechanism of haemophiliacs with coexistence of thrombophilic mutations,

possibly capable of preventing the high frequency and severity of bleeding episodes. These results were better confirmed using the TG test. The comparison between the different groups using the t-test distribution curve was as follows: a- Comparison between normal subjects (NS) and haemophiliacs with FV-Leiden mutations t-test, p<0.1 (ns), in contrary the comparison between the NS curve and all the rest groups was statistically different (p<0,05). **Conclusions.** The above results are in line with the observation that haemophilic patients bleed only after trauma, surgical intervention etc. It seems probable that during the million years of human life on earth one or more compensatory mechanisms have developed. These appear to play a significant role in the rate of bleeding episodes in haemophiliacs and may also be able to explain the increasing frequency of thrombotic events in these patients. FV-Leiden is the factor that most consistently appears to decrease severity of haemophilia.

0723

WEAK RELATIONSHIP BETWEEN SERUM LEVELS OF OXIDIZED LDL AND CORONARY ATHEROSCLEROSIS SEVERITY IN PATIENTS WITH NEWLY DIAGNOSED CORONARY ARTERY DISEASE

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Low-density lipoprotein (LDL) oxidation plays a pivotal role in the initiation and progression of atherosclerosis and eventually in the endothelial injury/ thrombogenesis process. **Aims.** To assess the relation between circulating levels of oxidized LDL and the severity of coronary artery disease (CAD) in patients undergoing coronary angiography. **Methods.** One hundred sixty four patients, <65 years old, 149 men and 15 women, admitted for CAD related symptoms of recent onset, had a coronary angiogram during their hospitalization. In addition to the number of diseased vessels, the extent of CAD was assessed with Hamsten scoring system. A full lipid profile was also acquired and oxidized LDL serum levels were measured with a commercially available sandwich ELISA kit. Bivariate correlations and multivariate regression models were used in the statistical analysis. **Results.** Twenty eight patients had normal coronary angiograms and were excluded from the analysis. In the remaining 136 patients, oxidized LDL significantly correlated with both the Hamsten score (r=0.264, p=0.009) and the number of diseased vessels (r=0.261, p=0.003). However, in the stepwise multivariate linear regression models, which included all the other lipid parameters in addition to oxidized LDL, only apolipoprotein B-100 was an independent predictor of the number of diseased vessels (p<0.001), while both apolipoprotein A-I and apolipoprotein B-100 were predictors of Hamsten score (p=0.001 and p=0.016 respectively). **Conclusions.** Circulating levels of oxidized LDL are weakly related to the severity of CAD. Since oxidized LDL is mainly confined to the atherosclerotic plaques, its few circulating molecules may not accurately reflect its atherogenic potency.

0724

PHARMACOKINETIC PROFILE OF MOLECULAR EXTENDED DIPETARUDIN

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Background. Dipetarudin is a direct thrombin inhibitor with a Ki of 446 fM and a molecular mass of 7.5 kDa. It is composed of the N-terminal head structure of dipetalogastin II and the exosite 1 blocking segment of hirudin. Because of its biochemical and pharmacokinetic characteristics, dipetarudin could be therapeutically applied as anticoagulant, but also in the antitumor therapy (1). However, it has a short elimination half-life of

about 30 minutes. It has been extensively described that the pharmacokinetic behavior of proteins can be enormously improved by conjugation to PEG derivates. *Aims.* The aim of this study is to achieve a better pharmacokinetic profile of dipetarudin, enhancing its anticoagulant efficacy via coupling to polyethylene glycol (PEG) chains. *Methods.* Dipetarudin and PEG of 20 kDa were incubated in 1 ml of borate buffer (100 mM, pH 8.0). The final dipetarudin:PEG ratio was 1:6. After 3 hours at 25°C, the reaction was stopped by loading into a pre-packed Superdex 75 column. The dissociation constant(K_i) of each inhibitor was determined by nonlinear regression analysis according to the theory of slow, tight binding. Pharmacokinetics of PEGylated dipetarudin was studied in rats after administration of 0.5 mg/kg of body weight. The pharmacokinetic parameters were calculated from the blood concentration time curve after intravenous administration of inhibitor by means of a two compartment model. *Results.* Dipetarudin was efficiently coupled to 20kDa PEG-chains and purified by gel filtration chromatography. Like dipetarudin, the PEGylated form of this inhibitor acts as a slow, tight-binding inhibitor of thrombin, but with a dissociation constant(K_i) for the interaction with thrombin 2-fold higher than that of dipetarudin (874.24 fM). Pharmacokinetic analysis revealed that PEGylated dipetarudin has longer distribution and elimination half-lives, higher area under the time concentration curve (AUC) and lower clearance than non-PEGylated dipetarudin. *5. Conclusions.* These results suggest that lower doses of PEGylated dipetarudin could produce the same anticoagulant/antitumor effect than unmodified dipetarudin which is a desirable attribute to future therapeutic application.

1) López and Nowak (2004). *Anticancer Drugs* 15:145-9.

0725

THE G20210A PROTHROMBIN GENE MUTATION AND THE PLASMINOGEN ACTIVATOR INHIBITOR (PAI) 5G/5G GENOTYPE INCREASE THE RISK OF PREMATURE ONSET OF SEVERE PREECLAMPSIA

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Background. and *Aims.* Obstetrical complications such as severe preeclampsia and HELLP syndrome are associated with abnormal placental vasculature and inadequate placental maternal-fetal circulation. Hereditary risk determinants of venous thrombosis have been reported to be associated with these obstetrical complications. So far there is no data to support whether these risk determinants are related to the time of onset of severe preeclampsia. *Methods.* We used a case-control design, studying 97 women with severe preeclampsia (blood pressure higher than 160/100 mm Hg; urinary protein excretion greater than 5 g per day, platelet count of less than 100.000/μL; combination of hemolysis, high serum aminotransferase concentration, and platelet count below 100.000/μL; or eclampsia) in previous pregnancies and 277 normal women to assess hereditary risk factors of venous thrombosis as risk determinants for severe preeclampsia. Moreover, a case-only design comprising the 97 women with a history of preeclampsia was used to evaluate these risk factors as risk determinants for early onset of severe preeclampsia. We determined the genetic risk markers factor V Leiden G1691A (FVL), the G20210A prothrombin gene mutation, the methylenetetrahydrofolate reductase polymorphism (MTHFR C677T), and the PAI 4G/5G polymorphism. None of the women had a combined or homozygous defect of the FVL or the G20210A prothrombin gene mutation or deficiencies of antithrombin, protein C, or protein S. Women with antiphospholipid syndrome were excluded. *Results.* Using the case-control design, there was no significant risk association of the hereditary risk factors with severe preeclampsia (factor V Leiden, odds ratio (OR) 0.9, 95% confidence interval (CI) 0.4-2.2; prothrombin mutation, OR 1.9, 95%CI 0.5-7.0); methylenetetrahydrofolate-reductase 677TT genotype, OR 0.8, 95%CI 0.4-

1.8; plasminogen activator inhibitor (PAI-1) 4G/4G genotype, OR 1.2, 95%CI 0.7-2.1; PAI-1 5G/5G genotype, OR 1.0, 95% CI 0.5-1.8). However, the onset of severe preeclampsia was significantly earlier in women with the G20210A prothrombin gene mutation (24.5 weeks vs. 30.1 weeks, p=0.046) and in women with the PAI-1 5G/5G genotype (25.7 weeks vs. 30.8, p=0.024). *Conclusions.* Hereditary risk factors for venous thrombosis do not predispose for severe preeclampsia. However, women who are carriers of the G20210A prothrombin gene mutation and the PAI-1 5G/5G genotype are at risk for early onset of severe preeclampsia. It appears that these risk factors do not induce the pathomechanism but accelerate the course of preeclampsia.

0726

NEW APPROACH FOR EVALUATION OF QUANTITATIVE RISK FACTORS OF VENOUS THROMBOEMBOLISM USING INDIVIDUALIZED REFERENCE VALUES

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Background. The evaluation of quantitative risk factors of venous thromboembolism is characterized by several unsolved problems. The majority of quantitative components of hemostasis are dependent on age, sex, and hormone intake. To calculate the relative risk associated with age-dependent variables, age-matches patient-control-pairs are formed and cut-off values according to the 5% or 95% percentile of the group under study are used. This conventional procedure has the disadvantage that even in large studies, in addition to age, no matching for hormone intake is possible since the resulting subgroups are too small for detailed evaluation. The cut-off value given depend on the specific patient/control group and can not be generalized on individuals with other characteristics. Furthermore, the approach does not give reference values dependent on age, sex, and hormone intake which are necessary for risk estimations in clinical practice. *Aim and Methods.* To overcome these disadvantages, we used a multiple regression analysis to create a system of reference values which change continuously depending on age, sex, and hormone intake according to the parameter distribution in healthy controls and calculated the relative risk of hemostatic components. In 1404 individuals (729 patients with first VTE and 675 healthy controls), we determined the activity of the coagulation factors I, VIII, vWF, IX, X, XI, XII, antithrombin, protein C, and protein S, and free protein S concentration. *Results.* A significantly increased risk for venous thrombosis was associated with deficiency of protein S activity (odds ratio (OR) 2.8 to 6.1, p=0.0007), free protein S concentration (OR 2.7 to 20.4, p=0.0001), protein C activity (OR 3.2 to 9.4, p=0.0001), antithrombin activity (OR up to 75, p=0.0001), and increased levels of fibrinogen (OR 3.8, p=0.0001), factor VIII:C (OR 3.3, p=0.0001), factor IX (OR 2.3, p=0.0001), factor XI (OR 2.9, p=0.0001), vWF activity (OR 2.4, p=0.0001), and vWF antigen (OR 3.5, p=0.0001). *Conclusions.* In contrast to previously published studies, this new approach gives clinically important cut-off values dependent on age, sex, and hormone intake and allows to identify patients at increased risk for venous thrombosis on an individualized basis. Particularly parameters which are highly dependent on age and sex, like protein S activity, can be characterized more precisely using the described procedure. This comprehensive analysis demonstrates that, apart from well-known risk determinants, deficiency of protein S and increased values of FI, FVIII:C, FIX, FXI, vWF, and vWF-Ag are risk determinants predicting venous thrombosis.

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0727

METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISM AND RISK OF VENOUS THROMBOSIS: DESCRIPTION OF 6 PEDIATRIC CASES

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Background. The venous thromboembolism is a serious and complex pathological condition especially for its potential complications. It is related to both congenital and acquired factors. Among the congenital factors, different mutations have been identified leading to a reduced activity of enzymes (even higher than 50%) involved in the homocysteine-methionine metabolic pathway, such as the methylenetetrahydrofolate reductase enzyme (MTHFR). We describe 6 clinical cases of children presenting a serious and complex thrombosis. The only risk factor found was the MTHFR mutation. **Aims.** The aim of this study is to define the real role of MTHFR mutations and increased risk of thrombotic events. **Methods. and Results.** Between december 2002 and december 2004 six children (median age 7, range 3-11) were admitted into our Department. They were affected by deep venous thrombosis. The MTHFR polymorphism C677T was found in all the patients and also in their parents. The polymorphism was identified by polymerase chain reaction (PCR) followed by restriction enzyme digestion and separation on a 3% agarose gel. Four of the children were homozygous for the genetic mutation, the other two were heterozygous. Two heterozygous and two homozygous patients presented increased plasmatic and urinary levels of homocysteine. The thrombotic events were diagnosed by CT angiography, echocolor doppler and digital cavography. One patient showed venae cavae thrombosis involving the renal veins bifurcation. Two patients showed a mesenteric thrombosis followed by an intestinal infarction requiring a bowel resection. One patient presented a thrombosis of left lower limb, one of suprahepatic vein, one of the spinal cord vascular funnel. Initially, five patients were treated with low molecular weight heparin. Afterwards, they continued with oral anticoagulant therapy lasting from 6 to 12 months; after stopping this therapy, three of them started a treatment with folic acid and B group vitamins. Two children are event free since more than two years, one showed two months ago a new thrombotic event. **Conclusions.** The association between the homozygous MTHFR mutation and thrombosis is still not clear. It seems that not always this polymorphism leads to high plasmatic level of homocysteine. On the other hand, hyperhomocysteinaemia spikes could occur in particular circumstances that might not be detectable at all times but clinically significant. Recent data demonstrated that MTHFR C677T homozygosity with hyperhomocysteinemia is not necessarily associated with VTE (Fredriksen J. et al.-2004). Nevertheless, our cases don't seem to confirm this considering that two patients presented both homozygosity and high level of plasmatic homocysteine with significant thrombotic events. Further investigations are required in order to understand the real link between MHTFR mutation and the increased risk of thrombosis.

0728

BREAST CANCER-INDUCED ENDOTHELIAL PROTHROMBOTIC AND ANGIOGENIC PROPERTIES ARE SENSITIVE TO HEPARINS IN VITRO

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Background. Recent clinical studies suggest a potential role for the anticoagulant heparin, particularly low-molecular-weight heparins (LMWHs), in improving survival in patients with cancer. The underlying mechanisms are under investigation, with

particular attention focused on the influence of heparins on vascular endothelial cell hemostatic properties. In this setting, tissue factor (TF) can play a key role, because it is the main activator for blood coagulation and also possesses pro-angiogenic capacity. The aim of this study was to: (1) evaluate the effects of two LMWHs (dalteparin and enoxaparin) compared to an unfractionated heparin (UFH) on TF expression in endothelial cells induced by tumor cell-derived products; and (2) determine whether the TF regulation mirrored the modulation of angiogenic features in endothelial cells. **Methods.** TF analysis: Microvascular endothelial cells (HMEC-1) were incubated with a conditioned medium (CM) obtained from the human MDA.MB.231 breast cancer cell line, in the absence or presence of increasing concentrations of each heparins (i.e. 0.01, 0.1, 1.0, and 10 IU/mL) or of vehicle (control). After 4h incubation, TF expression by HMEC-1 was assayed both as activity (by the one-stage clotting assay) and antigen (by ELISA method). Angiogenesis: HMEC-1 were seeded onto Matrigel in the presence of MDA.MB.231 CM or of different human recombinant pro-angiogenic factors (i.e. VEGF, FGF-2 and TNF-alpha)±heparins (0.01–10 IU/mL). After 24h, tube formation was examined under phase-contrast microscopy and tube length determined by image analysis software. **Results.** CM from MDA.MB.231 significantly ($P<0.01$) increased the expression of TF, and strongly stimulated new vessel formation by HMEC-1. Heparins dose-dependently inhibited (up to 100% for all three heparins) the increase in CM-induced TF activity and antigen expression. The three drugs also significantly ($P<0.01$) counteracted breast cancer-induced angiogenesis; however, this effect was less marked with UFH compared with LMWH. Furthermore, the two LMWHs significantly ($P<0.01$) inhibited tube formation induced by standard cytokines, whereas UFH did not. **Conclusions.** These data demonstrate that: 1. heparins modulate TF procoagulant expression in EC elicited by breast cancer cells; 2. this effect parallels the downregulation of EC pro-angiogenic activities; and 3. LMWHs are more effective than UFH in this experimental system.

0729

PREDICTORS OF LEFT VENTRICULAR THROMBUS FORMATION IN PATIENTS WITH DILATED CARDIOMYOPATHY: ROLE OF ACTIVATED PROTEIN C RESISTANCE

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Aims. The aim of this study was to investigate the association between left ventricular thrombus formation and natural anticoagulant systems including the protein C, protein S and antithrombin in patients with dilated cardiomyopathy. **Methods.** Sixty patients with dilated cardiomyopathy who met the inclusion criteria were included in the study. Patients were divided into two groups: group I consisted of 22 patients with left ventricular thrombus and group II consisted of 38 patients without left ventricular thrombus. Our main inclusion criteria were ejection fraction $\leq 35\%$ and left ventricular end-diastolic diameter ≥ 6.0 cm. These two groups were compared for clinical and hematologic parameters (activated protein C resistance, protein S and antithrombin). **Results.** There were no statistically significant differences between patients with or without left ventricular thrombi with respect to left ventricular end-diastolic and end-systolic dimensions, ejection fraction, fractional shortening and left atrial diameter. There were no statistically significant differences between patients with and without left ventricular thrombus with respect to platelet count ($252 \pm 64/\text{mm}^3 \times 10^3$ compared with $260 \pm 74/\text{mm}^3 \times 10^3$ respectively, $p=0.68$), prothrombin time (12.94 ± 1.9 s compared with 12.86 ± 1.3 s respectively, $p=0.82$), activated partial thromboplastin time (32 ± 5 compared with 30 ± 4 s respectively, $p=0.32$) and fibrinogen levels (36 ± 9 mg/dL compared with 34 ± 8 mg/dL respectively, $p=0.41$). None of the patients had protein S and antithrombin deficiency. Activated protein C resistance was found in 12 patients (12 out of 22, 54%) in group I and four

patients (four out of 38, 9.5%) in group II ($P < 0.01$). It was also shown to be an independent predictor of left ventricular thrombus ($P < 0.05$). **Conclusions.** Activated protein C resistance is found to be an independent predictor of left ventricular thrombus in patients with dilated cardiomyopathy who have ejection fractions less than 35% and left ventricular end-diastolic dimensions > 6.0 cm.

0730

CORRELATION OF MARKERS OF INFLAMMATION WITH CORONARY SINUS BLOOD TEMPERATURE IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background. C-reactive protein (CRP) and fibrinogen (FIB) are significant markers of inflammation. Recent studies have shown that patients (pts) with coronary artery disease (CAD) have increased blood temperature and inflammatory markers in the coronary sinus (CS), possibly due to a widespread inflammatory process. The aim of the present study was to measure temperature difference (ΔT) between CS and right atrial (RA) in pts with CAD and to correlate these findings with CRP and FIB. **Methods.** We enrolled 51 pts with CAD and 23 healthy subjects, who underwent coronary angiography for the evaluation of chest pain. In all pts we measured CRP and FIB. CS and RA blood temperature measurements were performed by a 7F thermography catheters. At the tip of the catheter is attached a thermistor probe. Proximally a steering arm enables the positioning of the thermistor probe. Proximally a steering arm enables the positioning of the thermistor in the CS and the RA. ΔT was calculated by subtracting the RA from the CS blood temperature. **Results.** The procedure was performed successfully without any complication. ΔT was increased in pts with CAD compared to healthy subjects (38.09 ± 0.28 vs $38.19 \pm 0.27^\circ\text{C}$, $P < 0.001$). The levels of CRP and FIB, were well correlated with ΔT ($R = 0.29$, $p = 0.02$ for CRP; $R = 0.26$, $p = 0.04$ for FIB). **Conclusions.** Levels of CRP and FIB in the peripheral blood were well correlated with ΔT , indicating that an inflammatory process possibly is the underlying mechanism for increased heat production from the myocardium.

0731

ELEVATED SERUM SOLUBLE CD40 LIGAND IN ACUTE CORONARY SYNDROMES ON THE EFFECT OF DUAL ANTIPLATELET THERAPY ON PLATELET ACTIVATION ON THE PATIENTS

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Background. Activated platelets as well as plaque inflammation participate in the pathogenesis of unstable angina or non ST elevation myocardial infarction (ACS). Serum soluble CD 40 ligand (sCD-40L) reflects both platelet activation and inflammation which are involved in ACS. Furthermore, an increase risk for adverse outcome has been observed in patients (pts) with ACS if soluble CD40L is elevated (> 5.0 $\mu\text{g/L}$). While aspirin is an effective therapy for UA the addition of clopidogrel an ADP receptor inhibitor of platelets, further improves clinical outcome. However, soluble P-selectin (s-PS), a marker of platelet activation has not been predictably altered. **Aims.** We evaluated the effect of clopidogrel on s-PS in association with sCD-40L levels in pts with UA. **Methods.** From 80 consecutive pts who were admitted for UA, 40 pts (mean age 70 ± 4 years, 33 males, 7 females) were randomized to receive aspirin (325 mg, daily) and clopidogrel (300 mg loading dose followed by 75 mg daily) (group: Asp+Clop), and 40 pts (mean age 67 ± 6 years, 31 males,

9 females) to receive aspirin (group: Asp), additionally to usual medical therapy. Blood samples were collected at 0, 8, 48 hours and on day 6. sCD-40 L and s-PS were determined by enzyme-linked immunosorbent assay. **Results.** Pts on Asp+Clop compared to pts on Asp alone had at baseline similar clinical characteristics. Overall, s-PS levels were similar among the two groups at 0, 8 and 48 hours and at day 6 (Asp+clop vs Asp: 55.6 ± 23 vs 53.75 ± 22.9 ng/mL $p = 0.4$, 52.41 ± 16 vs 54.14 ± 20 ng/mL $p = 0.5$, 50.17 ± 13.7 vs 60.8 ± 37.9 ng/mL $p = 0.1$, 50.53 ± 17.5 vs 54.64 ± 18.97 ng/mL $p = 0.4$). When the effect of clopidogrel on s-PS was examined in relation to sCD-40 L serum levels, pts with high levels of sCD-40 L above 5.0 $\mu\text{g/dL}$ had a significant increases in s-PS at 8 hours, if they received Asp only (48 ± 12 to 69.41 ± 21 ng/mL, $p = 0.047$), and levels of s-PS were greater than in pts on Clop+Asp (Clop+Asp vs Asp: $43.6 \pm 6 \pm 10$ vs 65.41 ± 11 ng/mL, $p = 0.01$). This difference was not maintained at 48 hours and 6 days. **Conclusions.** In pts with UA and high sCD-40 L, treatment with Asp+Clop is better than Asp alone in prohibiting early P-selectin elevation. This could be related to the greater intensity of platelet activation and inflammation in pts with ACS and higher sCD-40L levels.

0732

EVALUATION OF THE EFFECT OF CLOPIDOGREL ON PLATELET ACTIVATION ASSOCIATED WITH INCREASED CRP LEVELS IN PATIENT WITH ACUTE CORONARY DISEASE

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Background. In patients (pts) presenting with unstable angina and acute myocardial infarction without ST elevation (ACS), an increase risk for adverse outcome has been observed if C-reactive protein (CRP), a marker of inflammation, is elevated. Activated platelets as well as plaque inflammation play an important role in the pathogenesis of ACS. While aspirin is an effective therapy for ACS the addition of clopidogrel an ADP receptor inhibitor of platelets, further improves clinical outcome. However, soluble P-selectin (s-PS), a blood marker of platelet activation has not been predictably altered. Other factors, such as the severity inflammation may be important too. **Aims.** We evaluated the effect of clopidogrel on s-PS in association with CRP levels in pts with ACS. **Methods.** From 86 consecutive pts who were admitted for ACS, 43 pts (mean age 70 ± 4 years, 36 males, 7 females) were randomized to receive aspirin (100 mg to 325 mg, daily) and clopidogrel (300 mg loading dose followed by 75 mg daily) (group: Asp+Clop), and 43 pts (mean age 67 ± 6 years, 34 males, 9 females) to receive aspirin (group: Asp), additionally to usual medical therapy. Blood samples were collected and centrifuged at 0 hours, 8, 48 hours and on day 6, for measurements of CRP and s-PS by immunoturbidimetric assay and immunoassay accordingly. **Results.** Pts on Asp+Clop compared to pts on Asp alone had at baseline similar clinical characteristics and Tnl levels. Overall, s-PS levels were similar among the two groups at 0, 8 and 48 hours and at day 6 (Asp+clop vs Asp: 56.6 ± 23 vs 52.75 ± 22.9 ng/mL $p = 0.4$, 51.41 ± 16 vs 54.14 ± 20 ng/mL $p = 0.5$, 49.17 ± 13.7 vs 60.8 ± 37.9 ng/mL $p = 0.1$, 50.53 ± 17.5 vs 54.64 ± 18.97 ng/mL $p = 0.4$). When the effect of clopidogrel on s-PS was examined by CRP quartiles (CRP < 0.3 mg/dL, CRP 0.3 mg/dL to 0.5 mg/dL, CRP 0.5 mg/dL to 1.3 mg/dL, CRP > 1.3 mg/dL), pts that had CRP levels at the highest quartile had a significant increase in s-PS at 8 hours, if they received Asp only (53 ± 12 to 68.41 ± 21 ng/mL, $p = 0.043$), and levels of s-PS were greater than in pts on Clop+Asp (Clop+Asp vs Asp: 47.6 ± 14 vs 68.41 ± 21 ng/mL, $p = 0.01$). This difference was not maintained at 48 hours and 6 days. **Conclusions.** In pts with ACS and high CRP, treatment with Asp+Clop is better than Asp alone in prohibiting early P-selectin elevation. This could be related to the greater intensity of platelet activation in ACS in the presence of a more severe inflammation as indicated by higher CRP levels.

0733**CENTRAL VENOUS CATHETERS RELATED THROMBOSIS IN HEMATOLOGICAL PATIENTS**

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Background. Central venous catheters (CVC) are at risk of infection and thrombosis occurrence. The majority of patients who need subcutaneous catheters has oncologic or hematologic diseases. **Aims.** Aim of this study is the evaluation of thromboembolic events in haematological patients affected by haematological diseases with CVC. **Methods.** Here we report the incidence of catheter related venous thrombosis in 698 patients (406 males (58.1%), 292 females (41.8%) aged between 13.5 and 82 years (mean 48 years) affected by hematological diseases and treated at the Department of Biotechnology and Hematology, University La Sapienza Roma between April 1999 and May 2004. A total of 838 catheters (Groshong-Bard 7 Fr) were placed; in 123 patients, catheters were placed more than once. 674 catheters were placed in the right subclavian vein, 147 in the left subclavian vein, 13 in the right jugular vein and 4 in the left jugular vein. The patients were affected by LANL (n 289: 41.4%), NHL (n 151: 22.0%), MM (n 86: 12.3%), ALL (n 65: 9.3%), HL (n 43: 6.4%), CML (n 24: 3.3%), CLL (n 17: 2.1%), and other hematological diseases (n 23: 3.2%). **Results.** Twenty-five (2.98%) clinically manifested thrombotic events were recorded: 19 (80%) in the right subclavian vein; 4 (12%) in the left subclavian vein; 1 (4%) in the right jugular vein and 1 (4%) in the left jugular vein. The diagnosis of thrombosis has been confirmed by echodoppler examination. Median time from CVC insertion and thrombotic events was 44 days (range 9-87 days). Screening for congenital and acquired thrombophilic factors (AT, PC, PS, FV Leiden, Prothrombin G20210A mutation, Lupus anticoagulant, Hyperhomocysteinemia) was negative in all CVC related thrombotic patients. Mean platelet count in thrombotic patients was $112 \times 10^9/L$ (range $16-320 \times 10^9/L$). **Conclusions.** The incidence of symptomatic CVC-related thrombotic complications in hematological patients is not negligible, but did not seem to be related to congenital or acquired thrombophilic factors.

0734**EFFECTS OF THE C242T P22PHOX POLYMORPHISM AND ITS INTERACTION WITH HYPERHOMOCYSTEINEMIA ON THE RISK OF DEEP VEIN THROMBOSIS IN SPANISH PATIENTS**P. Carrasco¹, J.V. Sorli¹, M. Labios², O. Portoles¹, F. Frances¹, A. Boned¹, C. Saiz¹, F. Gabriel²¹Fac. Medicine. University of Valencia, VALENCIA, Spain; ²University Clinic Hospital, VALENCIA, Spain

Background. Oxidative stress in the vasculature induced by reactive oxygen species (ROS) has been implicated in the pathogenesis of cardiovascular diseases. Deep vein thrombosis (DVT) is a complex pathology in which ROS may influence several processes. Recently, it has been reported that NADPH oxidase has an important role in generating ROS in platelets and then altering the bioactivity of platelet NO, platelet aggregation and thrombus formation. P22phox, one of the electron transfer elements of NADPH oxidase is expressed vascular cells is a critical component for ROS generation. The C242T p22phox polymorphism, which substitutes histidine-72 by tyrosine located in the potential heme-binding sites, might modulate the activity and regulation of NADPH oxidase which leads to a decrease of oxidative stress in the vasculature. In addition, hyperhomocysteinemia (HHcy) increases ROS generation in vessels and can interact with NADPH activity in determining the risk of thrombus formation. **Aims.** Therefore, this study was undertaken to investigate the potential interaction between the C242T p22phox polymorphism and HHcy on DVT risk in Spanish patients. **Methods.** A case-control study was conducted in a thrombosis treatment program with bemiparine in a Valencian

Hospital. 237 consecutive patients (129 men and 108 women) aged 20-92 years (mean age 68 y.) with confirmed DVT by phlebography were selected. Then, 253 unrelated healthy controls, matched by age and sex with cases, were recruited from the same area. DNA was isolated from a blood sample and PCR was performed for genetic analysis of C242T p22phox polymorphism. Plasma homocysteine concentration and other clinical, biochemical and life-style variables were determined. Logistic regression analysis was performed to estimate crude and adjusted odds ratios (ORs). **Results.** Prevalence of the C242T polymorphisms was: 43.6%CC, 44.1%CT and 12.2%TT in cases versus 40.2%CC, 42.4%CT and 17.4%TT in controls; $p=0.336$. As previous studies have demonstrated that TT subjects lower levels of stress oxidative parameters, CC and CT individuals were grouped and compared with TT subjects. After grouping, the risk of DVT in carriers of the C allele compared with TT subjects was OR: 1.5 (95%CI: 0.8-2.7; $p=0.17$). On the other hand, the risk of DVT associated with HHcy (plasma homocysteine $>15 \mu\text{mol/L}$) in this population was estimated to be OR: 1.8 (95%CI: 1.2-2.8; $p=0.005$). Further, the joint interaction between HHcy and the C242T polymorphism was analyzed. We found that the C242T polymorphism could modulate the risk of DVT associated with HHcy. So, in C carriers, the risk of DVT associated with HHcy was OR: 2.1 (95%CI: 1.2-3.5; $p=0.007$), whereas in TT subjects, the risk decreased to OR: 1.6 (95%CI: 0.5-5.5; $p=0.47$). **Conclusions.** Our data show that although the C242T polymorphism do not constitute an independent factor for DVT in this population, this variant could modulate the DVT risk associated with HHcy, decreasing its risk in TT subjects.

0735**ELEVATED PROTHROMBIN FRAGMENT F1+2 LEVELS DURING PREGNANCY IN WOMEN WITH PREVIOUS VENOUS THROMBOEMBOLISM**

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Background. Changes in blood coagulation and fibrinolysis during pregnancy create a state of hypercoagulability. This phenomenon predisposes to venous thromboembolism. Indicators of hypercoagulation in normal pregnancy are circulating thrombin-antithrombin complexes and increased levels of prothrombin fragment 1+2. A significant positive correlation between gestational age and elevated prothrombin fragment 1+2 has been shown. **Aim and Methods.** We hypothesized that women with previous venous thromboembolism are at a higher hypercoagulable state during subsequent pregnancies than women without prior thrombotic complications. In a prospective study, we determined prothrombin fragment F1+2 over pregnancy among 109 women (175 measurements) with previous venous thromboembolism, and among 75 pregnant women (75 measurements) without previous venous thromboembolism. The prothrombin fragment F1+2 levels were statistically analyzed over time using a Mixed Model. This model allows a longitudinal analysis of the influence of a between-subjects factor (e.g. history of thrombosis) on prothrombin fragment F1+2 levels, the influence of a within-subjects factor (weeks of gestation) on prothrombin fragment F1+2 levels, and the interaction of the history of thrombosis and weeks of gestation representing a change of risk factor-dependent differences over time (weeks of gestation). **Results.** Among women with a previous history of venous thrombosis, prothrombin fragment F1+2 values were significantly higher during the course of pregnancy than among pregnant women without venous thromboembolism ($p=0.0014$). The results were adjusted for the physiological increase of prothrombin fragment F1+2 over pregnancy and were independent of heparin prophylaxis. **Conclusions.** Thus, determination of indicators of hypercoagulation like prothrombin fragment F1+2 represent an additional approach independent of known and unknown risk determinants of thrombosis to identify women at risk for venous thromboembolism during pregnancy.

0736**THE POLYMORPHISM OF PLATELET MEMBRANE INTEGRIN ALPHA2BETA1 (ALPHA2807TT) IS ASSOCIATED WITH PREMATURE ONSET OF FETAL LOSS**

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Background. and **Aims.** Inherited thrombophilia could increase susceptibility to adverse pregnancy outcomes such as fetal loss. As shown in preliminary studies, two platelet receptor genotypes, the HPA1-b allele of the beta3 subunit of platelet integrin alpha2bbeta3 and the 807TT genotype of the alpha2 subunit of integrin alpha2beta1 are risk factors for increased platelet thrombogenicity. In accordance with this observation, these genetic variants may lead to thrombotic occlusion in the presence of a predisposing vascular alteration resulting in an increased rate of fetal loss or premature fetal loss. **Methods.** We determined the G1691A mutation of the factor V gene (FVL), the G20210A mutation of the prothrombin gene, the C677T polymorphism of the methylenetetrahydrofolate-reductase (MTHFR) gene, the HPA-1 polymorphism, and the C807T polymorphism in 104 women with fetal loss and 277 normal women. Inclusion criteria for the women with fetal loss were either recurrent early fetal loss (three or more consecutive fetal losses at < 12 weeks gestation and no late fetal loss) or at least one late fetal loss (> 12 weeks gestation). All women had a detailed investigation which was negative for potential causes of fetal demise. **Results.** In a subgroup analysis of women with recurrent early fetal loss (n=34), the prevalence of the genetic markers did not differ significantly between the women with early fetal loss and the normal women. However, in this subgroup of patients, the onset of fetal loss was significantly earlier in women with the alpha2807TT genotype (5.4±0.9 vs. 8.1±1.4 weeks p=0.001; earliest fetal loss (n=34) as event time). No such significant difference was observed in carriers of the other genetic markers. These results were confirmed using a mixed-model-analysis for evaluation of all early fetal losses (n=132) over time (7.1±1.9 vs. 8.8±1.5 weeks, p=0.001). The alpha2807TT genotype was significantly associated with premature fetal loss using univariate and multivariate analysis (including age, factor V Leiden, 677TT MTHFR genotype, HPA-1 genotype, and alpha2807TT genotype). There was no significant influence of the number of fetal losses and no significant interaction between any risk factor and the number of fetal losses on the week of fetal loss. In the subgroup analysis of women with late fetal loss (n=70), only the prevalence of heterozygous FVL was significantly associated with late fetal loss (odds ratio 3.2, p=0.002). There was no significant association of any genetic risk factor with premature fetal loss in the subgroup analysis of women with at least one late miscarriage. **Conclusions.** This study demonstrates a significant association of the alpha2807TT genotype of the platelet membrane integrin alpha2beta1 with premature onset of early fetal loss. Based on our results it appears that this risk factor does not induce the pathomechanism but modulates the course of fetal loss. Furthermore, our study confirms the association of FVL with late fetal loss.

0737**SPLENECTOMY IN CHRONIC RELAPSING ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA**G.A. Giuffrida¹, A. Figuera², C.M. Rocca¹, M.C. Maccarrone Santo¹, R. Giustolisi¹¹P.O. Ferrarotto, CATANIA, Italy; ²Istituto di Ematologia, CATANIA, Italy

Thrombotic thrombocytopenic purpura (TTP) is a rare syndrome characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, renal failure and neurological manifestation. It is caused by a severe decreased of Von Willebrand factor cleaving protease activity (ADAMTS-13), leading to per-

sistence of unusually ultra-large Von Willebrand multimers (ULVWF) in the circulation that bind to platelets, causing platelet aggregates, microangiopathic hemolysis and thrombocytopenia. A lack of ADAMTS-13 activity can be caused by autoimmune inhibitors or may be due to a constitutional deficiency of this protein. Recently, the ADAMTS-13 gene that encodes for the ADAMTS-13 protein was found. It was mapped to chromosome 9q34 and consists of 29 exons. Several mutations has been identified in the ADAMTS gene in patient with the congenital form of TTP. Although TTP usually occurs as an acquired form due to autoantibodies against ADAMTS-13. The determination of the activity of ADAMTS-13 and of antibodies against ADAMTS-13 are important part in the workup of patients with TTP. Plasma exchange (PE) with fresh frozen plasma replacement is the standard treatment. The efficacy of PE is likely due to the removal of both antibodies and ULVWF and the infusion of ADAMTS-13. Additional treatment modalities include glucocorticoids, splenectomy, vincristine, cyclophosphamide, azathioprine, cyclosporin A, combination chemotherapy, intravenous immunoglobulins and, recently, rituximab. We report a case of chronic relapsing acquired idiopathic TTP successfully treated with splenectomy. The patient, an 50-year old woman, developed her first episode of TTP in May 2001. Remission was achieved after 12 sessions of PE, four dose of vincristine at dose of 0,02 mg/kg/die, corticosteroids at dose of 1 mg/kg/die and increased dose of prociclide from 10 to 60 mg /kg/die. From 2001 to 2004, she had six relapses responding to treatment with PE, vincristine, and corticosteroids. The relapse in 2004 was followed by a protracted course despite the addition of cyclosporine A and she become dependent on PE. On May 2004 she was treated with splenectomy. The postoperative course was uneventful. ADAMTS-13 activity and inhibitor levels were monitored. ADAMTS-13 activity was initially, pre-splenectomy, <6% (n.v. 46-160%) and inhibitor's titre against ADAMTS-13 was 12 U/mL (n.v. <1 U/mL). After splenectomy, the inhibitor against ADAMTS-13 disappeared rapidly after one month., while ADAMTS-13 activity has remained very low (<6%). After six months from splenectomy there wasn't full recovery of ADAMTS-13 activity. Follow up is now 10 months. ADAMTS-13 activity has remained <6% and inhibitors have not reappeared. Our experience with splenectomy in a patient with chronic relapsing acquired TTP, suggest that splenectomy, by eliminating an important source of B-lymphocytes producing inhibitory ADAMTS-13 autoantibodies, may be useful treatment option in patient with chronic relapsing acquired TTP.

0738**SEVERE HYPERHOMOCYSTEINEMIA RESULTING IN RECURRENT VENOUS THROMBOEMBOLISM, IN A PATIENT WITH MTHFR C677T HOMOZYGOSITY AND COMBINED VITAMIN B12 AND FOLATE DEFICIENCY**D. O Donghaile¹, S. Yap², M. McCauley², B. Fowler³, B. Nolan¹¹St. James' Hospital, DUBLIN, Ireland; ²NCIMD, Children's Hospital, DUBLIN, Ireland; ³University Childrens Hospital, BASEL, Switzerland

Homozygosity for MTHFR C677T results in mild hyperhomocysteinaemia (HHcy), with Vitamin B12 and folate as cofactors in the remethylation pathway of methionine. Severe HHcy is characteristic of classical homocystinuria (HCU) due to cystathionine β -synthase (C β S) deficiency with a clinical syndrome of mental retardation, skeletal abnormalities, ectopia lentis and premature vascular disease. HHcy is now an established vascular risk factor. A total homocysteine (tHcy) rise of 1SD above the normal population mean confers a 60% rise in relative risk of cardiovascular disease (Boushey et al 1995). We present the case of a 40 year old male with recurrent venous thromboembolism referred for thrombophilia screen. He initially presented with a spontaneous right DVT and bilateral pulmonary emboli, managed with enoxaparin 40 mg/kg BD. Other problems include severe polyarticular gout, obesity with poor diet and alcoholism. There was no prior personal or family history of venous thromboembolism. Ophthalmology examination and his habitus were normal. Eight months later, after two days of excessive drinking

and high protein intake, he represented with a further left pulmonary embolism while therapeutically anticoagulated on Enoxaparin. A Greenfield filter was inserted. Initial investigations: tHcy 235 mmol/L (4-12). Serum folate 1 µg/L (2.7-20). Red cell folate was 45 µg/L (150-1000) and Vitamin B12 of 100ng/L (150-1000). A thrombophilia screen was normal including activated Protein C resistance and Prothrombin G20210A. He was homozygous for MTHFR C677T. Skin fibroblast studies were performed to assess his transsulfuration and remethylation pathways. Normal C₁S activity out ruling homozygous C₁S deficiency with normal methionine and serine formation. On the second thrombotic episode, tHcy was 351 mmol/L; methionine 28 mmol/L (6-40). Initial management: Normalisation of the cofactor deficiencies were achieved by supplementation with Vitamin B12 IM and folic acid, with a resultant drop of tHcy from 235 to 57 mmol/L. Pyridoxine (B6) was commenced while awaiting C₁S enzyme assay. At the second thrombotic episode, acute homocysteine lowering treatment included stopping dietary protein intake and supplementing with a methionine free amino acid mixture. Betaine was started to remethylate homocysteine to methionine with the continuation of all three cofactors and anticoagulation. This regimen reduced tHcy to 21 within 36hours. Dietary protein was introduced gradually titrated with tHcy levels. Betaine and synthetic amino acid mixture were eventually stopped with his final protein intake restricted to 1 g/kg/day pending investigation for HCU. Once stabilised, mean tHcy was 9.1 mmol/L (range: 5-21; n=12) and mean methionine 25.8 mmol/L (range:12-39). He was reverted back to a normal diet and B6 stopped once HCU has been ruled out. He remains well with normal tHcy while on regular serum B12 and folate level surveillances. **Conclusions.** This case demonstrated that in the presence of combined B12 and folate deficiency, MTHFR C677T homozygosity resulted in severe hyperhomocysteinaemia causing recurrent thromboembolisms. Appropriate homocysteine lowering therapy can result in a good outcome. It is important to maintain normal B12 and folate levels in patients with MTHFR C677T.

0739**COMPENSATED RENAL INSUFFICIENCY DOES NOT PROTECT AGAINST VENOUS THROMBOSIS**

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Because of its alleged hypo-coagulable state elevated serum creatinine level (>150µM) has been considered in several clinical trials of venous thromboembolism (VTE) prophylaxis as an exclusion criterion. The aim of our study was to investigate whether there is or is not a need for VTE prophylaxis in patients with mild (I°- II°) chronic renal insufficiency. **Methods.** A group of 40 patients aged 56 to 91 (mean 71,6 years) with compensated renal insufficiency (creatinine level > 150 µM) with at least one risk factor for VTE was included into the study. Hypercoagulable state was defined as an increase of thrombin-antithrombin complexes (TAT) and/or peptide F1+2. Homocysteine level was measured with HPLC method. All patients had on the day 7±2 triplex Doppler of leg veins and if positive angioCT or perfusion lung scan. **Results.** table. Four patients from the study group developed deep vein thrombosis, two of them asymptomatic. One patient died of massive pulmonary embolism. **Conclusions.** Patients with compensated renal insufficiency manifest a hypercoagulable state with an increased risk for VTE, which may be partly due to hyperhomocysteinemia. Antithrombotic prophylaxis should indeed be considered in these patients.

Test	Study group	Control group	P
TAT (µg/l)	15.6 ± 8.2	2.6 ± 4.1	< 0.0
F1+2 (nmol/l)	1.64 ± 8.2	0.75 ± 1.1	< 0.0
Homocysteine (µmol/l)	36.3 ± 18.6	12.1 ± 4.5	< 0.0

0740**SCREENING FOR HYPERCOAGULABILITY IN PATIENTS WITH CANCER USING THROMBOELASTOGRAPHY**

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The high frequency of venous thrombosis and systemic activation of coagulation in malignancy is well established. However, there is little agreement regarding the most useful test for defining the coagulability of cancer. In this study; we aimed to assess hypercoagulability in patients with cancer, using thromboelastography (TEG) analysis. Seventy-three untreated cancer patients (27 gastrointestinal, 26 lung and 20 miscellaneous group of ovarian, renal, nasopharyngeal and unknown origin) were enrolled the study. 16 healthy individuals constituted the control group. Platelet count, PT, aPTT, fibrinogen and D-dimer were measured. CT (clotting time =s), CFT (clot formation time=s) and MCF (maximum clot firmness=mm) were determined by rotation thromboelastography (ROTEG) using four methods. (Integ, Exteg, Fibteg, Apteg) We evaluated the intrinsic pathway by Integ, the extrinsic pathway by Exteg, the fibrinogen status by Fibteg and hyperfibrinolysis by Apteg. Hypercoagulability was defined as any parameter above (MCF) and/or below (CT, CFT) our laboratory normals. CFT was negatively correlated with platelet number and fibrinogen levels while MCF was positively correlated with these parameters (p<0,01). CFT was positively correlated with hemoglobin and hematocrit levels while MCF was negatively correlated with these parameters (p<0,01). A significant decrease in CFT and an increase in MCF were established in the study group by all methods, in respect to the control group (p<0,001). When the cancer patients were categorised into 3 subgroups; gastrointestinal system tumours (group 1), respiratory system tumours (group 2) and miscellaneous tumours (group 3), a decrease in CFT by Exteg and an increase in MCF by Exteg and Apteg were seen in group 2 in respect to other groups (p<0,05). A significant increase in platelet and fibrinogen levels was also determined in group 2 when compared with other groups (p<0,01). These findings suggest that; 1. TEG is a rapid and sensitive means of screening for hypercoagulability in patients with cancer. 2. The potential risk for occurrence of thromboembolism is higher in cancer patients with increased platelet and fibrinogen levels and decreased hemoglobin and hematocrit levels.

0741**PROTHROMBIN TIME (PT) MEASUREMENTS-REQUIREMENTS FOR OBTAINING COMPARABLE INR VALUES BETWEEN LABORATORIES**

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Background. Prothrombin time (PT) measurements are clinically important, especially for the monitoring of oral anticoagulant treatment. In order to obtain comparable results from various PT methods the results should be converted to International Normalized Ratio (INR). However, a reliable conversion to INR requires a standardized calibration procedure of each local instrument-reagent combination [1] in order to obtain comparable results from various laboratories. **Methods. and results.** In 1999, a simplified procedure for local INR calibration of the PT assays was introduced in Sweden by means of only two lyophilised plasma calibrators with assigned INR values. A three-year follow-up [2] showed a significant reduction of the interlaboratory variation for both hospital based laboratories and smaller laboratories in the primary health care system. Typ-

ically, the mean coefficient of variation (CV) is less than 6% in the therapeutic INR range 2-4 for both types of laboratories. The low variation seen in Sweden is highly competitive in an international comparison. A likely explanation for the successful introduction of local INR calibration and the favourable laboratory variation is that all laboratories in Sweden use the same type of PT test system. The PT reagent is a combined thromboplastin, also called Owren-type reagent, which is characterized by its high specificity for the coagulation factors II, VII and X and that it is possible to use diluted plasma. In the Nordic countries, most laboratories use Owren type of reagents. All laboratories perform local calibrations, but the plasma calibrators used in the different countries, have their INR values assigned according to various protocols. However, a recent inter-Nordic study has indicated that there is no bias between the Nordic countries, despite differences in the calibration procedures. **Conclusions.** Standardized and regular calibrations of the local instrument-reagent combinations are the best way to obtain low inter-laboratory variation. The major factor for obtaining a conformational performance of PT assays is most likely the use of a robust assay procedure, i.e., the use of a modern Owren PT assay reagent allowing testing on diluted plasma samples.

1) Lindahl TL, Egberg N, Hillarp A, Ödegaard OR, Edlund B, Svensson J, Sandset PM, Rånby M. INR calibration of Owren-type prothrombin time based on the relationship between PT% and INR utilizing normal plasma samples. *Thromb Hemost* 2004; 91: 1223-31.

2) Hillarp A, Egberg N, Nordin G, Stigendal L, Fagerberg I, Lindahl TL. Local INR calibration of the Owren type prothrombin assay greatly improves the intra- and interlaboratory variation. A three-year follow-up from the Swedish national external quality assessment scheme. *Thromb Haemost* 2004; 91: 300-7.

0742

ASSOCIATION OF PRETREATMENT PLASMA LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR WITH FIBRINOLYSIS ACTIVATION MARKER IN PATIENTS WITH MALIGNANT DISEASE

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Background. Vascular endothelial growth factor (VEGF) is the most important proangiogenic cytokine, regulator of normal and pathological, as well tumor-related, angiogenesis. Plasma levels of VEGF are elevated in patients with multiple myeloma, acute myelogenous leukemia and the other malignant diseases and often correlate with clinicopathological variables of disease. Activation of coagulation and fibrinolytic system in patients with malignant disease has been well recognized for many years and thought to be related with tumor angiogenesis, being a first step before formation of new vessel. VEGF is thought to be a link between activation of hemostasis and angiogenesis. **Aims.** The purpose of this study was to investigate the relationship between plasma levels of marker of fibrinolysis activation (D-dimer) and plasma levels of marker of angiogenesis (VEGF) in patients with untreated or progressive metastatic breast cancer. **Methods.** The plasma levels of D-dimer (Dimertest Gold EIA, American Diagnostica Inc) and VEGF (Human VEGF Immunoassay, R&D Systems) were measured in 79 female patients with newly diagnosed or progressive metastatic breast cancer, before starting any treatment and in 25 healthy women. The clinical stage of disease was I in 8 patients, II in 22 patients, III in 23 patients and IV in 26 patients. **Results.** Median plasma levels of D-dimer and VEGF were significant higher in patients with breast cancer than in healthy women (70,63 vs 7,31 ng/L, $p=0,006$ and 86,89 vs 41,9 pg/L, $p=0,04$ respectively). The levels of both markers correlate with stage of disease and were highest in patients with metastatic breast cancer. The level of D-dimer positively correlated with level of VEGF ($p<0,001$, rang Spearman correlation 0,52). **Conclusions.** The correlation between marker of fibrinolysis activation and marker of angiogenesis in patients with breast cancer and the correlation both types of markers with stage of the malignant disease confirms the association between hemostasis disturbances and angiogenesis in tumor growth.

0743

C-1T POLYMORPHISM IN THE ANNEXIN V KOZAK SEQUENCE AND THE RISK OF ACUTE MYOCARDIAL INFARCTION

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Background. The acute myocardial infarction (AMI) is one of the most common pathologies in industrialized countries and is defined as myocardial cells death or necrosis that occurs when myocardial ischemia exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms. Myocardial oxygen and nutrients interruption supply occurs when a thrombus is formed on an ulcerated or unstable atherosclerotic plaque, resulting in coronary occlusion. The pathogenesis of AMI involves an interaction between environmental factors and genetic predisposition. Genetic factors associated to blood coagulation may contribute to the atherosclerosis pathogenesis and localized occlusive thrombus formation. Annexin V (ANV) is a member of the annexin protein family with vascular anticoagulant activity and has a physiological relevance in the haemostatic system, probably due to its capacity to bind negatively charged phospholipids in the presence of Ca²⁺ ions. In vitro, ANV, inhibiting the prothrombinase and tenase complexes formation, acts as a potent anticoagulant, preventing thrombosis in normal conditions of blood arterial flow. Recently, a C to T transition has been described in ANV gene Kozak region, affecting the nucleotide preceding the initiation codon. The role of this polymorphism in ANV plasma levels remains to be elucidated, but some authors suggest that it may increase ANV gene translation efficiency, increasing the ANV protein level and consequently the thrombotic/hemorrhagic imbalance. The aim of this study was to evaluate (1) the ANV gene (-1C>T) transition prevalence in the centre Portuguese population, (2) the risk of AMI associated with this polymorphism. **Material and Methods.** The study involved 100 patients (P) with AMI, documented by angiography, and 104 controls matching age and sex (C). ANV gene amplification of exon 2 and flanking regions was performed by PCR and ANV -1C>T polymorphism was screened by PCR products NcoI restriction analysis. Statistical analysis was performed with the Qui-square test. **Results.** The -1C/C genotype was the most frequent in both groups (P 75%, C 78,8%), 20,2% of the patients and 19,2% of the controls were heterozygous -1C/T; the remaining 1,0% of the patients and 1,9% of the controls were -1T/T. In both groups the allelic frequencies of -1C and -1T alleles were 0,885 and 0,115, respectively. **Conclusions.** These results suggest that in the centre Portuguese population, the -1C/T polymorphism, by itself, is not a risk factor for AMI, as described Van Heerd et al, nor play a protective role against AMI, as González-Conejero et al described in 2002. The frequency of the -1C/t polymorphism found in this study is similar to that found in Spanish population. The few studies in the literature present contradictory results; it could be important to evaluate the relevance of this polymorphism in haemostatic balance and the potential role in the thromboembolic or hemorrhagic disorders.

Miscellaneous

0744

NEUTROPENIA AND/OR ANEMIA ASSOCIATED WITH T-CELL LARGE GRANULAR LYMPHOCYTE LEUKEMIA: EVALUATION OF RESPONSE AND APOPTOSIS TO LOW DOSE CYCLOSPORINE THERAPY. A SINGLE CENTER EXPERIENCE

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T-Cell Large Granular Lymphocyte Leukemia (T-LGL) is a rare clonal disorder of T-cells, clinically indolent, but is associated with severe neutropenia and/or anemia in approximately

50% of cases. Until now they have been experienced several medication like Corticosteroids, Purine analogues and Cyclosporine, with various results and toxicities. We present six cases with proven T-LGL leukemia, four females and two males, with ages ranging from 47 to 80 years. Five of them presented with neutropenia and the other with anemia. Four patients received cyclosporine therapy 25mg bd and the other two 50 mg bd. The five neutropenic patients had acute neutrophil count ranging from 20 to 1000/uL, except from one (1400/uL) and the patient with anemia presented with Hgb 8.5g/dL. The immunophenotypic profile was CD3+ CD8+ CD16+/-CD56+/-CD57+ with TCR $\alpha\beta$, clonality, as the latter proved by PCR for TCR γ in three, Southern blot in one and V β repertoire for the rest two patients. The percentage of clonal T-cell apoptosis with flow cytometry analysis (after Annexin V binding assay), appeared very low in all patients and the difference was not statistically significant after cyclosporine therapy. The infiltration by leukemic T-cells of bone marrow in bone marrow biopsy, ranged from 18 to 30%. The time to response varied from 30 to 90 days from the beginning of therapy. Follow up period plots between 6 months to seven years and during this period the patients did not meet any treatment side effects like infections, renal insufficiency or cardiotoxicity. Despite the successful recovery of neutropenia in five and anemia in one patient, the absolute count of T-LGL lymphocytes and the percentage of apoptosis remained stable. The latter observation, together with the disproportionate degree of neutropenia or anemia in relation with the percentage of marrow infiltration, confirm the probability of the presence of an underlying immune mechanism in which cyclosporine, in low doses, intervenes very efficiently. This is probably the first study which depicts that cyclosporine can be used in lower doses than usual, in order to treat neutropenia and or anemia in patients suffering from T-LGL.

0745

EFFECTS OF HYPERHOMOCYSTEINEMIA ON THE SYSTEM OF HAEMOSTASIS UNDER STRESS

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Background. A mild to moderate elevation of plasma homocysteine is a risk factor for atherosclerosis and arterial and venous thrombosis especially under stress. Using a rat model we show that dietary folic acid deficiency under stress condition is associated with changes in anticoagulant system and platelet activities. **METHOD AND Results.** A total of 40 male Vistar rats, weighing 200-250 g were divided into 2 dietary groups (control and test) receiving standart folate-deficient diet for 6 weeks. The control group were fed a diet supplemented with 750 μ g/kg folic acid. The control and test diets contained 0.5% of succinylsulfathiazole. Stress was induced by free swimming of rats in water at 22°C for 40 min. The stress reaction was evidenced by increase in plasma catecholamines. Platelet aggregation, synthesis of thromboxane B2 (TxB2), activities of protein C and antithrombin III were studied in control and test rats after stress. Total plasma homocysteine was analyzed by using high-performance liquid chromatography with fluorescence detection. The reduced plasma folate concentration on the 40% was observed in test versus control animals. Test group animals that consumed folate-deficient diet had total plasma homocysteine concentration on the 120-140% more than in control group. After stress platelet aggregation induced by ADP, collagen or thrombin was higher in test versus control rats ($p < 0,05$). There were decrease in activities of protein C and antithrombin III, and increase in synthesis of TxB2 in folate-deficient animals under stress conditions in comparison with control animals. **Conclusions.** These findings suggest that mild hyperhomocysteinemia evoked by folate depletion may depress protein C activation and antithrombin III activity under stress conditions by inhibition of expression of thrombomodulin and heparin sulfate, and enhanced peroxidation of arachidonic acid with the subsequent increased platelet activation and aggregation.

0746

A PROSPECTIVE STUDY TO EVALUATE THE ROLE OF ENTONOX DURING BONE MARROW EXAMINATION

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Bone marrow aspiration and biopsy is a procedure of central importance in the diagnosis of haematological diseases and is one that can be safely performed in the outpatient clinic using only local anaesthesia. However many patients find the procedure painful and distressing with some requiring sedation with short acting benzodiazepines during bone marrow examination. Sedation, whilst being effective, can be associated with adverse side effects such as respiratory depression, so we sought to evaluate the role of inhaled nitrous oxide (Entonox) as an adjuvant to local anaesthesia (LA) in patients requiring bone examination. Entonox is a short acting, inhaled anaesthetic that has powerful analgesic properties in sub-anaesthetic concentrations. We prospectively studied the role of Entonox analgesia in patients attending our outpatient department who required bone marrow examination. All patients received LA with injected 2% lignocaine but patients were then assigned to either LA alone or LA plus inhaled Entonox. Following the procedure patients were given a questionnaire and asked to score the discomfort associated with the procedure (0 - no pain, 1 - mild pain, 2 - moderate pain, 3 - severe pain). 28 of 85 patients (33%) who received LA alone scored their pain as 3 (severe) compared with 7 of 51 patients (14%) who received LA plus Entonox, with this difference being significant ($p=0.025$, chi square). No adverse events were associated with Entonox use. 43 of the 51 patients who received Entonox stated that they would prefer to use this method of anaesthesia again. We conclude that inhaled Entonox is a useful adjuvant to local anaesthesia for patients undergoing bone marrow examination. Entonox can be easily administered and is an inexpensive and safe alternative to sedation with short-acting benzodiazepines.

0747

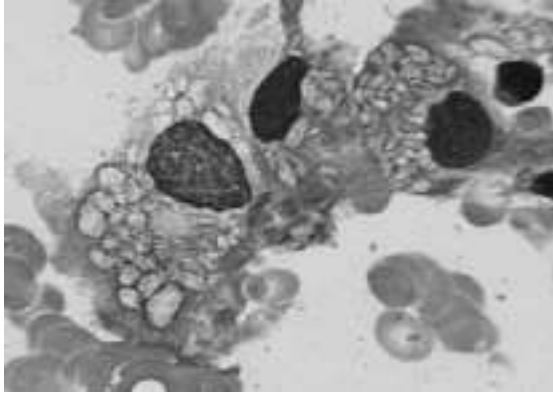
MYCOBACTERIUM GENAVENSE INCLUSIONS MIMICKING GAUCHERY'S CELLS

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A 45 years old man with C3 stage human immunodeficiency virus (HIV) infection and antiretroviral treatment since 1991 was admitted to the hospital because of fever and diarrhea. On physical examination 1.5-2 cm peripheral nodes were palpable and the spleen was felt 2-3 cm below the left costal margin. Mesenteric, retroperitoneal and iliac enlarged lymph nodes were detected in the thoracoabdominal scanner. The following complementary tests were abnormal: leucocytes $2.1 \times 10^9/L$, Hb 82 g/L, platelets $87 \times 10^9/L$, total proteins 4.5 g/dL, CD4+ cells $6/mm^3$ (32%), CD8+ cells $132/mm^3$ (62%), CD4/CD8 ratio 0.05% and HIV viral load of 316.227 copies/mL. Blood cultures were negative. The bone marrow aspirate showed histiocytic hyperplasia. The histiocytes had pseudo-Gaucher morphology with foamy and striated cytoplasm full of white inclusions and white bacillus-like structures. These inclusions and structures, also present on the background of the smear, seemed not to take Giemsa's stain. Ziehl's stain of marrow biopsy confirmed that this image was due to the accumulation of numerous acid-fast bacilli. Auramine stain of sputum was positive and Mycobacterium genavense was identified. The patient was treated with etambutol and azytromicin with a good response. **Conclusions.** The foamy and striated macrophages (pseudo-Gaucher's cells) as well as the Giemsa negative images, within the macrophages and on the background of the smears, should make us suspect the diagnosis of atypical mycobacteriosis. Special staining techniques such as PAS and Ziehl-Neelsen are useful in corroborat-

ing this. Pseudo-Gaucher cells have been previously described in patients with *Mycobacterium avium* complex but not with *Mycobacterium genavense*. Here we present the first case associated with this microorganism.



0748

IDENTIFYING HEMOGLOBIN VARIANTS BY AN AUTOMATED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD IN ISRAEL

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More than 700 hemoglobin variants have been described and about half of these variants are clinically silent. Gel electrophoresis is amongst the most widely utilized method for hemoglobin analysis, but with a limited resolution. Some variants of hemoglobin may behave similarly during gel electrophoresis and share several common biochemical or functional properties. Some hemoglobin variants are difficult to differentiate on gel electrophoresis but are clearly identified by cation-exchange high-performance liquid chromatography (HPLC). Israel is characterized by an extremely heterogeneous population as to ethnic origin with a high degree of consanguinity over a lots of generations. This suggests the possibility of lots of hemoglobin variants which could be confirmed by HPLC. For practical reason if becomes therefore justified to use methods like HPLC instead of gel electrophoresis. We are presently in the process of mapping known hemoglobin variants to establish their location on HPLC, in addition to the classical known variants as Hb A, A2, F, S, C, D. We have up to now localized specific peaks on HPLC: Hb Setif, Hb O-Arab, Hb Rambam, as well as combinations like (Hb Setif+ Hb O-Arab) or (Hb S + Hb O-Arab), Hb (-α3.7/αSetifα). All these hemoglobins were genetically confirmed.

0749

MUTATIONS OF ENDOGLIN GENE IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA TYPE 1 IN GREECE

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HHT is an inherited autosomal dominant vascular dysplasia found at a frequency of 1:10.000 adults and exhibits age-related penetrance with variable phenotype. It is characterized by mucocutaneous telangiectasia frequently complicated by serious hemorrhage in the gastrointestinal tract, in the lungs, the brain etc. There are at least 3 genetic loci. The first has been defined as the endoglin gene (chr. 9q33-34). The second has been defined as the ALK-1 (activin-like kinase receptor 1) at 12q13 and an unidentified gene on chromosome 13. The aim of this study is to inves-

tigate the mutations of the endoglin gene that is responsible for the Hereditary Hemorrhagic Telangiectasia type 1 (HHT-1). *Methods.* We studied 8 families from Crete and Thessalia with HHT Type 1. We amplified the exons of endoglin sequencing with flanking primers and performed direct sequencing on the PCR products. *Results.* We found 3 mutations in the studied families. The four families from Crete had the same mutation (39bp at exon 7, 882point), suggesting common origin. Two families from Thessalia had on common mutation (2bp del. at exon 11, 1553point), and the other family from Thessalia had a private mutation (21bp del. at exon 5, 855point).

0750

ADMINISTRATION OF SUSTAINED RELEASE INTRATHECAL CYTARABINE FOR NEOPLASTIC MENINGITIS-A SINGLE CENTRE EXPERIENCE

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Background. Sustained release intrathecal cytarabine (S-RITara-C) effectively treats neoplastic meningitis (NM) in patients with solid tumours, leukaemias and lymphomas, allowing reduction in frequency of intrathecal (IT) administrations whilst producing equal or better responses compared to IT methotrexate (MTX) or cytarabine (ara-C). Headaches and arachnoiditis are the main side effects. We report 3 cases of NM treated with S-RITara-C. Patient 1: 54y female, AML. In remission for 20 years after 3 courses of chemotherapy with daunorubicin, ara-c and thioguanine. Then presented with left upper limb nerve compression, found to have paraspinous mass. Histology c/w granulocytic sarcoma. Bone marrow was clear. Received radiotherapy to c5-c6. Developed progressive symptoms with right leg weakness power grade2. CSF positive cell count 922 for AML blasts. Received 3 doses of IT MTX with partial cytological response, but too unwell for further IT treatment after intracranial bleed. MRI brain showed meningeal thickening. Received S-RITara-C 2-weekly for 2 months. MRI brain showed complete resolution. S-RITara-C response is difficult to evaluate since overlapping systemic therapy. Headaches and arachnoiditis requiring admission followed each S-RITara-C injection despite dexamethasone prophylaxis. IT therapy changed to monthly cytarabine alone but side effects remained. The patient had an exceptional response and remains free of clinical or cytological CNS progression. Systemic treatment with high dose ara-C is likely to have affected her CNS disease and long-term response. Patient 2: 24y. female, Stage IVb B-Lymphoblastic NHL. Diagnosis later revised to ALL. Received CODOX-M/IVAC and CNS prophylaxis, then received cyclo/TBI sibling transplant in CR1. Relapse in CNS 3 months post transplant, rapidly followed by systemic relapse limiting treatment options and survival. Achieved neurological and cytological CR with initial high dose dexamethasone and IT MTX. Received 2 doses of S-RITara-C consolidation therapy, developed progressive systemic disease, treated with oral idarubicin. Too unwell to tolerate further lumbar punctures, palliative care only. With pre-terminal recurrent CNS disease. Aggressive relapsing disease responded well initially to Steroids and IT MTX. She was already in neurological CR when S-RITara-C started. The discrepancy between neurological and cytological response is noteworthy. Although the CSF cell count had risen by the time of her second S-RITara-C, she remained symptom free for another 3 weeks. Patient 3: 69y. female. Stage IVb DLBCL presenting with b-symptoms and high LDH. Commenced R-CHOP and developed progressive mental status changes. Diagnosis of NM based on CSF analysis and clinical examination. MR Brain was unhelpful. Given IT MTX with clinical and cytological PR. Showed initial further response to S-RITara-C but relapsed rapidly. Received ongoing R-CHOP resulting in a radiological PR after 3 cycles. Developed septic illness with cavitating lung lesions and ultimately CNS progression. *Summary.* No patient received S-RITara-C as induction. All showed some response to prior therapy. No high grade toxicities were observed with S-RITara-C. Treatment was associated with clinical benefit in all 3. We conclude that administration of S-RITara-C is safe and feasible, but more experience is needed.

0751**TELEHAEMATOLOGY: DEVELOPMENTS IN DIGITAL IMAGING FOR THE UK NEQAS (H) BLOOD CELL AND BONE MARROW MORPHOLOGY SCHEME**

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The collaborative partnership between the UK National External Quality Assurance Scheme for Haematology (NEQAS (H)) and the Department of Haematology at Manchester Royal Infirmary has provided a series of national pilot exercises investigating the incorporation of digital images and WEB access for quality assessment in morphology. We have now developed electronic (virtual) slides of blood and bone marrow smears comprised of multiple sequential images taken of high power fields and 'stitched' together using commercial software. The composite image is then presented via a viewing package which offers the capability of navigating within the image and zoom to examine any area at high magnification. The electronic slides can be viewed via the internet or distributed through a range of storage media. The aim is to complement traditional glass morphology slides by reproducing skills used in haematological cell identification and interpretation whilst allowing users in different locations to view identical material. Four cases from previous UK NEQAS (H) peripheral blood morphology glass slide surveys were chosen for imaging. Each of the final stitched images was constructed from 40 separate high power fields to be viewed using QuickTime software. Participants were invited to log on to the UK NEQAS (H) web site, view the images and report the five most significant morphological features per case as they would for standard glass slide surveys. The images had been coded and participants were not informed that the original smears had been part of a previous survey; cases chosen were: Hb SS, Chronic Myeloid Leukaemia, Polymorphocytic leukaemia and malaria: plasmodium ovale. Participants were also asked to comment on the process. Returns were received from 166 laboratory centres (37% of total scheme participants). Of the responders 92% found ease of navigation within the image acceptable and 72% thought the size of image presented was adequate for morphological evaluation. We found a clear level of agreement between the five most significant morphological features reported on the glass slide surveys compared with the digital images including diagnosis of the malaria. The pilot exercise with electronic slides has established remarkable comparability with glass slides and an acceptance and enthusiasm by participants for their use in a range of external quality assessment (EQA)-related applications. Developments in digital imaging provide the opportunity for tutorial based learning, highlighting complex morphology cases and building consensus opinion. In response to participant feedback from this exercise and to promote the educational aspects an Image Library of previous UK NEQAS (H) morphology surveys has been made available via the internet. This Library contains multiple images displayed alongside clinical details with both expert and consensus opinion from the original survey and can be viewed from the UK NEQAS (H) web site www.ukneqas-haem.org.uk (entering via the morphology exercise) or from the Manchester web site www.manlab.co.uk (select the Department of Morphology). Furthermore the Library has been successfully registered for 2005, by UK NEQAS (H), with the Executive of the Institute of Biomedical Scientists for the CPD scheme.

0752**ANALYSIS OF EPSTEIN-BARR VIRUS INFECTIONS IN PRIMATE CELL LINES**

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Epstein-Barr virus (EBV, human herpesvirus type 4) is ubiquitously distributed in all human populations, reaching infection rates of more than 90%. EBV is known to infect B lymphocytes and mucosal epithelium cells and to establish latent or productive infections. The virus is the causative agent of infectious mononucleosis and closely associated with the endemic form of Burkitt lymphoma (BL). EBV has also been associated with a various lymphoid and epithelial malignancies, such as Hodgkin, T cell, and AIDS-related lymphomas, and lymphoepithelioma-like carcinomas of several organs. In vitro, B lymphocytes are transformed by EBV into permanent lymphoblastoid cell lines (B-LCL). We investigated the EBV infection status of primate cell lines by PCR. This method detects EBV genomes integrated into the eukaryotic chromosomes, non-integrated EBV episomes, and linear genomes of active EBV particles. The analyses revealed that 38/410 cell lines contain the EBV genome. All EBV+ cell lines were established from B lineage leukemia/lymphoma cells (13/52 B cell lines, 10/13 BL cell lines, 2/2 hairy cell leukemia cell lines, 1/6 plasma cell leukemia cell lines) or are B-LCLs (9/9), natural killer cells (2/2), and one monkey cell line. No cell lines from other tissues were found to be EBV+. To further examine the production of EBV particles in the PCR+ cell lines, we analyzed the expression of the BZLF1 protein by Western blotting applying a ZEBRA monoclonal antibody. The cell lines were analyzed untreated as well as treated with the phorbol ester TPA for 3 days to induce the lytic phase of the EBV infection. Four cell lines exhibited a BZLF1 specific band. After stimulation with TPA, 4 further cell lines expressed BZLF1 protein to various extents. To distinguish between linear DNA of herpesviruses (DNA form of active viruses) and covalently closed circles of episomal DNA, we performed Gardella gels applying crude lysates from cell cultures. Except for cell line NAWALWA and its subclones, DG-75, DOHH-2, and OCI-LY19, all EBV-PCR+ cell lines showed at least one band of episomal genomes. Some cell lines showed two episomal bands pointing to a double infection or to mutated episomes. The amount of linear DNA does not correlate with the number of episomes. Southern blots of genomic DNA revealed different genotypes of EBV except for those cell lines which were established with B95-8 virus particles. To determine distribution of EBV genomes in single cells, we established a fluorescence in situ hybridization (FISH) method with a Cy3-labeled cosmid clone containing a genomic EBV fragment. The method showed for various cell lines that only a few culture cells contain high amounts of EBV genomes (several hundred) whereas the vast majority harbors only a few genomes in the nuclei. FISH appears to be superior to other methods allowing for EBV analysis at the single cell level to determine the cellular permissiveness.

0753**ADULT ONSET OF HEMOPHAGOCYTOTIC SYNDROME: REPORT OF EIGHT CASES FROM A SINGLE INSTITUTION**V. Rossi¹, C. Cattaneo¹, A. Re¹, O. Barozzi¹, B. Antonini¹, M. Luppi², G. Rossi¹¹Spedali Civili, BRESCIA, Italy; ²Ematologia, Policlinico, MODENA, Italy

Background. Hemophagocytic syndrome (or lymphohistiocytosis HLH) is a rare disorder of early infancy. Two distinct forms of HLH are recognized: familial (autosomal recessive disorder) and secondary (in association with systemic infection, underlying malignancy or autoimmune disorder). The diagnosis of adult HLH is often underestimated because of its rarity and heterogeneous clinical presentation. Fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia, hypofibrinogenemia and hyperferritinemia are often present. Diagnostic criteria, based on clinical and laboratory features, have been recently proposed. Aim, patients and **Methods.** we retrospectively evaluated eight cases (M/F 3/5, median age 64, range 31-71) fulfilling the diagnostic criteria, admitted to our Institution since February 2003 for cytopenia and fever, to clarify common clinical characteristics and underlying disease. **Results.** at presentation 7/8 patients had fever, 5/8 splenomegaly, 8/8 cytopenia, 5/8 hypertriglyceridemia and/or hypofibrinogenemia, 7/8 histological hemophagocytosis. Median hemoglobin level was 8.3 g/dL (range 7.7-11.7), platelets $29.5 \times 10^9/L$ (12-166), ANC $0.76 \times 10^9/L$ (0.09-4.2); triglycerid value 636 mg/dL (75-775), fibrinogen 168 mg/dL (35-746), ferritin, evaluated only in five cases, > 40000 microg/L (1687->40000). In 6 patients an underlying haematological disease was diagnosed (2 autoimmune haemolytic anemia [AHA], 1 Castleman disease, 1 diffuse large B cell lymphoma, 1 anaplastic null lymphoma, 1 T cell lymphoma). In 4 of 7 evaluated patients serum HHV8 DNA was identified. In one of them a concomitant haematological disease was not shown, the other three having AHA and Castleman disease. Two of the 8 patients were positive for CMV-DNA (1 T-cell lymphoma, 1 AHA); 3 of the 3 evaluated patients had detectable EBV-DNA (1 Castleman, 2 AHA). In one case, in which no haematological concomitant disease was shown, a low intracytoplasmic perforin expression was found, suggesting a possible genetic ethiopathogenesis. Two patients did not receive any therapy because of early death; Ig ev infusion was administered in 4/8, corticosteroid therapy in 5/8, anti-CD20 monoclonal antibody in 3/8, antiviral therapy in 4/8. One case, without evidence of haematological disease, was treated according to HLH 04 protocol (CyA+DMZ+VP16). All the patients died with a median survival of 9 days (range 2-24). **Conclusions.** a diagnosis of HLH has to be considered in all cases presenting with fever, cytopenia and hyperferritinemia, irrespective of any malignancy or infection. Neoplasia, viral infections, autoimmune disease might be the underlying conditions, but a genetic cause should also be considered. A tempestive diagnosis is mandatory in order to start adequate therapy trying to prevent a rapidly fatal outcome.

0754**MIGLUSTAT (NB-DNJ) WORKS AS A CHAPERONE FOR MUTATED B-GLUCOSIDASE IN CELLS TRANSFECTED WITH SEVERAL GAUCHER DISEASE MUTATIONS**P. Alfonso¹, P. Giraldo², S. Pampin³, J.C. Rodriguez-Rey³, M. Giral², M. Pocovi¹¹Zaragoza University, ZARAGOZA, Spain; ²Miguel Servet University Hospital, ZARAGOZA, Spain; ³Cantabria University, SANTANDER, Spain

Background. Gaucher disease (GD), one of the most common lysosomal storage disorders, is caused by a deficiency of glucocerebrosidase (GBA). The glucose analogue, N-butyldeoxy-nojirimycin (NB-DNJ, miglustat, Zavesca[®]) is an inhibitor of the ceramide-specific glucosyltransferase which catalyses the first step of glycosphingolipids (GLS) biosynthesis. Miglustat has

shown to be an effective and safe therapy for the treatment of type 1 GD and is currently approved in EU and USA in this indication. Nojirimycin derivatives have been shown to work as chemical chaperones for lysosomal enzymes. **Aims.** To get stable transfectants were expressing GBA from the most frequent mutations **Methods.** Using site directed mutagenesis we constructed several pCR3.1-GBA plasmids containing GBA mutants (N370S, L444P, S364R, M123T, V15M, P266L, 465delSer, L336P) and wild type. The pCR3.1-GBA plasmids were transfected into a COS7 cell line using DEAE-dextran-mediated transfection. Stable transfected cell lines were obtained by geneticin (G418) selection. Northern blot analysis confirmed that the stable transfectants were strongly expressing GBA at mRNA level. Stable transfectant COS7 cells were cultured during 6 days in Dulbecco's Modified Eagle's Medium (DMEM) /10% fetal calf serum (FCS) at 37°C in 5% CO₂ and 10 mM NB-DNJ. Culture medium was replaced every 3 days with fresh media supplemented with or without 10 mM NB-DNJ. Cells were harvested and the pellet was lysed by sonication. The β -Glucosidase (β -Glu) assay was performed using 4-methylumbelliferyl β -D-glucoside as substrate. **Results.** The addition of NB-DNJ to COS cell medium leads to an 1.3-, 3.6- and 9.9-fold increase in the activity of S364R (1.3 fold), V15M (3.6 fold), N370S (1.8 fold), and M123T (9.9 fold) and WT (2.0 fold) β -Glu, respectively. No significant changes in the L444P, 465delSer and L336P β -Glu activity were observed. Although the precise molecular basis is left unknown, these results suggest that NB-DNJ, in addition to the inhibitory effect on glucosyltransferase, also works as a chemical chaperone, increasing the activity of β -Glu of the N370S, M123T, V15M, and S364R GBA mutants and WT. **Conclusions.** This increase on the activity of the β -Glu would imply that miglustat is not only a substrate reducer but also an enhancer of the glucosylceramide degradation, with very promising clinical implications for the treatment of GD patients.

This work has been supported by a grant REDEMETH G03/054

0755**TYPE 1 GAUCHER'S DISEASE. PRELIMINARY DATA OF AN HOMOGENEOUS NEUROLOGICAL STUDY PERFORMED IN SPANISH PATIENTS**J.L. Capablo¹, A. Saenz de Cabezón², P. Alfonso³, J. Fraile¹, M. Pocovi³, M. Giral², P. Giraldo²¹Miguel Servet Univ Hospital, ZARAGOZA, Spain; ²Miguel Servet University Hospital, ZARAGOZA, Spain; ³Zaragoza University, ZARAGOZA, Spain

Background. Classically, type 1 Gaucher disease (GD) is characterized by organomegaly, haematological manifestations as anaemia and thrombocytopenia and frequent skeletal complications in absence of neurological manifestations. However, there is growing evidence for an association between GD and Parkinson's disease and tremors, peripheral neuropathy and others. These manifestations recently have been described in type 1 GD patients. The aim of our study has been to evaluate systematically a group of type 1 GD patients in order to assess about the presence of neurological manifestations. Design and **Methods.** In this study, we have included 18 type 1 GD Spanish patients in follow-up for five years or more (male 6/female 12; mean age at diagnosis 24 ± 16.88 years; SSI 6.5 ± 3.2 ; genotype N370S homozygous) 5 patient under enzyme replacement therapy (ERT) every two weeks at mean dose of 45 U/kg for ten years or more, 5 received therapy since four years ago and three under ERT since three years ago, all of them with satisfactory response (reduction in visceral size and normalization of haematological parameters). Five patients had not received any pharmacological therapy. All patients accept to participate in the study and the neurological exam and neurophysiological tests were performed by the same specialists. The protocol included a detailed inquiry about symptoms and concomitant medications and a neurological exam by a modified total neuropathy score to determine neurological signs. A superficial electroneurogram in sural and peroneal nerve was performed. As a

cognoscenti test we used the memory impairment screen for dementia. **Results.** From a total of 18 patients, 6 patients (33.3%) showed some neurological deficits: an 8 years old child (GBA genotype G377S/R463C) develop ocular alteration and psychomotor delayed and he was reclassified as type 3 GD patient; two females siblings of 64 and 61 years old (GBA genotype N370S/ IVS4-2A>G +c. (-203)A>G) has evident neurological symptoms. One of them presented parkinsonism since 40 years old and she did not response favourably to treatment with L-Dopa. The other sister carrier a joint hip replacement, has been under ERT since 1994 and she has developed dementia and Parkinson's disease at 68 years old. Another splenectomized patient (age at diagnosis: 15 years, genotype N370S/L444P) was classified with mild or moderate and stable disease without ERT the neurological exam showed saccades ocular movements. One male 25 years old (genotype N370S/N370S) has been under ERT three years ago and presents abnormalities of eye movement. The last patient a 32 years old woman, (genotype N370S/L444P) had peripheral neuropathy in arms. Comments: Due to the high incidence of neurological complaints in patients with GD type 1 it is necessary a precise and detailed clinical study to discard concurrent and medical problems and/or side effects from concomitant medications or true neurological problems related to GD. This work has been partially supported by a grant FIS 042476

0756

SAFETY EXPERIENCE WITH TRISENOX® (ARSENIC TRIOXIDE) INJECTION

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Background. Trisenox® (arsenic trioxide) injection was approved in the United States in Sept 2000 and in the EU in March 2002 for treatment of patients (pts) with relapsed or refractory acute promyelocytic leukemia (APL). **Aims.** The aim of this analysis is to assess the safety of Trisenox® in the total exposed patient population, both in clinical trials and post-marketing studies. **Methods.** Safety information was gathered from monitored clinical trials, investigator-sponsored trials, and spontaneous reports in postmarketing studies. **Results.** As of Dec 2004, approximately 4330 pts had received commercial Trisenox® for a variety of hematologic and nonhematologic cancers. Since the beginning of the Trisenox® development program, 923 pts [with APL (362), multiple myeloma (102), MDS (189), and other hematologic cancers or solid tumors (270)] have been treated in clinical trials or compassionate programs. Overall safety experience for Trisenox® is therefore based on exposure of approximately 5250 pts. Postmarketing Experience: By February 2005, 318 spontaneous postmarketing adverse events (AEs) had been reported to CTI; 146 of these were serious AEs (SAEs), with 16 deaths. Ten deaths unrelated to Trisenox® were due to complications of disease in pts with APL, AML, CML, NHL, MM, or ALL. Five potentially related deaths were due to possible tumor lysis syndrome (pt with MM), convulsions (MM), cardiac failure (MDS), intracranial hemorrhage (MDS), and unknown cause (APL). A pediatric pt with unknown disease died of renal failure; attribution of this death is unassessable. Most reported AEs were expected events, listed in Trisenox® product labeling. Trisenox®-related SAEs reported for >10 pts (including spontaneous, literature, and independent investigator study reports) were cytopenias (45 reports; neutropenia, anemia, febrile neutropenia, thrombocytopenia, leukopenia, pancytopenia), dyspnea/hypoxia (32 reports), infections (31), pyrexia (26), edema/wt gain/fluid retention (22), differentiation syndrome (20, including reports in pts with diseases other than APL), lung infiltration/pleural effusion (19), ventricular tachycardia (13), confusion/delirium (11), leukocytosis (11), prolonged QT/QTc interval (11), and renal failure/impairment(11). No postmarketing reports of torsade de pointes have been received. Clinical Trial Experience: With 309 pts enrolled in CTI-sponsored

clinical trials since US approval, 274 SAEs have been reported for 174 pts. This larger proportion of pts with SAEs likely reflects the monitored data in clinical trials compared with spontaneous postmarketing reports. Most of these SAEs are events listed in the Trisenox® label. AEs considered by investigators to be related to Trisenox® in 5% or more of the 278 pts with data received by CTI since US approval are nausea/vomiting (29%/12%), fatigue/asthenia (21%/6%), diarrhea (23%), rash/pruritus (14%/13%), headache (18%), neutropenia (12%), peripheral neuropathy (12%), anorexia (11%), prolonged QT/QTc interval (10%), thrombocytopenia (9%), edema (9%), pyrexia (8%), increased LFTs (7%), leukopenia (7%), dyspnea (6%), abdominal pain (6%), and in 5%: tachycardia, anemia, and cough. **Conclusions.** With >5250 pts exposed to Trisenox®, the postmarketing AEs reported are generally similar to those observed in clinical trials and listed in the product labeling. When pts are managed in accordance with the product label, the risk/benefit profile of Trisenox® remains acceptable.

0757

ENUMERATION OF T-CELL, B-CELL AND NK-CELL FRACTIONS WITH THE CELL-DYN 4000 HEMATOLOGY ANALYSER

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Cell-Dyn 4000 Abbott analyser has the capability of measuring FL1 and FL2 fluorescence available for two-colour immunophenotyping. It has also got the possibility of CD3/CD4/CD8 populations automated analysis. The aim of the study was process whole blood samples for analysing T, B and NK populations using the Cell-Dyn 4000 CD3/CD4/CD8 system and compare results with flow cytometry (FC) reference procedure analysis. **Methods.** 100 microl per tube obtained from 66 EDTA-anticoagulated samples (with range of absolute lymphocyte counts 0.2 to 33.3 x 10⁹/L) were stained with 10 microl of the following monoclonal antibodies: FITC-CD3/PE-CD56 (tube 1); FITC-HLA-DR/PE-CD19 (tube 2). Tubes were then incubated for 10 minutes at room temperature and processed using the CD3/CD4/CD8 mode. Compensation was set for FL1 to FL2 and FL3 to FL2 using the automatic procedure with WinList 4.0 (BD). Gates were set for CD3+CD56; CD3+CD56+; CD3-/CD56+ (tube 1) and HLA-DR+CD19-; HLA-DR+CD19+ (tube 2) for Cell-Dyn 4000 and FC methods. Assessment of agreement between the Cell-Dyn 4000 immunophenotype techniques and the FC reference procedure was by the method of Passing & Bablock. Bland-Altman test was used in order to compare automated lymphocyte percentage and Cell-Dyn 4000 immunosum (CD3+CD56-, CD3-CD56+, CD3+CD56+/HLA-DR+CD19+ fractions). **Results.** High correlation was observed between CD3+ T-cell counts obtained by flow cytometry and Cell-Dyn 4000 analysis (R²=0.95 y =1.01x) and HLA-DR+CD19+ B-cell counts (R²=0.99 and agreement y= 1.08x) **Results.** indicated a good correlation between the absolute CD56+ T/NK cell (R²= 0.83 y= 0.53x) and CD56+ NK-cell (R²= 0.52 y= 0.81x) counts obtained by FC and Cell-Dyn 4000 analysis. Bland-Altman test showed a good agreement between automated lymphocyte differential and immunosum comparisons except for an outlier which was further diagnosed from acute myeloid leukemia. **Conclusions.** The results obtained by Cell-Dyn 4000 immunophenotyping analysis of T-cell; B-cell and NK-cell populations showed good agreement with the results obtained by flow cytometry. Furthermore Cell-Dyn 4000 screening analysis can improve the procedures to selecting samples for further FC studies.

0758

QUALITY MANAGEMENT IN THE HAEMATOLOGY LABORATORY: REPORTING A RELIABLE BLAST CELL COUNTS. Jacobsson¹, H. Johansson²¹Sahlgrenska University Hospital, GÖTEBORG, Sweden; ²Sahlgren's University Hospital, GÖTEBORG, Sweden

Background. A correct determination of the blast cell fraction in bone marrow and/or blood or other body fluids is essential for the correct management of patients with haematological malignancies. The WHO classification of tumours of haematopoietic and lymphoid tissues states a requisite blast percentage for the diagnosis of many disorders. The true value of a reported blast percentage of 20 from a 500 cell differential count performed on a perfect sample lies anywhere between 16 and 24 percent (ninety-five percent confidence limits), provided that no cells are misidentified by the human observer. Each laboratory should therefore implement a quality control system intended to minimize observational and other errors. **Aims.** In our haematology laboratory encompassing morphology, flow cytometry, immunocytochemistry, cytogenetics and molecular genetics, we have developed a quality management system for the morphological methods. **Methods.** A structured method for educating and training personnel in morphological methods is used. The established reliability of the precision of differential counting is ensured through various internal quality control systems. The accuracy is guaranteed by the participation in four different proficiency testing schemes. **Results.** The laboratory has authorized and enrolled in the internal quality control system 7 morphologists for bone marrow cytology and 48 for blood leukocyte differential counting. The use of well established criteria for corrective actions in case of suboptimal observer performance has led to continuous improvement of the diagnostic service. More than satisfactory performance goals have been reached in national and international proficiency testing schemes. The laboratory, as the first in Sweden, has been accredited by SWEDAC, The Swedish Board for Accreditation and Conformity Assessment. **Summary:** It is feasible for haematology laboratories to implement a quality management system for the morphological services. The level of performance can hereby be established, maintained and mediated to clinicians and researchers.

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INFORMATION NEEDS IN THE FIELD OF LEUKEMIA: RESULTS FROM A QUESTIONNAIRE BY THE EUROPEAN LEUKEMIA INFORMATION CENTER (ELIC)N. Gökbuget¹, R. Arnold², P. Brück¹, H. Horst³, S. Herrmann¹, J. Atta¹¹J.W.Goethe University, FRANKFURT, Germany; ²University Charité Mitte, BERLIN, Germany; ³University Hospital, KIEL, Germany

In January 2004 the European LeukemiaNet(ELN) was founded by 116 partner organisations from 22 European countries coordinated by R.Hehlmann (Mannheim) and obtained funding by the EU. It includes 18 projects on clinical research in leukemic disease entities (ALL, AML, CLL, CML, MDS and CMPD), expert groups on diagnostics, supportive care and stem cell transplantation. All major European leukemia study groups joined the network. The major aim is to structure European research durably and to spread scientific excellence in the field of leukemia. This can only be achieved by regular information flow within the network but also with the public-mainly physicians and patients all over Europe. Therefore the European Leukemia Information Center (ELIC) which is located at the University of Frankfurt is one of the central projects of the network. ELIC is responsible for coordination of information exchange between network members and for setup of the network's website (www.leukemia-net.org). To estimate general and special needs for information on leukemias questionnaires

were sent to all network participants. 70 opinion leaders from European research groups answered the questionnaire. 99% of the respondents considered the internet as highly important for their project. 97% wanted to present their project within one central website. The detailed results of the first questionnaire are shown in table 1. Since information on ongoing studies was considered as particularly important, the participants were asked which study information should be provided. 90% wanted to present short protocols, 87% overview plans of study design, 90% flow-sheets on European trials and 79% complete protocols. Participants were furthermore asked whether information on complete study protocols should be limited. The majority voted for access limits for complete protocols (50%) to physicians. With a second questionnaire, ELIC identified 'advisors' within the network to support the accrual of important local information from different European countries such as addresses/links of hematology associations, patient organisations, self-help groups etc. Based on these results, the concept for the website was finalised. One major element of the website is the planned online study-database which finally may become a European leukemia study-registry. It shall provide information on ongoing leukemia trials including short-protocols, documentation material, patient information etc. Furthermore information on all major leukemia topics shall be provided online and ELIC prepares this information individually for different user-groups e.g. physicians, patients, nursing staff or press & media. A regular e-mail newsletter informs the network members about new studies or contents, interesting links, meetings etc. Providing treatment information via Internet is a main precondition to optimize the treatment of leukemia patients in Europe and to support clinical research. According to the experience from a German leukemia network, this may contribute to higher patient recruitment for clinical trials and improved treatment quality. Information on innovative and experimental protocols provides additional treatment options for European patients with advanced diseases. In view of the varied preconditions and requirements within European medical institutions, a central information center that structures and coordinates the information-exchange is indispensable for a successful network.

Table 1. Results from the 1st questionnaire.

Should your Partner contribute the following (yes/no)?	Yes	very important	Total
General project-information	98%	55% / 41%	96%
Contents for joint study registry	95%	40% / 44%	81%
Standards, SOPs, consensus doc.	99%	48% / 30%	81%
Cases & meetings	97%	48% / 30%	84%
Links	95%	38% / 43%	81%
Publications related to project	90%	21% / 32%	73%
Contents for newsletters	81%	21% / 21%	72%
Education & training material	81%	36% / 34%	70%
Publications by project members	78%	17% / 33%	66%
Information for media	66%	16% / 32%	54%
Information for patients	67%	22% / 31%	72%
Counseling for physicians	64%	15% / 35%	56%
Education / Discuss on forum	53%	10% / 40%	56%
Counseling for patients	44%	13% / 23%	42%

0760

IDENTIFICATION OF A NEW ALLELE IN A SICILIAN INDIVIDUAL: HLA-DPB1*0302

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We report here the identification and characterisation of a novel HLA-DPB1 allele that was subsequently named HLA-DPB1*0302† by the WHO Nomenclature Committee. HLA-DPB1*0302 was identified in a single Sicilian individual by a combination of sequence specific primers (SSP), reverse line sequence specific oligonucleotide probing (SSO) and sequence based typing (SBT). The DPB1*0302 allele is most similar to the DPB1*3101 allele, differing by a single mismatch at nucleotide position 301 (T to G) We report herein the identification of a novel HLA-DPB1 sequence, DPB1*0302. Genotyping technologies employed were PCR-SSP and reverse line PCR-SSO. The specific amplification pattern obtained did not correspond to any known DPB1 allele. Further HLA genotyping revealed this individual's HLA genotype to be: HLA-A*0301,*6801; B*1402,*3801; Cw*0802,*1203; DRB1*1104,*1301; DRB3*0101,*0202; DQB1*0301,*0603; DQA1*0103,*0505 The new HLA-DPB1 allele was characterized by direct sequencing of exon 2 in both directions, following separation of the DPB1*0302 allele from the DPB1*0401 allele using the allele-specific and sequencing primers described below.

Exon 2 sequence of DPB1*0302 compared to DPB1*3101



HLA-DPB1*0302 differs only from DPB1*3101 at position 301 (boxed). Homology with the consensus sequence is indicated with hyphens.

The complete exon 2 sequences of both DPB1 alleles were amplified with the following primers: 'DPB1F' 5'TGTAAAC-GACGGCCAGTGAGAGTGGCGCCTCCGCTCA and 'DPB1R' 5'CAGGAAACAGCTATGACCCCGGCC-CAAAGCCCTCACTC The bold sequence in the forward primer matches HLA-DPB1 intron 1 (41-20 bases 5' from exon 2 i.e. 4491-4511 in intron 1), whereas the bold sequence in the reverse primer matches HLA-DPB1 intron 2: 3-23. Confirmation of the novel allele DPB1*0302 was performed by allele-specific amplification that separated DPB1*0401 from DPB1*0302. Amplification for DPB1*0401 utilized the forward 'DPB1F' as described above, in conjunction with the following reverse sequencing primer: 'DPB0401R' 5'CTGCAGGGTCATGGGCC DPB1*0302 was amplified utilizing the same forward primer 'DPB1F' in conjunction with the following primer: 'DPB3101R' 5'CTGCAGGGTCACGGCCT The amplification and sequencing were based on methods previously described from DRB1 sequencing by Sayer et al using an ABI 3100 capillary sequencer (Perkin Elmer, Applied Biosystems, Warrington, Cheshire, UK) Sequencing confirmed the presence of HLA-DPB1*040101, and identified the subsequently named DPB1*0302 allele. As shown in Figure 1, the DPB1*0302 allele most similar to DPB1*3101 with a 'T' to 'G' substitution at nucleotide 301. This single base

change results in a coding change in codon 72 from leucine to valine. This change is located on the exposed alpha-helix and thus may have some functional relevance. DPB1*0302 is identical to DPB1*040101 up to nucleotide 280 and thereafter identical to DPB1*030101 and so DPB1*0302 may have arisen from a gene conversion event involving commonly found alleles such as DPB1*040101 and DPB1*030101. Alternatively, DPB1*0302 allele may have arisen by point mutation from the similar DPB1*3101 allele. The frequency of DPB1*0302 in the general population is unknown, but we can speculate that as DPB1*0302 was readily detected with popular, commercially available DPB1 typing systems, so it follows that this new allele is likely to be uncommon for it not to have been previously discovered.

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MULTIMEDIA CASE HISTORY USE FOR DECISION SUPPORT AND STATISTICAL ANALYSIS

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Background. A user-friendly computerized method for monitoring medical events has been developed to implement computer-aided monitoring diagnostic procedures, treatment protocols and clinical outcomes. The method is known as Multimedia Case History, or MMCH, for short [1]. The study was partly supported by Russian Foundation for Basic Research with grants RFBR 99-07-90314 and RFBR 04-01-00401. **Aims.** To develop computer-aided Decision Support System, or DSS, for case-to-case and case-to-cluster comparative and multivariate statistical analysis based on modeling and generalization of verified clinical information on individual patients as stored in MMCH. **Methods.** MMCH comprises data integrated on a single time axis with normalized graphics for Laboratory data set according to standard protocols selected by the user and diagnostic images integrated as needed [1]. It automatically detects variables that fall outside established limits and violations of protocols, and generates 'alarm signals' or 'blocking inquiries' (demanding an obligatory user's reaction) [1]. The limits create a sort of 'rectangular' domains in sub-spaces of parameters. The DSS makes individual MMCH data pair-wise comparable using normalization procedures in individual time-and-subspace time domain and interpolation techniques for multimedia data. Rather complicated data components like X-ray images can be described in a more simple way using a system of indexing [2]. Important advantage of the normalization algorithm is that subspace domain is not obligatory 'rectangular', so correlations between parameters can be taken into account. **Results.** The approach made it possible to define a measure of similarity on a set of individual MMCH. That has been done through pair-wise comparison of MMCH using parameter-space-and-time-domain normalization of MMCH. Furthermore, it allowed applying multivariate statistical analysis for MMCH clustering and modeling (generalization) of patterns within the clusters with purpose of better individual patient monitoring and treatment. Evidence-Based Medicine rules were obeyed. **Summary/Conclusions/Results.** obtained can be used as background for a unified language to meet needs of WHO in quantitative description, comparison and generalization of individual patients' patterns. The generalized patterns serve as models of clusters comprising similar MMCH patterns. The predictive models are combined with goal-oriented evidence-based findings to draw diagnostic conclusions and to generate optimal prescriptions (treatment) [2]. Thus, the DSS preserves all the advantages in handling individual cases pro-

vided with MMCH approach [1]. On the other side, DSS makes it possible to compare the cases quantitatively. Moreover, DSS is a tool for creation of generalized MMCH patterns. 6.

1. Nikita Shklovskiy-Kordi. *Multimedia Case History as a Tool for Management of Cancer Patients and Clinical Trials. UICC Abstracts. - Dublin, 2004.*

2. Vsevolod V. Shakin, et al. *Life Support Systems in Medicine and Biology. - Operations Research International Conference ORM-2004, Proceedings. - Moscow, Dorodnicyn Computing Center of the Russian Academy of Sciences, 2004. *Nikita@blood.ru*

0762

THE EFFECT OF GNIDILATIMONOEIN ON VIABILITY, DIFFERENTIATION AND APOPTOSIS OF K562, U937 AND NB4 LEUKEMIA CELL LINES

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Among plant-derived agents, daphnane-type diterpene esters have found to possess potent anti-leukemia activity. Gnidilatimonoein is a recently characterized diterpene ester from *daphne macronata* (thymelaeaceae) with strong anti-proliferative and anti-metastatic activities in both *in vitro* (cell culture)

and *in vivo* (animal tests) studies. Our previous studies have also shown that the new drug significantly inhibited DNA biosynthesis in the treated cells. However, the RNA biosynthetic pathways were not significantly affected by Gnidilatimonoein. In this investigation, we evaluated the effect(s) of this new compound on induction of differentiation and apoptosis in three different cancer cell lines (K562, U937 and NB4). Upon treatment of these cells with a single dose of the drug at 15µM, almost 60-70% of the cells showed morphological changes (wright-giemsa test) and the number of the NBT positive cells increased from 20% (after 2 days of treatment) to almost 70% after 4 days. The growth of the cells was also inhibited by about 50% by 24 h of exposure without substantial loss of cell viability (<10%). The extent of the cell growth inhibition by the drug increased to 85% after 3 days of treatment, and the viability was also enhanced to about 50%. Our data clearly indicated that the drug, at high doses (≥30µM), has potential cytotoxic effects. Fluorescence microscopic examination of the double stained drug-treated U937 and NB4 cells with acridine orange/ethidium bromide showed that at low drug doses (15µM) longer exposure times to the drug were required to observe apoptosis among the cells, and apparently, apoptosis occurred after differentiation. The occurrence of apoptosis in the treated cells was further confirmed by DNA fragmentation analysis and flow cytometry detection of sub-G1 population among the cells after 3 days. Based on these data, it might be concluded that gnidilatimonoein has the potential of being considered as a valuable candidate for leukemia chemotherapy.

SIMULTANEOUS SESSION II

Cellular and molecular control of hematopoiesis

0763

THE ONCOPROTEIN SCL/TAL-1 ASSOCIATES WITH THE CO-REPRESSOR ETO-2 IN MULTIPROTEIN COMPLEXES IN ERYTHROID CELLS AND MEGAKARYOCYTES

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Background. The bHLH transcription factor SCL/Tal-1 is required for specification of hematopoietic stem cells and maturation of definitive erythroid cells and megakaryocytes. Additionally, ectopic SCL expression is the most common pathogenetic event in T-cell acute lymphoblastic leukaemia (T-ALL) (up to 60%). However, the mechanisms underlying SCL action are not understood although evidence suggests that protein-protein interaction play a critical role in the modulation of SCL function. **Aims.** The aim of the current study was therefore to isolate SCL-containing protein complexes in definitive erythroid cells and megakaryocytes. **Methods.** To this, we used a single step protein purification strategy whereby SCL is tagged to a sequence that can be biotinylated *in vivo* (biotag-SCL), and SCL-containing complexes are purified by streptavidin beads and identified by mass spectrometry. **Results.** First, to check that biotinylated SCL was functional, we ensured that both primitive and definitive haematopoietic development could be rescued from SCL^{-/-} ES cells *in vitro* with biotag SCL. Then, we expressed biotag-SCL in mouse erythroleukemic (MEL) and megakaryoblastic (L8057) cell lines and pulled-down SCL-containing protein complexes. Western Blot analysis of precipitated proteins showed that known partners of SCL (including the transcription factors E2A, GATA1, LMO2 and Ldb1) were present in the pulled-down fractions, thereby validating the technique. Subsequent mass spectrometry analysis led to the identification of several new candidate partners in MEL and L8057 cells. Two such proteins include ETO-2 (human homologue: MTG16), a member of the ETO family of co-repressor proteins involved in the pathogenesis of acute myeloid leukaemia, and SSDP-2, previously identified as a partner of Ldb1. Several approaches were then used to characterise the SCL/ETO-2 interaction. First, the interaction between the endogenous proteins was validated by several different approaches including reverse co-immunoprecipitation and co-localisation in both MEL and L8057 nuclear extracts. Besides, the interaction was confirmed in primary erythroid cells and megakaryocytes. Second, immunodepletion experiments demonstrated that the composition of the SCL/ETO-2 complexes is different in MEL and L8057 cells including the corepressor Gfi-1b in MEL cells only. Consistent with the repressor role of ETO-2, transactivation experiments in heterologous cells revealed that ETO-2 expression represses the activator function of a pentameric complex consisting of GATA1/SCL/E2A/LMO2/Ldb-1 on GATA-1 regulatory elements. Finally, using *in vitro* differentiation of d12.5 fetal liver cells, we show that the SCL/ETO-2/Gfi-1b complex present in primary erythroid progenitors dissociates with terminal erythroid differentiation. Gain- and loss-of-function-studies are in progress. Summary Taken together, these data establish for the first time that SCL interacts with ETO-2 in erythroid cells and megakaryocytes, that the composition of SCL/ETO-2-containing complexes differ in these two lineages and that a complex consisting of SCL/ETO-2/Gfi-1b may have a repressor activity on genes critical for erythroid differentiation. Interestingly, we also detect SCL binding to ETO-2 in an SCL-expressing T-ALL cell line. It might therefore be plausible that ectopic SCL contributes to leukaemia by repressing genes indispensable for normal T cell differentiation. If confirmed, this would

shed new light on the mechanism and possible therapeutics for SCL expressing T-ALL.

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THE 'CYTOKINE STORM' AT THE ERA OF REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT)

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In the setting of standard myeloablative allo-SCT, perturbation of the cytokine network may function as a final common pathway of target organ damage, and the rapid onset of severe acute GVHD (aGVHD) can be considered a 'cytokine storm'. However, the use of RIC regimens has modified the natural history of GVHD after allo-SCT. Here, we investigated the 'cytokine storm' in 113 patients who received a RIC regimen before allo-SCT from an HLA-identical sibling. Our aim was to assess whether aGVHD is still a paradigm disease of systemic cytokine dysregulation at the era of nonmyeloablative and less toxic RIC regimens. Thus, 10 different cytokines (IL-1beta, IL-6, IL-8, IL-10, IL-12, IL-18, TNF-alpha, IFN-alpha, IFN-gamma, and FasL) were serially assessed in plasma samples collected from patients before allo-SCT, at day 0 and at 1, 2 and 3 months after allo-SCT. Median age of recipients was 49 (range, 18-63) years. 42 patients (37%) had a myeloid malignancy, 45 (40%) had a lymphoid malignancy and 26 (23%) had a metastatic non-hematological malignancy. 90 patients (80%) had an advanced disease. In addition to fludarabine, the RIC regimen included busulfan and ATG in 80 patients (71%) and low dose irradiation in 33 (29%) patients. In this cohort, the overall cumulative incidence of grade 2-4 aGVHD was 45% (95%CI, 36-54%). In a univariate analysis, advanced disease stage (p=0.04) and a high IL-12 level (P<10e-4) measured around the first month after RIC allo-SCT were significantly associated with the development of severe grade 2-4 aGVHD. Of note, to the contrary of myeloablative allo-SCT, TNF-alpha levels did not show a significant correlation with the risk of aGVHD. IL-12 levels were significantly correlated (r=0.69) to the severity of aGVHD: grade 0-1, median 468 pg/mL; grade 2, median 2538 pg/mL; grade 3-4, median 4615 pg/mL (P<10e-4). Interestingly, IL-12 levels significantly decreased after appropriate aGVHD treatment. In multivariate analysis, IL-12 level measured around the first month after RIC allo-SCT was the strongest predictive factor for aGVHD development and severity (P<10e-4; RR=10.8). In all, these results suggest that the use of RIC regimens modifies the pattern of cytokine network involved in the pathogenesis of aGVHD. Monitoring of IL-12, a key cytokine in the induction of Th1 and cytotoxic immune responses, appears to be a useful indicator of aGVHD, and anti-IL-12-based therapy might represent a potential tool for controlling alloreactivity.

0765

APAF-1 AND CASPASE 9 REGULATE EARLY AND LATE STAGES OF MK DIFFERENTIATION

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There is increasing evidence that caspases have apoptotic as well non apoptotic functions. The use of caspase inhibitors in *in vitro* cultures has afforded evidence that caspase activation plays an important role in proplatelet formation. To explore the precise role of the mitochondrial pathway of caspase activation in megakaryocyte (MK) differentiation, we have induced megakaryocytic differentiation in mouse ES cells, which were

either wild type, Apaf-1^{-/-} or caspase 9^{-/-}. MKs derived from wild type ES cells displayed well developed demarcation membrane system (dms) and typical α -granules containing usual secretion proteins such as von Willebrand factor: it showed polar intragranular distribution pattern, like in α -granule from mature bone marrow MKs. These mature MKs often displayed platelet formation signs, eg. widening of dms and elongated proplatelets. Consistent with the postulated role of caspases in platelet formation, we found that both Apaf-1^{-/-} and caspase 9^{-/-} MKs exhibited a defect in proplatelet formation which was in the same order of magnitude than that of wild type MKs treated with caspase inhibitors. This defect in proplatelet formation was also associated with marked abnormalities in α -granule development: indeed, α -granule were scarce or absent, whereas they were replaced by numerous microvesicular bodies where α -granule proteins were accumulating. Unexpectedly, a marked quantitative defect in the compartment of MK progenitors and in the number of MKs generated *in vitro* was observed, whereas erythroid progenitors and erythroid differentiation were greatly increased. In conclusion, these results suggest that the mitochondrial pathway of caspase activation is involved in early stages of hematopoiesis and presumably in the commitment regulation between erythroid and megakaryocytic differentiation. It also regulates late maturation events including platelet formation.

0766

TREATMENT RESPONSE AND LONG TERM SURVIVAL IN CHILDHOOD B- PRE-CURSOR ALL IS DETERMINED BY THE NOVEL PARAMETER CRAC (CYTOCHROME C RELATED ACTIVATION OF CASPASES)

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Background. Deficient activation of apoptosis signaling pathways may be responsible for treatment failure in acute leukemia and is involved in leukemogenesis. Several studies have been performed to analyze the expression of apoptosis molecules with respect to their prognostic value for treatment outcome but could not establish a correlation with response to treatment and prognosis or resulted in contradictory findings. **Aims.** We addressed the importance of intact apoptosis signaling for response to treatment and long-term survival in 78 patients with pediatric B-cell precursor lymphoblastic leukemia. **Methods.** The relation between the two key apoptogenic events, caspase-3 activation and cytochrome c release were analyzed in primary leukaemia cells upon induction of apoptosis by factor withdrawal. Active caspase-3 and released cytochrome c was analyzed in leukemia cells identified by surface staining flowcytometrically and correlated to clinical data. **Results.** Only in the group of good responding patients and patients in continuous remission a significant correlation between both parameters was found, suggesting that an intact apoptosis signaling system is important for patients achieving long-term remission. By combining both parameters, we could identify a novel indicator, cytochrome c related activation of caspase-3 (CRAC). This parameter connects the extent of caspase activation to cytochrome c release in single cells in a individual patient sample, resulting in positive values indicating proficient apoptosis signal induction while negative values identify deficient apoptosis signaling. Classification of the patients according to this parameter revealed that proficient apoptosis signaling (CRAC positive values) is correlated with good response to treatment. Patients exhibiting high numbers of persisting leukaemia cells on day 15 were exclusively found in the CRAC negative group (neg.: N= 36, mean 22.6%; pos.: N= 27, mean 6.0%; p= 0.003). Most interestingly, 14 out of 17 patients with relapse in this study were found to have negative CRAC values (p=0,013). Overall, positive CRAC values resulted in the probability of 91,4% of relapse free survival in contrast to 67,4% for patients with negative CRAC values (p=0,0194). **Conclusions.** Our data

show, that a functional intact apoptosis signaling system is important for efficient treatment and achievement of relapse free survival in childhood B precursor ALL. Assessment and quantification of this relation for individual patients by the novel parameter CRAC may serve as potential factor for treatment stratification.

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A ROLE FOR FLT-1 IN ACUTE LYMPHOBLASTIC LEUKEMIA

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The VEGF receptors FLT-1 and KDR are expressed on a subset of leukemia cells. Earlier, we identified a VEGF/VEGFR-2 autocrine loop that supports growth and migration of a subset of leukemia, namely AML (Dias et al, 2000, 2001). More recently it was shown that FLT-1 activation is essential for hematopoietic stem cell recovery, mediating cell migration from the bone marrow microenvironment (Heissig et al, 2002). However, the role of FLT-1 in regulating leukemia biology within the BM is not known and is the subject of the present study. First we evaluated the expression of this receptor, by RT-PCR, in 10 cell lines representing different types of leukemia (ALL, AML and CML) and on 83 patient samples (30 CML, 23 ALL and 30 AML). Approximately 80% of the samples expressed FLT-1 (91% of CML, 71% of ALL, 78% of AML). In order to identify the function and regulation of this receptor we started by studying the response of 5 FLT-1 expressing ALL cell lines to its specific ligand, PlGF. One of the cell lines showed a significantly higher proliferation rate (p<0,05) and 3 migrate at least 3 folds more (p<0,05) in response to this growth factor. Next, we transfected an ALL cell line (with a basal expression of this receptor) with full length FLT-1 and determined its importance in regulating these properties. In response to VEGF, FLT-1 overexpressing cells show no differences in proliferation, but migrate significantly more (p<0,05), an effect that is blocked by an FLT-1 neutralizing Ab (p<0,05). The aggressiveness as well engraftment potential of FLT-1 expressing leukaemia cells was also determined *in vivo*. We observed that FLT-1 overexpressing cells are more aggressive (lower mouse survival, overall) than their untransfected counterparts. Treatment with an FLT-1 neutralizing Ab prolonged survival and prevented leukemia infiltration into other organs (ex lungs). In all *in vivo* assays, a preferential localization of FLT-1 expressing cells near the bone growth plate (epiphysis), was observed. Taken together these results indicate a role for FLT-1 in mediating migration of subsets of leukemia cells, and consequently in regulating malignant cell distribution within BM. Given the variation in leukemia aggressiveness associated with the expression of this receptor, the use of neutralizing FLT-1 agents should be considered as a therapeutic approach to treat subsets of acute leukemia.

Myelodysplastic syndromes

0768

DECITABINE, A 'HYPO METHYLATING AGENT', IS MORE EFFECTIVE THAN SUPPORTIVE CARE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS): RESULTS OF A PHASE III TRIAL

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Background. Epigenetic mechanisms of tumor suppressor gene inactivation, such as cytosine methylation, provide poten-

tial targets for treatment approaches. Decitabine (DacogenTM, DAC) is a cytosine analog that was first synthesized in Czechoslovakia. DAC reverses aberrant DNA methylation leading to re-expression of silenced tumor suppressor genes. It has demonstrated efficacy in several early phase trials. AIM To evaluate the safety and efficacy of DAC vs supportive care (SC) in a pivotal Phase III, randomized, open-label trial. *Methods.* MDS patients (pts) with International Prognostic Scoring System (IPSS) Intermediate (Int)-1 (31%), Int-2 (44%) and high-risk disease (26%), those with secondary MDS (14%) and those previously treated (27%) were included. Adjudicated final results (independently judged by a blinded reviewer) are presented. *Results.* 170 pts (mean age, 68 yrs) received either DAC plus SC (n = 89) or SC alone (n = 81). DAC was administered as a 3 hr infusion (15mg/m²/hr q 8 hrs x 3 days q 6 wks) although alternative 1 hr infusion schedules under evaluation have also shown efficacy (Kantarjian, ASH 2004). Both groups were comparable for baseline characteristics. Response rates according to International Working Group (IWG) MDS criteria following a blinded, centralized bone marrow review, were 17% for DAC (9% CR, 8% PR) vs. 0% for SC (p < 0.001). Responses occurred in all IPSS groups, in pts with *de novo* and secondary MDS and in pts who were or were not previously treated. Responses were durable, lasting a median of 9 months. DAC responders vs all non-responders had a median of 18 vs. 10 months before AML transformation or death (TTAML/D) and a median survival of 24 vs. 14 months. All responders remained or became red cell and platelet transfusion independent. 47% of responders had a major cytogenetic response and 6% (1 pt) a minor cytogenetic response. Median TTAML/D in all pts was 12 months for DAC vs. 8 months for SC (p = 0.043 Wilcoxon, 0.160 Log-rank) and 9 vs 3 months in high-risk pts. Using a Cox proportional hazards model, the probability of progression to AML or death was 1.72-fold greater for SC than DAC (p=0.017). Global health status, physical functioning, fatigue and dyspnea was significantly better for DAC-treated pts than SC pts. As expected, the primary toxicity was myelosuppression with the major Grade 3-4 toxicity being febrile neutropenia. SUMMARY/CONCLUSION DAC is a promising novel treatment approach for MDS patients with predictable and manageable toxicity.

0769**ICL670, A ONCE-DAILY ORAL IRON CHELATOR, IS EFFECTIVE AND WELL TOLERATED IN PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) AND IRON OVERLOAD**

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Background. Although blood transfusions are an important therapy for MDS, the resultant chronic iron overload can impact survival, if left untreated. Deferoxamine (Desferal[®], DFO), a standard iron chelation therapy, effectively treats iron overload. However, DFO's subcutaneous, slow infusion over an 8–12-hour period, five to seven times weekly is a demanding regimen. Many MDS patients also have difficulty tolerating subcutaneous infusions. ICL670 (deferasirox) is a once-daily, oral iron chelator in Phase III clinical development that has demonstrated efficacy and acceptable tolerability in patients with transfusional iron overload. *Aims.* To evaluate the efficacy and tolerability of ICL670 in a cohort of transfusion-dependent MDS patients, a sub-population of the rare anemias in the trial C1CL670A0108. *Methods.* In this 52-week, open-label, multicenter Phase II trial, 47 MDS patients were recruited from seven countries (Belgium, Canada, France, Germany, Italy, UK and USA). Daily ICL670 doses were assigned based on a patient's liver iron content (LIC) at baseline. The iron chelation efficacy of ICL670 was evaluat-

ed based on changes in LIC (baseline versus end of study; defined as success or failure according to the degree of change or value at end of study) and serum ferritin measured monthly. *Results.* Based on baseline LIC, patients (median age 66 years; range 20.0–81.0 years) received either ICL670 5, 10, 20 or 30 mg/kg/day (n=4, 7, 12 and 24, respectively). Median duration of study drug exposure was 51.6 weeks (range 0.7–58.6). At baseline, the mean LIC measured by biopsy or SQUID was 15.6±11.9 mg Fe/g dw which decreased by 5.7 mg Fe/g dw (SD±6.3) after 52 weeks' ICL670 treatment. The overall success rate was 56.4%, with 95% confidence intervals of [40.8, 72.0]. Median serum ferritin level at baseline was 2674 µg/L (range 537–9099, and fell by 331 µg/L (range -5857–4834). The median number of transfusions during the study was 22.5 (25–75% percentiles; 16–40), while the median amount of blood transfused was 0.26 mL RBC/kg/day (25–75% percentiles; 0.16–0.35). The mean rate of iron excretion (0.45±0.24 mg/kg/day) was greater than iron intake (0.28±0.14 mg/kg/day). Twenty-nine patients (61.7%) completed the study. Non-completion included: four deaths, all considered due to the patients' underlying disease; six patients withdrew consent; one patient no longer required study drug; seven patients discontinued due to adverse events (AEs) - of which only one (nausea and vomiting) was considered drug-related. Forty-five patients reported AEs, the majority were mild or moderate in severity. The most common drug-related AEs were mild, transient gastrointestinal disturbances such as diarrhea, nausea, vomiting and abdominal pain. Dose adjustments, mostly reductions, were required in 28 patients, commonly due to AEs or laboratory test abnormalities. Mild serum creatinine increases >33% baseline were seen in 17 patients at 20 and 30 mg/kg, but never >1.5 x ULN. There were no incidences of drug-induced neutropenia or arthralgia during the study. *Conclusions.* Once-daily, oral ICL670 appears to be a convenient, effective and well-tolerated iron chelation therapy in iron overloaded MDS patients.

0770**RESULTS OF AN INTERNATIONAL PHASE 2 STUDY OF THE ORAL FARNESYL TRANSFERASE INHIBITOR (FTI) TIPIFARNIB (ZARNESTRA[®], R115777) IN PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SYNDROME (MDS)**

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Background. Tipifarnib (ZARNESTRA[®], R115777) is an orally administered selective FTI with antiangiogenic, antiproliferative, and apoptotic effects. Single-agent activity of tipifarnib in patients with hematologic malignancies, including MDS, with manageable toxicity, was previously reported in phase 1–2 studies. *Aims.* To assess tipifarnib in patients with high-risk MDS. *Methods.* A multicenter study was conducted to assess the response rate (International Working Group criteria) and survival of patients with high-risk MDS treated with tipifarnib. MDS was defined by French-American-British (FAB) classification as refractory anemia with excess blasts in transformation (RAEB-t), refractory anemia with excess blasts (RAEB), or chronic myelomonocytic leukemia (CMML) with ≥10% bone marrow blasts. *Results.* Starting dose of tipifarnib was 300 mg BID for the first 21 days of every 28-day cycle. Eighty-two patients were enrolled (68% male; 77% age ≥65 years; 49% Eastern Cooperative Oncology Group [ECOG] performance status = 0) with RAEB-t(28%), RAEB (49%), and CMML (23%). By International Prognostic Scoring System (IPSS) score, 44% of patients had IPSS score high, 39% intermediate (INT)-2, and 17% INT-1. Fifty-two patients (63%) were therapy-naïve, while 30 patients (37%) had received prior therapy for MDS. Patients were treated for a median of 3 cycles (range, 6 to 723+ days), with 72% relative dose intensity. Patients discontinued treat-

ment primarily for progressive disease (48%) or for adverse events (24%; 13% drug-related), with 6 patients still on therapy. Responses were seen in 28 patients (34.2%; 7 complete responses (CR), 4 complete responses with incomplete platelet recovery (CRp), 2 partial responses (PR), 15 hematologic improvements [HI]), both in therapy-naïve and pretreated patients. Objective responses (CR, CRp, and PR) were durable, with median duration of 10.1 months (range, 3.9+ to 19.4+ months) from attaining bone marrow response to loss of bone marrow response or worsening of peripheral counts. In 68 patients with at least 1 on-study bone marrow assessment, bone marrow response (either normalization or $\geq 50\%$ decrease in bone marrow blast count) was observed in 35 patients (51%). Median time to leukemic transformation for patients with RAEB-t was 3.7 months; median could not be calculated for other classes. Overall median time to leukemia or death (mTT-LoD) was 7.4 months and median overall survival (mOS) was 11.7 months. Results (in months) by risk-group have been tabulated. Myelosuppression was the most common drug-related side effect, with 20% grade (G) 3–4 neutropenia, including 6% neutropenic fever, and 34% G3–4 thrombocytopenia. Drug-related nonhematologic toxicity was mostly G1–2, rarely G3, and no G4. Most frequently reported were nausea (26%; 1% G3), fatigue (32%; 2% G3), diarrhea (20%; 1% G3), fever (5%, 4% G3), dyspnea (6%; 1% G3), purpura (10%; 2% G3), and rash (11%; 4% G3). Summary/conclusions: In a large cohort of patients with high-risk MDS as defined by FAB classification and IPSS score, clinical activity with durable responses and limited toxicity was confirmed in patients treated with tipifarnib. Further study is warranted.

Table. Results by Risk Group.

	By FAB classification			By IPSS score		
	RAEB-t	RAEB	CMML	High	INT-2	INT-1
n	23	40	19	36	32	14
mOS (months)	9.2	12.2	14.5	10.4	15.0	15.1
mTTLoD (months)	3.4	7.8	11.9	3.7	7.4	11.9

0771

HIGH-RESOLUTION GENOMIC PROFILING OF MYELODYSPLASIA (MDS) BY MICROARRAY COMPARATIVE GENOMIC HYBRIDISATION (CGH)

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Background and Aims. Myelodysplasia (MDS) is a heterogeneous group of clonal disorders of haematopoietic stem cells characterised by ineffective haematopoiesis and a variable risk of transformation to acute myelogenous leukaemia. Approximately, 50% of MDS patients have a normal bone marrow karyotype according to metaphase cytogenetic findings. Two major implications follow from this observation: first for diagnosis-it may be problematic to distinguish patients with Refractory

Anaemia (RA) with normal karyotypes from patients with reactive anaemias, second for research - without a cytogenetic abnormality there may be no genetic 'clue' on which to base molecular studies of pathogenesis. We have used Comparative Genomic Hybridisation (CGH) microarray analysis, a technology that represents a significant improvement in resolution over conventional cytogenetic analysis, to screen genomic DNA from MDS patients for the identification of genome-wide Copy-Number Changes (CNCs). **Methods.** We have studied genomic DNA obtained from the neutrophil population of 21 MDS patients. Seven patients (4 RA, 2 RAEB, 1 RARS) had a normal karyotype, while 14 (of which 9 had 5q- syndrome) had an aberrant bone marrow karyotype. Comparative Genomic Hybridisation (CGH) microarray analysis was performed using microarrays containing 3500 BAC clones at 1Mb intervals over the whole human genome. The patient DNA and a pool of normal reference DNA was labelled with different fluorochromes and cohybridised to the microarray. The normalised ratio of signal intensities was calculated and log₂ ratios between -0.4 and 0.4 were considered normal. Ratios below or above the normal range were interpreted as loss or gain of genetic material, respectively. **Results.** Copy-number changes (CNCs) that escaped conventional cytogenetic detection were identified in the 7 MDS patients originally reported with normal bone marrow karyotypes. In these cases, submicroscopic deletions were detected at several genomic loci, including 2p11, 12q11, 17q11 and 22q12, while gain of genetic material was found at 8q11 and 19p13. Microarray CGH data confirmed all abnormalities reported by conventional cytogenetic analysis in the 14 MDS patients with aberrant bone marrow karyotypes. The deletions on chromosome 5q were precisely mapped by array-CGH in the nine patients with the 5q- syndrome but no additional CNCs were detected. Of the remaining five patients with aberrant karyotype, four showed additional CNCs. The majority of the additional CNCs were deletions of single clones with three examples of single clone amplification. Several genes involved in either the initiation or progression of haematological malignancies are known to map within the cryptic abnormalities identified in the patients studied. For example, one patient with an apparently normal karyotype showed a small deletion at 17q11 which encompasses the NF1 gene. **Conclusions.** Further work will determine whether particular abnormalities detected by microarray CGH are recurrent and the nature of the genes involved. However, the promise of microarray CGH in the diagnostic work up of MDS particularly in those patients with normal karyotypes is clear. This study has been funded by Leukaemia Research Fund

0772

RESULTS OF THE MDS-002 AND -003 INTERNATIONAL PHASE II STUDIES EVALUATING LENALIDOMIDE (CC-5013; REVLIMIDA) IN THE TREATMENT OF TRANSFUSION-DEPENDENT (TD) PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS)

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Background. Lenalidomide is a thalidomide analog that modifies cell response following receptor:ligand engagement. In a phase I/II study in MDS, lenalidomide yielded an erythroid response and/or sustained transfusion-independence (TI) in 10/11 patients (pts) with del5q31 and in 45% of pts with a normal karyotype or alternate cytogenetic (CTG) abnormality (NEJM 2005;352:11). **Aims.** To further evaluate the erythropoietic activity of lenalidomide in MDS, we performed two mul-

ticenter efficacy studies in pts with Low/Int-1 IPSS TD-MDS with either del5q31 [MDS-003] or alternate CTG abnormalities [MDS-002]. *Methods.* Eligible pts had TD-anemia (>2 units RBC/8 wks), ANC >500/mL, and platelets >50,000/mL. Lenalidomide was administered at 10mg/dx21 q4 wks or 10mg/d with response assessment by IWG criteria after 24 wks supported by blinded central CTG and pathology review. RBC TI was defined as absence of transfusion for any consecutive 8-wk period and >1 g/dL Hgb-. *Results.* Median age in both trials was 72 years [range, 27-95]. The following table summarizes demographic and erythroid response data according to an Intent-to-Treat (ITT) analysis as of June 15 [MDS-002] or September 15, 2004 [MDS-003]. The frequency of TI was significantly higher in pts with del5q31 (P<0.001), with a median 5.2 g/dL Hgb- (range, 1.1-11.4). TI rate was higher in pts with isolated del5q (67% vs 57%), associated with CTG response in 79% of TI del5q31 patients and 55% CTG CRs. Pathologic CR was documented in 32/110 (29%) evaluable del5q pts. After a median follow-up of 9.3 mos (4.2 to 14.8+), median response duration is not reached with only 14 vs 13 TI pts failing on the 003 and 002 studies, respectively. Neutropenia (39% [003] vs 19% [002]) and thrombocytopenia (35% vs 15%) were the most common AEs necessitating tx interruption or dose adjustment. Study deaths were associated with either disease progression (9 [003] vs 8 [002]) or infection (3 vs 4). *Conclusions.* These multicenter phase II studies confirm the erythropoietic activity of lenalidomide in lower risk MDS, and indicate that lenalidomide has unprecedented hematologic and CTG remitting activity in pts with del5q31.

Study	# TD Pts	IPSS Low/Int-1	Duration (yrs)	Median # RBCs/8wk	Erythroid Response	No.Pts TI
002	214	169 (79%)	2.7	4 Units	73 (43%)	42 (25%)
003	146	122 (82%)	3.4	5 Units	110 (75%)	95 (65%)

Quality of life / Chronic disorders

0773

NON-HODGKIN'S LYMPHOMA AND OTHER NON-HEPATIC MALIGNANCIES IN SWEDISH PATIENTS WITH HEPATITIS C VIRUS INFECTION

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Background. The incidence of non-Hodgkin's lymphoma (NHL) has for not yet identified reasons increased during the last decades. Several case-control studies has suggested an increased risk for NHL correlated to infection with hepatitis C virus (HCV). Aim The aim of this study was to evaluate the association between HCV infection and NHL, multiple myeloma (MM), thyroid cancer (TC), chronic lymphatic leukaemia (CLL), acute lymphatic leukaemia (ALL), and Hodgkin's lymphoma (HL). *Methods.* The Swedish cohort of 27 150 HCV infected persons, notified during 1990-2000, was included in the study. The database was linked to other national registers to calculate the observation time, expressed as person-years, and to identify all incident malignancies in the cohort. The patients were stratified according to assumed prior time of HCV infection. The relative risk of malignancy was expressed as a standardised incidence ratio (SIR), the observed number compared to the expected number. *Results.* During 1990-2000 there were totally 50 NHL, 15 MM, 14 ALL, 8 TC, 6 CLL and 4 HL diagnosed in the cohort. Altogether 20 NHL, 7 MM, 5 TC, 4 CLL, 1 ALL and 1 HL

patients fulfilled the criteria to be included in the statistical analysis. The observation time was 122 272 person-years. The risk of NHL and MM was significantly increased in the stratum with more than 15 years of infection, SIR 1.89 (95% CI, 1.10-3.03) and 2.54 (95% CI, 1.11-5.69). For TC and CLL the association was not significant. *Conclusions.* This is the first study of the incidence of these malignancies in a nationwide cohort of HCV infected persons. Although the delayed diagnosis of HCV probably has made us underestimate the risk, this study showed a significantly increased risk of NHL and MM.

0774

A MODIFIED S-303 PATHOGEN INACTIVATION PROCESS ELIMINATES IMMUNOREACTIVITY OF S-303 RBC DETECTED IN PIVOTAL CLINICAL TRIALS

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Background. S-303 inactivates viruses, bacteria, protozoa, and leukocytes in red blood cell concentrates (RBC). S-303 is a modular FRALe compound (FRangible Anchor Linker Effector) designed to bind to nucleic acids with its Anchor, to react through its Effector, and form nucleic acid cross-links that inactivate pathogens. The treatment process was optimized to maintain RBC function and maximize pathogen inactivation (PI). Pre-clinical animal studies showed no detectable antibodies (Ab) to S-303 RBC (original method; O-SRBC). Clinical studies in healthy volunteers, demonstrated that autologous O-SRBC had post-transfusion recovery and life span comparable to control RBC (CRBC). O-SRBC supported acute anemia comparable to CRBC in a pivotal clinical trial. In pivotal trials evaluating allogeneic O-SRBC support of chronic and acute anemia, low titer Ab against O-SRBC were detected in 2 of 17 chronic patients exposed to O-SRBC, and 2 of 74 control acute patients exposed only to CRBC. Hapten inhibition studies demonstrated Ab specificity for the Anchor group of S-303. *Aims.* An improved S-303 treatment process (modified method; M-SRBC) was developed and characterized for PI, *in vitro* function and reduction of immunoreactivity. *Methods.* The original PI process utilized 200 µM S-303 and 2 mM unbuffered GSH. The modified process uses 10-fold more neutralized GSH (20 mM) added to RBC up to 10 min prior to addition of S-303 (200µM). High titer rabbit anti-anchor sera (RaS) and monoclonal mouse Ab (mmAb) were elicited by immunization with a stable Anchor-KLH construct. FACS assays to detect decoration of RBC with S-303 were developed using RaS and FITC goat anti-rabbit(GAR) IgG or a FITC mmAb. Indirect Antiglobulin Tests (IAT) were performed with two high titer RaS and mmAb were tested with buffer gel cards (MTS), SRBC suspended in low ionic strength solution (LISS) and GAR IgG. Reactive patient sera were tested with SRBC and anti-IgG gel cards (MTS). *Results.* O-SRBC prepared with the original clinical process were positive for IAT for both the RaS (1:100) and for 4 of the 4 pivotal patient sera (1:3). FACS analysis using RaS (1:100) with FITC GAR IgG (1:64) demonstrated a high level of labeling. Under improved conditions M-SRBC exhibited negative IAT and minimal FACS signal above background with RaS or FITC-mmAb. Sera from the 4 patients with positive IAT against SRBC were negative against M-SRBC. Potent inactivation of bacteria (*S. epidermis*, *S. marcescens*, and *Y. enterocolitica*), viruses (Vesicular stomatitis virus) and leukocytes was retained in M-SRBC. Storage of M-SRBC for 42 days exhibited hemolysis and K+ levels comparable to CRBC and ATP levels higher than CRBC. The higher ATP levels were attributable to the higher GSH concentration and pH increase of M-SRBC. *Conclusions.* An improved PI process was developed that significantly reduces RBC decoration by S-303, while maintaining PI and *in vitro* M-SRBC properties. The new process eliminated the immunoreactivity observed with O-SRBC and sera from 4 patients in the pivotal clinical trials.

0775

EFFECT OF TREATMENT WITH RITUXIMAB IN PATIENTS WITH REFRACTORY AUTOIMMUNE CYTOPENIAS

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Background. The autoimmune cytopenias encompass the disorders of immune thrombocytopenia purpura (ITP), autoimmune haemolytic anaemia (AIHA), autoimmune neutropenia (AIN), pure red cell aplasia (PRCA) and various combinations of these conditions. AIMS We describe 32 patients with severe autoimmune cytopenias resistant to standard immunosuppression treated with the anti-CD20 monoclonal antibody Rituximab. 16 patients had ITP, 6 AIN, 2 AIHA, 1 PRCA, 3 AIHA and ITP (Evan's syndrome), 2 AIHA and AIN, 1 PRCA and ITP, and 1 AIN and ITP. 25 patients had primary autoimmune cytopenias and in 7 patients, the cytopenia was secondary to an underlying disorder (3 B-NHL, 1 T-NHL, 1 B-CLL, 1 NK-LGL and 1 SLE). **Methods.** Rituximab was administered intravenously at a dose of 375mg/m² weekly for 4 weeks although in one patient treatment was discontinued after 3 infusions due to a hypotensive syncopal episode. No other major infusion related side effects were observed. **Results.** Responses were seen in 10/16 patients with ITP, 2/2 patients with AIHA and 3/3 patients with AIHA and ITP. Responses were sustained in 8/10 ITP patients with median follow up of 28.4 months (range 9–64 months). In contrast, there was no response in 5/6 patients with AIN and in both patients with PRCA. In the 2 patients with a combination of AIN and AIHA there was no response in the AIN and in the patient with AIN and ITP there was also no response in the AIN but response observed in the ITP. Two patients died of cytopenia related complications: one with AIN unresponsive to treatment died of pneumocystis pneumoniae infection, the other with ITP died of gastrointestinal haemorrhage and respiratory failure. **CONCLUSIONS** In conclusion, Rituximab appears to be a valuable therapeutic agent in ITP and AIHA inducing sustained responses with minimal toxicity. In contrast, no responses were seen in refractory AIN and PRCA. fareedaj@hotmail.com

0776

HEMOGLOBIN LEVEL AND OTHER FACTORS THAT INFLUENCE FACT-AN ASSESSMENT OF QUALITY OF LIFE IN CHEMOTHERAPY-TREATED LYMPHOMA PATIENTS

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Background. Anemia and fatigue can significantly diminish quality of life (QOL) in cancer patients. The Functional Assessment of Cancer Therapy-Anemia (FACT-An) subscale has been used successfully to evaluate effects of anemia and fatigue on

QOL in cancer patients. Studies in anemic patients with various non-myeloid malignancies have shown a relationship between hemoglobin (Hb) levels and FACT-An scores (Crawford, 2002; Fallowfield, 2002; Lind, 2002). A cross-sectional survey in patients with multiple myeloma demonstrated—in addition to Hb level—age, sex, chemotherapy response, staging, and comorbidity may significantly predict FACT-An scores (Palumbo, 2002). **Aims.** Examine the relationship between FACT-An scores and several factors, including Hb level, in lymphoma patients with or without anemia, before and after chemotherapy. **Methods.** Patients with non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma (HL) were enrolled in this prospective multicenter study conducted in Italy. Hb level and other characteristics were recorded at entry (before 1st chemotherapy cycle), and patients completed the 13 fatigue and 7 non-fatigue items of the FACT-An subscale. At final visit, Hb determination and QOL assessments were repeated and response to chemotherapy was recorded. Multiple logistic regression analyses estimated the effect of age, sex, disease phase and stage, International Prognostic Index [NHL only], and Hb at entry on FACT-An scores while adjusting for the effect of all other factors. For final FACT-An scores, chemotherapy type, response to therapy, final Hb, age, sex, disease phase, and stage were considered. A cross-sectional model that predicts post-chemotherapy FACT-An scores in terms of characteristics existing at final visit alone, and a longitudinal model that predicts final FACT-An scores using baseline scores and baseline Hb, as well as Hb changes during the study were developed. **Results.** The study enrolled 357 patients (NHL, 245; HL, 112) from 15 centers; 330 remained in the study after chemotherapy. Mean baseline Hb was 12.57±1.85 g/dL for NHL patients and 12.09±1.31 g/dL for HL patients. For both groups, cross-sectional analysis showed older age was significantly associated with lower FACT-An scores, indicative of poorer QOL (P = 0.0085-P = 0.0006); response to chemotherapy was significantly associated with higher FACT-An scores, indicative of better QOL (P = 0.034-P = 0.030). In NHL patients, higher baseline and final Hb levels were significantly associated with higher baseline and final FACT-An scores, respectively (P = 0.0001). In HL patients, only baseline Hb was significantly related to FACT-An scores (P = 0.013), possibly reflecting the greater effect of age and tumor response on FACT-An in HL than NHL patients. In the longitudinal analysis, baseline FACT-An score, age, baseline Hb, Hb change, response to chemotherapy, and bleomycin chemotherapy were identified as factors that may significantly predict subsequent FACT-An scores; effect of size and direction (+ = better, - = worse), as well as significance of the factors, are shown in the table by diagnostic group. **Conclusions.** These results provide further evidence of Hb as predictive for FACT-An scores and QOL and show other factors must be considered when evaluating impact of Hb level on QOL.

Table. Predictors of QOL in Lymphoma Patients.

Factor	Non-Hodgkin Lymphoma (N=245)		Hodgkin's Lymphoma (N=112)	
	Mean effect (95% CI)	P	Mean effect (95% CI)	P
Baseline FACT-An score (age-adjusted)	-0.15 (-0.12, -0.17)	0.006	-0.15 (-0.24, -0.06)	0.001
Age, yr	-0.06 (-0.12, -0.01)	0.017	-0.15 (-0.25, -0.05)	0.011
Baseline Hb, g/dL	0.17 (0.04, 0.18)	0.001	-	-
Hb change, g/dL	0.32 (0.25, 0.28)	0.001	-	-
Response to CT	-	-	-	-

0777**HOW SATISFIED ARE PATIENTS IN ITALY WITH THE TREATMENT OF THEIR HAEMOPHILIA? RESULTS OF THE COCHE STUDY**S. von Mackensen¹, A. Gringeri², L. Scalone³, L. Mantovani³, COCHE Study Group⁴¹Institute for Medical Psychology, HAMBURG, Germany; ²Haemophilia & Thrombosis Centre, MILAN, Italy; ³Centre of Pharmacoeconomic, MILAN, Italy

Background. Treatment of patients with haemophilia requires a substantial amount of economical and human resources and therefore it is mandatory to investigate treatment satisfaction of haemophilic patients, which is important as well for their quality of life and self-management abilities. In the COCHE Study patients with moderate and severe haemophilia without inhibitors have been investigated in Italy concerning cost of care, quality of life and treatment satisfaction. **Aims.** Evaluation of patients experience of different treatment regimens. Description of treatment satisfaction in haemophilia patients in Italy across treatment options and clinical conditions. Identification of relevant determinants of treatment satisfaction. **Methods.** Prevalence-based, longitudinal retrospective and prospective observational study of cost of care and quality of life assessment. Quality of life is assessed with generic (SF-36 and EQ-5D) and haemophilia-specific (Haem-A-QoL) questionnaires. Treatment satisfaction is assessed with the first haemophilia-specific treatment satisfaction scale for adults (Hemo-SatA). In order to obtain socio-demographic, clinical, and economic information, a detailed semi-structured medical documentation is filled out by physicians. In addition every enrolled patient fill in a daily diary any medical event occurring during the follow-up period. **Results.** 230 patients without inhibitors with a median age of 34 years (18-74 years) were enrolled in the COCHE Study. 86.5% had haemophilia A, and 73.4% of the patients were severely affected. Psychometric results from the pilot testing of the newly developed Hemo-SatA could be confirmed. The Hemo-SatA questionnaire consisting of 34 items pertaining to 6 dimensions (ease & convenience, efficacy, burden, specialist/nurses, centre/hospital, general satisfaction) showed excellent psychometric characteristics in terms of reliability (Cronbach's alpha ranging from .71 - .90) and validity. Significant differences were found for treatment options. **Conclusions.** Treatment satisfaction is an important outcome criterion in the treatment of patients with chronic diseases. For the adequate assessment of treatment satisfaction disease-specific measurements are necessary. The Hemo-SatA questionnaire proved to be a valid and reliable instrument and is currently included in the ESCHQoL Study in more than 1500 patients with haemophilia from 22 European countries.

Bleeding disorders**0778****GENE MUTATION AND ITT RESPONSE IN THE ITALIAN COHORT OF PATIENTS WITH HAEMOPHILIA A**G. Tagariello¹, R. Salviato¹, D. Belvini¹, P. Radossi¹, E. Zanon², G. Castaman³, G. Gandini⁴, R. Sartori¹¹Centro Reg. per le Malattie del Sangue, CASTELFRANCO VENETO, Italy; ²Centro Emofilia Azienda Osp. Padova, PADUA, Italy; ³Ospedale San Bortolo Vicenza, VICENZA, Italy; ⁴Centro Emofilia Osp. Policlinico Verona, VERONA, Italy

Background. administration of daily high doses of factor VIII concentrates by intravenous infusions, known as immune tolerance therapy (ITT), can induce tolerance to the exogenous protein and eradicate the antibody response to FVIII, which still represents the main complication of replacement therapy in

haemophilia A (HA) severe cases. **Methods.** we have investigated 71 HA patients with inhibitors (sixty-nine severe, one moderate and one mild cases) for the causative mutations using LR-PCR for the intron22 inversion, multiplex-PCR for intron1 inversion and conformation sensitive gel electrophoresis (CSGE) followed by DNA sequencing for other mutation types in the F8 gene. **Results.** diverse genetic defects were detected in the severe cases with a predominance of null mutations: F8 gene inversions, large deletions and nonsense mutations account for 68% of the mutations, whereas specific missense mutations were identified in the two non severe patients. ITT has been used in 16 HR patients of this cohort and it failed in 5 cases. **Conclusions.** we confirmed that the presence of inhibitors correlates well with the presence of null mutations, as firstly reported by Schwaab et al (Thromb Haemost 1995). Several predictive factors for ITT outcome have been so far taken into consideration but no correlation has been made between F8 genotype and ITT response. In our cohort two large deletions, two intron22 inversions and a nonsense mutations failed to respond. For the remaining 2 large deletions no ITT were attempted so no information are available. Large deletions appear to be a high risk genetic factor not only for inhibitor development but also for long term inhibitors persistence and ITT unresponsiveness. The identification of these patients at very high risk of inhibitor development and ITT unresponsiveness by mutation analysis soon after the diagnosis should therefore be strongly recommended, even because of the high cost of the therapy.

0779**THE EUROPEAN ACQUIRED HAEMOPHILIA (EACH) REGISTRY**F. Baudo¹, P. Collins², A. Huth-Kühne³, H. Levesque⁴, P. Marco⁵, L. Nemes³, L. Tengborn⁶¹Niguarda Hospital, MILAN, Italy; ²University of Wales Coll. of Medicine, CARDIFF, UK; ³Haemophilia Centre, HEIDELBERG, Germany; ⁴Department of Internal Medicine, ROUEN, France; ⁵Hospital General Avda, ALICANTE, Spain; ⁶Universitetssjukhuset MAS, MALMÖ, Sweden

Acquired haemophilia (AH) is a rare bleeding disorder characterized by the development of autoantibodies to factor VIII, and less often factor IX. The bleeding is usually severe and often fatal. The sudden onset of bleeding can be either spontaneous or induced by surgery or trauma. Treatments of AH are diverse and currently there is no standardized therapeutic modality. The European Acquired Haemophilia (EACH) Registry (www.eachregistry.org) has been established to collect data on patients with AH across Europe. Data are collected from patients who were treated from 1 January 2003 onwards. The information collected includes underlying condition, cause of bleeding (spontaneous or surgery/trauma related), site and severity of bleeding, type and titre of inhibitor, haemostatic treatment and immunosuppressive therapy (agents used, regimens, results and adverse events). Up to December 2004, 105 patients (59 males and 46 females) have been registered from 13 countries across Europe. The median age was 67 years (range 13-93). The details of the underlying conditions (no. of patients) were as follow: autoimmune diseases (14), neoplasm (12), post-partum (17), and other conditions (13). The development of autoantibodies was idiopathic in 49 patients. The median inhibitor titre was 20 BU (range 1-816). Overall, there were 122 bleeding episodes (95 spontaneous, 27 surgery/trauma-induced). Sixty-four bleeding episodes required one or multiple therapeutic modalities (36 rFVIIa, 13 APCC, 8 FVIII, 7 fresh frozen plasma, 4 immunoadsorption, 3 DDAVP). Seventy-four patients received immunosuppressive therapy (steroid alone or in combination with other drugs). In total, 13 patients died (3 of bleeding and 10 of the underlying conditions). Based on the registry data, bleeding mortality rate appears to be lower than that reported in the literature. The outcome of the EACH Registry will provide a basis for the standardization of the treatment of AH in the future. A full analysis of the data has been planned by the end of May 2005.

0780**THE USE OF FIBRINOGEN CONCENTRATE IN ACQUIRED HYPOFIBRINOGENAEMIC STATES**

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Background. Acquired hypofibrinogenemic states such as disseminated intravascular coagulation (DIC) are associated with bleeding. Cryoprecipitate is the standard product used for fibrinogen replacement. Concerns about viral and prion infection have led to interest in the use of recombinant or purified plasma products. A purified, virally-inactivated fibrinogen concentrate derived from pooled human plasma is available, and has been used on an unlicensed basis to treat a number of patients with acquired hypofibrinogenemic states in our centre. **Aims.** We aimed to assess the indications, dosage, efficacy, safety and cost of fibrinogen concentrate in patients with acquired hypofibrinogenemic states. **Methods.** Using inpatient hospital notes and laboratory records, we undertook a retrospective single-centre study of patients treated with fibrinogen concentrate (Haemocomplettan, ZLB Behring) for acquired hypofibrinogenemic states. **Results.** 33 adult patients were treated between 1 January 1999 and 10 October 2004; median age was 40 years (range 17-85). Indications for fibrinogen replacement were: placental abruption (8 patients); massive blood loss and transfusion (8 patients); liver failure (6 patients); post-cardiothoracic surgery (5 patients); sepsis (2 patients); other (4 patients). Median transfusion requirements during the 24 hours prior to and 72 hours following fibrinogen replacement were: 13 Units red cells (range 0-66); 2 pools platelets (range 0-11); 9 Units fresh frozen plasma (range 0-41); 0 bags cryoprecipitate (range 0-55). The median initial dose of fibrinogen concentrate was 4g (range 2-14g). 39% of patients received a median of one further dose during their inpatient stay. Median subsequent dose of fibrinogen concentrate was 4g (range 2-12g). Mean Clauss fibrinogen level pre-treatment was 0.81 g/L (range 0.17-1.97 g/L). Mean fibrinogen increment was 0.30 g/L per 1g fibrinogen concentrate administered (range 0.07-0.83). Mean Clauss fibrinogen following treatment was 2.10 g/L (range 0.92-3.55). 79% of patients were bleeding at the time of fibrinogen administration. Of these patients, 46% stopped bleeding following product replacement alone (using fibrinogen concentrate, blood components and plasma products), 30% stopped bleeding after both product replacement and intervention (surgical or endoscopic), and 23% did not stop bleeding. Overall inpatient mortality was 37%. No venous thromboses were documented. Three ischaemic cerebrovascular accidents were observed, all of which occurred in patients with strong cardiovascular risk factors, bleeding and DIC. None could be attributed to over-replacement with fibrinogen concentrate. Before taking costs of storage, laboratory time and administration into account, the cost of fibrinogen concentrate was similar to that of cryoprecipitate. **SUMMARY/Conclusions.** Fibrinogen concentrate may represent an efficacious and cost-effective alternative to cryoprecipitate in the management of bleeding in acquired hypofibrinogenemic states such as DIC. The fibrinogen increment following a given dose of fibrinogen concentrate varies, possibly reflecting the degree of ongoing fibrinogen consumption and concurrent administration of fresh frozen plasma. Clauss fibrinogen levels must be monitored to determine the need for further fibrinogen replacement.

0781**VESSEL WALL DISCONTINUITIES AND INCREASED EXPRESSION OF VEGF-A AND VEGF RECEPTORS 1 AND 2 IN ENDOMETRIAL BLOOD VESSELS OF MENORRHAGIA PATIENTS**M. Mints¹, E. Zetterberg¹, R. Rogers², K. Hultenby¹, J. Palmblad¹¹Karolinska Institutet, STOCKHOLM, Sweden; ²Harvard School of Publ. Health, BOSTON, USA

Background. Angiogenesis is required for growth and repair of injured tissues as well as for growth of tumors, leukemias etc.

One example of dramatic angiogenesis is the normal cycling human endometrium (EM), where regrowing glandular tissue needs new vessels after every period. Perturbation of this process has been associated with idiopathic menorrhagia (IM). **Aims.** We investigated whether the structure or regulation of the growth of EM blood vessels might be abnormal in women with IM. **Methods.** EM biopsy specimens, obtained from 18 healthy women and 24 patients with IM, were analyzed by immunohistochemical staining and morphometry. **Results.** EM vessels in both patients and controls manifested an unusual morphology characterized by focal discontinuities, or gaps, in endothelial staining for CD34, CD31, and von Willebrand factor. Electron microscopy revealed that perivascular cells covered these gaps in the vessel wall. The relative size of the gaps was significantly greater in IM patients than in controls (median values, 8.0 and 4.7% of the perimeter of the vessel wall, respectively; $P = 0.000002$). Vessel perimeter was also larger and more vessels were positive for vascular endothelial growth factor-A (VEGF-A) as well as for VEGF receptors 1 and 2 in patients than in controls. Moreover, gap size was significantly correlated with the number of vessels expressing VEGF-A or VEGF receptor 1. **Conclusions.** These results suggest that EM blood vessels possess a specific morphology characterized by cytoplasmic discontinuities in the endothelium, and that these gaps are more pronounced in women with IM, are related to overexpression of VEGF-A and VEGF receptor 1, and might contribute to the excessive blood loss associated with menorrhagia. The human endometrium offers unique possibilities to study intense angiogenesis under normal and pathologic conditions.

0782**PROSPECTIVE EVALUATION OF LOW PAI-1 ACTIVITY AS A RISK FACTOR FOR HEMORRHAGIC DIATHESIS**

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Background. A majority of investigations of patients with a bleeding diathesis turn out negative. Several cases with deficiency of plasminogen activator inhibitor type 1 (PAI-1) have been described, but no large-scale prospective study of the epidemiology and clinical significance of low PAI-1 activity has been performed. **Aims.** This study was performed to evaluate the clinical significance of a low PAI-1 activity to provide a guideline for possible inclusion of this analysis in a routine investigation for bleeding tendency. **Method:** In 586 consecutive patients, referred due to a bleeding diathesis, the analysis of PAI-1 activity was added to the routine investigation. In 512 of the patients the tissue plasminogen activator complex with PAI-1 (t-PA-PAI-1) was measured with an immunoassay. The bleeding history was classified as clinically significant or not. As controls 100 blood donors and 100 age- and sex-matched healthy individuals were tested for PAI-1 activity, and the latter were also evaluated regarding previous bleeding episodes. **Results.** Criteria for a clinically significant bleeding history were fulfilled in 75% of the patients and 18% of the healthy controls. The laboratory investigation of the patients revealed a platelet function defect in 143 (24.4%), von Willebrand Disease in 59 (10.1%), thrombocytopenia in 7 (1.2%) and various vascular or coagulation factor defects in 39 (6.7%), but it was negative in 336 patients (57%). A low PAI-1 activity, defined as <1.0 IU/mL, was found in 23% of the patients and in 13% and 10% of the blood donors and healthy controls, respectively; odds ratio and 95% confidence interval: 2.04; 1.11-3.77 and 2.75; 1.39-5.42, respectively. The proportion of PAI-1 <1.0 IU/mL was higher among females, was inversely correlated with body mass index but did not correlate with age, when adjusted for oestrogen use. No specific symptom predicted for low PAI-1, and the latter did not aggravate the clinical picture when combined with other defects. Low tPA-PAI-1:Ag was not associated with increased bleeding tendency. **Conclusions.** Low PAI-1 activity is seen in a substantial proportion of patients with a bleeding tendency, but is a risk factor of minor clinical importance and it is not associated with any specific bleeding manifestation.

Non-Hodgkin lymphoma - Clinical

0783

HIV-RELATED NON-HODGKIN'S LYMPHOMAS BEFORE AND AFTER HAART: FINAL ANALYSIS ON 485 PATIENTS TREATED WITH RISK-ADAPTED INTENSIVE CHEMOTHERAPY FROM THE FRENCH-ITALIAN COOPERATIVE GROUP (GELGICAT)

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Background. Treatment of AIDS-related lymphoma (ARL) is still controversial, as intensive chemotherapy could exacerbate immunodeficiency, with subsequent adverse effects for patients. We aimed to compare the effectiveness of risk-adapted intensive chemotherapy in patients with ARL treated in the NHL-HIV 93 randomized trial, before and after the use of highly active antiretroviral therapy (HAART). **Methods.** 485 patients aged from 18 to 67 years were randomly assigned to CHOP based chemotherapy with a pretreatment stratification according to the HIV score which is based on performance status, prior AIDS and CD4+ cell counts <0.1 G/L. Chemotherapy was administered in conjunction with HAART for the 187 patients enrolled after June 1996. Three main histologic types of NHL were represented in the study population: diffuse large cell lymphoma (54%), Burkitt lymphoma (20%) and immunoblastic lymphoma (17%). **Results.** With 6 years median follow-up, in the low risk group (HIV score=0, n=218) the OS was estimated at 51% for high-dose CHOP (ACVBP) versus 47% for CHOP (p=0.85). In the intermediate risk group (HIV score=1, n=177), it was 28% for CHOP versus 24% for Id-CHOP (p=0.19) and in the high risk group (HIV score=2-3, n=90), 11% for Id-CHOP versus 3% for VS (vincristine, steroid) (p=0.14). CR rate of CHOP was significantly different of the Id-CHOP (49% vs. 32%, p=0.02) but not of the ACVBP (51% vs. 61%, p=0.17). Patients in the post HAART area experienced the same CR rate and toxicity during chemotherapy than patients in the pre-HAART area. After adjusting for the follow up, OS was higher in the post HAART area (21% vs. 37% at 3 yrs, p<0.001) but there was no difference between the therapeutic effects of the chemotherapy arm in each risk group. Time dependent Cox model demonstrated that the only significant factors were HAART (RR 1.6, p=0.0002), HIV score (RR 1.7, p=0.0001) and IPI score (RR 1.5, p=0.0012). **Conclusions.** Our findings indicate that benefit of ACVBP in ARL is not improved over the standard CHOP chemotherapy even in the post HAART area. On the other hand, Id-CHOP is not an alternative for CHOP in higher risk patients because it led to lower CR rate and poor survival.

0784

TREATMENT WITH RITUXIMAB, CHOP AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN AIDS-RELATED DIFFUSE LARGE B-CELL LYMPHOMAS (DLBCL). STUDY OF 48 PATIENTS

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Background. and aims. Rituximab and CHOP (RCHOP) is the most employed treatment for DLBCL, but patients with AIDS-related lymphomas are usually excluded from clinical trials. The

objective of this open, prospective multicenter observational trial is to evaluate the feasibility, efficacy and toxicity RCHOP and HAART in AIDS-related DLBCL. **Patients and methods.** Between April 2001 and November 2004, 48 consecutive HIV-infected patients with newly diagnosed DLBCL were included in 19 Spanish hospitals. HAART was given to all patients from diagnosis if they were not already receiving it. Six cycles of RCHOP were administered, IT CNS prophylaxis (MTX, ARA-C and hydrocortisone) was given in every cycle to all patients. G-CSF support was recommended Response to chemotherapy, toxicity, OS and DFS for complete responders were recorded. **Results.** Median age of the patients was 42 (range 26-64) years, 39 (81%) were males, 29 (60%) had a previous diagnosis of HIV infection. Median CD4 lymphocyte counts was 1547 μ L (range 0-1,500) and a median viral load of 28x10³ copies/mL (range 0-2x10⁶). Extranodal involvement was present in 32 (66%) patients, 36 (75%) had stages III-IV and 23 (48%) had intermediate-high or high age-adjusted IPI score. Six patients are still under treatment. A complete response (CR) was achieved in 30 of 42 evaluable patients (71%). The death rate during treatment cycles was 10% (5 patients, infection in 3, hepatic failure in 2) and 5 additional patients (10%) died of progressive disease. Patients with advanced (III/IV) stage presented significantly lower CR rates (19/36, 53%) than patients with localized DLBCL (11/12, 92%) (p=0.016). After a median follow-up of 1.3 yr, the probability of 1-yr. survival was 71% (CI95% 52-83). The probability of remaining alive and in first CR at 1 year for complete responders was 88% (CI95% 76-98). Three patients died in first CR (hepatic failure, sudden death and violent death) and no relapses have occurred to date. Virologic response to HAART at 6 months after the completion of treatment was maintained or achieved in 19/30 (66%) of patients. **Conclusions.** In patients with AIDS-related DLBCL the combination of HAART and RCHOP is feasible and effective. At this moment the response rate and survival are comparable to those obtained in immunocompetent patients treated with RCHOP.

0785

THERAPEUTIC MANAGEMENT, PROGNOSTIC FACTORS AND CLINICAL OUTCOME OF 416 PATIENTS WITH PRIMARY EXTRANODAL HEAD AND NECK NON-HODGKIN'S LYMPHOMA (IELSG23)

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Background. Head and Neck (HN) is the second most common site of localised extranodal presentation of non-Hodgkin's lymphoma and it is at high risk of CNS recurrence. **Aims.** To evaluate the clinical outcome, prognostic factors and the rate of CNS recurrence in patients with HN lymphoma. **Methods.** From December 1990 to June 2004, 416 patients (median age 60, range 18-85) were referred to 11 international centers. The most common sites were Waldeyer's ring (65%), parotid and salivary glands (12%) and nose and paranasal sinuses (8%). The prevailing histology subgroups were DLBCL (74%) and MALT (10%). Adverse prognostic features included: stage II (65%), elevated LDH (15%), elevated beta 2-microglobulin (14%), bulky disease (10%), No of extranodal sites>1 (9%), B symptoms (8%), ECOG-PS>1 (6%), and stage modified IPI (MIPi)>1 (48%). Two hundred fifteen patients were treated with chemotherapy (n=160), surgery (n=15), or radiotherapy (n=40) alone, while 157 received CHOP or CHOP like regimens + IFRT. Only 34/348 (10%) patients received CNS prophylaxis (Methotrexate 12 mg

i.t.; median cycles 3, range 1-6). **Results.** Three hundred fifty-six patients (87%) achieved a complete remission, 22 a partial remission and 31 were resistant to therapy. Acute toxicity (G2-3) mostly consisting in xerostomia occurred in 6.5% of patients and treatment-related mortality was 0.5%. Among 356 responders, 91 (26%) eventually relapsed, 40% in the same site, 54% in other sites and 6% in both. Only 1/234 patients (0.4%), who did not receive prophylaxis, relapsed in CNS. After a median follow-up of 41 months (range 6-220), 5-year estimate of OS, EFS, DFS was 72%, 54% and 69%, respectively. EFS at 5 years varied according to the site of presentation (66% in oral cavity vs 30% in thyroid) and the histology (60% in DLBCL vs 40% in MALT). Combined treatment was superior to single therapy (5-yr EFS, 64% vs 45%; $p=0.0000$). By Cox multivariate analysis, a risk factor >1 according to MIPI predicted a poor EFS. **Conclusions.** The present study showed that the site of disease, histology, MIPI and a combined treatment influence the outcome of patients with primary HN lymphoma. Moreover, in the present series a very low rate of CNS recurrence occurred in high risk patients, who did not receive adequate prophylaxis, suggesting that CNS prophylaxis could not be mandatory in HN patients. This should be confirmed by prospective studies of clinical outcome.

0786

YTTRIUM 90 (90Y) IBRITUMOMAB TIUXETAN (ZEVALIN®) INDUCES LONG-TERM RESPONSES IN PATIENTS WITH RELAPSED OR REFRACTORY FOLLICULAR LYMPHOMA (FL)

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Background. Radioimmunotherapy (RIT) is an emerging clinical treatment option for patients with non-Hodgkin's lymphoma (NHL). Yttrium 90 (90Y) ibritumomab tiuxetan (Zevalin®) RIT was approved by the FDA in February 2002 for the treatment of patients with relapsed or refractory low-grade, follicular, or transformed B-cell NHL, including patients with rituximab-refractory follicular NHL. In 4 registrational trials of 90Y-ibritumomab tiuxetan conducted between 1996 and 1999, 211 patients with B-cell NHL were treated. Of these 211 patients, 153 patients (73%) had follicular lymphoma (FL). With ongoing follow-up, long-term durable responses have been observed, but until now have not been more fully characterized. **Methods.** Responding patients with time to progression (TTP) of ≥ 12 months were identified and characterized as long-term responding (LTR) patients. **Results.** In the 4 registrational trials, 78 of the 211 patients (37%) were identified as LTR patients. Characteristics of these LTR patients were as follows: median age 58 years (range, 24 to 80), 44% >60 years old, 55% male, 76% with FL, and 41% with lymphomatous marrow involvement. LTR patients had a median of 2 prior regimens (range, 1-7): 59% had ≥ 2 prior therapies, 33% had ≥ 3 prior therapies, and 37% had no response to their last prior therapy. Thirty percent of LTR patients had bulky disease (tumor size >5 cm) and 83% had stage III/IV disease. At the time of this analysis, the median duration of response (DR) and TTP for LTR patients were 28 months (range 11-80) and 29 months (range 12-82), respectively, with a median follow-up of 50 months (range 13-82). The median DR to the last prior therapy for LTR patients was 12 months. The complete response rate (confirmed [CR] and unconfirmed [CRu]) among LTR patients was 65%, and the median DR and TTP were 29 and 31 months, respectively, for CR/CRu patients. In ongoing responders the median DR is 52 months (range 48-80). Among the 153 patients with FL, 59 (39%) were identified as LTR patients. Compared to the overall LTR patients, LTR patients with FL had similar disease characteristics, DR, TTP, and CR/CRu rates. **Conclusions.** Ongoing

follow-up indicates that 90Y-ibritumomab tiuxetan frequently produces durable long-term responses (TTP ≥ 12 months) in patients with relapsed or refractory B-cell NHL. Failure to respond to the therapy immediately prior to treatment with 90Y-ibritumomab tiuxetan does not appear to affect the ability to achieve long-term responses with 90Y-ibritumomab tiuxetan. Durable long-term responses were achieved in 37% of all patients and 39% of patients with FL treated in 4 registrational trials of 90Y-ibritumomab tiuxetan at 30 centers in the US. Of these LTR patients, a high proportion were >60 years old and had received ≥ 3 prior therapies.

0787

INCIDENCE AND RISK FACTORS OF CENTRAL NERVOUS SYSTEM RECURRENCE IN AGGRESSIVE NHL-A SURVEY OF 1693 PATIENTS TREATED IN PROTOCOLS OF THE GERMAN HIGH-GRADE LYMPHOMA STUDY GROUP (DSHNHL)

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Central nervous system (CNS) relapse is a devastating and usually fatal complication of aggressive lymphoma. In earlier series the incidence of CNS recurrence for patients (pts.) not receiving prophylactic intrathecal (i.th.) treatment was about 5%. Risk factors reflecting the extent of the disease, the proliferation rate or the site of extranodal involvement have been discussed, but up to date there is no general agreement neither on subgroups of patients with increased risk of relapse nor on the optimal prophylaxis. We analyzed the patients treated on DSHNHL-Studies between 1990-2000, evaluated the rate and prognostic factors for CNS-recurrence and developed a risk model trying to identify subsets of patients for future prophylactic strategies. From 1993-2000, 1500 patients (< 60 yrs. with normal LDH and > 60 yrs. irrespective of LDH) were randomized to receive 6x CHOP-21/14 vs. 6x CHOEP-21/14 in the NHL-B Studies. 312 patients < 60 yrs. with an elevated LDH were randomized from 1990-1997 to 5 cycles CHOEP + IF radiotherapy or three cycles CHOEP followed by high-dose BEAM and autologous stem-cell transplantation (NHL-A Study). In all, 1693 patients were evaluable, of whom 37 developed relapse or progression to the CNS (2.2%). I.th. prophylaxis was mandatory for lymphoblastic lymphoma only ($n=9$), but additional 62 pts. (total 71/1693 = 4.2%) received prophylaxis by decision of the treating physicians. Multivariate Coxregression analysis identified increased LDH ($p = 0.004$) and in the NHL-B study population involvement of more than one extranodal site ($p=0.002$) as independent predictors of CNS recurrence. Treatment without Etoposide significantly increased the risk of CNS involvement (relative risk of 2.458 ($p=0.017$)), whereas the time between chemotherapy (21 vs. 14 days) did not influence the risk of CNS relapse. Extranodal sites such as sinuses, kidneys, adrenals or the liver were associated with an increased risk for CNS disease. Elderly patients presenting with both an elevated LDH and lymphoma involvement in one of these sites had an up to 10-fold risk of spread of disease to the CNS. In conclusion, the incidence of CNS relapse in 1693 pts. treated for aggressive lymphomas on DSHNHL protocols from 1990-2000 was low, especially considering the small proportion of pts. (4.2%) that had received i.th. prophylaxis. Multivariate regression analysis identified an increased LDH and involvement of more than one extranodal site as independent risk factors of CNS recurrence. Specific extranodal sites will have to be integrated into risk models for future selection of patient subgroups for whom CNS prophylaxis may be useful.

Anemia

0788

SUSTAINED CONTROL OF HEMOLYSIS AND SYMPTOMS AND REDUCED TRANSFUSION REQUIREMENTS OVER A PERIOD OF 2 YEARS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) WITH ECULIZUMAB THERAPY

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by intravascular hemolysis and venous thrombosis. Deficiency of the terminal complement inhibitor CD59 from PNH red cells results in complement-mediated hemolysis. Eculizumab, a humanized monoclonal antibody that inhibits terminal complement by binding to C5, is effective at controlling intravascular hemolysis in PNH. **Aims.** To present follow-up for over 2 years of eculizumab therapy in 11 transfusion-dependent PNH patients. **Methods.** All patients continued to receive 900mg eculizumab i.v. over 30 minutes 2 weekly. Assessed clinical and laboratory measures of hemolysis as well as transfusion requirements during therapy. **Results.** 10 of the 11 patients continued to receive eculizumab every other week for 2 years. The remaining patient stopped eculizumab after 23 months despite effective control of intravascular hemolysis, as the patient continued to be transfused even after erythropoietin therapy. This patient had the most severe hypoplasia at the start of eculizumab therapy with a platelet count below $30 \times 10^9/L$, suggesting that the ongoing transfusions were due to the bone marrow failure and not continuing hemolysis. No SAEs were attributable to eculizumab. The dramatic improvement in various parameters of hemolysis persisted during the 2 year treatment period for all patients. Mean LDH levels decreased from 3111 ± 598 U/L over the 12 months prior to treatment to 634 ± 34 U/L up to 24 months following treatment ($p=0.002$). PNH Type III red cells progressively increased during the treatment period from a mean of 36.7% to 58.9% ($p=0.001$) while PNH Type II red cells increased from 5.3% to 8.7% ($p=0.01$). There has been no change in the proportion of PNH neutrophils during eculizumab therapy indicating that the increase in the proportion of PNH red cells is due to a reduction in hemolysis and transfusions rather than a change in the PNH clone (s) itself. The mean and median transfusion rates decreased from 2.1 and 1.8 units/patient/month to 0.4 and 0.3 units/patient/month respectively ($p<0.001$). Three patients remained transfusion independent during the whole 2 year treatment period and the other 7 patients remaining on eculizumab all had at least a three-fold reduction in transfusion requirements. No patients have had a thrombosis during the study period, including 5 patients who were not on warfarin therapy. Following the first dose of eculizumab there has been a complete and sustained resolution of symptoms related to haemolysis including abdominal pain, dysphagia, erectile dysfunction and lethargy. These classical PNH symptoms have been attributed, at least in part, to the release of free hemoglobin (Hb) during intravascular hemolysis which overwhelms the protective Hb scavenging mechanisms. Free Hb efficiently absorbs and depletes nitric oxide (NO) which is a critical regulator of smooth muscle contraction, vascular tone and platelet activation. The potential role of NO in the symptomologies of PNH are currently under investigation. **Summary.** eculizumab is well tolerated and has persisting efficacy as demonstrated by decreased hemolysis and transfusions, as well as improvement in the symptoms of PNH, for over 2 years of therapy.

0789

INSULATOR LIKE ELEMENT IS DISPENSABLE FOR ALPHA GLOBIN FUNCTION

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Background. The eukaryotic genome is thought to be organized into self-contained functional units with specific levels and patterns of gene expression called chromosomal domains. Some evidence indicates that these domains are delimited by elements called boundaries or insulators. Insulators can block enhancer-promoter interactions when interposed between them and protect transgene from chromosomal positional effects. Nuclear protein CTCF associates to most boundaries/insulators described in vertebrates. The best characterized is the chicken beta-globin 5'HS4. In this element, enhancer blocking is associated with CTCF, but protection against position effect depends on several independent sites. One of these binds USF1 and 2. USF has been considered responsible for the recruitment of histone acetylation and dimethylation of lysine 4 of histone H3 (diMeK4 H3) that is necessary for 5'HS4 barrier activity. The major regulatory element and other cis-acting elements lie upstream of the alpha-globin cluster within the intron of a widely expressed gene providing a challenge to the insulator model. **Aims.** To investigate the presence of insulators at the alpha-globin cluster and to assess their role at their natural environment. **Methods.** DNase1 Hypersensitive sites were analyzed as previously (Genes Dev 1990; 4: 1588-1601). Chromatin immunoprecipitation (ChIP) assay was performed as described (EMBO J 2004; 23: 2841-2852) with antibodies against CTCF, anti-acetylated histone 3, anti-tetra-acetylated histone 4, anti-dimethyl H3 K4 (all from Upstate biotechnology), USF1, USF2 (Santa Cruz). Primers and 5'FAM-3'TAMRA labeled probes were selected from unique sequences in the alpha-globin locus and external controls using Primer Express. Input and immunoprecipitated material were analyzed by real time PCR as described (EMBO J 2004; 23: 2841-2852). Gene targeting was performed as described (Blood 2002; 100: 3450-3456). **Results.** We analyzed CTCF and USF binding at the alpha-globin cluster, finding elements that bind these proteins at conserved positions in human and mouse. These putative insulators localize with DNase 1 HSs at the ends of the previously described acetylation domain. These sites are also enriched in histone H3 and H4 acetylation and diMeK4 H3. Natural deletions affecting human 3' CTCF binding sequence have no phenotype. Targeted deletion of the mouse most 3' USF binding site produced normal mice and no change in histone acetylation pattern. On the other hand both human and mouse 5' CTCF binding site localize between erythroid specific DNase 1 HSs that bind erythroid specific transcription factors *in vivo*. **Conclusions.** There is evidence that in human and mouse beta and alpha globin clusters enhancers function by DNA looping. Insulator-like sequences could mediate this. In the insulator natural chromosomal environment the long distance between enhancer and promoter could provide sufficient freedom to allow interaction at both sides of the loop, this would enable a cis-acting element to interact with the alpha globin promoter even with an 'insulator' in between. On the other hand, in the absence of one of these structural elements, another similar element could substitute this function. This could be the case for the 3' element. In fact we have recently detected another CTCF binding site downstream the mouse cluster.

0790**IDENTIFICATION OF A NEW FAMILY OF SYNTHETIC HISTONE DEACETYLASES INHIBITORS THAT STRONGLY REACTIVATES [GAMMA]-GLOBIN GENE EXPRESSION BY A NOVEL SCREENING STRATEGY INVOLVING PRIMARY HUMAN ERYTHROBLASTS**

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Background. The most recent experience of gene therapy for genetic diseases has re-evaluated the importance of studies on post-natal pharmacological reactivation of HbF production for the cure for beta-thalassemia and Sickle Cell Anemia. Among the many compounds identified so far in these studies, of particular interest are chemicals capable to inhibit the catalytic activity of histone deacetylases (HDACs), a family of enzymes involved in chromatin remodelling and transcription regulation. The potential clinical use of the HDACs inhibitors identified so far, however, is unclear because of their modest effects on HbF synthesis and high toxicity. **Aim.** To identify new HDACs inhibitors for the treatment of beta-thalassemia and Sickle Cell Anemia using a novel two step strategy based on a first rapid screening on a cell line stably transfected with a dual luciferase reporter driven by either the human gamma- or beta-globin promoter followed by a second screening on synchronized primary human erythroblasts differentiated *in vitro* from normal donors. **Methods.** Seventeen different members of a new family of synthetic HDACs inhibitors, the Aroyl-pyrrolyl-hydroxy-amides (APHAs), were tested for their ability to change the ratio between the reporter activity driven by the human A-gamma- and beta-globin promoter in GM979 cells. Compounds active in this assay were then tested for their ability to raise gamma-globin expression (measured by quantitative Real Time RT-PCR) during four days of *in vitro* differentiation of primary adult erythroblasts. **Results.** As expected, the majority of the compounds tested did not affect the ratio between the two reporter activities expressed by GM979 cells. One of the compounds, however, MC1575, increased by 3-fold (from 0.09 to 0.30) the reporter ratio at a concentration, 2 microM, that modestly affected the proliferation of the cell line. When tested on primary erythroblasts, MC1575, at a concentration of 2-10 microM, increased by three-fold (from 0.04 to 0.12) the gamma/(gamma+beta) globin mRNA ratio determined by quantitative RT-PCR. At this concentrations, MC1575 had no appreciable effect on the viability of the primary cells. Among the compounds tested in this study, MC1575 did not represent the most potent inhibitor of total HDAC activity but was the chemical that most selectively inhibited the activity of the maize homologue of mammalian class IIa HDAC. **Conclusions.** We have identified a new compound, MC1575, capable to reactivate gamma-globin expression in primary adult erythroblasts. The chemical properties of this compound are consistent with the hypothesis that each class of histone deacetylases might have a specific biological function and indicate that HDAC of class IIa might represent those more specifically involved in globin gene regulation. We suggest that, by targeting the ADAC inhibitors toward the catalytic domain of the enzymes of class II, it might be possible to identify more specific, more potent and less toxic compounds for pharmacological treatment of beta-thalassemia or Sickle Cell Anemia.

0791**THALASSAEMIA SURVIVAL IN CYPRUS: EVIDENCE FOR A PROTECTIVE EFFECT OF COMBINATION CHELATION THERAPY WITH DESFERIOXAMINE AND DEFERIPRONE**

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Background. Thalassaemia births in Cyprus have dramatically reduced after implementation of a pre-marital carrier screening programme. Since the late 1970's, the remaining patients have received standard medical treatment with regular hypertransfusion (maintaining Hb>9.5g/dL) and iron chelation with desferrioxamine 20-50 mg/kg 5-6 per wk. In 1999-2000, in view of an increasing cardiac mortality, a combination chelation therapy protocol was introduced using desferrioxamine 30-50 mg/kg 2-5 nights per week and deferiprone 75 mg/kg/day for patients with high serum Ferritin (>2500µg/L) or with clinical or echocardiographic evidence of heart dysfunction. **Aims.** This study assesses the changes in survival trends over the period 1980-2003. **Methods.** A register of all patients under regular treatment in Cyprus since 1980 was created, and data collected on genotype, treatment, causes and dates of death. Statistical analysis was done using multi-variate analysis of hazard ratios, censoring data at 1st January 2004. **Results.** 656 patients were included, 158 of whom were treated with combination chelation therapy after the year 2000. There were 74 deaths, 39 of which were related to cardiac iron overload. The estimated survival to age 25 in those born 1970-4 was 94.8%, and in those born 1975-9 was 95.8%. There was a significant decrease in incidence of death after the year 2000. In multivariate analysis, significant independent risk factors for death were: male gender (2.3 times increased), year of birth before 1974 (3.3 times increased), and the time period 1995 to 2000 (2.3 times increased). **Conclusions.** This study shows excellent survival for younger thalassaemics in Cyprus. We interpret these results as showing a high risk of death due to 'treatment-adherence fatigue' with desferrioxamine during the period 1995-2000, and an improvement in survival brought about by more effective chelation with a combination regime including oral deferiprone

0792**CHARACTERISATION OF THE BIOCHEMICAL AND GENETIC DEFECT IN CONGENITAL GLYCOSYLPHOSPHATIDYLINOSITOL DEFICIENCY**

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Background. The glycosylphosphatidylinositol anchor is required for cell surface expression of multiple proteins with diverse functions. In paroxysmal nocturnal haemoglobinuria (PNH) blood cells lack surface expression of GPI-linked proteins because the initial step in GPI biosynthesis, i.e., addition of N-Acetyl glucosamine (GlcNAc) to a phosphatidylinositol (PI) base, is disrupted in haemopoietic stem cells as a result of somatic mutation (s) of the X-linked PIG A gene. Further sequential reactions in the biosynthesis of GPI involve de-acetylation to N-glucosamine, acylation of the inositol moiety, the addition of three mannose to N-glucosamine and subsequent modification with phosphoethanolamine (EtnP). Following covalent binding of proteins to terminal EtnP the intact structure is transferred to the cell membrane. To date PNH is the only human phenotype in which defective GPI biosynthesis has been

characterised. *Methods.* Biosynthesis of GPI was studied by radiolabelling and separation of intermediates by thin layer chromatography (TLC). Labelling with ³H-inositol, ³H-UDP-Glc-Nac and ³H-mannose was performed on EBV-transformed lymphoblastoid B cell lines generated from the affected children and unaffected family members. A B cell line harbouring a PIG-A mutation and its GPI+ counterpart and mouse thymoma cell lines with blocks at the addition of the third mannose residue (TB) and terminal EtnP (TF) were employed as controls. GPI intermediates were extracted and purified then separated on a silica gel by TLC and visualised by autoradiography. Homozygosity mapping was performed employing microsatellite markers flanking all 23 genes known to date to be involved in the biosynthesis of the GPI anchor. *Results.* We have identified two unrelated consanguineous families with congenital GPI deficiency. In both cases the propositus presented at an early age with hepatic vein thrombosis and exhibited neurological manifestations with partial seizures. In one family a sibling diagnosed presymptomatically and treated with anticoagulation to prevent thrombosis developed similar neurological complications. Flow-cytometry revealed partial deficiency of GPI and GPI-linked proteins. Metabolic labelling and TLC analysis showed that formation of PI and the addition and de-acetylation of GlcNAc were intact in the affected cell lines. Labelling with ³H-mannose revealed the accumulation of a mannose-containing intermediate, identical in both families. GPI-PLD digestion confirmed that the accumulated structure was a GPI intermediate. This structure was found to comigrate with that derived from TF cells in which there is accumulation of H6, a GPI intermediate possessing three mannose residues but no terminal EtnP modification. These findings indicate the block in GPI biosynthesis in both families arises from failure in addition of terminal EtnP. Homozygosity mapping showed that PIG-F and PIG-O, i.e., the genes known to regulate GPI biosynthesis at the site of block were not candidate disease genes. This was confirmed by direct sequencing and/or cDNA transfection experiments. *Conclusions.* Through combined biochemical and genetic approaches we have identified the biochemical defect responsible for congenital GPI deficiency and have shown that this defect involves a novel, yet to be characterised, gene required for EtnP modification and assembly of the GPI anchor in homo sapiens.

Stem cell transplantation

0793

BONE MARROW TRANSPLANTATION IN ADULT THALASSEMIA: LONG-TERM FOLLOW-UP

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Chronic blood transfusion programme and chelation have improved survival of patients with thalassemia which resulted in increasing the proportion of adult patients. However most adult patients have disease and treatment related complications progressing over time which cause severe morbidity and shortened life expectancy. Stem cell transplantation is the only cure currently available for thalassemia with the best results in young patients who have been regularly transfused and chelated. *Results.* of transplant in adult patients are inferior mainly due to transplant related toxicity. Therefore new approaches aiming to reduce transplant related toxicity in adult patients are warranted. Between November 1988 and September 1996, 107 consecutive patients with median age of 22 years (range 17- 35 years) received bone marrow transplantation (BMT) from HLA-identical related donors following BU14/16 CY120/160 regimen.

The probability of overall survival, event-free survival, mortality and rejection were 66%, 62%, 37% and 4% respectively. The incidence of grade 2-4 acute GVHD was 24,5%, and moderate or severe chronic GVHD was 5.6%. The presence of chronic active hepatitis at the time of transplant was the only factor associated with poor survival (RR 2.5; p=0.05). Sixty eight patients survive with a median follow-up of 12 years (range 8.3-16.2 years), and 66 of them are free of thalassemia. In April 1997 we started a new reduced dose intensity conditioning for adult patients with BU14CY90 preceded by a preparative regimen with hydroxiurea 30 mg/kg/day, azathioprine 3 mg/kg/day from day -45 through day -11, and fludarabine 20 mg/m²/day from day -17 through day -13 along with hypertransfusion and continuous chelation aimed to reduce hyperplastic bone marrow and gradually increase immunosuppression (Protocol 26). Fifteen high risk patients with thalassemia (n=14) or sickle cell anemia (n=1) with median age of 21 years (range 17-31 years) were given BMT from HLA-identical siblings after preparation with Protocol 26. The probability of thalassemia-free survival was 67% in this small group of patients. In conclusion current myeloablative BMT in adult patients is characterised by higher transplant related toxicity due to advanced phase of disease. Although our new approach to transplant adult patients with a reduced dose intensity conditioning regimen has improved thalassemia-free survival, transplant related mortality in these high risk patients still remains elevated.

0794

INOLIMOMAB IN THE TREATMENT OF SEVERE ACUTE GRAFT VERSUS HOST DISEASE

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From June 1998 to November 2004, 158 patients received inolimomab, a membrane-bound CD25 monoclonal antibody, for the treatment of acute GvHD (aGvHD) following allogeneic hematopoietic stem cell transplantation (HSCT) in 20 institutions. One hundred and twenty five patients (86 males and 39 females) were analysed (16 non steroid-resistant GvHDs, 8 grade I and 6 post-DLI GvHDs were excluded from analysis of efficacy and safety). Median age, at transplantation, was 37.6 years (2 months- 61 y). Underlying disease was: hematological disease for 105 patients (24 ALL, 20 AML, 16 CML, 13 NHL, 11 MDS, 7 MM, 7 CLL, 4 MPS, 2 AA, 1 HD), severe immune deficiency (11 patients), other inherited disease (4 patients), solid tumor (5 patients). Sixty-three patients received HSC from HLA-matched sibling donors, 44 from HLA-matched unrelated, 11 from mismatched unrelated donors and 7 from mismatched related donors. Fifty-seven patients received BMT, 65 PBSCT and 3 cord blood transplantation. Conditioning regimen was myeloablative for 87 patients and non-myeloablative for 37 patients. One patient (paediatric) did not receive any conditioning regimen. One hundred and thirteen patients received ciclosporine for GvH prophylaxis, combined with methotrexate (MTX) for 74 patients. One patient received methotrexate alone. Prophylactic ATG was administered to 3 patients, mycophenolate mofetil (MMF) to 26 patients. Twelve patients received CD34+ -selected HSC. Acute GvHD was diagnosed at a median of 27 days (6-143 d) after HSCT (grade II for 30, grade III for 42 and grade IV for 53). First-line aGvHD treatment con-

sisted of steroids starting from 1 mg/kg/day up to 12 mg/kg/day. Inolimomab was administered for steroid-resistant aGvHD at a median of 14 days (0-163 d) after onset of aGvHD at a median dosage of 0.34 mg/kg/day (0.05-1.5 mg/kg/d) during a median period of 22 days (1-156 d). Some patients received inolimomab as front-line therapy for GvHD relapse. This period includes an induction regimen corresponding to daily infusions (median duration: 9 days (1-69 d)), followed by a maintenance regimen (infusions twice or thrice weekly, with the same dosage for most of the patients). The total number of inolimomab infusions varies from 1 to 72 infusions (median: 16 infusions). **Results.** Twenty nine patients were in complete response (29 CR), 45 were in partial response (45 PR), and 51 were non responders (51 NR). Overall response rate was thus 59%. Using the Kaplan-Meier method, three-month and twelve-month survivals are estimated at 75% and 35% respectively. In a multivariate analysis, III-IV intestinal grades seemed associated with a weaker response rate. **Conclusions.** Inolimomab seems to be effective in the treatment of severe steroid resistant aGvHD, both in pediatric and adult populations and deserves to be evaluated in second-line treatment. Further data on additional patients will be presented.

0795

REDUCED-INTENSITY CONDITIONING FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTATION IS AN EFFECTIVE SALVAGE TREATMENT FOR AGGRESSIVE B- AND T-CELL NON-HODGKIN'S LYMPHOMA

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Reduced intensity conditioning (RIC) followed by allogeneic stem cell transplantation (allo-SCT) is associated with durable engraftment, low transplant-related mortality (TRM) and clinical remissions in hematologic malignancies. Several studies of myeloablative allo-SCT showed that aggressive non-Hodgkin lymphoma (NHL) treated for relapsed or refractory disease had a high transplant-related mortality (TRM) with a still significant relapse risk. The conducted a prospective phase II trial in which relapsed NHL were enrolled. In the present report, we compare the outcome of patients with peripheral T-cell (PTCL) (n=21) and diffuse large B-cell lymphoma (DLBCL) (n= 26). Transformed low-grade lymphomas were excluded from the analysis. All patients received several courses of debulking chemotherapy (usually 3 DHAP followed by high-dose cytarabine) followed by the same RIC regimen containing thiotepa (10 mg/kg), fludarabine (60 mg/ms) and cyclophosphamide (60 mg/kg). GVHD prophylaxis consisted of cyclosporine A and short-course methotrexate. Patients median age was 50 years (range 20-67). The two groups (PTCL versus DLBCL) had similar characteristics in terms of: previously failed auto-SCT (52% versus 61%, p=ns), chemosensitive disease (66% versus 65%, p=ns), complete remission at transplant (19% versus 42%, p=ns), number of previous lines of chemotherapy <2 vs > 2 (66% versus 80%, p=ns). The time from diagnosis to transplantation was comparable in the two groups: 19 versus 21 months. All patients grafted. De-novo acute GVHD (II/IV) developed in 38% and 31% of patients, and chronic GVHD in 42% and 38% for PTCL and B-DLBCL, respectively. TRM at 3 years was 10% and 15% respectively. The median follow-up of surviving patients was 31 months (range, 5-68) for PTCL vs 25 months (range, 6-70) for DLBCL. Three-year overall survival and progression free survival were 78% and 59% for PTCL, and 80% and 64% for DLBCL (p=ns). In conclusion, our data indicate: 1) RIC followed by allo-SCT can induce long-term disease control in patients with aggressive NHL; 2) TRM is lower than reported with myeloablative conditioning; 3) although, this is not a randomized study, the outcome of PTCL is encouraging and seems not different from DLBCL.

0796

DONOR NATURAL KILLER CELL ALLOREACTIVITY DOES NOT PREVENT ACUTE GRAFT-VERSUS-HOST DISEASE IN ADULTS UNDERGOING UNRELATED DONOR CORD BLOOD TRANSPLANTATION

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Background. Incompatibility in the graft-versus-host direction for killer cell Ig-like receptor (KIR) ligands has demonstrated in haploidentical hematopoietic stem cell transplantation to have a potent graft-versus leukemia effect, avoid rejection and protect from graft-versus-host disease (GVHD). **Aims.** To analyze the effect of KIR ligand incompatibility on outcomes of unrelated donor cord blood transplantation (UD-CBT). **Methods.** From May 1997 to February 2005, 71 consecutive adult patients with hematologic malignancies underwent UD-CBT. The median age was 30 years (range 18-46). Patients were divided according to their KIR ligand incompatibility in the GVH direction, as described by Ruggeri et al (Science 2002;295:2097). Incompatibility was assigned if a donor KIR ligand class I was not present in the recipient. GVHD prophylaxis consisted of cyclosporine and prednisone. Conditioning was based on thiotepa, busulfan, cyclophosphamide and antithymocyte globulin in 70 patients, and on thiotepa, fludarabine and antithymocyte globuline in 1 patient. **Results.** KIR ligand incompatibility was observed in 17 patient-donor pairs, whereas 54 patients did not present this incompatibility. No differences were found in patients with or without KIR ligand incompatibility in the incidence of GVHD grade I to IV (88% versus 81%, p = 0.7), grade above I (41% versus 43%, p = 0.9) or above II (18% versus 21%, p = 0.8). After a median follow-up of 36 months (range 0.3-89), the 4-year overall, disease-free, and relapse-free survival rates according to the KIR ligand incompatibility were 52% versus 38% (p = 0.6), 39% versus 35% (p = 0.5), and 67% versus 80% (p = 0.9), respectively. **Conclusions.** These data indicate that KIR ligand incompatibility is not associated with better outcome in adult recipients of UD-CBT and does not prevent the occurrence of clinically significant acute GVHD.

0797

STEM CELL TRANSPLANTATION FOR PHILADELPHIA-CHROMOSOME POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ALL) IN FIRST COMPLETE REMISSION AFTER IMATINIB-BASED INDUCTION THERAPY-RESULTS OF A SMALL TRIAL

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Background. Allogeneic stem cell transplantation is widely accepted as the only curative treatment option in adults with Ph+ALL. Best results are achieved with alloSCT in CR1; in addition, pediatric studies of ALL suggest more favorable outcome when minimal residual disease levels pre-transplant are low or undetectable. Accordingly, treatment outcome in patients with newly diagnosed Ph+ALL will depend on the efficacy of induction therapy, the frequency of alloSCT, and on post transplant relapse and mortality rates. These parameters may be affected by pre-transplant therapy. Recently, inclusion of Imatinib in

first-line therapy for Ph+ALL has improved the results of induction therapy, but its impact on the proportion of patients transferred to alloSCT and on treatment outcome after transplantation have not been established. **Objectives.** To evaluate transplant related mortality (TRM), disease free (DFS) and overall survival (OS) in Ph+ALL pts. following allogeneic (n=92) or autologous (n=5) SCT in CR1 after induction therapy with (n=74) or without(n=23) Imatinib. Patients and **Methods.** Two patients cohorts were treated successively in a prospective, multicenter GMALL study: cohort A encompassed 40 pts. [median age: 45 (23-62) yrs.] who received a 4 week-course of single agent Imatinib that was started after achieving a CR with induction phase I and continued up to the first cycle of consolidation chemotherapy. Cohort B included 34 pts. [median age: 39 (19-62) yrs.] who received phase II of induction combined with Imatinib irrespective of remission status. Post transplant outcome was compared with a historical control group (cohort C) encompassing 23 pts. [median age 42 (18-61) yrs.] known not to have received Imatinib prior to SCT. At the time of SCT all pts. were in CR1. TBI-based myeloablative conditioning was recommended, but transplantation procedures were performed according to individual center's policies. **Results.** Median post-transplant follow-up was 10.7 (0.8-39) mos. (cohort A), 5.2 (0-16) mos. (cohort B) and 23 (0,6-52) mos. (cohort C). Outcome is shown in the table below. Median post transplant DFS in the three groups was 8.5, 9.8 and 11.5 months (n.s.) and estimated DFS was 32% at 39 mo., 30% at 14.5 mo. and 39% at 51 mo. (n.s.), respectively. Estimated OS was 34% at 46 mo., 26% at 20 mo. and 56% at 42 mo. (n.s.), respectively. There was a trend towards improved remission duration but not DFS in patients with low or negative versus high bcr-abl transcript levels. TRM was particularly high (approaching 70%) in patients receiving a mismatched unrelated donor transplant. **Conclusions.** Incorporation of imatinib into the GMALL induction regimen enables a high percentage of patients to undergo SCT in CR1. TRM, DFS and OS after SCT are not significantly different in the 3 patient cohorts, receiving different induction schedules with or without imatinib, demonstrating that imatinib pretreatment is not detrimental for subsequent alloSCT for Ph+ALL. For the greater efficacy of imatinib-based induction regimens to translate into improved overall survival, both the relapse rates after alloSCT and TRM will have to be reduced.

Table. Outcome after SCT in relation to pre-SCT therapy.

	Cohort (pre-transplant therapy)		
	Imatinib after Ind. I (cohort A)	Imatinib+Ind. I (cohort B)	no Imatinib (cohort C)
Pts. in CR1	n=47	n=44	n=24
med. age (yrs.)	45 (23-62)	39 (19-62)	42 (18-61)
no SCT in CR1	7	10	13
Outcomes (SCT in CR1)			
CR	40 (85%)	34 (77%)	23 (96%)
Relapse	15 (32.5%)	19 (46%)	9 (39%)
TRM	11 (27.5%)	7 (20.5%)	9 (22%)
TRM	14 (36%)	9 (22.5%)	9 (29%)

Chronic lymphoblastic leukemia and related disorders - Biology

0798

NEWLY ESTABLISHED MONOCLONAL ANTIBODY AGAINST CD47 INDUCES CELL DEATH IN LYMPHOID MALIGNANT CELLS IN VITRO AND IN VIVO

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Background. CD47 belongs to immunoglobulin superfamily, and is expressed as a 50 kDa cell surface antigen. CD47 associates with integrins of beta1, beta2, and beta3, and it serves as a receptor for thrombospondin (TSP) and also as a ligand for transmembrane signal regulatory protein SIRP-alfa. CD 47 has a number of different functions such as platelet activation, cell motility, leukocyte adhesion and migration, and phagocytosis. Recently, it was reported that the ligation of CD47 rapidly induces cell death in T-cells and chronic lymphocytic leukemic B cells (B-CLL) in a caspase-independent manner. B-CLL is the most common hematological malignancy in Western countries. Although new chemotherapeutic agents including fludarabine and 2-chlorodeoxyadenosine have been introduced into the clinic, B-CLL is not curable, and therefore, there is a strong need of new effective drug. **Aims.** We generated a murine monoclonal antibody against an extracellular domain of human CD47 (designated MABL). We analyzed the anti-tumor effect of established antibodies *in vitro* and *in vivo*. **Methods. and Results.** Soluble MABL (10 micro g/mL) induced apoptosis in CD47-positive CCRF-CEM and MOLT-4 cells but not in CD47-negative cells as judged by annexin V staining. Caspase 3 was not activated by MABL, confirming that the cell death mediated by CD47 was caspase independent. In addition, administration of the F(ab')₂ of MABL (200 micro g/mouse twice a day on the 3, 4 and 5) significantly prolonged the survival of the SCID mice inoculated CCRF-CEM (>150 days in MABL-treated group vs. <50 days in vehicle control group), indicating that even *in vivo* MABL caused tumor cell death independently of ADCC and CDC. Because both MABL and F(ab')₂ of MABL caused hemoagglutination, we created a disulfide-stabilized dimer of a single-chain antibody fragment(scFv/S-S) of MABL to get rid of such an adverse effect. scFv/S-S indeed did not cause hemoagglutination, whereas MABL and the F(ab')₂ of MABL did. Furthermore, proapoptotic effects in primary B-CLL cells by MABL was significantly augmented by goat anti-mouse IgG (GAM) (percentage of annexin V-positive cells was 36.3% by 10 micro g/mL MABL, and it was 68.1% by 10 micro g/mL MABL plus 50 micro g/mL GAM), and the same degree of apoptosis was achieved by scFv/S-S of MABL alone. These results demonstrate that scFv/S-S of MABL induced the ligation of CD47 more efficiently than original MABL without causing hemoagglutination, and therefore, scFv/S-S can be used as a therapeutic agent. In order to gain insight into the mechanism underlying the cell death by the ligation of CD47, we carried out gene expression profile. Gene expression profiling with Affymetrix gene chips of MOLT-4 cells revealed that several proapoptotic genes were upregulated by the treatment of scFv/S-S and MABL plus GAM. Involvement of these genes in cell death mediated by the ligation of CD47 is speculated. **Conclusions.** CD47 could become a molecular target of hematopoietic lymphoid malignant cells. Our newly established antibodies against CD47 may have a potential as a novel therapeutic agent for the treatment of incurable lymphoid malignancies including B-CLL.

0799

THE LPL/ADAM29 EXPRESSION RATIO IS A NOVEL PROGNOSIS INDICATOR IN CHRONIC LYMPHOBLASTIC LEUKAEMIA

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Background. By allocating CLL cases into three major risk groups (low, intermediate and high), according to tumor burden and the presence of anemia and thrombocytopenia, the classical Rai or Binet staging systems provided a basis for therapeutic stratification. However, both staging systems fail to initially identify among low risk patients, those who will progress. Recent reports indicate that patients expressing unmutated (UM) IgVH genes display a poor prognosis when compared to mutated (MT) ones. Thus, IgVH mutational status is a strong prognostic indicator in CLL, which in addition provides early detection of progressive cases among low risk patients. However, the difficulty to extend this study to routine practice has led to a search for surrogate markers. While ZAP-70 has been shown to be overexpressed in patients displaying UM IgVH genes, a previous microarray study from our group showed overexpression of LPL and ADAM29 genes among UM and MT CLLs respectively. **Aims and Methods.** We try to define the role of LPL and ADAM29 gene expression in CLL prognosis and as surrogate markers for the IgVH mutational status.

Multivariate analysis of event-free survival (EFS) and overall survival (OS) in CLL

A (including IgVH)		B (without considering IgVH)	
Factor	P	Factor	P
EFS for all stages			
Age	0.730	Age	0.20
Sex	0.239	Sex	0.45
Stage	0.012	Stage	0.039
IgVH	<0.0001	ZAP-70	0.36
		L/A ratio	0.064
EFS for stage A			
Age	0.66	Age	0.20
Sex	0.20	Sex	0.32
Stage	0.002	ZAP-70	0.019
		L/A ratio	0.029
OS for stages B and C			
Age	0.026	Age	0.11
Sex	0.90	Sex	0.32
IgVH	0.06	ZAP-70	0.30
		L/A ratio	0.30

* Given the high correlation between IgVH mutational status and both L/A ratio and ZAP-70, it may not be appropriate to use prognostic factors including simultaneously these 3 parameters.

To assess the prognostic value of these genes, we quantified their expression by QO-PCR in a cohort of 127 CLL patients, and correlated this with clinical outcome, IgVH mutational status and ZAP-70 protein expression, as assessed by flow-cytometry. **Results.** IgVH mutational status, ZAP-70 and the LPL and ADAM29 mRNA ratios (L/A ratio) were predictive of event-free survival for the whole cohort and for stage A patients. In stage B and C patients, the L/A ratio was an independent prognostic factor while ZAP-70 did not predict survival. Simultaneous usage of the L/A ratio and ZAP-70 expression allowed an almost perfect (99%) assessment of the IgVH status in the 80% of patients with concordant results (L/A+, ZAP-70+ or L/A-, ZAP-70-). LPL and ADAM29 gene expression could also be determined by a simple competitive multiplex RT-PCR assay. **Conclusions.** Quantification of LPL and ADAM29 gene expression is a strong prognostic indicator in CLL, providing better prognostic assessment than ZAP-70 in advanced stages of the disease (Oppezzo et al, Blood, in press).

0800

LOWER LEVELS OF SURFACE B-CELL RECEPTOR EXPRESSION IN CHRONIC LYMPHOBLASTIC LEUKEMIA ARE ASSOCIATED WITH GLYCOSYLATION AND FOLDING DEFECTS OF THE μ AND CD79A CHAINS

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Background. Low levels of B-cell receptor (BCR) expression are the hallmark of tumoral B lymphocytes in B-cell chronic lymphocytic leukemia (B-CLL). This receptor is expressed on the plasma membrane as a disulfide-bonded complex of heavy and light immunoglobulin chains associated with the CD79a/CD79b heterodimer. We previously reported that: 1) Intracellular synthesis of BCR components, including the transcription of the corresponding genes was normal and 2) Nascent IgM was not processed to give a mature glycosylated chain in most CLL patients. Proteins comprising several subunits, such as the BCR, require folding and assembly. These processes take place in the endoplasmic reticulum (ER), where the proteins are modified (cleavage of signal peptide, N-glycosylation, formation of disulfide bonds) and folded before they pass into the Golgi apparatus. If folding and maturation are defective then the quality control system of the cell makes use of several different mechanisms to prevent the production of non-native proteins: 1) a retention of the proteins in the ER and several resident chaperones are responsible for refolding the molecules correctly, 2) a degradation or accumulation as aggregates of improperly folded or assembled proteins or 3) a venture into the Golgi apparatus, from which proteins may be retrieved by the ER. In the case of BCR, several chaperones, including calnexin, calreticulin, BiP and GRP94, have been shown to associate with the μ , CD79a and CD79b chains. **Aims.** We investigated the glycosylation status and cellular localization of μ , CD79a and CD79b chains in 10 CLL patients differing in surface IgM expression. Moreover, we look for structural defects in BCR components and chaperone proteins involved in the folding of this receptor. **Methods.** The processing and surface expression of the BCR were investigated in the B cells of 10 CLL patients by a combination of flow cytometry, immunofluorescence or electron microscopy, western-blotting and immunoprecipitation studies. **Results.** Our data clearly indicate that: 1) The level of IgM expression on the surface of B cells in CLL patients is related to the extent to which the glycosylation of μ and CD79a chains is impaired, 2) The retention of the μ and CD79a chains in the ER contrasts with the Golgi-processed glycoform patterns of CD79b, 3) Only mature glycoforms of BCR components were found to be associated whereas a major defect in the glycosylation of CD79a and μ chains may result in the aggregation of μ chains into megavesicles, and 4) The impaired surface expression of IgM could not be accounted for by structural defects in BCR components and chaperone proteins. **Conclusions.** These data show, for the first time, that lower levels of BCR surface expression observed in CLL are accounted for by an impaired glycosylation and folding of the μ and CD79a chains. These defects were associated with the retention of both chains in the ER. In contrast, no clear impairment of glycosylation and folding was observed for CD79b. No sequence defects were identified for BCR components and for the chaperone proteins involved in BCR folding processes (Vuillier et al, Blood, in press).

0801**TELOMERE LENGTH HAS PROGNOSTIC IMPACT IN B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA (B-CLL)**

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Background. Germinal Center (GC) experience is a basic prognostic feature in B-CLL. Patients with VH-mutated GC-experienced CLL have a good prognosis while those with VH-unmutated GC-inexperienced CLL have a poor prognosis. In a recent study we demonstrated that telomere length (TL) of lymphoproliferative disorders strongly correlates with GC, pre-GC or post-GC origin (Ladetto M et al, Blood 2004). AIMS. To further define the relationship between TL and VH mutational status in B-CLL and correlate both these parameters with clinical outcome. **Methods.** 109 B-CLL patients have been analyzed for telomere restriction fragments (TRF) length and VH mutational status. All samples were taken at diagnosis or during the 'watch and wait' phase. Male were 68, females 41. Median age was 62 years (range 34-87). Fifty-three patients were in stage A, 30 patients were in stage B and 16 were stage C according to Binet staging system. Our patient population has been monitored for a median time of 60 months (range 7-290). Sixty-three patients have been already treated for their disease while 46 have not required treatment, so far. TRF length was evaluated by Southern blot and VH mutational status by direct sequencing. The standard cut-off of 2% deviation from any germ line VH sequence was employed to define VH mutational status. Survival analyses were performed using the Kaplan-Meier method. **Results.** Overall, median TRF length was 5898bp (range 1737-14837bp). There was no correlation between TRF length and patient age, sex or stage. A cut-off of 4500bp discriminated two subgroups of patients characterized by different clinical outcome in terms of time to first treatment (TTFT) and time to disease progression (TTP) following first line treatment. Patients with TL < 4500bp had a median TTFT of 16 months and a median TTP of 14 months while patients with TL > 4500bp had a median TTFT of 36 months and a median TTP of 50 months (p<0.05 and p<0.005, respectively). A comparison between TRF length (using the previously defined 4500bp cut-off) and VH mutational status showed the following: a) 100% concordance between VH-mutated status and TRF length >4500bp; b) 62% concordance between unmutated VH-status and TRF length <4500bp; c) the 10 discordant patients with VH-unmutated status and TRF >4500bp had a clinical outcome similar to that observed in patients with VH-mutated status (median TTFT: 22 months; median TTP: 80 months). **CONCLUSIONS** Our data demonstrate that: 1) TL in B-CLL has a good correlation with VH mutational status; 2) TRF length has prognostic significance in B-CLL in terms of TTFT and TTP; 3) when discordance exists between these two parameters, the clinical behavior seems to be better predicted by TRF length compared to VH mutational status.

0802**TELOMERE LENGTH AS A PROGNOSTIC PARAMETER IN CHRONIC LYMPHOBLASTIC LEUKEMIA WITH SPECIAL REFERENCE TO VH GENE MUTATION STATUS**

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B-cell chronic lymphocytic leukemia (CLL) consists of two prognostic entities where cases with mutated immunoglobulin VH genes have better outcome than unmutated cases. VH

mutated CLLs display longer telomeres compared to unmutated cases and telomere length has been indicated to predict outcome, although the prognostic value of telomere length has not been fully established in CLL. We analyzed telomere length, VH gene mutation status and clinical parameters in a large series of CLL. Telomere length was assessed by quantitative PCR, giving a very good correlation to telomere length estimated by Southern blotting (p<0.001). The prognostic information given by mutation status (n=282) and telomere length (n=246) was significant (p<0.001 respectively). Telomere length was a prognostic factor for stage A (p=0.021) and stage B/C (p=0.018) patients, whereas mutation status predicted outcome only in stage A patients (p<0.001). Furthermore, mutated CLLs were subdivided by telomere length into two groups with different prognosis (p=0.003), a feature not seen for unmutated cases (p=0.232). Interestingly, the VH mutated group with short telomeres had an overall survival close to unmutated cases. Thus, by combining VH mutation status and telomere length an improved subclassification of CLL was achieved identifying previously unrecognized patient groups with different outcome.

Platelets**0803****VIRULENCE PROFILE OF HELICOBACTER PYLORI STRAINS IN INFECTED PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA**

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Background. Recent data have shown that the eradication of *Helicobacter pylori* (HP) may persistently increase the platelet count in a fraction of infected patients with chronic immune thrombocytopenic purpura (ITP), strongly suggesting that HP may be one of the causes of ITP, at least in some countries (Italy and Japan); instead poor platelet response rates have been reported in two American and Spanish cohorts. The pathogenic mechanisms of HP-associated ITP have still to be elucidated. **Aims.** To investigate the possible prevalence of some HP virulence factors in ITP patients compared with those non-ITP, with gastro-duodenal disease. Also to study the possible gastric B-cell clonality. **Methods.** In the last two years we enrolled 32 consecutive patients with chronic ITP, Caucasian from Northern Italy, 15 males, 18 females, median age 54 years (range 26-83). All patients were tested for HP infection by 13C Urea Breath Test (UBT) and, when infected, eradicated by a triple therapy. Platelets were counted before and at least 1 and 6 months after eradication treatment. Eight ITP patients HP-positive and eight non ITP patients with gastric disease HP-positive, as control, underwent gastric biopsy for biological studies. To determine the gastric B-cell clonality, we analyzed immunoglobulin heavy chain gene rearrangement using genomic DNA extracted from biopsy of the gastric mucosa (corpus and antrum) by semi-nested PCR amplification. The same extracted DNA was also used for the PCR amplification of HP virulence genes such as *cagA*, *vacA*, *iceA1*, *iceA2* and *babA2*. **Results.** At UBT, 14 patients (44%) were found positive for HP infection, 13 of infected (92%) were eradicated and 11 of eradicated (79%) showed marked increase of platelet count. The responsive by intention to treat patients were=34%. The median follow-up was 19 months. At histologic examination, all 8 infected ITP patients were found to be suffering from asymptomatic gastritis, while all controls were suffering from symptomatic gastritis. The B-cell clonality study showed no differences between ITP patients and controls; 5 ITP patients and 5 controls were mono- or oligoclonal, while the remaining ones were polyclonal. Concerning the virulence profile, 5 ITP patients showed the expression of at least three genes, *cagA*, *vacA* s1/m2 and *ice A1* ('triple positive' strains) in comparison with only 1 control. Only 1 ITP

patient with triple positive strains, also expressed the babA2 gene, in respect to 3 controls (1 with and 2 without triple positive strains). The only ITP patient expressing babA2 gene was non responsive to HP eradication treatment. **Conclusions.** Our data suggest that infected ITP patients have a gastritis, even if asymptomatic and confirm that a significant proportion of ITP show a sustainable recovery in platelet count after eradication of the bacterium. In respect to virulence profile, considering that HP strains are highly diverse with a variable host immune response; which awareness that the data have to be confirmed in larger series of patients, our findings seem, anyway, provide a useful insight into the pathogenesis of ITP HP-related.

0804

LOW-MOLECULAR-WEIGHT HEPARIN CAUSES LESS HEPARIN-INDUCED THROMBOCYTOPENIA THAN UNFRACTIONATED HEPARIN: FURTHER EVIDENCE FROM A STUDY IN 311 NEUROLOGIC PATIENTS

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Background. Low-molecular-weight heparin (LMWH) has widely replaced unfractionated heparin (UFH) for prophylaxis and treatment of thrombosis, however heparin-induced thrombocytopenia (HIT) may occur in LMWH treated patients too. Incidence of HIT varies between different patient populations. So far only few prospective studies have evaluated the frequency of heparin-induced antibodies (HABs) and HIT in LMWH treated patients. **Aims.** We assessed the incidence of HABs and HIT in neurologic patients treated with UFH or LMWH for prophylactic or therapeutic purpose. Patients and **Methods.** A total of 311 patients with neurologic disorders (44.4% cerebrovascular, 20.3% neuroinflammatory, 16.4% neurodegenerative/neoplastic, 19.0% miscellaneous; m/f 143/168; mean age 57.2±15.8 y) requiring antithrombotic therapy were included in the study. 111 patients received LMWH (nadroparin, Sanofi-Synthelabo, France) s.c. for thromboprophylaxis, 200 were treated with UFH (Liquemin N, Roche, Germany), administered either i.v. in high therapeutic dose for cerebrovascular disease (n=94) or s.c. in low dose for thromboprophylaxis (n=106). Inclusion criterion was heparin treatment for at least five days. Blood for HAB search was taken before/at the onset of heparin treatment and thereafter every five days. All HAB determinations were done after end of heparin treatment from stored samples (-70°C) unless platelet count dropped > 50% or thrombocytopenia (< 120 G/L) developed. Heparin-platelet-factor-4-ELISA (Roche, Germany) and heparin-induced-platelet-activation-assay were used for detection of HABs. Platelet counts were measured before and every 2-3 days during heparin treatment. **Results.** None of the LMWH patients, but five of the UFH patients (2.5%) developed HIT (p=0.17). The frequency of patients with HABs was lower in the LMWH than the UFH group (1.8 vs 20.5%; p<0.001). Logistic regression analysis revealed an influence of heparin type (p< 0.05) and treatment indication (p<0.01) on the development of HABs. No other factor influenced occurrence of HABs (age, p=0.47; sex, p=0.29; previous heparin treatment, p=0.51; duration of heparin treatment, p=0.09; diagnosis of cerebrovascular disease, p=0.24). The rate of HABs was higher in patients receiving UFH for therapeutic anticoagulation as compared with patients receiving LMWH or UFH for thromboprophylaxis (each p<0.001). Additionally, HAB frequency was higher in patients with UFH vs LMWH thromboprophylaxis (p<0.01). Incidence of thrombosis was higher in HAB positive compared to HAB negative patients (11.6% vs. 1.9%; p<0.01). **Conclusions.** LMWH compared with UFH treated neurologic patients are at lower risk to develop HABs and HIT. HAB formation is associated with an increased risk for subsequent thrombosis.

0805

POST-TRANSCRIPTIONAL REGULATION OF GATA-1 IN A TPO-DEPENDENT SUBCLONE OF THE MURINE 32D CELL LINE

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Background. Megakaryocytic (Mk) differentiation is regulated by both extrinsic (the growth factor thrombopoietin, TPO) and intrinsic (the transcription factor GATA-1) pathways. How the two pathways interact with each other to define the final megakaryocyte output is not yet understood. The observations that Mk from mice with targeted deletion of the regulatory sequences that reduce GATA-1 mRNA express low levels of mpl, the receptor for TPO, while those from normal mice being treated with TPO express reduced levels of GATA-1 protein (by immunohistochemistry) and mRNA (by RT-PCR) (Vannucchi et al, Blood pre-published on line January 2005), have suggested the existence of a feed-back loop between the two pathways. The difficulties, however, to isolate Mk in numbers sufficient for biochemical studies has hampered the identification of such interaction thus far. **Aim.** To isolate TPO-dependent differentiation mutants from the IL-3-dependent multipotent 32D cell line and to identify whether TPO would affect GATA-1 expression in these cells. **Methods.** TPO was used as selective pressure to isolate 32D TPO, a nearly tetraploid (modal chromosome no. 66) subclone of the 32D cell line, that depends on TPO for survival and proliferation. 32D TPO cells retain the capacity to grow in IL-3 but do not respond to either EPO, G-CSF or GM-CSF. 32D TPO cells were starved for 4 hrs and exposed to increasing concentration of TPO (10-100 ng/mL) for up to 24 hrs. At 4 and 24 hrs after growth factor stimulation, the cells were harvested to evaluate cell viability (by Annexin staining), level of GATA-1 mRNA (by quantitative RT-PCR) and protein (by Western blot). **Results.** The 32D TPO cell line express levels of GATA-1 mRNA similar to those expressed by freshly isolated Mk (Delta Ct = 4,7±0.3 in both cases). All of the 32D TPO cells express Mk-specific markers by FACS (CD41, CD61 and 2D5) but are highly heterogeneous in size ranging from 15 µm (small cells with proliferation activity) to 50-75 µm (cells with a clear Mk morphology releasing pro-platelets from their surface, see figure). 32D TPO cells are highly sensitive to growth factor starvation and more than 80% of them become Annexin-positive after 8 hrs of growth factor deprivation.



TPO-treatment of 32D TPO cells induced a modest concentration-independent decrement in GATA-1 mRNA (Delta Ct from 4.0 to 4.7 and 5.0 at 4 and 24 hrs of TPO stimulation, respectively) but a consistent concentration-dependent decrease in GATA-1 protein that became barely detectable after only 4 hrs of treatment with 100 ng/mL of TPO. **Conclusions.** 32D TPO is a new cell line that grows in response to TPO and mature up to the stage of Mk releasing pro-platelets from the surface. TPO affected the level of GATA-1 expressed by this cell line by modestly reducing the mRNA and profoundly affecting the protein. We propose that 32D TPO might represent a valid tool to detail how TPO regulates GATA-1 expression in megakaryocytopoiesis.

0806**DEVELOPMENT OF A NEW EXPERIMENTAL ANIMAL MODEL OF HUMAN MEGAKARYOPOIESIS USING IMMUNODEFICIENT NOD/SCID/GAMMA CHAIN NULL (NOG) MICE**

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Background. We have developed small molecules which have thrombopoietin mimetic activities *in vitro*. These small molecules stimulates the cells expressing human thrombopoietin receptor, c-Mpl, but not murine c-Mpl. However, the lack of experimental animal models of human megakaryopoiesis has hampered us to evaluate whether these molecules can increase the number of human platelets *in vivo*. As the growth of megakaryocytes were slower than other lineage cells, it was difficult to reconstitute human megakaryopoiesis in immunodeficient NOD/SCID mice without human fetal tissues. As the usage of human fetal tissues is accompanied by the ethical problems and difficulties for reproducibility, it is necessary to develop the new experimental animal models of human megakaryopoiesis. [Aims] In this study, we develop the new experimental animal model of human megakaryopoiesis using immunodeficient NOD/SCID/gamma chain null (NOG) mice. [Methods] After 2.4-Gy X-ray irradiation, 1x10⁵ CD34-positive hematopoietic cells from human cord blood, bone marrow and peripheral blood were intravenously injected into NOG mice. The engraftment of human megakaryocytes and platelets in NOG mice was analyzed by flow cytometry and immunohistochemistry for up to 6 months. [Results] Four weeks after transplantation, we found that around 1.5% of platelets in these mice were positive for human CD41 antigen (fibrinogen receptor) (n=8). We also confirmed 30-40% of megakaryocytes in the murine bone marrow were positive for human-specific megakaryocyte markers by immunohistochemistry. The percentage of human platelets in the murine blood was maintained around 2% for 6 months after transplantation with human CD34-positive cord blood cells. In contrast, the engraftment of human platelets was transient and disappeared after 3 months, when CD34-positive cells from human bone marrow and peripheral blood were injected into NOG mice. Interestingly, we found around 20% and 40% are human CD45-positive cells in the bone marrow of NOG mice after transplantation with peripheral blood- and bone marrow-derived CD34-positive cells, three months after transplantation, respectively (n=7). These results indicated cord blood cells have higher potential to maintain human megakaryopoiesis than bone marrow and peripheral blood cells in NOG mice. [Conclusions] We succeeded in developing the new experimental animal model of human megakaryopoiesis and thrombopoiesis using NOG mice. Currently, we are evaluating *in vivo* efficacies of our new thrombopoietin mimetic compounds using this model.

0807**ADAMTS-13 ACTIVITY IN THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP) PATIENTS: EXPERIENCE AT A SINGLE CENTRE**

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Background. The VWF-cleaving protease (ADAMTS-13), a metalloprotease of the ADAMTS family, is important for maintaining the normal size distribution of VWF multimers. A severe deficiency of ADAMTS-13 activity (i.e. <5% of that of normal plasma), caused by either mutation of the ADAMTS-13 gene or by inhibitory auto-antibodies to ADAMTS-13, is associated to TTP. **Aims.** To prospectively follow the levels of ADAMTS-13 activity and inhibitors in a group of 19 consecutive TTP patients admitted to our Institution between Jan. 2001 to Feb. 2005.

Methods. ADAMTS-13 activity (by Gerritsen's method) and inhibitors (by mixing study) was measured in plasma samples of 19 TTP consecutive patients (14 in the acute phase, 5 in complete remission) upon admission and at different time points during the follow-up. The patients were classified as: idiopathic TTP (n = 12; 9 acute, 4 in remission); drug-associated TTP (n=3; 2 thienopyridine-associated and 1 fludarabine-associated TTP, acute), bone marrow transplantation (BMT)-associated TTP (n=2, acute), and pregnancy-associated TTP (n=1, in remission). All patients were treated with plasmapheresis and plasma infusion, and, if refractory, with immunosuppressive therapy. All the acute patients were followed with serial blood sampling until the clinical and haematological remission. **Results.** In the acute idiopathic TTP group (n=9) the prevalence of complete ADAMTS-13 activity deficiency was 100%. An inhibitory activity against ADAMTS-13 was found in 8/9 patients. One patient was identified as a possible carrier of a true constitutive ADAMTS-13 deficiency; indeed no plasma inhibitory activity was found by mixing studies, and the ADAMTS-13 activity was detectable only during plasmapheresis/infusion, but not on clinical remissions and upon relapses (n= 4). Differently, in the remaining 8 patients, the levels of plasma ADAMTS-13 activity were severely reduced in the acute phase with a significant inhibitory activity, and variable behaviour on remission (i.e.: normal [> 50%] = 4 cases; complete deficiency [<5%] = 2 cases; severe deficiency [7-20%] = 2 cases). No severe plasma ADAMTS-13 deficiency was found in the 2 patients with BMT-associated TTP, whereas all the 3 patients with drug-associated TTP, showed negligible ADAMTS-13 activity in the acute phase and normal levels on remission. Finally, plasma ADAMTS-13 activity was normal in the patient with pregnancy-associated TTP in complete remission. **Summary/conclusions:** In conclusion, in our experience, the measurement of plasma ADAMTS-13 during the acute phase could not discriminate idiopathic TTP from drug-induced TTP, but distinguished the BMT-associated TTP. The patient with congenital deficiency was the most refractory to therapies. Interestingly, at remission, in 44% of idiopathic TTP patients, the resolution of microangiopathy, haemolytic anaemia and thrombocytopenia was not associated with a significant ADAMTS-13 level restoration.

Acute myeloid leukemia**0808****RAPID RISK PROFILING INCLUDING FAST DONOR SEARCH ALLOWS FOR EARLY ALLOGENEIC STEM CELL TRANSPLANTATION AS PART OF INDUCTION THERAPY IN PATIENTS WITH HIGH-RISK ACUTE MYELOID LEUKEMIA**

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Background. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment option for most high-risk patients with acute myeloid leukemia (AML). However, many high-risk patients regenerate with blasts or relapse early after induction therapy. Thus, consolidation with allogeneic HSCT in first CR is often not possible. Performing early allogeneic HSCT as part of induction therapy has the potential to circumvent these problems and might furthermore reduce cumulative toxicity in high-risk patients. **Aims.** Therefore, the prospective randomized treatment trial AML2003 for patients ≤ 60 years was set up, to investigate the feasibility and value of an intensified treatment strategy, i.e. early allogeneic stem cell transplantation as part of induction therapy, for high risk AML patients in a multi-center setting. **Methods.** To achieve this goal, rapid analysis of cytogenetics, FLT3 status and HLA-DNA-typing of the patient and possible family donors is of utmost importance. This 'fast search diagnostics' together with routine analy-

ses of morphology and immunophenotyping is accomplished centrally in all enclosed patients. Furthermore, an unrelated donor search was initiated right after completion of risk profiling in all high-risk patients without a family donor. **Results.** Within the first 13 months 183 AML patients with a median age of 48 (17-60) years were included in the study. 'Fast search diagnostics' was complete within a median of 14 days after arrival of the bone marrow samples for all patients. 91/183 patients were randomized into the intensified treatment arms. Out of these 41 (46%) patients with high risk characteristics have been identified with 35 patients being evaluable at this point of time. A suitable related or unrelated donor was found for 29 (83%) of those evaluable high-risk patients. Thirteen (45%) of those high-risk patients with a donor received early allogeneic stem cell transplantation in aplasia after one or two cycles of conventional induction therapy. Five were transplanted with stem cells of related and eight of unrelated donors. The dose-reduced preparative regimen consisted of melphalan 150mg/m² and fludarabine 150mg/m². So far 12 patients are in CR and only one treatment associated death had to be recognized. **Conclusions.** These encouraging preliminary results show that rapid risk profiling and fast donor-search is feasible in a large multi-center study. This leads to a significant proportion of early allogeneic stem cell transplants as part of the induction therapy within the group of high-risk AML patients, which may improve the disastrous prognosis of this group of patients in the future.

0809

CLADRIBINE ADDED TO THE STANDARD AML TREATMENT IMPROVES LONG-TERM OUTCOME IN HIGH TUMOR BURDEN AND OLDER THAN 40 YEARS ACUTE MYELOID LEUKEMIA PATIENTS. FIVE-YEAR FOLLOW-UP OF THE DAC VS. DA STUDY

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The goal of the study was to evaluate long-term outcome of acute myeloid leukemia (AML) patients treated within the PALG 1999 DAC vs. DA Study. Between 1999-2002, 445 patients, aged 18-60 years, were randomized to the induction DAC-7: daunorubicin 60 mg/m²/d iv 1-3; cytarabine 200 mg/m²/d ci 1-7; cladribine 5 mg/m² 2h inf. iv d 1-5 or standard DA-7 regimen (the same regimen without cladribine). Patients achieving CR received two courses of subsequent intensive consolidation: 1) HAM (HD AraC, mitoxantrone) 2) HD AraC with or without cladribine in the DAC-7 or DA-7 arm, respectively. In case of PR after the first induction course the same regimen was repeated, NR patients received CLAG (2-CDA, HD-AraC, G-CSF), regardless the randomization arm. Post-consolidation therapy was in both arms comparable. As previously reported, a single course of DAC-7 induction resulted in 17% higher CR rate compared to the DA-7 treatment. The difference was particularly pronounced in a population of patients >40 years and for those with initial WBC >100x10⁹/L. In the latter subgroup also the overall CR rate (achieved after >=1 induction course) was higher in the DAC-7 arm (71% vs. 43%). [Leukemia. 2004 May;18 (5):989-97] In the present report we analyzed long-term outcome (median follow-up 3.2 years) in the whole study group and in pre-defined subgroups taking into account initial tumor burden, age, cytogenetics, FAB subtype, and preceding myelodysplasia. At five years the overall survival (OS) rate equaled 31% for DAC-7 and 25% for DA-7 arm (p=0.42) and leukemia free survival (LFS) 32% vs. 29% (p=0.38), respectively. The subgroup analysis revealed higher probability of the OS in patients with initial WBC ≥100 G/L assigned to DAC-7 compared to DA-7 arm (39% vs. 11%, p=0.02). The LFS rate and the probability of relapse equaled 50% and 32% (p=NS) and 36% and 68% (p=0.0497), respectively. In patients aged >40 years, the therapy containing cladribine was

associated with improved LFS (30% for DAC-7 vs. 20% for DA-7, p=0.01), and a tendency to improved OS (28% vs. 18%, p=0.09). In this subgroup DAC-7 therapy resulted in reduced relapse incidence (60% vs. 69%, p=0.04). We conclude that addition of cladribine to induction/consolidation therapy of AML may improve long-term outcome in higher age ranges patients and in those with high tumor burden. The improvement results mainly from reduced risk of relapse, and, in patients with high initial WBC, from higher CR rate.

0810

THE ROLE OF REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): A DONOR VERSUS NO DONOR COMPARISON

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Standard myeloablative allo-SCT is a well established therapy for patients with AML. However, because of the high incidence of procedure-related toxicity, this procedure is often limited to younger patients in good medical condition. Using a genetic randomization through a 'donor' versus 'no donor' comparison, the aim of this analysis was to assess the real benefit of RIC-allo-SCT among 95 adult high risk AML patients. In an intention-to-treat analysis, the Kaplan-Meier estimate of LFS was significantly higher in the 'donor' group as compared to the 'no donor' group (p=0.01; 54% versus 30% at 4 years). When restricting the analysis to patients who could effectively receive the RIC-allo-SCT, the difference in LFS was also significant between this group of 25 patients ('transplant' group) and the remaining 70 patients ('no transplant' group) who did not receive allo-SCT (p=0.001; 62% versus 31% at 4 years). In the 'transplant' group, RIC-allo-SCT was performed at a median of 209 (range, 119-413) days after diagnosis. No grade 3 or 4 toxicities were encountered during RIC administration, and only 3 patients died from transplant-toxicity, for an overall cumulative incidence of TRM of 12% (95%CI, 3-32%). This relatively low TRM translated towards a significantly higher overall survival (OS) in the 'transplant' group as compared to the 'no transplant' group (p=0.01) Overall, 41 patients (43%; 95%CI, 33-53%) had relapsed at a median of 295 (range, 116-823) days after diagnosis, with the 4-year cumulative incidence of relapse being significantly higher in the 'no transplant' group as compared to the 'transplant' group (p=0.0002; 54% versus 12%). After controlling for all relevant factors [demographic characteristics, leukemia features (FAB subtype, leukemia origin (secondary vs. *de novo*), cytogenetics risk group, history of prior high dose cytarabine or autologous transplantation, and number of chemotherapy induction courses to achieve first CR), identification of an HLA-identical sibling donor, and effective performance of RIC-allo-SCT], in the multivariate analysis, only an intermediate cytogenetic risk group (p=0.01; RR=1.2; 95%CI, 1.2-4.7) and effective performance of RIC-allo-SCT (p=0.001; RR=4.0; 95%CI, 1.7-9.6), were significantly predictive of an improved LFS. We conclude that if a matched related donor is identified, RIC-allo-SCT should be proposed for AML patients not eligible for standard myeloablative allo-SCT.

0811

BAALC EXPRESSION AND FLT3 INTERNAL TANDEM DUPLICATION MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS WITH NORMAL CYTOGENETICS: PROGNOSTIC IMPLICATIONS

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Background. Approximately half of acute myeloid leukemia

(AML) patients lack clonal chromosome aberrations at diagnosis, and while this group has an intermediate prognosis only 40% are long-term survivors. The identification of molecular markers that more precisely differentiate a patient's risk could improve treatment outcome by the use of sophisticated risk adaptive treatment strategies. BAALC (Brain And Acute Leukemia, Cytoplasmic) is a gene implicated in leukemia. High mRNA expression levels of BAALC have been shown to be an adverse factor in AML patients (n=86) with normal cytogenetics and a more favorable FLT3 mutation status treated on the Cancer And Leukemia Group B (CALGB) protocol 9621. *Aims.* To evaluate the impact of BAALC with normal cytogenetics, and FLT3 internal tandem duplication (ITD) mutations as independent prognostic factors in a larger cohort of AML patients treated on the AML'96 protocol of the Deutsche Studieninitiative Leukämie (DSIL). In addition, the value of allogeneic and autologous stem cell transplantation (SCT) consolidation was assessed. *Methods.* BAALC expression was determined by real-time RT-PCR in pretreatment blood samples of 307 adults ≤60 years of age with AML with normal cytogenetics. Patients were dichotomized at BAALC's median expression into low and high expressers. The FLT3 ITD mutant-wild-type-ratio was determined by fragment analysis. We defined patients with either FLT3 wildtype or a low FLT3ITD/WT-ratio as low risk FLT3, and patients with a high FLT3ITD/WT-ratio (i. e. > 0.8) as high risk FLT3. *Results.* Compared to low BAALC, high BAALC patients had a lower complete remission rate (62% vs. 73%; p=0.038) and a higher rate of primary resistant leukemia (16% vs. 6%; p=0.006). High BAALC expression was associated with a higher cumulative incidence of relapse (CIR, p=0.018) and an inferior overall survival (OS, 3-years OS: 36% vs. 54%; p=0.0012). On multivariable analysis high BAALC was independently predictive of resistant disease (p=0.019) and confirmed high BAALC as well as a high FLT3 mutant-wild-type-ratio as the only factors predicting a high CIR (BAALC: p=0.0096; FLT3 p=0.002) and inferior OS (BAALC: p=0.001; FLT3: p=0.012). Whereas autologous transplantation did not influence the adverse impact of high BAALC, allogeneic SCT resulted in comparably low 3-year CIR for high (16%) and low (14%) BAALC patients. The same was true for patients within the FLT3 low risk group where allogeneic SCT led to a low 3-year CIR for high and low BAALC patients (BAALC high/FLT3 low: 8%; BAALC low/FLT3 low 11%). *Conclusions.* High BAALC expression is established to be one of the most important independent risk factors associated with resistance to standard induction chemotherapy, a high CIR and inferior survival in AML patients with normal cytogenetics. Modulation of induction and intensification of the post-remission therapies may be critical to improve outcome for these high risk patients. A prospective validation is necessary to confirm the beneficial effect of consolidation with allogeneic SCT for high risk BAALC patients. claudia.baldus@charite.de

0812

BONE MARROW RESPONSE RATE IN ELDERLY PATIENTS WITH POOR-RISK AML FOLLOWING TREATMENT WITH THE ORALLY AVAILABLE FARNESYL TRANSFERASE INHIBITOR TIPIFARNIB (ZARNESTRA®, R115777)

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Background. Acute myeloid leukemia (AML) is characterized by the accumulation of clonal neoplastic cells from the myeloid series. In the 25 European Union member states, the annual

incidence of AML is approximately 12,300. The incidence of AML increases with age, which is a major factor influencing patient outcome. Elderly patients with poor-risk AML are generally undertreated or untreated because they do not qualify for or cannot tolerate standard induction chemotherapy. Tipifarnib (ZARNESTRA®, R115777) is an orally available farnesyl transferase inhibitor that offers a new treatment option for this patient population. *Aims.* To analyze overall survival data in elderly patients with poor-risk AML according to clinical response and bone marrow status. *Methods.* A phase 2, open-label, single-arm, multicenter study assessed the overall response rate to tipifarnib in previously untreated elderly patients with poor-risk AML (age ≥75 years or age ≥65 years with AML preceded by MDS). Tipifarnib 600 mg was administered orally twice daily for 21 days of each 28-day cycle. Overall survival was estimated by Kaplan-Meier survival curves plotted according to patient response. Complete response (CR) was defined as <5% myeloblasts, absolute neutrophil count(ANC) ≥1,000/μL, and a platelet count ≥100,000/μL; partial response (PR) was defined similar to CR, except with 5% to 19% blasts and ≥50% decrease in blasts from baseline; hematologic improvement(HI) was defined similar to PR, except with recovery of ANC to 500 to 1,000/μL and platelet count to 20,000 to 100,000/μL. Stable disease (SD) was defined as anything other than CR, PR, HI, or progressive disease. *Results.* A total of 171 patients with poor-risk hematologic malignancies (AML, n = 158 [92%]); elderly poor-risk AML, n = 136 [80%]) completed enrollment to this study. The median number of cycles received was 2. In the poor-risk AML cohort(n = 136), the CR rate was 15%; CR was associated with prolonged survival. Median survival was 433 days (95% confidence interval [CI], 279-572 days) for the 20 patients who achieved a CR and 136 days (95% CI, 88-167 days) for the non-responders. The estimated 12-month survival rate was 68% for the complete responders. Disease control (PR, HI, and SD) also appeared to be associated with a survival advantage, albeit smaller than that observed in patients who achieved a CR. Additionally, morphologic response (ie, leukemia-free state [LFS]) had a survival impact similar to that of complete response. The median overall survival of the 25 subjects who reached a LFS (<5% bone marrow blasts) was 395 days (95% CI, 257-564 days); estimated 6-month and 12-month survival rates were 81% and 62%, respectively. A reduction in tumor burden also appeared to be associated with improved patient survival. *Summary/conclusions:* Patients who responded (CR, PR, or HI) to tipifarnib or achieved SD all appear to have an improved survival rate. Furthermore, patients who achieved a LFS also appear to have a survival benefit. Tipifarnib produces clinical response and improves survival in elderly patients with poor-risk AML who otherwise might be offered palliative treatment or supportive care only.

Non-Hodgkin Lymphoma: Biology

0813

LONG-TERM MONITORING OF CANCER-FREE SUBJECTS CARRYING NON-LYMPHOMA ASSOCIATED BCL2/IGH REARRANGEMENTS (NLABR): PERSISTENCE OF CLONAL POPULATIONS RELATED TO FOLLICULAR LYMPHOMA (FL)

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Introduction: NLABRs are frequently observed in cancer free-subjects. We recently observed that NLABR+ clones can persist up to 60 days (Ladetto et al, J Clin Oncol 2003). However the long-term kinetics and potential pre-neoplastic role of NLABR-carrying cells are unknown. To define the natural history of NLABR+ clones, long term monitoring of cancer-free subjects carrying these lesions has been performed. *Methods.* 118 subjects

undergoing periodical blood examinations for warfarin therapy were screened for the bcl-2/IgH translocation. PCR-positive subjects underwent subsequent monitoring at least once every three months. NLABR+ clones were monitored using both nested and real time-PCR according to previously published approaches (Ladetto et al Exp Hematol 2001). Sequence homology of NLABRs has always been confirmed by direct sequencing of nested PCR products. **Results.** 15 NLABR-positive subjects were identified out of 118 (12.7%) subjects. NLABR+ subjects were monitored for a median time of 18 months (mos) (range 6-36 mos) for a total number of 60 timepoints. In eight subjects (53%), NLABRs detected at study initiation were not detected again in follow-up samples. These eight subjects have been monitored for median period of 18 mos (range 6-28 mos). Follow-up samples in this group were usually PCR-negative, although transient PCR-positivity due to unrelated NLABRs were noticed in two samples. In seven subjects (47%), the same NLABR observed at study initiation was detected one or more times at follow-up. In four subjects, NLABRs detected at diagnosis were amplified in every available follow-up sample (four to eight samples were available for each subject). In three, NLABRs detected at diagnosis were amplified only in a fraction of follow-up samples while the remaining were PCR-negative. Overall, persistent NLABRs were followed on these subjects for a median time of 18 months (range 6-36). The median burden of persistent NLABRs assessed by real-time PCR was 33 rearrangements (rg)/106 diploid genomes (dg) (range <10-760), while the median burden of short-lived NLABRs was <10rg/106 dg (range <10-330) ($p < 0.05$). The number of NLABR+ cells appeared to be stable in subjects with persistent NLABR+ clones. In none of these subjects we could detect differences greater than 1 log among available follow-up samples. Subjects having mixed PCR-positive and PCR- results had a smaller tumor burden compared to those constantly PCR+. This is consistent with the presence of a small though persistent clonal population. Studies on selected populations showed that NLABR+ cells were CD19-positive. **Discussion.** CD19+ NLABR+ clones are long-lived cell populations in approximately 50% of cases. Based on this finding it is reasonable to hypothesize the existence of a follicular lymphoma (FL)-related lymphoproliferation of undetermined significance. Since NLABRs occurs in more than 10% of healthy subjects, this condition is expected to be highly prevalent in the general population (as observed in MGUS and CLUS) and of potential relevance for the pathogenesis of FL.

0814

THE MECHANISM OF ACTION OF RITUXIMAB IN VIVO DEPENDS ON THE TUMOUR MODEL

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Background. Rituximab is an anti-CD20 antibody used for the treatment of B-NHL and B-CLL. Its mechanism of action *in vivo* may include complement dependent cytotoxicity (CDC), antibody dependent cytotoxicity (ADCC) and/or induction of apoptosis. **Aims.** To determine the role of CDC, of different immune cell populations and of apoptosis in the mechanism of action of rituximab in two murine models of lymphoma in mice. **Methods.** Tumour cell growth was analysed by PCR and by measurement of subcutaneous tumours. Complement was depleted *in vivo* with cobra venom factor. NK cells and neutrophils were depleted by repeated treatments with the TM- β 1 and RB6-8C5 antibodies and macrophages by clodronate-liposome injections. **Results.** We have set up two B lymphoma models to investigate the mechanism of action of rituximab *in vivo*. The first model was a fast growing syngeneic B lymphoma, the 38C13 cell line, homing in lymph nodes and other hematopoietic organs. The 38C13 murine B lymphoma was stably transduced with the human CD20 cDNA and gave rise to tumours in bone marrow,

spleen and lymph nodes in syngeneic mice. Treatment with 250 μ g rituximab i.p. cured 100% of animals. Depletion studies demonstrated that complement alone, and not NK cell, PMN or macrophages, is responsible for the therapeutic activity of rituximab. Rituximab maintained efficacy when given up to 10-15 days after tumour inoculation, when tumour load was however still minimal. In order to study rituximab in a bulky lymphoma, a second tumour model was set up and characterised after subcutaneous inoculation of the BJAB human B lymphoma in athymic mice. Rituximab, given weekly after tumour had reached 250 mm², led to complete disappearance of the lymphoma within 2-3 weeks. Complement, as well as NK cells, PMN and macrophages were required for the therapeutic activity of rituximab in the BJAB model. **Conclusions.** These data demonstrate that complement is consistently required for the therapeutic activity of rituximab *in vivo*, to cure both minimal syngeneic tumours as well as bulky slow growing xenografts, but that different tumours may vary in their requirement for immune cells mediating ADCC and phagocytosis. Differential leukocytes infiltration, susceptibility to ADCC as well as tumour bulk may contribute these differences. We thank Roche Italia, AIL-sezione Paolo Belli-Bergamo, AIRC and MIUR (Project FIRB to JG) for their support

0815

THE PATTERN OF IGV GENES INDICATES THAT MANY POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS DERIVE FROM B-CELLS THAT HAVE FAILED THE GERMINAL CENTER REACTION

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Background. Posttransplant lymphoproliferative disorders (PTLD) represent a heterogeneous group of lymphoid proliferations arising in organ transplant recipients receiving immunosuppressive therapy. PTLD are classified into early reactive lesions, polymorphic PTLD (P-PTLD), and monomorphic B-cell lymphoma comprising diffuse large B cell lymphoma and Burkitt/ Burkitt-like lymphoma. Although recent studies have shown that the majority of PTLD derive from mature, antigen-experienced B-cells, a detailed molecular characterization of immunoglobulin heavy chain (IgVH) and light chain variable genes (IgVL) is currently lacking. **Aims.** To define the molecular profile of IgV genes throughout the spectrum of PTLD. **Methods.** We investigated 54 PTLD for usage, mutation frequency and mutation pattern of clonal IgVH and IgVL rearrangements. IgV rearrangements were amplified by multiple PCR approaches and sequences were aligned to the V-BASE sequence directory. The distribution of mutations among CDR and FR gene segments was investigated by the binomial (Chang-Casali) and the multinomial distribution models. **Results.** We identified a functional IgVH rearrangement in 47/54 (87.0%) cases. Four cases yielded only out of frame IgVH rearrangements or rearrangements rendered nonfunctional by crippling mutations. Three cases showed hybrid Ig VDJ rearrangements, namely a V-V fusion rearrangement and a J-J fusion rearrangement, suggesting a failed attempt of heavy chain receptor revision in the germinal center (GC). Despite extensive investigation by multiple PCR strategies, a functional IgVL rearrangement was found in only 25/54 (46.3%) cases. Eleven out of 25 (44.0%) cases harbored IgV kappa rearrangements and 12/25 (48.0%) cases harbored IgV lambda rearrangements. Among PTLD carrying solely nonfunctional IgVL rearrangements, 7/54 (13.0%) cases showed a crippled rearrangement and 11/54 (20.4%) cases harbored only an out of frame and/or inactivated IgV kappa gene. Inactivation occurred by rearrangement involving the kappa-deleting element (KDE). In 11/54 (20.4%) cases, no IgVL rearrangement was identified. Overall, only 23/54 (42.6%) PTLD displayed both a functional IgVH and a functional IgVL rearrangement. Analysis of somatic

hypermutation showed the presence of somatically hypermutated IgVH and/or IgVL genes in 45/54 PTLD (83.3%). Conversely, IgV rearrangements of 9/54 (16.6%) PTLD were in germline configuration, suggesting a derivation from B-cells that have not experienced the GC-reaction. Among mutated cases, the average mutation frequency was 8.83% (median 8.43%, range 2.10%-24.1%) for IgVH genes and 7.37% (median 6.71%, range 2.30%-26.0%) for IgVL genes. Thirty-two cases (71.1%) showed highly mutated (mutation frequency >6%) IgVH and/or IgVL genes, a condition that, in normal B-cell, results in lower affinity for antigen and apoptosis. Analysis of the distribution of replacement and silent mutations in functional IgVH and/or IgVL sequences showed tendency to conserve FR sequences and maintain antigen binding in 20/34 (58.8%) cases. Selection for high affinity antigen binding occurred in 14/34 (41.2%) cases. **Conclusions.** Our data suggest that most PTLD arise from B-cells that have experienced the GC-reaction and frequently display impaired B-cell receptors. Since a functional receptor is required for normal B-cell survival during GC transit, PTLD development may implicate rescue from apoptosis and expansion of B-cells that have failed the GC-reaction.

0816**HUMORAL IMMUNE RESPONSES AGAINST THE ONCOFETAL ANTIGEN-IMMATURE LAMININ RECEPTOR PROTEIN IN PATIENTS WITH CHRONIC LYMPHOBLASTIC LEUKEMIA-AN IMMUNOLOGICAL CONTROL OF TUMOR CELLS?**

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Background. The oncofetal antigen immature laminin receptor (OFA/iLR) is strongly overexpressed on malignant cells of chronic lymphocytic leukemia (CLL) but is not present on the cell surface of normal differentiated tissues. **Aims.** We analyzed specific humoral immune responses towards OFA/iLR in 64 patients with CLL (28 Binet A, 24 Binet C, 12 allogeneic stem cell transplantation) and 40 healthy individuals. **Methods.** Sera obtained from patients with OFA-iLR-expressing CLL were analyzed in enzyme-linked immunosorbent assay (ELISA). In a next step, anti-i-OFA/iLR reactive sera were tested in an antibody dependent cellular cytotoxicity (ADCC) assay for their ability to lyse CLL cells. **Results.** Immunoglobulin IgG and IgM OFA/iLR antibodies were detected in 30 of 64 (47%) patients, whereas none of the healthy volunteers had IgG or IgM OFA/iLR antibodies. Interestingly, in 22 of 28 (78%) patients with Binet A and in 8 of 12 (66%) patients receiving an allotransplant a significant level of anti-OFA/iLR antibodies are detectable, but not in patients with progressive disease (Binet C). To determine epitope specificity of the antibody response to OFA/iLR 62 peptides deduced from the OFA/iLR amino acid sequence were synthesized. All patient samples that were positive for antibodies to OFA/iLR protein were also reactive with at least one OFA/iLR peptide. Furthermore, patient sera reacting to OFA/iLR protein were able to induce complement-mediated lysis and ADCC of primary OFA/iLR expressing CLL cells. **Conclusions.** For the first time, these data suggest that anti-OFA/iLR antibodies might be involved in the immunological control of OFA/iLR expressing CLL cells *in vivo*.

0817**IDENTIFICATION OF CHROMOSOMAL ABERRATIONS AND DELINEATION OF A COMMONLY DELETED REGION ON 9P21 IN AGGRESSIVE NON-HODGKIN LYMPHOMAS USING ARRAY BASED COMPARATIVE GENOMIC HYBRIDIZATION**

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Background. Aggressive non-Hodgkin lymphomas harbour characteristic chromosomal aberrations such as t(14;18) and imbalances on 2p, 3q, 6q, 12q, 17p and 18q. However, the precise genomic extension as well as pathogenetic and prognostic implications of most of these alterations are largely unknown. **Aims/Methods.** To further elucidate the genotype of aggressive NHL, we applied high resolution array-CGH to analyze 151 lymphoma samples histologically reviewed by the reference panel of pathologists within the German cancer aid network project 'molecular mechanisms in malignant lymphoma'. Diagnosis were DLBCL (n=92), FL Grade III (n=2), atypical BL (n=18), typical BL (n=10). For 29 additional analyzed cases a panel diagnosis is pending. A genomic DNA-chip was set up using 2.800 target clones comprising (i) contigs mapping to genomic regions of possible pathogenetic relevance in lymphoma (n = 610 target clones); (ii) selected oncogenes and tumor suppressor genes (n = 686) potentially relevant in B-cell neoplasms; and (iii) a genome-wide set of 1502 target clones covering the genome at a distance of approx. 2 Mbp (part of the golden path clone set). This chip represents approximately 10% of the human genome at a median resolution of ~1.5 Mbp. **Results.** The most frequent genomic gains were found on chromosomes 1q (31%), 18q (27.1%), 3q (22.5%), 11q (21.2%), 12q (19.8%), 2p (16%) and 7q (14.5%) whereas deletions frequently affected 6q (30.5%), 17p (21.2%), 9p (20.5%), 13q (15.9%) and 1p (12.5%). Additionally, 35 DNA amplifications recurrently involving 18q (n=5), 2p (n=5), 12q (n=4), 11p (n=4) and 7q (n=3) were found. The majority of low copy alterations affected large chromatin segments (>10 Mbp) or complete chromosomal arms. However, in 30 lymphoma samples (19.8%) we were able to delineate a consensus region of approximately 0.3 Mbp on 9p21. The region is so far only sporadically known to be deleted in aggressive NHL and encompasses the tumor suppressor genes CDKN2A and CDKN2B. **Conclusions.** Our data demonstrate the usefulness of array-CGH for genomic screening in aggressive NHL and pinpoint towards the susceptibility of 9p21 for genomic alterations and the involvement of CDKN2A and CDKN2B in the pathogenesis of aggressive NHL. A detailed evaluation of the data set regarding physical mapping and prevalence of aberrations according to histological subgroups is in process and will be presented.

Genomics and molecular targeting in hematological malignancies**0818****SIRNA-MEDIATED SUPPRESSION OF MLL-AF4 COMPROMISES THE LEUKAEMIC STATUS IN VITRO AND IN VIVO**

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Background. Fusion genes have been shown to be involved in the development of leukaemia. However, much less is known about their roles in the maintenance of leukaemia. If such a fusion gene was essential for leukaemic maintenance, it might be an ideal target for molecularly defined therapeutic approaches. However, particularly those fusion genes acting as transcriptional modulators are difficult to target by small molecule inhibitors. An alternative way of specifically inhibiting fusion gene function is its suppression by RNA interference. **Aims.** We have examined the consequences of depleting the leukaemic fusion gene MLL-AF4 for the leukaemic status. **Methods.** t(4;11)-positive leukaemic cell lines were transiently transfected with siRNAs complementary to the corresponding fusion sites. Effects of fusion protein depletion on the leukaemic status was examined *in vitro* (proliferation, clonogenicity) and *in vivo* (leukaemic engraftment). **Results.** Reduction of MLL-AF4 levels also resulted in severely reduced colony formation and proliferation and an increased rate of apoptosis in t(4;11)-positive cells. Transient fusion gene suppression prior to xenotransplantation of t(4;11)-positive leukaemic cells significantly diminishes leukaemia-associated

morbidity and mortality. Summary/conclusions: In summary, we could demonstrate that MLL-AF4 is essential for the maintenance of leukaemia. Furthermore, because of their high specificity and efficiency, siRNAs targeting the MLL-AF4 fusion gene may be promising agents for a molecularly defined antileukaemic therapy approach.

0819

QUANTITATIVE MEASUREMENTS OF MICRORNAS REVEAL A PATTERN OF CORRELATION TO HOX GENE EXPRESSION IN AML

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Background. MicroRNAs (miRNAs) are single stranded RNAs, approximately 22 nucleotides in length, that repress translation of target mRNAs by complementarity-binding the 3' untranslated region. Although miRNAs are not fully understood, they appear to play key roles in a number of regulatory functions including modulation of haematopoiesis and cell differentiation in mammals. There are several indications that miRNAs might be a new class of genes involved in human tumorigenesis, with 50% of human miRNA genes being located at genomic regions involved in cancers. **Aims.** To measure the expression level of 10 miRNAs, in a set of 30 primary adult AMLs with normal karyotypes. Previous expression profile analysis, using the U133A and U133B Affymetrix arrays, showed that these leukaemias fell into two distinct groups according to HOX gene expression level. **Methods.** MicroRNAs were quantitated by real-time PCR using TaqMan® -MicroRNA assay (Applied Biosystems). The assays, which are specific to the mature miRNAs and do not detect the precursor species, were provided as part of a collaboration agreement with Applied Biosystems (expected to be commercially available later this year*). The measurement included 6 miRNAs located within the HOX clusters (miR-10a and miR-196a-1 on 17q21, miR-196a-2 at 12q13, miR-10b at 2q31, and miR-148 and miR-152 located within 1 Mb from the HOX clusters at 7p15 and 17q21, respectively) and 4 miRNAs known to be involved in haematopoietic development, or having as targets homeobox genes (miR-181a, miR181c, miR-223, and miR-19a). Data were normalised against miR-223, equally expressed across the set of samples. **Results.** MicroRNAs located within the HOX clusters (miR-10a, miR-10b, miR-196a-1, and miR-196a-2) followed an expression pattern that was correlated with that of some of the genes in the clusters. The correlation was more paralogous than position related. For each miRNA, a distinct list of both correlated and anti-correlated genes was identified from the microarray expression profile analysis. **Conclusions.** This is the first study performed in a set of AML patient samples reporting a quantitative measurement of miRNA expression and the computational discovery of genome-wide associations. The independent modulation of the miRNAs from that of the neighbouring HOX genes indicates a more complex regulatory mechanism between them. The use of this new quantitative assay will allow a more detailed examination of their role in leukemogenesis.

0820

TWELVE DIFFERENT LEUKEMIA SUBTYPES CAN BE DIAGNOSED USING GENE EXPRESSION PROFILING WITH A VERY HIGH ACCURACY

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Background. Diagnosis of leukemia requires a combination of different methods. **Aims.** Develop a diagnostic tool using gene expression profiling to predict clinically relevant subtypes of leukemia. **Methods.** We analyzed samples from 1337 untreated patients at diagnosis and healthy donors using HG-U133A+B, and plus 2.0 microarrays (Affymetrix). 13 subgroups were included:

620 AML (42 t(15;17); 38 t(8;21); 49 inv (16); 47 t(11q23); 75 complex aberrant karyotype (CA); 193 normal karyotype (NK); 176 other cytogenetic abn.) 152 ALL (26 Pro-B-ALL/t(11q23); 12 ALL-t(8;14); 32 T-ALL; 82 c-ALL/Pre-B-ALL); 75 CML, 45 CLL, and 45 bone marrows from healthy volunteers. (nBM). Class prediction was performed using support vector machines (SVM). Prediction accuracy was estimated by 10-fold cross validation (CV) and assessed for robustness in a resampling approach. A second prospective series comprised 400 unselected cases which were hybridized to HG-U133 Plus 2.0 microarrays. To validate the diagnostic accuracy of our approach these cases were processed blinded in parallel to routine diagnostic work-up and classified based on the gene expression signatures discovered in the first series described above. **Results.** 891 of the 937 samples (95.1%) were correctly classified (10-fold CV). A resampling approach with 2/3 training and 1/3 test cohort (100 runs of SVM) confirmed this high accuracy (median, 93.8%). 100% sensitivity and specificity was achieved for AML with t(15;17), t(8;21), and inv (16), as well as Pro-B-ALL/t(11q23), and CLL. Applying the first classification step as described above to the second cohort of 400 pts. the 13 different diagnoses were predicted again with an accuracy of 94.5%. Failures were mostly due to misclassification into biologically related subgroups, e.g. AML with del (5q) aberrations classified as AML with complex aberrant karyotype. **Conclusions.** We identified distinct expression profiles for 12 relevant adult leukemia subtypes and their discrimination from nBM. Accuracy, sensitivity, and specificity were higher than achieved with each of the gold standard techniques alone used today. Thus, gene expression patterns analyzed by microarrays qualify as a diagnostic tool in a routine setting for leukemia diagnosis and classification and may guide relevant therapeutic decisions.

0821

DETAILED ASSESSMENT OF GENOME-WIDE COPY NUMBER ALTERATIONS USING ARRAY-CGH IN MANTLE CELL LYMPHOMA AND RELATION TO IGVH GENE USAGE AND SURVIVAL

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Background. Mantle cell lymphoma (MCL) is characterized by an over-expression of cyclin D1 as a result of the characteristic t(11;14) (q13;q32). However, this translocation alone has proven not to be sufficient for lymphomagenesis, suggesting the involvement of additional alterations. **Aims.** The aim of this study was to analyze MCL tumors on a genome-wide basis with a high resolution array-CGH in order to detect regions of copy number gain or loss. **Methods.** Thirty-five cases of MCL were characterized by array comparative genomic hybridization (array-CGH) with an average resolution of 0.97 Mb distributed over the complete human genome. **Results.** Using this approach, new recurrent chromosomal alterations were detected in this material, such as losses in 1p and 22q. Furthermore, two homozygously deleted regions were identified at 1p, covering the p18/INK4c locus, and at 13q, respectively. Overall, the most common alterations were losses in 1p13.2-p31.1 (29%), 6q16.2-q27 (31%), 8p21.3 (17%), 9p13.2-p24.3 (29%), 9q13-q31.3 (31%), 11q14.3-q23.3 (40%), 13q14.13-q21.31 (46%), 13q33.1-q34 (51%), and 22q11.23-q13.33 (26%) and gains involving 3q21.2-q29 (31%), 7p12.1-p22.3 (29%), 8q24.13-q24.23 (17%), and 18q21.33-q22.3 (17%). For most regions, the data allowed further submapping of common target regions. The most recurrent changes were losses in 13q (54%), where two main regions could be recognized, i.e. a proximal at 13q14-q21 and a distal at 13q33-q34, in which deletions were identified in 51% and 46% of the cases, respectively, 11q (40%), 6q (31%), and 9q (31%) as well as gain in 3q (31%). Importantly, gain in 3q was

significantly associated with shorter survival ($p=0.047$). When compared to VH gene usage, the VH3-21+ subset displayed a lower number of alterations compared to those utilizing other VH genes (median 4 vs. 8, $p=0.032$). Furthermore, 9p losses, encompassing p16/INK4a, were exclusively found in cases with a non-VH3-21 utilization ($p=0.0056$). **Summary/Conclusions.** High-resolution array-CGH is a powerful tool in the detection of chromosomal alterations at high resolution. New chromosomal alterations appearing as recurrent in this material were losses of 1p and 22q. Homozygously deleted regions were identified as overlapping at 1p and possibly overlapping at 13q, both encompassing a region of approximately 1 Mb. Moreover, losses of 9p were restricted to patients with non-VH3-21 usage and showed a tendency to negative impact on prognosis. Furthermore, gains of 3q were associated with an unfavorable outcome. Detailed assessment of genetic alteration will thus further allow us to reveal genes of pathological as well as clinical significance in the field of MCL.

0822

SHORT CHAIN FATTY ACID-HDAC INHIBITORS INDUCE SPECIFIC CHROMATIN MODIFICATION AND TRANSCRIPTIONAL ACTIVATION IN CBF/AML CELLS

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Acute myeloid leukaemia (AML) characterized by the presence of AML1/ETO fusion protein, or by other rearrangements involving core binding factors, share a common leukemogenic mechanism, determining transcriptional repression of target genes important for myeloid maturation. The oncogenic fusion proteins, in fact, determine the permanent recruitment to the bound portion of DNA of protein complexes containing HDACs, DNMTs and co-repressor molecules. We demonstrated that butyrates are able as single agents to restore histone acetylation and to reinduce gene expression allowing granulocytic maturation in AML1/ETO pos Kasumi-1 cell line, as well as in primary CBF-AML blasts. Core histone proteins can be acetylated by histone acetyl transferases (HAT) or methylated by histone methyltransferases (HMT) at N-terminal lysines, at different residues which are specifically associated to the transcriptional state of the nearby chromatin. Transcriptionally active chromatin is characterized by histones with acetylated lysine tails, and methylated lysine 4 of histone H3, whereas dimethylated lysine 9 of H3 and deacetylated lysines in H4 and H3 seem associated with transcriptional silence. We investigated the acetylation and di-methylation pattern of lysine residues of histone H4 and H3 in our AML1/ETO pos cell model, prior and after exposure to butyrate as HDAC inhibitor. Cells were lysed after 6-12-72 and 96 hrs of culture and whole cell lysate was analysed by SDS-PAGE electrophoresis and Western blot, with antibodies specific to different lysine residues of the two histones. K5 and K16 residues of histone H4 were not acetylated in the absence of butyrate and their acetylation strongly increased after 6 h of butyrate administration. K8 and K12 residues showed a basal acetylation which was slightly enhanced by the treatment with the short chain fatty acid HDAC inhibitor. Lysine methylation of histone H3 was also evaluated. Butyrates determined a significant reduction of dimethylated K9 H3. Di-methylated K4 H3 was, on the other hand, unmodified after butyrate administration. These histone epigenetic modifications were paralleled by an increase of p21/cip1 protein expression and a decrease of p27/kip1 protein expression. Appearance of a conspicuous amount of p57/kip2, an as yet uncharacterised oncosuppressor, after 72-96 h treatment with butyrates was also observed. Moreover, CEBP family expression and phosphorylation were induced. In parallel, AML1/ETO mRNA and protein expression was markedly

reduced after D1 exposure. Our findings are consistent with the hypothesis of a specific direct activity of HDAC inhibitors in removing the corepressor complex which causes the block of transcription in CBF/AMLs.

PUBLICATION ONLY

0823

HOPELESSNESS AND DEPRESSION AS BASIS FOR CHILDREN'S SELF-ESTIMATION FOR QOL WHEN CHRONIC DISEASE IS PRESENT

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Anxiety and depression are not a *privilege* only for patients with malignant hematological conditions. For our study they are characteristic also for children with hemophilia and thrombocytopenia - hematological conditions that are presumed not as a life-threatening anymore, grace to the new therapies advances. We tried to answer the simple question *Why is that so?* We found the causes in the therapy management; complications connected with the medicament and with the age and in the reduction model of life. The base data for the study included results from 15 children with malignant hematological conditions and 15 children with hemophilia or thrombocytopenia. The method used was WHO's QoL questionnaire and anxiety and depression test. So far QoL was compared between healthy and ill people. We grade children's self estimation for QoL in chronic diverse diseases and in different gravity of their condition.

0824

PSYCHOLOGICAL BURDEN OF CHELATION THERAPY WITH DESFERRIXAMINE IN PATIENTS WITH MAJOR THALASSAEMIA IRAN IN 2004

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Background. In addition to the chronic physical manifestations of thalassaemia, its treatment places an additional psychological burden on the patients. Chelation therapy with desferrioxamine is difficult and burdensome. Moreover, the benefit of chelation therapy accumulate over the long-term so that neither the positive effects of adhering to therapy nor the negative effects of failure to adhere are readily apparent to the patients. These combinations leaves the temptation towards reduced compliance with desferrioxamine. There are some factors contributing to low compliance in these patients. **Aims.** Assessment of existence of depression and other barriers to adherence to desferrioxamine in patients with Major thalassaemia **Methods.** 205 patients with major thalassaemia older than 6 years were. Included. For assessment of depression: Beck Depression questionnaire forms were given to the patients older than 18 years old and *Coax* questionnaire -for depression assessment in Children -were given to the rest. Compliance was measured by compliance index was measured as No. of days of treatment per one month/No. of treatment days prescribed by physician. $CI > 70\%$ was considered good and $CI < 70\%$ was poor, the third group was the patient without compliance. **Results.** Among 172 patients who completed the forms 22% of children and 14% of Adults had mild to moderate depression. 15% of children and 10% of adults had severe depression. 24% of patients had poor compliance but there was no association between compliance and depression. The list of causes of incompliance mentioned by the patients were as follows: local reactions (firmness and lumps) in injection site (83%), painful injection due to improper method or device specially poor quality needles (70%), expensive infusion devices (16%), restriction of participation in certain activities among peer groups (10%). **Conclusions.** Long term compliance with chelation therapy has significant effect on prognosis of tha-

lassemic patients and as there isn't yet any substitute for chelation therapy with desferrioxamine, therefore this method of chelation therapy must be encouraged. Compliance is a multifaceted problem, so the solution to which requires close interaction between the patient, the family and community.

0825

CYCLIN D1 EXPRESSION IN ACUTE LEUKEMIA

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Disorders of the cell cycle regulatory machinery play a key role in the pathogenesis of cancer. The gene encoding the cyclin D1 protein, a positive regulator of the progression from the G1-to S-phase, is found disrupted in the cancer cell genome by the processes of chromosome translocation or gene amplification. Over expression of cyclin D1 protein has been reported in several solid tumors and certain lymphoid malignancies, but little is known about the involvement of cyclin D1 in acute leukemia. In this study, we analyzed the expression of cyclin D1 at protein level in, 40 AML, 10 ALL, and 11 normal controls using flow cytometry. The expression of cyclin D1 was not significantly different in AML group as compared to normal controls. On the other hand, over expression of cyclin D1 was evident in ALL group (4/10) as compared to that in healthy control. The ALL cases with cyclin D1 over expression were significantly correlated to blast cell counts in the peripheral blood and bone marrow but not with hemoglobin level, WBCs, and platelets count. The ALL group with lymphadenopathy and organomegaly express significantly higher cyclin D1 over expression as compared to those without. In conclusion; the biological value of cyclin D1 over expression might be different in AML and ALL.

0826

THE PREVALENCE OF MTHFR MUTATION IN IRANIAN DEEP-VEIN THROMBOPOETIC PATIENTS

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Thrombophilia has both genetic and acquired origins. The most important genetic causes are Anti-thrombin, Protein S and Protein C deficiencies, resistance to activated protein C, prothrombin G20210A mutation and hyper-homocysteinemia. Hyper-homocysteinemia is caused by mutation in its gene especially in nucleotides 677 (C → T) and related variants known as thermo-labile variants. This besides increased total plasma homo-cysteine will culminate to endothelial cell damage in vessel lining wall by diverse mechanisms and finally contribute to thrombosis. This study is a descriptive and cross-sectional one which was performed for referral patients with confirmed (clinically approved) thrombosis by the hemostasis and thrombosis reference laboratory from the Iranian Blood Transfusion Organization. Using PCR-RFLP method with mutagenic primers patients were screened. All precautions were performed to exclude false negative and false positive results. The average age for patient was around 38.99±13.54 years, 47.2% males vs. 52.8% females. The mutation frequency in patients was 30.8% in which 23.2% were heterozygote and 7.6% were homozygote. 41.6% of the gene mutated patients were male and 58.4% were females. Between the frequency of mutations and thrombosis in lower extremity organs (p=0.024) and thrombosis at unusual sites (port, splenic- mesenteric vein, retina) a meaningful relationship were investigated. This report is the first report from the Iranian patients which is comparable with the Italian and Canadian thrombophilic populations. These results could be attributed to direct causes and predisposing factors in thrombotic patients. zahra@nrcgeb.ac.ir

0827

PREVALENCE OF PROTHROMBIN G20210A MUTATION IN IRANIAN THROMBOTIC PATIENTS

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Thrombo-embolism is a prevalent and terrible disorder. Etiologically both genetic and acquired factors are involved in its pathogenesis. Mutations in 3' un-translated region of prothrombin gene (G20210A) which culminate an increase in prothrombin level have been described earlier. For patients harboring this mutation a 3 fold increase in DVT risk factor has been reported. It seems that increased pro-thrombin level could cause an imbalance between coagulation, anticoagulation and fibrinolytic systems. Prevalence of this mutation in Caucasians populations and in patients with DVT history is 2% and 6% respectively. Its frequency in patients with familial history is up to 18% according to the reports. These cross sectional descriptive studies have been designed to determine the prevalence of this mutation in Iranian DVT patients. 299 patients, 38.8% males and 61.2% females with an average age of 36.37 years and at least a history of DVT or Idiopathic abortion have been examined. Complete evaluation for Protein C, S, and APC-R by clotting and colorimetric method (Diagnostics stago 2000 France) have been done. By a novel PCR reaction with mutagenic primers, 2 restriction sites for Hind III were introduced to pro-thrombin gene. Then by RFLP method it could be traced on both wild-type and mutant genes. In this reaction one of the restriction sites acts as an internal control to find out any inhibitions in both wild-type and mutant phenotypes. For DVT 1.7% prevalence of this mutation has been evident. 40% of patients harboring this mutation were males and 60% were females. 40% of patients had evidence of DVT in lower extremities, 20% of them exhibited CVA and the other 40% had idiopathic abortions.

0828

EFFECTS OF MIXED CHIMERISM ON GRAFT-VERSUS-HOST DISEASE, DISEASE RECURRENCE AND SURVIVAL AFTER HLA-IDENTICAL MARROW TRANSPLANTATION IN IRAN

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Background. The co-existence of recipient's and donor's hematopoietic systems after allogeneic marrow transplantation is called mixed chimerism. **Methods.** The association of mixed chimerism with acute graft-versus-host disease (GVHD), disease recurrence, survival, and relapse free survival was investigated in 91 patients who underwent either bone (12) or peripheral blood (79) HLA-identical marrow transplantation. Chimerism was assessed using multiplex amplification of short tandem repeats (STR-PCR) with 12 STR markers. Cases included thalassemia (19), AML (29), ALL (20), CML (18) and others (5). Median age was 21 years (3-50). There were 38 female (41.8%) and 53 male (58.2%). Conditioning was busulfan plus cyclophosphamide in 34 patients, busulfan plus fludarabine in 51 patients and busulfan plus fludarabine plus anti-thymocyte globulin in 6 patients. Median time of follow up was 13 months. **Results.** On day +30, mixed chimerism (MC) was observed in 15 patients (16.5%), complete donor chimerism (CC) in 72 patients (79%), and no chimerism in 4 patients. The incidence of acute GVHD was significantly lower in mixed chimeras than in complete chimeras (p=0.01) but there was no significant difference in acute GVHD grade (I, II vs. III, IV) between two groups. The incidence of relapse was 17.6% and there was no difference in relapse rate between MC and CC. Overall survival was 88.9% and there was no signifi-

cant difference in overall survival between MC and CC (95.6% vs. 85.1% respectively). Relapse free survival was 80.2% and not significantly different between two groups. *Conclusions.* Despite some previous reports, we found no significant difference in survival and relapse rate between patients with MC and CC. It may be because the percentage of chimerism in most of our patients was between 80-95% and it was very near to CC.

0829**ASSESSMENT OF CHIMERISM AFTER ALLOGENEIC MARROW TRANSPLANTATION IN THALASSEMIA**

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Background. Using multiplex amplification of short tandem repeats (STR-PCR) permits sensitive assessment of chimerism after marrow transplantation for thalassemia. *Methods.* Chimerism was assessed in 19 thalassemia patients using a set of twelve STR markers in multiplex PCR on day +30 and day +60. Eleven patients received peripheral blood and 8 patients received bone marrow transplantation. Median age was 8 years (3-28). There were 7 female (36.8%) and 12 male (63.2%). Conditioning regimen was busulfan plus cyclophosphamide in 15 patients, busulfan plus fludarabine in one patient and busulfan plus fludarabine plus anti-thymocyte globulin in 3 patients. Median time of follow up was 13 months. *Results.* On day +30, mixed chimerism (MC) was observed in 7 patients (36.8%), complete donor chimerism (CC) in 9 patients (47.4%), and no chimerism in 3 patients (15.8%). The incidence of acute GVHD was significantly lower in mixed chimeras than in complete chimeras ($p=0.01$) but there was no significant difference in acute GVHD grade (I, II vs. III, IV) between two groups. There was no statistically significant difference in age, sex, conditioning regimen, transplanted cell type, GVHD prophylaxis and CMV status between mixed chimeras and complete chimeras. STR analysis was done for 9 patients on day +60. Status of one patient changed from CC to MC, 2 patients from MC to CC, 5 patients remained MC and one patient remained CC. *Conclusions.* Assessment of chimerism may permit therapeutic interventions in thalassemia patients at high risk for rejection and/or relapse.

0830**THE DIAGNOSTIC VALUE OF CD14 AND CD64 IN THE SUBCLASSIFICATION OF ACUTE MYELOID LEUKEMIA WITH MONOCYtic DIFFERENTIATION**

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Background. Immunophenotyping by flow cytometry is widely used in the diagnosis and subclassification of acute leukemia (AML). CD14 is the monocyte associated antigen used to identify AML with monocytic differentiation (French-American-British classes M4 and M5). However several studies have indicated that CD14 expression is frequently diminished or absent in such cases and some studies showed that CD64 (FCAR1) is an early specific myelo-monocytic marker. *Aims.* We designed this study to identify monocyte associated antigens that might improve recognition of AML M4 and M5. *Methods.* 216 cases of AML that was classified according to FAB criteria were selected. We used Epics-xl flow cytometry and a panel of antibodies to distinguish cells of monocytic lineage. According to the references an antigen was considered positive if 20% or more of the cells were expressed. *Results.* CD14 was highly specific (96%) but was only moderately sensitive (31%) and CD64 had the best-combined sensitivity (67%) and specificity (88%) for monocytic differentiation with more than 95% confidence rate. *Summary/Conclusions.* Because of high sensitivity and specificity of CD64 as an early myelo-monocytic marker, it must be considered in all of the immunophenotyping protocols.

0831**β-CATENIN EXPRESSION ON BLASTIC CELLS AS A PROGNOSTIC FACTOR IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)**

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Background. β-catenin is a multifunctional protein, which plays a role as a component of the cell-cell adhesion apparatus. Deregulation of β-catenin has been implicated in the malignant transformation of cells of epithelial origin. The function for β-catenin in hematological malignancies has not been reported. Overexpression of β-catenin has been found in Jurkat cells, K562 cell line and HUT-102 cells. Reduction of β-catenin expression inhibited proliferation and clonogenicity in these cell lines. The data suggest that β-catenin can play a significant role in promoting leukemic cell proliferation, adhesion and survival. *Aim.* The aim of this study was to investigate β-catenin expression on leukemic cells in AML and ALL at presentation and to correlate obtained data with the treatment outcome. *Patients and methods.* Fifty-seven patients were included: 46 with AML, 8 with ALL and 3 with bifenotypic leukemia. Twenty patients reached complete remission (CR): 5 with ALL and 15 with AML. β-catenin expression was assessed on cytosmeareds of bone marrow mononuclear cells by indirect immunofluorescent method using anti-β-catenin antibodies from Santa Cruz and reporter antibodies from DAKO. Percentage of positive cells was estimated on 200 cells using fluorescent microscope. Statistical analysis was performed using the Mann-Whitney's test for independent data. *Results.* In the group of all patients reaching CR (n=20) the mean expression of β-catenin was $2.2 \pm 3.0\%$ and was significantly lower than in patients without CR: $9.1 \pm 6.3\%$, $p=0.000008$. There was no difference of mean β-catenin expression between AML and ALL patients. No correlation between mean β-catenin expression and the number of leukemic cells in the bone marrow has been observed. *Conclusions.* Our data indicate that β-catenin expression could be considered as the prognostic factor in acute leukemia.

0832**UPDATE OF ANEMIA IN PREGNANCY PATHOGENESIS**

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Background. It is estimated that iron deficiency is the main cause of anemia in pregnancy. That is why, iron therapy is the basic treatment of anemic pregnant women all over the world. However, resistance is often found to the treatment of anemia with iron. In our opinion an anemia in pregnancy must not be considered as the problem concerned with iron deficiency only. *AIM:* To show that anemia in pregnancy has a more complex pathogenesis, than ineffective erythropoiesis, caused by iron or folate deficiency, we investigated the adequacy of the erythropoietin (EPO) production for the degree of anemia in pregnant women. *Materials and Methods.* A total 172 anemic pregnant women were tested. Control group consisted of 19 non-pregnant women with iron-deficiency anemia (IDA). We determined Hb concentration, reticulocyte count, erythrocyte indexes, serum iron, total iron binding capacity and serum ferritin concentrations. EPO values were measured immunoenzymometrically by using ELISA-EPO (IBL, Germany) kits. Soluble receptors I to TNF (RI-TNF) concentrations were evaluated by using commercial sets RI-TNF (R&D, USA). *Results.* All anemic women were divided into 4 groups on the basis of key laboratory tests: 1-IDA, n=55; 2-IDA + infection, n=17; 3-anemia of chronic diseases (ACD), n=13 and 4-anemia with normal iron status (ACD-like), n = 87. Mean EPO level was similar only in

pregnant women with IDA as compared with control group. All other groups showed significantly lower EPO levels, than non-pregnant women with IDA (20.1±2.8 U/L, 27.2±4.7 and 17.3±1.7 versus 58.7±9.1 U/L in IDA + infections, ACD, ACD-like and control groups, respectively). As compared with anemic controls, the mean O/E (log EPO) ratio was significantly lower in all groups of anemic pregnant women. Serum RI-TNF concentrations in mixed group of anemic pregnant women were 2311.2±152.1 ng/L and in ACD-like pregnant women were 2554.6±211.9 ng/L. In sera of women in 6-8 weeks after delivery RI-TNF concentrations were decreased to normal values-1916.4±190.0 ng/L and 1950.0±180.3 ng/L, respectively. **Conclusions.** Inadequately low production of EPO for the degree of the anemia is important mechanism in pathogenesis of anemia in pregnancy. Blunted erythropoiesis in anemia during pregnancy is obviously related to increased production of inflammatory cytokines. We suppose some causes of proinflammatory cytokines increased production in anemic pregnant women. Firstly, infections during pregnancy may lead to elevated serum levels of inflammatory cytokines. Secondly, it is known that reduced O₂ stimulates placental production of inflammatory cytokines. That is why hypoxia may lead to decreased production of EPO in the group of anemic pregnant women with IDA. Thirdly, there is increased serum level of estrogens during pregnancy. It is well known that estrogens inhibit the erythropoiesis. Our own and other investigators data about high efficacy of a recombinant human EPO therapy of anemic pregnant women are good confirmation of such comprehension of anemia in pregnancy pathogenesis.

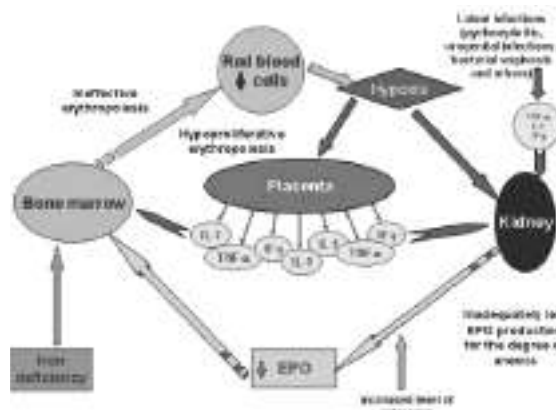


Figure. Pathogenesis of anemia in pregnancy

0833

EXPRESSION OF HOMING -ASSOCIATED CELL ADHESION MOLECULES CD44 AND CXCR4 ON EXPANDED CORD BLOOD CD34+ HEMATOPOIETIC PROGENITOR CELLS WITH MESENCHYMAL STEM CELLS

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We investigated the expression of CD44 and CXCR4 molecules on expanded CD34+ hematopoietic progenitor cells in coculture with human bone marrow mesenchymal stem cells. **Background.** A number of potential cell adhesion molecules, which mediate essential cell-to-cell or cell-to-matrix interactions, are expressed on the surface of CD34+ HPCs. such as integrins, CD44 and CXCR4. These molecules are essential to the homing process. **Material and Methods.** Using human bone marrow mesenchymal stem cells and cytokines (TPO, SCF, Flt-3 and IL-3), was expanded cord blood CD34+ cells. then expression of CD44 and

CXCR4 were evaluated on CD34+ cells by immunofluorescence analysis. **Results.** after 2 week of suspension culture, expression of CXCR4 was decreased 3.4 fold on CD34+ cells that expanded in serum free media. In contrast, expression of CXCR4 on CD34+ cells that expanded on hMSCs was increased. The results indicate that co-culture of cord blood stem cells and MSCs significantly increases CXCR4 expression on CB CD34+ cells.

0834

³¹P MR SPECTROSCOPY AND MULTIDRUG RESISTANCE (MDR) OF BLAST CELLS FROM PATIENTS WITH ACUTE LEUKEMIA (AL)

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Background. Multidrug resistance (MDR) due to overexpression of several proteins eg. ABC (ATP-binding cassette) proteins associated with P-glycoprotein (MDR1) and negative for P-gp MRP1 (multiresistance protein), LRP (lung resistance protein) and BCRP (breast cancer protein resistance) has been implicated in refractoriness to chemotherapy in acute leukaemias (AL), especially acute myeloid leukaemia (AML). These proteins can also translocate certain lipids across membranes: MDR3 - only phosphatidylcholine (PC), MDR1 - besides analogs of PC - translocates phosphatidylethanolamine (PE), sphingomyelin (SM), and glucosylceramide and MRP1 can translocate sphingolipid analogs. The dysfunctional metabolism of ceramide plays also a role in MDR. Aim of our pilot study was to examine the concentration of some phospholipids in blast cells from peripheral blood (PB) and bone marrow (BM) and relate these findings to the presence of some MDR proteins. **Methods.** Five AL patients, 3 with AML and 2 with ALL newly diagnosed patients with AL were included into the study, ³¹P MR spectra of phospholipids' extracts from PB and BM blast cells were obtained simultaneously at diagnosis. Cellular phospholipids (isolated from 60 mln cells by Ficoll buffy coat centrifugation) underwent methanol-chloroform extraction. AMX 300 Bruker spectrometer 7,05 T was applied. FCS analysis associated with application of specific monoclonal antibodies and leukaemic blasts gated with leukemia cell-specific antibodies in patients allowed for simultaneous assay of MDR proteins expression, e.g. MDR1, MDR3, MRP1, BCRP, and LRP. Results are shown in the Table.

Table. Phospholipids [10⁻⁶ mole/l] and MDR proteins.

Cell	State	Phospholipids				MDR proteins					O/E ratio	
		PC	CPLAS	SM	PE	MDR1	MDR3	MRP1	BCRP	LRP		
AML	PB	143	53	0	144	20	0	-	-	-	-	CR
	BM	745	40	32	434	47	0	-	-	-	-	CR
AML	PB	413	77	133	243	302	35	-	-	-	-	CR
	BM	297	27	549	172	151	34	-	-	-	-	CR
ALL	PB	287	8	0	47	0	0	-	-	-	-	CR
	BM	135	8	0	98	0	0	-	-	-	-	CR
AML	PB	754	153	338	473	31	146	-	-	-	-	CR
	BM	488	52	715	284	45	51	-	-	-	-	CR
		MDR during chemotherapy										
ALL	PB	211	8	90	132	0	0	-	-	-	-	CR
	BM	473	8	40	271	128	0	-	-	-	-	CR
		MDR during relapse										
		-	-	-	-	-	-	-	-	-	-	CR

Results. ³¹P spectrum of phospholipids' extracts from blasts consists of 8 peaks due to: phosphatidylcholine (PC), plasmalogen of phosphatidylcholine (CPLAS), lysophosphatidylcholine (LPC), sphingomyelin (SM), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), cardiolipin (CL). Two first AML patients in CR did not express any MDR proteins and the phospholipids concentration in blasts, both from PB and BM were within normal limits (with exception for a null SM for pt 1). In all three cases, who relapsed after

5-12 months of CR, the concentration of phospholipids in PB and BM blasts was lower than normal, but though MDR proteins were not expressed at diagnosis they appeared in two of them during chemotherapy; in AML (M5a) MDR3, MRP1 and LRP, and in ALL common MDR1. *Conclusions. Results.* of our pilot study suggests the relationship between phospholipids' metabolism and MDR.

0835

THE INFLUENCE OF GLYCIL-PROLINE, PROLIL-GLYCINE AND THEIR OXYPROLINE DERIVATIVES ON THE AGGREGATION OF RAT BLOOD IN VITRO

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Background. It was shown that such small peptides as Pro-Gly, Gly-Pro and Pro-Gly-Pro could demonstrate anticoagulant, fibrinolytic and antithrombotic effects both *in vitro* and *in vivo*. *Aims.* The comparison of the influence of four dipeptides Pro-Gly, HyPro-Gly, Gly-Pro and Gly-HyPro on the activity of rat thrombocytes in the whole blood *in vitro*. *Methods.* Blood was taken from male Vistar rats. Final concentration of the dipeptides was 10-7-10-3 M. Aggregometer 'Model 560CA' ('Whole blood lumi-ionized calcium aggregation system'; 'Chrono-Log Corporation', USA) was used. Aggregation was induced in the whole blood by either collagen ('Sigma Diagnostics', USA) at the final concentration of 0.2 mg/mL or ADP ('Chrono-Log Corporation', USA) at the final concentration of 10 µM. *Results.* 10-3-10-4 M Pro-Gly could not demonstrate any statistically significant effect on the collagen-induced thrombocyte aggregation. However 10-5-10-7 M Pro-Gly could perform the statistically significant changes: the aggregation lag-time was increased by 20% and the aggregation velocity was increased by 60%. The amplitude of aggregation (the maximal level of aggregation) remained unchanged. 10-5-10-7 M HyPro-Gly had the same influence on the collagen-stimulated thrombocyte aggregation as 10-5-10-7 M Pro-Gly. 10-4 M HyPro-Gly caused the statistically significant increase of the aggregation lag-time by 25% and the increase of the aggregation velocity by 30% as well as the decrease of the amplitude of aggregation by 40%. 10-4-10-6 M Gly-Pro could not change the lag-time of collagen-stimulated aggregation but decreased both the velocity of aggregation (by 30-40%) and the amplitude of aggregation (by 25-50%). 10-4-10-6 M Gly-HyPro increased the lag-time of collagen-stimulated aggregation by 30-40% and decreased both the velocity of aggregation (by 30-35%) and the amplitude of aggregation (by 35-40%). 10-3-10-7 M Pro-Gly could perform the statistically significant changes in ADP-stimulated thrombocyte aggregation: the increase in the lag-time and the decrease both in the velocity of aggregation (by 40-60%) and the amplitude of aggregation (by 40-60%). 10-4-10-7 M HyPro-Gly could increase the lag-time of the ADP-stimulated aggregation and decrease both the velocity of aggregation (by 25-40%) and amplitude of aggregation (by 25-40%). Gly-Pro and Gly-HyPro had an especially strong influence on ADP-induced thrombocyte aggregation. 10-4 M-10-7 M Gly-Pro increased the lag-time by 110-120%, decreased the velocity of aggregation by 50-75% and decreased the aggregation amplitude by 40-60%. 10-4 M-10-7 M Gly-HyPro caused the increase in the lag-time by 40-100% and the decrease of both the velocity of aggregation (by 25-70%) and the amplitude of aggregation (by 25-70%). *Conclusions.* Therefore, HyPro-Gly and Gly-HyPro were more effective antithrombotic agents when the aggregation was stimulated by the collagen. At the same time Pro-Gly and Gly-Pro were more effective antithrombotic agents when the aggregation was stimulated by ADP.

0836

DETECTION OF NEONATAL ALLOIMMUNE THROMBOCYTOPENIA (NAIT) BY SCREENING OF CORD BLOOD SAMPLES

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Background. Fetal or neonatal alloimmune thrombocytopenia (F/NAIT) is caused by maternal alloantibodies due to fetomaternal incompatibility for human platelet (plt) antigens (HPA) crossing the placenta. The severity of clinical symptoms can vary from no signs of disease to severe intracranial bleeding possibly leading to neurological sequelae or even death. Most cases are diagnosed after birth, affecting about 1 in 2000 live births and being responsible for about 10% of neonatal thrombocytopenia with plt counts <100x10E9/L. As causative antigens HPA-1a (78%), HPA-5b (19%) and HPA-2, -3 and -4 (3%) are reported. In 40-60% of cases already the first pregnancy is affected. Yet there is no routine antenatal screening for the condition recommended to define pregnant women at risk for this disease and the management of affected fetuses is not standardized at all. *Methods.* Since October 2003 we have routinely analysed plt counts in umbilical cord blood samples by automated cell counting (Sysmex, SE-9500, Mueller). In cases of confirmed neonatal thrombocytopenia we have screened maternal serum for alloantibodies by commercial solid phase assay (Capture-P, Immucor) and ELISA (PakPlus, GTI). Furthermore HPA-genotyping of the parents has been performed by PCR-SSP (Protrans) to confirm a possible fetomaternal incompatibility. *Results.* Until January 2005 we screened 1929 cord blood samples, 744 samples had to be excluded due to clotting (n=146) and/or plt aggregates (n=596) or insufficient volume (n=10). In 15 samples we found a plt count below 100x10E9/L; other parameters of blood cells were normal. Only in 3 cases thrombocytopenia was confirmed by capillary blood count analysis. One newborn was thrombocytopenic because of sepsis. In one of the 2 residual cases (plt count 10x10E9/L in the cord blood, capillary count 82x10E9/L) screening for alloantibodies was positive, revealing an anti-HPA-1a alloantibody in an HPA-1bb genotyped mother. It had been her fourth pregnancy without former complications. Except petechiae the child did not develop clinical symptoms of haemorrhage. Post partum there was no need for plt transfusions, on day 3 plt count had normalised (200x10E9/L). *Summary.* Screening of cord blood samples for thrombocytopenia is a useful method to detect affected neonates with mild occurrence of NAIT and to define alloimmunised women as being at risk for FAIT during following pregnancies.

0837

COMPARATIVE STUDY OF TH1 AND TH2 CYTOKINES PRODUCTION BY T-CELL IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS AND HEALTHY CONTROLS

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Background. T-helper cells are an integral part of the effective immune response against various malignancies; however in tumor-bearing patients they are frequently functionally unresponsive. Several sets of data indicate on the important role of T-helper 1 subset in the control of tumor growth. *Aim.* In order to assess the influence of T-cell-response polarization on the course of the disease intracellular cytokines (IFN-gamma, IL-4) were investigated in T-cells of peripheral blood in AML patients. *Methods.* Th1 and Th2 cytokine production was detected in CD3+CD8- (considered as CD4) and CD3+CD8+ lymphocytes from AML patients by flow cytometry analysis. Lymphocytes were isolated from the whole blood by Ficol-Hypaque density centrifugation, activated with PMA (phorbol 12-myristate-13-acetate, 50ng/mL) and calcium ionophore (250 ng/mL) for 12 h in the presence of Golgi Plug (brefeldin A) and stained for surface CD3, CD8 antigens and intracellular IFN gamma, IL-4. Samples from AML patients were collected at diagnosis (n=10), duration

of remission (n=14, samples=30), and at relapse (n=4, samples=6). Nine healthy donors constituted the control group. Statistical data were computed by program 'Statistics for Windows 5.5'. **Results.** There were CD3+CD8+IFN-gamma 37,6±12,2%, CD3+CD8-IFN-gamma 14±3%, CD3+CD8+IL-4 5,4±4,4%, CD3+CD8-IL-4 5,4±1,8% in 9 healthy donors and there were 37,9±22%, 31,9±18,5%, 5,1±3,6%, 3,5±1,7% in 10 AML patients at diagnosis respectively. There were 42,7±16,3%, 27,5±9,3%, 5,9±4,5%, 6,4±3,4% in 14 AML patients at remission respectively and there were 38,1±18,7%, 18,8±5,1%, 11,9±6%, 13,6±2,2% in 4 AML at relapse respectively. The percentages of IFN-gamma producing CD3+CD8+ cells did not differ much in AML patients and in donors. The percentages of IFN-gamma-producing CD3+CD8- T cells in AML patients were similar at diagnosis and in remission and exceeded such counts in healthy donors (p<0,02, p<0,0001). The amount of IFN-gamma-producing CD3+CD8-cells decreased at relapse. Simultaneously prior to relapse and at relapse the increase of IL-4 producing cells, both CD3+CD8+ and CD3+CD8-, was observed (p=0,05, p<0,0001). **Conclusions.** The activation of pro-inflammatory cytokine response (Th1) was detected in AML patients at diagnosis and during remission. Increased level of Th2 cytokine IL-4 was registered before and at AML relapse. The data provide the evidence of altered cytokine secretion by T-cell subsets in AML patients at different time points of the course of acute leukemia.

0838

MYELODYSPLASTIC PATIENTS HAVE EVIDENCE OF FUNCTIONAL IRON DEFICIENCY

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1.Background. Elevated percentage hypochromic red cells (%HRC) (normal range 0-5%) may indicate iron deficiency anaemia or functional iron deficiency in dialysis patients on erythropoietin (EPO) therapy. In myelodysplastic syndrome (MDS) patients, we have observed elevated %HRC despite normal/elevated serum ferritin and marrow iron stores. **2.Aims.** To investigate the incidence of elevated HRC in low-risk MDS patients and look for evidence of functional iron deficiency. **3.Methods.** 30 patients with low-risk MDS (22 refractory anaemia (RA) [16/22 with multilineage dysplasia] and 8 refractory anaemia with ring sideroblasts (RARS) [6/8 with multilineage dysplasia]) had complete blood counts (CBC) and reticulocyte counts, including %HRC and haemoglobin content of reticulocytes (MCHr), performed on the Bayer ADVIA 120 automated blood count analyzer. Serum LDH, CRP, ferritin, iron and erythropoietin, as well as bone marrow cytogenetics, were performed at diagnosis. Erythrocyte zinc protoporphyrin (ZnPP) and serum lead levels were performed in 8 MDS cases. **4.Results.** For RA patients, median haemoglobin (Hb) level was 9.3g/dL (6.2-12.8) and median MCV was 97.2 fl (84.7-122.4). For RARS patients, the equivalent figures were 9.8g/dL (6.8-11.2) and 100.6 fl (87.8-110.8). %HRC was significantly higher in the RARS group (7.8% (2.9-12.5)) compared to RA patients (1.3% (0.1-12.2)) (p=0.0001), despite normal/raised bone marrow iron stores in all cases. %HRC was elevated at diagnosis in 6/8 RARS and in 2/22 RA. A further patient with RA subsequently developed elevated %HRC but later had a complete remission of MDS with normalisation of %HRC after a respiratory tract infection. There was no correlation between %HRC and any CBC parameters or CRP, LDH or ferritin. A positive correlation was found between %HRC (median 5.8%; range 0.1-23.2) and erythrocyte zinc protoporphyrin (ZnPP) (median 4.7 ug/g Hb; range 0.2-8.3) levels (r=.879; p<0.01). 4/6 patients (RA 2; RARS 4) with elevated %HRC responded to EPO therapy by a sustained rise in Hb: in 2 RARS, %HRC remained stable, whilst %HRC increased further in 1 RARS and 1 RA. **5.Summary/conclusions.** Elevated %HRC is found in most cases of RARS. ZnPP levels are described for the first time in MDS and correlate with %HRC, suggesting functional iron deficiency as a factor for ineffective erythropoiesis in MDS with raised %HRC. 4/6 patients with elevated %HRC responded to EPO, with further increase in %HRC in 2 cases.

0839

NEONATAL HAEMOGLOBINOPATHY SCREENING BY HPLC: RESULTS FROM PREMATURE INFANTS

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In order to ensure the early detection of all children with sickle cell disease, universal neonatal haemoglobinopathy screening is being instituted in England. Those babies in whom no HbA is detected or who have had a transfusion prior to testing are recalled for a confirmatory test. This has the effect of increasing workload in the central laboratory where testing is carried out and generating parental anxiety. In the case of premature infants, interpretation of the screening test is often difficult as there are low levels of HbA present and no clear guidance as to what level is appropriate for the gestational age. In addition, it is often difficult to determine whether an infant has received a transfusion prior to testing and clinical history is not always available. It is therefore important to have a range of HbA% for each gestational age in order to aid interpretation and prevent unnecessary recalls. We report the HPLC results of 1966 infants, born between 23 and 42 weeks gestation. The graph below shows the average percentage of HbA present at each gestational age. The above data will aid accurate interpretation of the screening tests and allow identification of those infants who have been transfused prior to testing for whom no clinical information is available. It may also draw attention to infants who have inappropriately low levels of HbA for their gestational age. This can arise in the context of abnormal haemoglobins which are under-expressed and yet are of clinical significance, such as Hb Lepore.



Figure. %HbA in different gestational ages.

0840

USEFULNESS OF CD45 DENSITY IN THE DIAGNOSIS OF B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS

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Background. Although many B-cell chronic lymphoproliferative disorders (BCLPDs) including B-cell chronic lymphocytic leukemia (B-CLL) have characteristic clinical and biological features, the overlapping morphologic and immunophenotypic profiles of various BCLPDs still is a main problem and remain to be disclosed. **Aims.** Our aim was to evaluate the usefulness of CD45 expression in the immunological classification of BCLPDs (CLL vs. non-CLL) using a panel including antibodies to CD2, CD3, CD5, CD7, CD10, CD16, CD19, CD20, CD22,

CD23, CD25, CD38, CD45, CD56, CD79b, CD103, CD138, FMC-7, TdT, and Smlg by flow cytometry. Setting and design; *Methods.* and Material 37 patients with BCLPDs including CLL, hairy cell leukemia (HCL), B-prolymphocytic leukemia (B-PLL), and B-non-Hodgkin's lymphoma (B-NHL) in leukemic phase, and 8 healthy donors entered this study. By combination of the CD45 intensity with right-angle light scatter (often termed CD45 gating); we quantitatively compared the expression of CD45 among BCLPDs and normal lymphocytes using mean, median, and peak channel fluorescence intensities to assess the diagnostic usefulness of anti-CD45. *Results.* Our data indicate that; low intensity CD45 is associated highly with typical CLL. Non-CLL cases had brighter CD45 expression in comparison to cells of typical CLL (p<0.0001) and atypical CLL (p<0.04). Atypical forms of CLL, non-CLL, and normal lymphocytes express CD45 significantly higher than typical CLL (p<0.0001, p<0.0001, p<0.0001) respectively. *Conclusions.* CD45 seems to be a useful marker, to discriminate the typical CLL from the non-CLL and also from atypical CLL.

0841

THROMBOPHILIA IN YOUNG ADULTS WITH ISCHEMIC STROKE NOT RELATED TO CARDIOVASCULAR RISK FACTORS

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Background. The role of thrombophilia for causing ischemic stroke in young adults is still debated. Aim. During a period of 2 years (2001-2003), we evaluated 73 consecutive patients (younger than 50 y.o) with an episode of ischemic stroke not related to common cardiovascular risk factors.

Table. Results.

	Patients	Controls	OR (95% Confidence Interval)	p value
No. of patients (n=73)	11 (15%)	18 (25%)		ns
Mean age, y	37.8 (SD 10.1)	38.2 (SD 11.1)		ns
Age at the first event	20-44 (12)			
Risk factors				
Current smoking	31.2%	38.9%	1.0 (0.4-2.3)	ns
Diabetes	11.4%	8.9%	1.3 (0.5-3.0)	ns
Hyperlipidemia	17.9%	8.9%	2.2 (0.9-5.0)	ns
Obesity	1.4%	1.4%	1.0 (0.5-1.5)	ns
Thrombophilia				
LAC	12%	1.4%	4.9 (1.5-16)	0.04
Other (Factor V and II, AT, APC)	27%	1.4%	1.9 (0.5-7.0)	ns
FV Leiden	0%	0.6%	1.0 (0.2-3.3)	ns
II mutation	1.4%	0.2%	8.8 (0.3-1.1)	ns
HTTII mutation	10.4%	0.6%	17.8 (3.2-9.1)	ns
Combined (LAC)	4.1%	0.6%	7.2 (0.8-1.3)	ns
Fasting homocysteine levels (mmol/L)	12.4 (SD 5.0)	9.2 (SD 5.0)		0.001
Homocysteinuria	11.4%	0.6%	17.8 (3.2-9.1)	ns
Hyperhomocysteinemia	7.7%	0.6%	13.8 (2.0-8.7)	ns
Defects	1.4%	1.4%	1.0 (0.5-1.5)	ns

Methods. The prevalence of coagulation abnormalities were determined; 138 healthy subject served as controls. *Results.* The results are shown in the table. The prevalence of common cardiovascular risk factors did not differ between case and controls. Fasting and PML (Post-Methionin Load) serum homocysteine levels and LAC were the only variables that significantly differed between patients and controls, respectively. *Conclusions.* We concluded that these two variables only should be investigated in young adults with ischemic stroke and no presence of common cardiovascular risk factors.

0842

EFFECTIVE TREATMENT OF HYPEREOSINOPHILIC SYNDROME WITH IMATINIB MESYLATE

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Idiopathic hyper eosinophilic syndrome (HES) is a myeloproliferative disorder characterized by persistent eosinophilia and organ involvement. Patients with HES have a poor prognosis. HES has been treated by corticosteroids, cyclophosphamide, vincristine, hydroxyurea and interferon-alpha. Imatinib mesylate is highly effective in chronic myeloid leukemia and recent data have suggested that it is also effective in the treatment of HES, regardless of the presence of the fusion tyrosine kinase, FIP1L1-PDGFR-alpha. We describe a patient with HES and severe multiple organ damage, including the heart and central nervous system. His bone marrow was hypercellular with markedly increased number of eosinophils and their precursors. There was moderate to severe myelofibrosis. Cytogenetic study of the bone marrow did not reveal any abnormality. FIP1L1-PDGFRa mutation was not examined. He was treated with corticosteroids and hydroxyurea without significant clinical and hematological response. He then received 100 mg imatinib mesylate daily and achieved complete hematological response that has lasted 8 months. There was marked improvement of his cardiac and neurological symptoms. Since most of the patients with HES respond promptly to imatinib, we believe, that short term trial with low dose of imatinib should be offered to all symptomatic patients regardless of the presence of the mutation FIP1L1- PDGFRA fusion gene.

0843

A FULMINANT COURSE OF SINUS HISTIOCYTOSIS WITH MASSIVE LYMPHADENOPATHY (ROSAL-DORFMAN DISEASE)

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Introduction. Sinus histiocytosis with massive lymphadenopathy (Rosai- Dorfman disease) is a rare disorder characterized by a nonmalignant proliferation of distinctive histiocytic-phagocytic cells within lymph node sinuses and lymphatics in extranodal sites. The disease is usually diagnosed in childhood and young adulthood. Only rarely had the disease been reported in elderly patients. *Case report.* We present an 83 year- old female patient who was hospitalized with weight loss, night sweats and muscular weakness. Physical examination revealed generalized lymphadenopathy, hepatomegaly and urticaria. She had severe muscular damage as reflected by laboratory tests and muscle biopsy. Laboratory results indicated autoimmune disease, including hemolytic anemia. The biopsy of the cervical lymph node showed marked dilatation of the sinuses by a proliferation of large histiocytes, showing emperipolesis (phagocytosis of lymphocytes and plasma cells). The cells expressed antigen CD 68 and S-100. These findings were compatible with diagnosis of sinus histiocytosis with massive lymphadenopathy (SHML). Her clinical condition improved spontaneously, however she later died of sepsis, related to her poor general condition. *Discussion.* SHML is a rare disease of childhood, presenting in 90% of the cases with painless cervical lymphadenopathy. Axillar, inguinal and paraaortic lymph nodes are also frequently involved. The most frequently involved extranodal sites are skin, respiratory tract, eye, CNS and soft tissues. Immunological abnormalities such as hemolytic anemia, rheumatoid arthritis, asthma and diabetes mellitus are frequent. The etiology is unknown; a pathological response of hematopoietic system to an undetermined immunological trigger had been postulated. Another mechanism might be cytotoxic activation by perforin pathway causing accumulation of cytotoxic T cells and inflammatory cytokines. Pathologic diag-

nosis is based on the presence of expanded sinuses filled with histiocytes and lymphophagocytosis. The histiocytes express CD 68 antigen and S 100 protein. Treatment with steroids, surgical debulking, radiotherapy, interferon and chemotherapy had been used with some response. Half of the cases resolved spontaneously. Our patient is one of the oldest patients reported with the disease. She had multiple organ involvement, including skin, muscles and lymph nodes as well as multiple autoimmune disorders. Due to the rarity of the disease, the clinical diagnosis was in favor of autoimmune disorder or lymphoma. Only after biopsy of the lymph node was the final diagnosis established. We conclude that diagnosis of SHML should be considered in patients with lymphadenopathy and multiple organ involvement.

0844

INCREASED SENSITIVITY OF BCR/ABL DETECTION BY RQ-PCR

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Background. Careful monitoring of minimal residual disease (MRD) is essential in CML patients. It can help in therapeutic decisions, such as timing of donor lymphocyte infusions (DLI's), prediction of relapse etc. The most sensitive and accurate method for that purpose is the real-time quantitative RT-PCR (RQ-PCR), which monitors the kinetics of residual BCR-ABL transcripts over time. Lately, a standardized protocol for TaqMan-based RQ-PCR analysis of BCR-ABL was established by the Europe Against Cancer (EAC) program (Gabert et al, 2003). **Aims.** Our aim was to increase the sensitivity of the RQ-PCR method to that of 'nested' qualitative RT-PCR. **Methods.** Patients and samples A total of 42 samples were obtained from 21 CML patients, 4 at diagnosis and 38 while follow up. RT-PCR analysis Mononuclear cells were isolated from bone marrow (BM) or peripheral blood (PB), followed by isolation of total RNA. 2-10 µg RNA were used for preparation of cDNA. Samples at diagnosis were subjected to one step RT-PCR, whereas MRD detection was performed using 'nested' RT-PCR. RQ-PCR In parallel, samples were evaluated using RQ-PCR according to the EAC protocol using Rotor-Gene 3000 (Corbett Research). 1µg RNA was used for cDNA preparation. β -2-microglobulin (B2M) was used as a control gene (one of the three control genes recommended by the EAC). All RQ-PCR reactions were performed in duplicates. **Results.** were determined as 'negative' only if BCR-ABL transcripts were below detection and the B2M transcripts were $>1 \times 10^5$. **Results.** 11 of 42 (26%) samples were positive using 1st RT-PCR. The 31 samples which were negative by 1st RT-PCR, were subjected to 2nd (nested) RT-PCR and RQ-PCR. Using 2nd RT-PCR we were able to detect BCR-ABL in 30 (96%) of the samples, while only 25 (81%) of the samples were positive using RQ-PCR. For the 5 samples which were 2nd RT-PCR-positive/RQ-PCR negative, new cDNAs were prepared from 3 µg RNA, and checked by RQ-PCR. With this setup, 3 out of 5 samples appeared to be positive. In order to assess the suitability of augmented RNA amount for samples with higher levels of BCR-ABL transcripts, we prepared two cDNAs (from 1 and 3 µg RNA) for each of 7 patients: 3 at diagnosis, 3 in remission and 1 in accelerated phase. RQ-PCR was performed on those cDNAs, and the ratio between BCR/ABL and B2M was calculated. This ratio is similar in each pair of cDNAs. **Conclusions.** By using higher amounts of RNA (3µg), we achieved higher sensitivity of RQ-PCR. The BCR/ABL detection in this setup was almost equal to that of nested RT-PCR. Since the increase in RNA amount had no significant influence on the ratio between BCR/ABL and B2M, this method is also suitable for usage with higher levels of BCR/ABL transcripts.

0845

EXPRESSION OF NEURAL CELL ADHESION MOLECULE (CD56) ON MALIGNANT PLASMA CELLS

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Background. It was shown that the absence of CD56 on malignant plasma cells (PCs) is a hallmark of plasma cell leukemia (PCL) and of a special subset of multiple myeloma (MM) (Pellet-Deceunynck et al. Leukemia 1998; 12: 1977-1982). There was also found that expression of CD56 correlates with the presence of lytic bone lesions in MM and distinguishes MM from monoclonal gammopathy of undetermined significance and lymphomas with plasmacytoid differentiation (Ely et al. Am J Pathol 2002; 160: 1293-1299). The aim of this study was to evaluate the intensity of CD56 expression on bone marrow (BM) and peripheral blood malignant PCs and to assess clinical correlations. **Material and Methods.** The study group consisted of 100 MM patients (52M 48F, median age 65, range 39-80yr; 15 at stage I, 22-II, 63-III acc. to D.S.; 65 had osteolysis; monoclonal protein IgG was in 70 patients, IgA-20, IgM-1, Bence Jones -9) and 15 PCL patients. Controls were 10 healthy subjects. Immunophenotyping was done on freshly collected BM and blood samples using triple staining combination of CD138/CD56/CD38 monoclonal antibodies analysed by flow cytometry (Cytoron Absolute and FACSCalibur-Becton Dickinson). Antigen expression intensity was calculated as relative fluorescence intensity (RFI) and for direct quantitative analysis the QuantiBRITE test was applied. Mean channels of phycoerythrin fluorescence were defined and antibody binding capacity (ABC) was then calculated using QuantICALC software. **Results.** In 69 MM patients (69%) PCs showed CD56 expression. Out of all CD38⁺⁺/CD138⁺ BM cells mean proportion of PCs with CD56 expression, was $77 \pm 21\%$, median 88%. RFI values ranged from 7,6 to 23,3 in individual patients ($15,9 \pm 3,6$, median 15,6) and the number of CD56 binding sites (ABC) on MM plasma cells ranged from 2255 to 58469 (14199 ± 15038 , median 8866). A correlation was found between RFI and ABC values ($r=0,76$; $p<0,05$). In 31 MM patients considered as CD56 negative myeloma mean proportion of all BM CD38⁺⁺ cells with CD56 expression was $4,8 \pm 4,1\%$, median 4,0%. A correlation was found between proportion of all BM CD38⁺⁺ cells with CD56 expression and ABC ($r=0,60$) and RFI ($r=0,61$) indices ($p<0,05$). Normal PCs did not express CD56. No differences among analysed cases were seen between occurrence of CD56 expression and presence of osteolysis, stage of disease and monoclonal protein isotype. Response rate to chemotherapy was similar in CD56 positive and CD56 negative plasma cell proliferation; 58 and 55% respectively. Of 15 PCL cases 8 showed CD56 expression on PCs in BM and on those in peripheral blood. **Conclusions.** In two thirds of MM patients CD56 molecule could be considered as a 'tumor associated antigen'. Intensity of CD56 expression on PCs varies among particular CD56 positive MM patients and differences in expression level may be as big as many times. There is relationship between proportion of BM CD56 positive PCs and density (ABC) and intensity (RFI) of expression of this molecule. PCL cases show heterogeneity in expression of CD56.

0846

EFFECTIVENESS OF ANAGRELIDE IN THE TREATMENT OF SYMPTOMATIC PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Patients with essential thrombocythemia (ET) are characteristically prone to the erythromelalgia as a result of the thrombocytosis and platelet coagulation activation. It has been demon-

strated that ET patients receiving cyto-reduction and antiaggregants show normal platelets but present still platelet coagulant activation and thrombosis. Anagrelide (ANA) is a platelet-lowering drug that inhibits platelet activation. Thus, we investigated platelets and markers of platelet coagulant activation such as platelet factor 4 (PF4), prothrombin fragment 1+2 (F1+2), plasmin/a2antiplasmin complex (PAP) and plasminogen activator inhibitor-1 (PAI-1) in patients with ET and erythromelalgia. It is known that platelet endothelial activation may release tissue factor pathway inhibitor (TFPI), which is a marker of microvascular damage. The study group included 17 patients (10 males and 7 females, mean age 48 years) affected by ET diagnosed according to PVSG criteria. All patients were on antiaggregants either aspirin (ASA) (9 patients) or indobufen (IND) (7 patients) and dipyridamole (DYP) (1 patient). Their mean duration of disease was 4 years. 9 of them had erythromelalgia. After a median time from diagnosis of 3 years all patients were started on ANA. ANA was initially administered in dose of 0.5 mg/day, with increases of 0.5 mg/day every 7 days until the platelets decreased below $500 \times 10^9/L$ and with a average maintenance dosage of 2.1 mg/day. None of studied patients had thrombotic risk factors. All measurements were performed before ANA and to complete response defined as platelets $< 500 \times 10^9/L$. Platelets were determined by automated analyser. PF4, F1+2, PAI-1, PAP and TFPI were measured by ELISA. Before ANA all patients had high platelets ($1.120 \pm 272 \times 10^9/L$) and increased PF4 and F1+2 (118 ± 42 IU/mL vs 4.9 ± 3.1 IU/mL and 3.9 ± 3.3 nmol/L vs 0.6 ± 0.3 nmol/L, respectively) ($p < 0.0001$ and $p < 0.0001$, respectively) as well as PAP, PAI-1 and TFPI (398 ± 248 ng/mL vs 128 ± 77 ng/mL and 136 ± 62 ng/mL vs 26 ± 8 ng/mL and 166 ± 69 ng/mL vs 81 ± 12 ng/mL, respectively) ($p < 0.0001$ and $p < 0.0001$ and $p < 0.0001$, respectively). After ANA all patients had platelets $< 500 \times 10^9/L$ ($394 \pm 63 \times 10^9/L$) and normal PF4 (8.5 ± 3.2 IU/mL), F1+2 (1.3 ± 0.6 nmol/L), PAP (189 ± 189 ng/mL), PAI-1 (35 ± 6 ng/mL) and TFPI (98 ± 54 ng/mL) and erythromelalgic symptoms disappeared. These measurements were repeated in a further sample, collected after 1 or 2 months, and showed concordance for PF4, F1+2, PAP, PAI-1 and TFPI concentrations. A positive correlation there was between PF4 and F1+2 and PAP and PAI-1 and TFPI ($p < 0.0001$ and $p = 0.045$ and $p = 0.039$ and $p = 0.002$, respectively). No correlation we found between platelets, PF4, F1+2, PAP, PAI-1 and thrombosis whereas a significant correlation there was between TFPI and thrombosis ($p = 0.015$). Our data suggest that erythromelalgia is associated with platelet-mediated endothelial activation and that the ANA may be efficacious in the treatment of symptomatic patients with ET.

0847

THROMBOCYTOPENIA IN TRANSFUSION-DEPENDENT BETA-THALASSEMIA

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Background. Thrombocytopenia developed in 9 of 89 patients with transfusion-dependent beta-thalassemia. Historically, the condition was principally attributed to hypersplenism, which was due to irregular transfusions and was accompanied by an increase in sequestration and the accelerated destruction of platelets in the pathologically enlarged and congested spleen. Nowadays, splenomegaly should be rare after adapting hypertransfusion regimen in our patients. **Aims.** To study the risk factors for thrombocytopenia in transfusion-dependent beta-thalassemic patients. **Methods.** Clinical data of 89 patients including age, sex, age at the first blood transfusion, age at the start of iron-chelation therapy, average serum ferritin level, previous splenectomy, existence of splenomegaly, existence of hepatitis B surface antigen, existence of hepatitis C virus (HCV) RNA, serum alanine aminotransferase (ALT) level, and average platelet count were collected from hospital files. Potential risk factors were identified using univariate logistic-regression analyses. Multivariable logistic-regression model was used to select the independent risk factors that best predicted thrombocy-

topenia. A two-tailed p value of < 0.05 was considered to be statistically significant. **Results.** The prevalence of thrombocytopenia was 10.1% (9 of 89). All are poorly compliant with iron-chelation therapy. Splenomegaly was found in 7 patients. Abnormal ALT level was observed in 8 patients. Five patients (55.6%) had HCV infection. One patient had previous splenectomy. The risk factor for thrombocytopenia found in transfusion-dependent beta-thalassemic patients was splenomegaly. **Conclusions.** Splenomegaly was 26.1% (18 of 69) in present study, and was the independent risk factor for thrombocytopenia. Splenomegaly in transfusion-dependent beta-thalassemia could be attributed to extramedullary hematopoiesis and/or portal hypertension due to chronic liver disease. However, the existence of a splenectomized patient with thrombocytopenia proved that splenomegaly is unable to fully explain thrombocytopenia in these patients. The patient was co-infected with hepatitis B virus (HBV) and HCV. She also had very high level of serum ferritin concentration. Low thrombopoietin (TPO) level and anti-platelet antibodies formation due to chronic liver disease and/or multiple transfusions might be implicated in the development of thrombocytopenia for her. Hence, in addition to search other causes for splenomegaly, such as serum erythropoietin and soluble transferrin receptor for unsuccessful erythropoiesis suppression, serum TPO level and anti-platelet antibodies should be measured for possible etiology of thrombocytopenia in the future.

0848

BENDAMUSTINE IN COMBINATION WITH THALIDOMIDE AND PREDNISOLONE IN PATIENTS WITH REFRACTORY OR RELAPSED MULTIPLE MYELOMA. PRELIMINARY RESULTS OF A PHASE I CLINICAL TRIAL

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Thalidomide is remarkably active as single agent in advanced relapsed or refractory multiple Myeloma (MM), but with a significant comorbidity due to side-effects such as polyneuropathy. We questioned whether lower doses of thalidomide in combination with weekly doses of bendamustine and prednisolone might be a more effective regimen with fewer side-effects especially in relation to neurotoxicity. The cytotoxic agent bendamustine combines a purine-like benzimidazol and bifunctionally alkylating nitrogen mustard group with low toxicity. The treatment consists of a fixed dose of bendamustine (60 mg/qm) i.v. day 1, 8, and 15 and prednisolone (100 mg) p.o. day 1, 8, 15, and 22. At the same time, thalidomide was given in patients cohorts with escalating doses, starting with 50 mg to a maximum of 200 mg daily. 4 patients were enrolled at each dose level. If 1 dose limiting toxicity (DLT) occurs, then additional 2 patients would be enrolled at that dose level. Cycles were repeated every 28 days for a minimum of 2 and a maximum of 10 cycles until a maximal response was achieved, a DLT or a disease progression were observed. 9 patients (4 in the first dose level with 50 mg thalidomide, 4 in the second dose level with 100 mg thalidomide and 1 patient in the third dose level with 200 mg) are enrolled until now. The number of prior treatment regimens was 2 or more in all patients. 4 patients were refractory for the last treatment. Median age was 69 years (range: 64-78). **Results.** All patients completed 2 cycles of BFT-treatment (4 completed 6 cycles, 1 completed 5 cycles and 4 completed 4 cycles) and were hence evaluable. Response was assessed using EBMT criteria modified to include near complete remission (nCR) and very good partial remission (VGPR). 7 of 9 patients responded after at least 2 cycles of chemotherapy with 1 CR, 2 VGPR and 4 PR. 2 patients had stable disease. No DLT was observed in the first 2 doses level. Most common site effects were constipation (5 patients WHO grade 1, 3 patients WHO grade 2) and somnolence (3 patients WHO grade 1). None of the 9 patients devel-

oped dose-limiting haematotoxicity as defined by an ANC $<1,0 \times 10^9/L$ for >7 days or an ANC $<0,5 \times 10^9/L$ for >3 days or platelet count $<25 \times 10^9/L$. Neutropenia was reported in 3 patients (WHO grade 2) but no thrombocytopenia was observed. No grade 3 or 4 non haematological toxicity was encountered and no dose modification was required. BPT with a dose of 50 or 100 mg thalidomide daily is well tolerated in patients with relapsed or refractory MM. Thus we are continuing the dose escalation with the third dose level of 200 mg thalidomide daily to evaluate the maximal tolerable doses of the BPT regimen.

0849

SIBLING UMBILICAL CORD BLOOD TRANSPLANTATION FOR 23 PATIENTS WITH HEMATOLOGICAL MALIGNANCY

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Background. Sibling umbilical cord blood transplantation is an effective way to treat hematopoietic malignancy, it has been used to treat the malignant hematopoietic disorders for more than a decade years, but only a few patients received this treatment worldwide and only less than 100 cases have been transplanted in China by now. Since 1998, we have done a pioneer work in China to treat the hematological malignant patients with sibling umbilical cord blood transplantation. **Aims.** To investigate the hematopoietic reconstitution and to observe the graft versus host disease and other complications related to sibling umbilical cord blood transplantation in the Chinese patients. **Methods.** 23 patients with hematological malignancy were transplanted with related umbilical cord blood; the stem cells were from the sibling of the patients. Of the 23 patients, 14 were male and 9 were female. The median age was 11 years (range 3-16). The median body weight was 38kg (range 15-65). These patients included 10 cases of ANLL, 5 cases of ALL, 6 cases of CML, 1 case of MDS and 1 case of SAA. Among the 23 patients, 19 patients received matched stem cells, 4 patients received 1-2 HLA loci mismatched stem cells. The median number of infused nucleated cells was $3.5-107/kg$ (range $1.5-10.0$). The conditioning regimen consisted of busulfan and cyclophosphamide. GVHD prophylaxis consisted of cyclosporine A. **Results.** 19 patients obtained hematopoietic reconstitution, 4 patients failed to reconstitute hematopoietic function. The median time to achieve an absolute neutrophil count in excess of $0.5-10^9/L$ was 17 days (range 11-42) after cord blood infusion. The median time to achieve platelet count greater than $20-10^9/L$ was 28 days (range 15-54) after the infusion of the cord blood. Complete chimera of DNA was found by monitoring microsatellite point at 21-90 days. 12 patients developed grade 1-2 aGVHD, 1 patient developed grade 3 aGVHD. 4 patient developed cGVHD. 3 patients died of infectious complication. 4 patients relapsed. 12 patients were EFS posttransplantation and the time of following-up was from 7 months to 72 months. **Conclusions.** Sibling UCBT is a safe and effective way to treat hematopoietic malignancy. The time of the hematopoietic recovery relates to the number of infused NCs. The conditioning regimen consisting of busulfan and cyclophosphamide is safe and suitable for children. GVHD prophylaxis with only CSA is effective.

0850

STUDY OF AUTO-PERIPHERAL BLOOD STEM CELL MOBILIZATION AND COLLECTION IN HEMATOPOIETIC MALIGNANCIES

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Aims. To study the strategies of mobilization-collection-preservation of auto-peripheral blood stem cell (APBSC) for hematopoietic malignancies and detection of APBSC activity. To explore the mutual relationship between reconstruction of hematopoietic system and the count & quality of APBSC col-

lection and preservation after auto-peripheral blood stem cell transplanting (APBSC) for hematopoietic malignancies. **Methods.** Eighteen patients with hematopoietic malignancies were administered with routine chemotherapy followed by Lenograstim (rhG-CSF, $5\mu g \cdot kg^{-1} \cdot d^{-1}$, from the day WBC $<1.0 \times 10^9/L$ until completion of leukapheresis). The APBSC of patients were collected by CS-3000 plus blood cell separator (Baxter corporation), persevered in -196° liquid nitrogen after treat with program controlled cryoprotection; infused immediately after recovered frozen in $37-40^\circ$ water bath; detected the percentage of tropochrome cell to trypanum, the positive rate of CD34, the collection rate of MNC & CFU-GM of APBSC before preservation and after recovered frozen. **Results.** All cases are successful collection. The time of collection was 9-19 day (average 12.6) after chemotherapy; the number of time was 1-3, (average 2.4); the mean collection rate of MNC was $138.6 \pm 52.32\%$; the mean MNC recovery rate of the recovered frozen stem cell was $91.96 \pm 1.37\%$, $CD34+85.94 \pm 0.64\%$, $CFU-GM 87.69 \pm 4.53\%$. The percentage of tropochrome cell to trypanum before preservation & after recovered frozen was $96.26 \pm 1.33\%$, $92.75 \pm 2.04\%$. No significant difference of the percentage of tropochrome cell to trypanum was observed between each group ($P > 0.05$). The collection rate of CFU-GM MNC and the positive rate of CD34 in multiple myeloma was lower than that of in leukemia and malignant lymphoma. The mean collection rate of MNC was lower and CFU-GM was dysgonic in 5 patients with more than 10 times chemotherapy and three of them were delayed erythropoiesis after APBSC. **Conclusions.** Chemotherapy combined with rh-GCSF is a safe and highly effective method for APBSC mobilization in hematopoietic malignancies. There was a significant individual difference in the count & quality of APBSC and it is correlated with the different kinds of disease and chemotherapy plan. It may be effect the count & quality of APBSC to increase the time of the chemotherapy before PBSCT.

0851

THE VALUE OF CHEMOTHERAPEUTIC REGIMEN CONTAINING PIRARUBICIN IN THE TREATMENT OF ADULT WITH HIGH-RISK OR REFRACTORY AND RELAPSED ACUTE LEUKEMIAS

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Aims. To Evaluate the therapeutic effects of chemotherapeutic regimen containing pirarubicin (THP) in the treatment of adult with high-risk or refractory and relapsed acute leukemias (AL). **Methods.** A total of 80 Adult with high-risk or refractory and relapsed AL enrolled in this study [the treatment group (n=40) and the control group (n=40)]. In the treatment group, all received treatment regimens with THP+ Cytosine-arabioside (Ara-C) in the acute myeloid leukemia (AML) or THP+ Ara-C +vincristine (VCR) +prednisone (Pred) in the acute lymphocytic leukemia (ALL) [ALL or mixed AL]. The regimens with mitoxantron (MIT)+ Ara-C (AML) or MIT+ Ara-C + VCR + Pred (ALL or mixed AL) were used in the control group. Only one pair was primary treatment with high-risk factor and another 39 pairs matched patients were high-risk or refractory and relapsed. Only 4 pairs had some difference and another 36 matched patients were in the same of subtype AL. **Results.** The effects were comparable between the treatment group and the control group. Complete remission (CR) rate was $47.5\% VS 45\%$ ($p=0.823$), Partial response (PR) was $25\% VS 20\%$, ($p=0.455$), Overall response (OR) was $72.5\% VS 65\%$ ($p=0.772$). Continuous CR days was significantly longer in the treatment group than the control group, $527.95 VS 463.4$ ($P < 0.05$). Marrow suppression was more serious in the treatment group. Patients in the treatment group has higher Incidence of infections ($p=0.045$); the times of used G-CSF has obviously increased ($P < 0.05$) and the platelet recovery was longer ($p=0.019$) than the control group. **Conclusions.** Regimens with THP is effectual in the treatment of adults with high-risk or refractory and relapsed AL. But marrow suppression is more serious and the incidence of infection is higher.

0852**PLASMA CELL LEUKEMIA IMMUNOPHENOTYPIC CHARACTERISTICS**

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The aim of the study was to determine expression of adhesion molecules CD11a (LFA- α), CD18 (LFA- β), CD11b, CD29, CD49d, CD44 (H-CAM), CD54 (ICAM-1), CD56 (N-CAM) and CD117 (c-kit) on peripheral blood (PB) and bone marrow (BM) lymphoid cells in 21 plasma cell leukemia patients (PCL), at diagnosis and in the control group of 10 healthy subjects. Immunophenotyping was performed on freshly collected blood and bone marrow samples by means of flow cytometry. Plasma cells were identified as showing high-density expression of CD38 and CD138 (syndecan-1). *Results.* of analysis were presented both as relative and absolute (omitted in abstract) values of numbers of cells with antigen expression and as relative fluorescence indices (RFIs) of studied antigens. Statistical analysis was performed using Wilcoxon's test. All below presented differences are statistically significant. The study revealed in PCL patients a significantly higher relative and absolute number of CD54+ cells (in brackets: means \pm SD of PCL pts vs control) both in BM (63 \pm 29% vs 13 \pm 5%) and PB (49 \pm 25% vs 8 \pm 3%) as well as that of CD38+ cells both in BM (84 \pm 12% vs 54 \pm 11%) and PB (74 \pm 11% vs 52 \pm 7%). In turn, PCL patients showed a decreased relative number of BM: CD11a+cells (40 \pm 28% vs 73 \pm 10%), CD18+cells (47 \pm 25% vs 88 \pm 7%), CD11a+CD18+cells (42 \pm 27% vs 72 \pm 10%), CD44+cells (71 \pm 26% vs 93 \pm 4%), CD11b+cells (17 \pm 12% vs 35 \pm 10%) and PB: CD11a+cells (58 \pm 28% vs 96 \pm 3%), CD18+cells (58 \pm 29% vs 99 \pm 0,2%), CD11a+CD18+cells (58 \pm 29% vs 96 \pm 3%), CD44+cells (86 \pm 15% vs 98 \pm 0,9%). In BM of PCL patients compared with the control there were found decreased RFIs of CD18 (15,0 \pm 1,3 vs 16,6 \pm 0,7) and CD29 (8,6 \pm 1,4 vs 10,4 \pm 0,8) and increased RFIs of CD54 (16,9 \pm 2,5 vs 13,0 \pm 0,5) and CD11a (18,4 \pm 1,5 vs 14,7 \pm 0,9). In PB of PCL patients RFIs of CD29 (10,4 \pm 1,2) was lower than this in control (11,6 \pm 0,9) while RFIs of CD38 (16,9 \pm 3,0 vs 14,8 \pm 1,3), CD54 (16,1 \pm 2,8 vs 12,3 \pm 0,3), CD11a (20,4 \pm 1,8 vs 18,3 \pm 0,3) were higher. In all examined cases PB and BM leukemic cells with high expression of CD38 and CD138 showed co-expression of CD54 antigens, in 10 cases-also CD56, in 5-CD117, in 7- CD29 and CD49d, in 4 (4 in PB, 1 in BM) out 13 - CD11b, in 5 out 9 - CD44. On the other hand, in all cases PB and BM leukemic cells showed lack or weak expression of CD18, CD11a, CD11a+CD18+. *Conclusions.* Immunophenotype of leukemic plasma cells characterizes mainly increased expression of CD38, CD54 and CD138 and disturbed expression of CD18, CD11a and CD11b. In one half cases tumor cells show expression of CD56 and CD117.

0853**RESOLUTION OF PSORIASIS AFTER ALLOGENEIC PERIPHERAL STEM CELL TRANSPLANTATION FOR CHRONIC MYELOGENOUS TRANSPLANTATION**

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Treatment of autoimmune disease with hematopoietic stem cell transplantation (HSCT) is under investigation. A few reports of patients undergoing allogeneic stem cell transplantation for malign conditions observed the resolution of psoriasis after HSCT, with minimal late morbidity. We describe a patient with chronic myelogenous leukemia (CML) whose psoriasis resolved completely after allogeneic HSCT. A 43 year old man underwent allogeneic HSCT in 2001, 6 months following a diagnosis of CML. His donor was his HLA matched healthy brother. The patient also had a history of long standing severe erythrodermic psoriasis for long years, which manifested as typical well demarcated erythematous plaques symmetrically distributed over

the elbows. During the years preceding the transplant, he was treated with corticosteroids, but with no significant response. The conditioning regimen for allogeneic HSCT included busulfan (16 mg/kg) and cyclophosphamid (120 mg/kg) followed by transplantation of HSC. Cyclosporine and methotrexate were given for GVHD prophylaxis. On day +12 he had complete engraftment of donor cells. His extensive psoriatic plaques responded rapidly after initiation of the conditioning regimen, and resolved completely by day + 12. The response of the psoriatic skin lesions was durable, with no evidence of recurrence of psoriasis through 3.1 years of follow-up post transplant. This report demonstrates that long term remission of immune mediated diseases can occur with HSCT.

0854**MULTIDRUG RESISTANCE GENE-1 EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKAEMIA, RELATION TO IMMUNOPHENOTYPING**

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Therapeutic resistance is a major obstacle in the treatment of acute lymphoblastic leukemia. Clinical resistance to anticancer agents may occur at the time of presentation as well as during the course of treatment and relapse. Although many patients with acute lymphoblastic leukemia can achieve complete remission with induction of chemotherapy, a large proportion will eventually relapse with disease resistant to broad spectrum of chemotherapeutic agents. The aim of the present work is to evaluate the expression of MDR1 gene in acute lymphoblastic leukemia patients and to find out its relation to immunophenotypic pattern and to treatment outcome. The present study included 30 subjects: 20 newly diagnosed ALL patients. Among them 9 (45%) was diagnosed as common ALL; 6 (30%) as Pre-B; 3 (15%) as T-ALL and 2 (10%) as B-ALL and 10 age and sex matched apparently healthy individuals as control group. All patients of this study were subjected to thorough history taking, Full clinical examination, routine laboratory investigations and immunophenotyping. Study of multidrug resistance-1 gene (MDR1) expression was performed by the method of real time reverse transcription-polymerase chain reaction (RT-PCR) to all 30 subjects of this study. The result of the present study showed: MDR1 gene was expressed on normal cells of the control group as well as blast cells of ALL patients. MDR1 gene was over expressed (MDR1-positive cases) in 30% (6/20) of the patients. The difference in the mean values of white cell count, hemoglobin level, platelet count, percentage of peripheral and bone marrow blast cells, as well as sex distribution and FAB subtypes was not statistically significant among MDR1 positive and negative cases. Among laboratory parameters, there was a significant positive correlation between MDR1 over expression only with advanced age in ALL cases. There was no significant association between MDR1 positive cases with hepatomegaly, splenomegaly or lymphadenopathy. In the whole group of the ALL patients, The number of MDR1 negative patients who entered into complete remission (CR) was significantly higher than those of the MDR1 positive patients.

0855**AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS COLLECTION FROM PATIENTS WITH NEOPLASTIC DISEASES AND BODY WEIGHT UNDER 30 KG- SINGLE CENTER EXPERIENCE**

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Intensive chemotherapy followed by autologous peripheral blood stem cell (PBSC) transplantation is the treatment of choice for children with recurrent or refractory malignant diseases. Successful mobilization and collection of PBSC affect the amount of infused stem cells and the outcome of the transplantation, respectively. There are many problems, especially technical, in

mobilization and collection of PBSC in children, and they are more frequent in patients (pts) with body weight less than 20 kg. The objective of this study was to define the safety and efficacy of PBSC collection in children with neoplastic diseases and body weight under 30 kg. From October 1997 to October 2004 we performed 24 mobilizations and 45 collections in 22 pts with malignant diseases and body weight under 30 kg. The pts characteristics were as follows: age range - 2-11 years; sex-8 females and 14 males; stage of disease-2 in II, 4 in III and 16 in IV; state of remission - 4 in complete remission (CR), 12 in partial remission (PR) and 6 with progression. The pts diagnoses were Acute Myeloblastic Leukemia (ANLL)-2 (9%), Hodgkin Lymphoma (HL)-2 (9%), NonHodgkin Lymphoma (NHL)-1 (4,5%), Rhabdomyosarcoma (RMS)-7 (32,2%), Ewing Sarcoma-2 (9%), Neuroblastoma-6 (27,3), Nephroblastoma-1 (4,5%) and Germ cells tumors-1 (4,5%). Two types of mobilization were conducted-the first with chemotherapy (according to type of disease) and G-CSF-5 mcg/kg body weight and the second with chemotherapy and G-CSF-10 mcg/kg body weight. The mean interval between last chemotherapy and mobilization was 35 days (10-58). After mobilization, the CD34+ cells in blood in all pts were upon 20/mcl until day 20. All collections were conducted at the day when CD34+ cells were upon 40/mcl (average on day 8 of mobilization) with blood cell separator Fresenius AS104, Germany, after priming the lines with compatible blood and human serum albumin 5%. Blood flow rate was 20-40 ml/min. according to the body weight of the patient. We collected 5x10⁶/kg patient's body weight with on average 2 aphereses (1-4) in all patients. There were no severe complications during manipulations. Mild hypocalcaemia was observed in 2 pts and skin allergy in 5 pts. Significant factors for successful collections of CD34+ cells are the stage of disease, state of remission, interval between mobilization and collection, consecutiveness of apheresis, pre-apheresis WBC-and CD34+ cells count and number of apheresis. The age and body weight in this group are not independent prognostic factors for the quantity of collected cells and are not an obstacle for this manipulation. The mobilization and collection of PBSC in small children are safe and effective procedures, when performed by experienced teams with respect of the exact principles ensuring patient's safety.

0856

SEVERE ACQUIRED APLASTIC ANEMIA PRESENTED IN COMBINATION WITH AUTOIMMUNE THYROIDITIS

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The autoimmune background of aplastic anemia was long ago established. It occurs its combination with other autoimmune diseases. We present a case with severe acquired aplastic anemia (ASAA) with autoimmune thyroiditis in diagnosing. A 30-year-old woman was admitted in our hospital because of skin hemorrhages. She also showed bilateral struma (2nd degree). Laboratory data revealed severe pancytopenia with WBC-1500/mm³, neutrophils-100/mm³, hemoglobin-75 g/L and platelets-5000/mm³. Bone marrow aspirate and biopsy specimens indicated severe hypocellularity. In addition several autoantibodies (TAT and MAT) were highly elevated. The diagnoses were ASAA and autoimmune thyroiditis. The patient received immunosuppressive therapy consisting of antithymocyte globulin (ATG) and cyclosporin A (CsA). Now, three months after this treatment the patient is with partial hematological response and remarkably decreased antibodies titers. We report this case as a rare combination of aplastic anemia and other autoimmune disease. The immunosuppressive treatment of ASAA improves substantially the autoimmune thyroiditis.

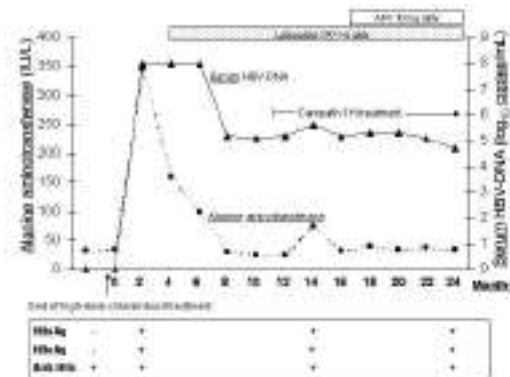
0857

INHIBITION OF HBV-REPLICATION BY COMBINED TREATMENT OF LAMIVUDINE AND ADEFOVIR DIPIVOXIL ALLOWS LONG-TERM CAMPATH-1H THERAPY IN REFRACTORY B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA

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Reactivation of hepatitis B virus infection in HBsAg-positive or HBsAg-negative/HBcAb-positive patients is a well known complication of immunosuppressive and cytotoxic therapies. A 49-year-old man, who tested HBsAg-negative/HBcAb-positive, was diagnosed as having Binet stage A B-cell chronic lymphocytic leukemia (B-CLL) in 1989. One year later, because of progressive leucocytosis, he started cytotoxic treatment with low-dose chlorambucil (5mg/day). CLL partial remission was obtained after 4 months of such therapy. Subsequently the disease was maintained under clinical control by repeated cycles of the same therapy during a period of 12 years. In March 2002, because of a new relapse, the patient started high-dose chlorambucil treatment (0.5 mg/Kg/day for 5 days monthly) for a total of 6 cycles and a new partial remission was obtained. At start of this treatment the only marker of HBV infection was again anti-HBc positivity. Two weeks after the end of alkylating therapy, liver function tests levels for the first time showed an abnormal increase with concomitant detection of HBsAg positivity, HBeAg positivity and HBV DNA levels of 8 log₁₀ copies/mL (by PCR assay, LLQ <3.3 log₁₀ copies/mL).



Ultrasound examination of the abdomen was otherwise unremarkable. The temporal relationship between hepatitis onset and chlorambucil withdrawal, and the exclusion of any other known factor for HBV infection indicate the reverse seroconversion of a silent HBV infection as the only responsible for the acute hepatic flare observed. In April 2003, due to the persistence of HBsAg and high levels of serum HBV-DNA, in order to prevent hepatic flares, lamivudine (200mg/day) was started. In the first 4 months, ALT levels normalized and the serum viral load decreased to 5.2 log₁₀ copies/mL. In November 2003, due to a further leukemic B-CLL relapse, we started treatment with low-dose alemtuzumab (Campath-1H) (10 mg three times a week subcutaneously). In March 2004, because there was not further reduction of virus load and because of the need of increasing dose cytostatic treatment to 30 mg three times a week, adefovir dipivoxil 10 mg/day was added to ongoing lamivudine. During 10 months of this combined antiviral lamivudine and adefovir dipivoxil treatment, ALT levels remained within the normal range and serum HBV-DNA progressively declined to 4.7 log₁₀ copies/mL. No adverse events were recorded. To the best of our knowledge, this is the first report that the association of lamivudine with adefovir dipivoxil could allow a safe and long-term Campath-1H treatment in patients with advanced B-CLL and chronic hepatitis B.

0858**ACTIVATION OF CASPASES DURING APOPTOSIS OF MALIGNANT CELLS IN B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA**

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B-cell chronic lymphocytic leukemia (B-CLL) is characterized by the accumulation of malignant CD5+ B cells, in which dysregulation of apoptosis rather than increased proliferation rate seems to play the crucial role. In this study we investigated the activation of caspases in B-CLL cells exposed to apoptosis both spontaneous and induced via external and internal pathway. The experiments were done in peripheral blood (PB) and bone marrow (BM) samples obtained from five newly diagnosed, previously untreated B-CLL patients. The cells were cultured without apoptosis inducers or were treated either with camptothecin (CPT) or with a combination of TNF-alpha and cycloheximide (CHX) that induce caspase activation via receptor pathway and via mitochondrial pathway, respectively. The experiments were done at 24 and 48h of cell culture. We used the method based on detection of activated caspase with use of FAM-VAD-FMK that is the fluorochrome labeled inhibitors of caspases being considered as a pan-caspase marker and flow cytometry technique. We detected the higher percentage of FAM-VAD-FMK positive cells in PB than in BM samples submitted to spontaneous (10.58 ± 8.79 vs 6.87 ± 4.79 at 24h, and 13.13 ± 7.06 vs 8.46 ± 5.25 at 48h) and CPT-induced apoptosis (9.04 ± 8.05 vs 5.86 ± 4.57 at 24h and 14.88 ± 11.16 vs 12.27 ± 10.97). There were no significant changes between PB and BM in apoptosis induced by TNF-alpha and CHX. Percentage of FAM-VAD-FMK positive cells in culture with CPT was higher than in culture with TNF-alpha and CHX both in PB and BM samples (14.88 ± 11.16 vs 10.87 ± 4.53 in PB and 12.27 ± 10.97 vs 10.57 ± 8.02 in BM at 48h) Our results indicate time-dependent caspase activation in apoptosis process of B-CLL cells obtained from both PB and BM. We detected the higher rate of caspase activation during spontaneous and mitochondrial induced apoptosis in PB than in BM, that may indicate the differences in apoptosis process depending on cell environment. We detected caspase activation both in receptor and mitochondrial induction of apoptosis, but with higher rate in mitochondrial one, that may suggest dominance of this mechanism in B-CLL, however, activation of caspases connected with receptor way cannot be excluded.

0859**ANALYSIS OF THE MANAGEMENT AND OUTCOME OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE IN NORTHERN IRELAND**K.M.A. Ramadan¹, M. Bowers², M.R. El-Agna², P. Kettle¹¹*Belfast City Hospital, BELFAST, UK;* ²*Ulster Hospital, BELFAST, UK*

Background. Post-transplant lymphoproliferative disease (PTLD) is a lymphoid proliferation or lymphoma that develops as a consequence of immunosuppression in recipients of solid organ or bone marrow allograft. Patients receiving renal transplant have the lowest incidence (1%). Cardiac and hepatic are at intermediate risk (1-2%). Eighty percent of PTLD patients are Epstein Barr Virus (EBV) positive. Prognosis is very poor with a mortality rate of 80% in many published series. Reduction of immunosuppression may induce regression of hyperplastic and polymorphic tumours whereas monomorphic tumours are more likely to be unresponsive. **Aims and Methods.** We reviewed the diagnosis and management outcome of four male patients diagnosed with post-transplant lymphoproliferative disease (PTLD), B cell type, over the past 5 years in Northern Ireland. **Results.** Two patients with liver transplant diagnosed with PTLD 8 and 10 years post-transplant. Both patients were on tacrolimus and had extra-nodal disease; one with monomorphic pathology and the other had polymorphic PTLD. They

received a combination chemotherapy and Rituximab. The patient with polymorphic PTLD also had central nervous system infiltration and responded very well to cranial radiotherapy. Overall survival was 24 months in the monomorphic case and 18 months in the polymorphic case. One patient had cardiac transplant 10 years before he presented with polymorphic PTLD. He was on cyclosporin and azathioprine. He had nodal disease and died 8 months later despite the reduction in his immunosuppression, combination chemotherapy and Rituximab. The fourth patient with renal transplant developed PTLD 8 years later. He was on cyclosporin and prednisolone and responded very well to reduction of immunosuppression alone. He is well and alive 24 months later. Despite what has been reported in literature in response of polymorphic tumors to reduction of immunosuppression only one out of three patients with polymorphic disease has responded. The optimal immunosuppression reduction regimen to ensure regression of disease is unknown. Three patients died with disease progression despite the treatment with combination chemotherapy. The average survival was 18 months. All patients in this series developed PTLD at 8 years or more post organ transplant which is unusual finding as most cases are developed in the first year when the immunosuppression is intense. **Summary.** PTLD is very rare yet a fatal disease in the majority of patients. Small percentage of patients responds to reduction of immunosuppression, which should be attempted when appropriate. This should be based upon the severity of the disease and the health risk associated with the possible loss of the allograft.

0860**THE ROLE OF SPLENECTOMY IN THE MANAGEMENT OF SELECTED HAEMATOLOGY PATIENTS**K.M.A. Ramadan¹, A. Moore², S. Kirk², M.R. El-Agna²¹*Belfast City Hospital, BELFAST, UK;* ²*Ulster Hospital, BELFAST, UK*

Background. Splenectomy can be beneficial in a variety of haematological conditions to control cytopenias and relieve discomfort. This procedure can be technically difficult and carries certain peri-operative and post-operative mortality and morbidity risks. The role of splenectomy in management of idiopathic thrombocytopenic purpura (ITP) is well established but its role in other haematological conditions such as myeloproliferative diseases and non-Hodgkin lymphoma is still controversial. **Methods.** A series of patients who had undergone splenectomy for different haematological conditions were identified. The hospital clinical records were reviewed to ascertain the haematological diagnosis, indication for splenectomy, administered vaccinations and blood products prior to surgery, length of in-patient episode, post-operative infection and prognosis with regard to disease outcome. **Results.** Fifteen patients underwent splenectomy for the management of haematological disease. The length of stay in hospital on average was 8 days (range 2-27) post-splenectomy. Eight patients with ITP were referred for splenectomy. All ITP patients gained satisfactory response. Seven were in complete remissions with normal platelet counts. Three patients with Non-Hodgkin's lymphoma (NHL) were referred for splenectomy. One patient had splenic lymphoma with villous lymphocytes (SLVL) and presented with left upper quadrant pain and pancytopenia secondary to splenomegaly. A further female patient with NHL complained of splenic pain and had symptomatic anaemia requiring repeated red cell transfusion, which was thought to be related to hypersplenism. Both patients gained normal blood counts and stable remission. A male patient with stage IVB low grade NHL presented with severe pancytopenia and massive splenomegaly. He was managed with chemotherapy prior to splenectomy. He had symptomatic relief although died 4 months after splenectomy with renal failure and disease progression. One elderly male patient with chronic lymphocytic leukaemia (CLL) with anaemia and thrombocytopenia (Binet stage C) had massive splenomegaly causing abdominal discomfort. He gained symptomatic benefit

and disease remission for 2 years post-splenectomy. He relapsed requiring further chemotherapy and subsequently died of disease progression. Three male patients with myeloproliferative disease were referred for splenectomy to control abdominal pain and cytopenias. They all gained symptomatic relief after removal of massive spleens but required on-going myelosuppressive therapy for control of their underlying disease. Two died, 8 and 10 months post-splenectomy, with acute myeloid leukaemia transformation. One is well at 60 months post-splenectomy and maintained on myelosuppressive therapy. It was recorded that 14 patients received pneumococcal vaccine and 13 patients received Hib (Haemophilus influenzae type b) vaccine prior to splenectomy. Twelve patients were commenced on penicillin V, one on amoxicillin, and two on erythromycin long term. *Conclusions.* This review indicates that there is still a role for splenectomy in the management of haematological diseases. In our cohort of patients there were no deaths due to immediate or late complications of splenectomy. It can alleviate abdominal pain, control cytopenias and provide disease remission in some cases. When patients are carefully selected and the operation is performed by an experienced surgeon mortality and morbidity tend to be very low.

0861

SYMPTOMATIC URINARY BLADDER INFILTRATION WITH CHRONIC LYMPHOCYTIC LEUKAEMIA: A VERY UNUSUAL COMPLICATION

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Background. Chronic lymphocytic leukaemia (CLL) is the most common type of leukaemia in the western world, accounting for 40% of all leukaemias in individuals over the age of 65 years. It usually presents with lymphocytosis and bone marrow infiltration. We report two patients with urinary bladder infiltration with CLL presented with urinary symptoms and treated as recurrent urinary tract infections (UTI) for several years before the CLL diagnosis was established. *Methods.* We reviewed the clinical notes of two cases with urinary bladder infiltration with CLL to ascertain the clinical presentation, urinary bladder biopsy findings and response to therapy. *Results.* Case 1: A 60-year-old female attended urology clinic for three years and treated as recurrent UTI. She had constantly negative urine cultures. She was referred to haematology with persistent lymphocytosis. Further investigations confirmed the diagnosis of pleomorphic CLL. CD38 was expressed on 60% of lymphocytes. Renal ultrasound was normal. Intravenous urogram (IVU) showed filling defects in the UB. Cystoscopy showed patchy erythematous areas. Biopsy revealed lymphoid infiltration which was CD5 and CD20 positive. She received chlorambucil which resulted in disappearance of haematuria. Case 2: A 67-year-old female referred from diabetic clinic with lymphocytosis. Further investigations confirmed the diagnosis of CLL. She was treated as recurrent UTI for several years. Renal ultrasound was inconclusive. IVU demonstrated dilatation in the right renal pelvis and ureter. Cystoscopy revealed flat papillary lesions in the UB. Biopsy showed lymphoid infiltration which was CD20, CD5 and CD23 positive. *Summary.* UB infiltration with CLL is extremely rare. In both cases the presentation with urinary symptoms has preceded the diagnosis of CLL with no evidence of other organ infiltration including liver and spleen (ie. Binet stage A). This shows the limitation of current staging systems (ie. Binet and Rai) to incorporate cases with extralymphatic infiltration. These two cases also highlight the difficulty in establishing the diagnosis of urinary bladder infiltration with CLL as symptoms are usually attributed to UTI. It seems that renal ultrasound is not useful to demonstrate any urinary bladder abnormalities in these cases. It appears that there is a diagnostic role for IVU. Cystoscopy and urinary bladder biopsies remain the mainstay for definitive diagnosis. Urinary bladder infiltration with CLL should be considered in patients with per-

sistent urinary symptoms and negative urinary cultures or no clinical response to antibiotics. In our opinion, treatment for CLL is indicated in patients with symptomatic infiltration of urinary bladder. To our knowledge these two cases are first reports of urinary bladder (UB) infiltration with CLL.

0862

MELANOMA-ASSOCIATED ANTIGEN-A (MAGE-A) GENE EXPRESSION BASED ON NESTED RT-PCR USING MAGE A1-6 COMMON PRIMER IN ACUTE MYELOID LEUKEMIA

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Introduction: Although the Melanoma-associated Antigen A (MAGE-A) family is expressed in various cancers, there have only been a few studies focused on acute myeloid leukemia (AML). Accordingly, the current study attempted to evaluate the role of MAGE-A gene expression as a prognostic factor in AML patients. *Methods & materials.* A common primer for the MAGE A1-6 subtype genes was used for the nested reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of 26 AML patients. The response rate, leukemia-free survival (LFS), and overall survival (OS) were then analyzed according to MAGE-A mRNA expression. *Results.* The MAGE-A gene was expressed in 8 out of 26 patients (31%), and an association with the CD19 positive immunophenotype implied ($p=0.040$). MAGE-A gene expression was not found to be associated with the treatment response ($p=0.581$) or survival ($p=0.3642$ for OS, $p=0.9649$ for LFS). *Conclusions.* MAGE-A mRNA was identified in 31% of the AML patients based on a nested RT-PCR using a common primer for the MAGE-A1 to A6 subtypes, however, this h

0863

PROTECTIVE ROLE OF INTERLEUKIN-10 PROMOTER GENE POLYMORPHISM IN THE PATHOGENESIS OF INVASIVE PULMONARY ASPERGILLOSIS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction: The current study attempted to evaluate the association between IL-10 promoter gene single nucleotide polymorphism (SNP) and invasive pulmonary aspergillosis (IPA) after allogeneic stem cell transplantation (SCT) in 105 patients. *Methods.* We analyzed 3 single-nucleotide polymorphisms in proximal region of IL-10 promoter gene (-1082/-819/-592). *Results.* In the current study, only two haplotypes (1082*A/819*T/592*A [ATA] and 1082*A/819*C/592*C [ACC]) were found, consistent with previous publication in Korean ethnicity. Overall incidence of IPA was estimated as $14.1\pm 4.5\%$ with median 186 days (62~405 days). An increased occurrence of IPA was noted dependent on the IL-10 haplotypes (0% vs $11.5\pm 6.4\%$ vs $19.7\pm 7.7\%$ in ACC/ACC vs ATA/ACC vs ATA/ATA haplotype, $p=0.0296$ when comparing ACC with non-ACC haplotype). In a multivariate survival analysis using Cox's proportional hazard model, the IL-10 promoter gene SNPs was identified an independent predictive factor for the development of IPA ($p=0.012$, hazard ratio [HR] 9.3), together with the HLA-identical donors ($p=0.005$, HR 16.3), the CD34+ cell dose transplanted ($p=0.004$, HR 26.5) and time-dependent chronic graft-versus-host disease (GVHD; $p=0.049$, HR 16.0). *Conclusions.* IL-10 promoter gene SNP was found to be apparently associated with the development of IPA after allogeneic transplantation regardless of HLA-disparity or chronic GVHD.

0864**PROGNOSTIC SCORING MODEL BASED ON MULTIDRUG RESISTANCE STATUS AND CYTOGENETICS IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA**D.H. Kim¹, N.Y. Lee¹, S.J. Oh², J.H. Baek¹, S.K. Sohn¹, J.S. Suh¹, K.S. Lee¹, K.B. Lee¹¹Kyungpook National University Hospital, DAEGU, South-Korea.²Sungkyunkwan University, SEOUL, South-Korea

Background. and objective: Clinical heterogeneity exist within acute myeloid leukemia (AML) patient group with the same cytogenetic risk. Multidrug resistance (MDR) is also regarded as one of potential prognostic factors for AML. Accordingly, the prognostic scoring model can be generated based on both consideration of cytogenetic risk and the MDR status for AML. Design and *Methods.* The CR rate, event-free (EFS) and overall survival (OS) were analyzed according to cytogenetic risk, MDR status and clinical factors. Prognostic score was calculated by the sum of MDR status (0 for negative, 1 for positive) and dichotomized scoring for cytogenetic risk (0 for favorable/intermediate and 1 for unfavorable cytogenetics). *Results.* MDR expression was noted in 36.6% of the patients and associated with a lower CR rate ($p=0.037$). MDR, cytogenetics and the use of SCT were identified as independent prognostic factors for EFS and OS. The CR rate of the group scored with 0, 1, and 2 was 81.4%, 66.7%, and 44.4%, respectively ($p=0.050$). The prognostic scoring model depicted a discriminating role in terms of EFS ($p<0.0001$) and OS ($p=0.0001$). *Conclusions.* The prognostic scoring model based on cytogenetic risk and MDR provided an improved method for evaluating the prognosis in AML and helped to stratify the risk of patients within same cytogenetic risk.

0865**ACUTE MYELOID LEUKEMIA PATIENTS WITH T (8;21) (Q22;Q22) HAVE SUPERIOR OVERALL SURVIVAL WHEN STEM CELL TRANSPLANTATION ARE ADDED AS POST REMISSION THERAPY**J.-Y. You¹, C. Chen², W.K. Kwang², C.H. Ho², C.H. Tzeng², W.K. Chau², H.C. Hsu², J.P. Gau², Y.-B. Yu²¹Lo-Tung Pohai Hospital, YI-LAN COUNTY, Taiwan (China); ²Veterans General Hospital, TAIPEI, Taiwan (China)

Background. The best post remission therapy for young patients with t(8;21) Acute myeloid leukemia remains poorly defined. Studies for t(8;21) AML have often been hampered by the limited number of patients reported, frequently collectively considered with those with AML carrying the inv(16)/t(16;16). Debate continues about whether the presence of t(8;21) karyotype remains prognostically significant for AML patients after stem cell transplantation (SCT) as consolidation modality. *Aims.* The purpose of this study was to compare the treatment outcome of t(8;21) (q22;q22) with normal karyotype, and the impact of stem cell transplantation as postremission therapy for patients with acute myeloid leukemia (AML) who have the t(8;21) (q22;q22) karyotype. *Methods.* We examined the cumulative incidence of disease-free survival (DFS), and overall survival (OS) for 55 AML patients with normal diploid karyotype and 22 patients with t(8;21) (q22;q22) in a single institute. Within AML t(8;21) (q22;q22) patients, the value of stem cell transplantation (autologous, allogeneic, or non-myeloablative) versus chemotherapy alone were also analyzed. *Results.* The treatment outcome of normal diploid karyotype and t(8;21) (q22;q22) AML patients were similar. Among t(8;21) (q22;q22) acute myeloid leukemia patients, univariate analysis revealed that significant prognosis factors for OS were platelet count, white cell count(WBC) index, and stem cell transplantation, but none being for DFS. In a multivariable analysis, stem cell transplantation and lower WBC index predicted for superior OS. *Conclusions.* These data demonstrate that disease-free survival and overall survival of patients with t(8;21) (q22;q22) AML may

be not a good prognostic factor compared with normal karyotype. The t(8;21) (q22;q22) AML patients would be compromised by treatment approaches that do not include stem cell transplantation.

0866**TREATMENT OF ACUTE PROMYELOCYTIC LEUKAEMIA WITH MODIFIED AIDA REGIMEN: A MALAYSIAN EXPERIENCE**

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Background. Acute promyelocytic leukaemia (APL) is the most curable subtypes of AML, characterised by t(15;17) resulting in fusion PML-RAR α gene and protein that plays a vital role in its pathogenesis. Great efforts have been made at molecular level to understand its genesis, diagnosis and disease monitoring. Simultaneously, therapeutic strategies have rapidly evolved with new mechanism-/gene-based targeted agents that have further improved its outcome. With limited resources in Malaysia, we treated our patients with modified AIDA regimen. *Aims.* We study the complete remission rate (CRR), the induction death, the relapse rate, the overall survival (OS) and event free survival (EFS). We also compare the outcome with different types of anthracycline-based induction regimen. *Methods.* A retrospective study among APL patients in 2 centres in Malaysia was done from 15.9.1998 till 15.9.2003 and followed them up till 15.12.2004. All patients received modified AIDA regimen and monitored mainly with morphological marrow study due to poor yield in the cytogenetic results (55% available). *Results.* 42 patients were identified. Two patients died from lethal haemorrhages before initiating treatment. The other 40 patients received therapy as above. Two induction deaths recorded. Five patients defaulted. Hence, we have 34 evaluable patients. The median age was 32.5 years (16-49 years). The gender and ethnic distribution are shown in. Our patients mainly fell into the intermediate and high risk groups. 65% of them were induced with ATRA/Idarubicin. All patients achieved complete remission after induction. All ATRA/Idarubicin and 4 ATRA/Daunorubicin patients were subsequently consolidated with Idarubicin. One ATRA/Idarubicin patient was switched to high-dose Cytarabine (HIDAC) when noted relapse. Two patients had upfront stem cell transplantation (SCT) (one with TWDC $>100 \times 10^6$ and additional chromosomal abnormality; one was intolerant to ATRA). As of 15.12.2004, after a median follow-up period of 50 months (15 to 72 months), the CRR (≥ 1 remission) was 97%. The OS and EFS were 85% and 82% respectively. The cumulative relapse rate was 41%, majority relapsed after more than one year (93%) with the median time of relapse at 22 months (15 to 40 months). Only one patient (7%) relapsed at 6 months. No statistically difference in relapse rate among the two different induction regimens. The only determinant for relapse was the patient's risk group. Mortality during first induction was 5%. The re-induction mortality was 8%. Following the approval of arsenic trioxide, our patients attained second remission without complication. Three patients in second remission underwent autologous SCT. One patient in third remission underwent an eventful allogeneic SCT. All survived with complete remission. One patient refused further treatment after attaining second remission, relapsed subsequently. *Conclusions.* APL is common in Malaysia, appears to come in clusters with female preponderance. It affects the young age group and is more common among the Chinese. Majority fall into the intermediate and high risk groups. Modified AIDA regimen is effective therapy for our patients with comparable OS and EFS. We expect a better outcome once more targeted new agents are made available in Malaysia plus improvements in our cytogenetic support for disease monitoring.

0867**SPLENECTOMY IN ADULT PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP) - A LONG-TERM FOLLOW UP**E. Johansson¹, P. Engvall¹, O. Landgren¹, S. Widell¹, S. Rezaei¹, M. Björkholm²¹Karolinska Hospital and Institutet, STOCKHOLM, Sweden; ²Karolinska University Hospital, STOCKHOLM, Sweden

Background. Splenectomy is the recommended treatment in adult patients with ITP who fail to respond to corticosteroid treatment or who require unacceptably high doses to maintain a safe platelet level. For these patients splenectomy may produce a good response in 60-80% of patients. However, the observation time after splenectomy has in most published studies been relatively short. Thus, the true long-term response to splenectomy still remains less well defined. **Aims.** The aims of the present study were to assess the long-term efficacy and safety of splenectomy in adult patients with chronic ITP and define the response to additional pharmacological therapy in those who failed splenectomy. **Methods.** We retrospectively reviewed a cohort of 59 ITP patients (M/F=25/34, median age at splenectomy; 39 yrs, range 14-75 yrs). The median time from diagnosis to splenectomy was 5.5 months (range 0.4-199 months). Criteria used to define response to splenectomy were: CR; platelet count $>150 \times 10^9/L$ lasting >4 weeks, PR; platelet count $>50-150 \times 10^9/L$ lasting >4 weeks and, NR; failure to achieve platelet counts $>50 \times 10^9/L$. The criterion for relapse in CR/PR patients was a decrease in platelet counts to $<50 \times 10^9/L$. **Results.** The median follow up time after splenectomy was 18 yrs (range 2-32 yrs). No serious complications related to the surgical operation occurred. The overall response rate was 78% with 59% CR and 19% PR. CR and PR patients were younger than NR patients at time of diagnosis (median age: 36 yrs vs 48 yrs, $p=0.03$) and at splenectomy (median age: 38 yrs vs 51 yrs, $p=0.02$). Among the 46 responding patients 17 eventually relapsed. A plateau in the progression free survival curve was identified after 12.1 and 7.3 years for patients in CR or PR, respectively. At follow up, three of 13 patients refractory to splenectomy and four of 17 patients relapsing after splenectomy were in CR. Septicemia was recorded in four patients during a total of 1023 patient years at risk. **Conclusions.** We conclude that splenectomy is an effective and safe treatment in adult patients with chronic ITP failing to respond to corticosteroid treatment. The complication rate was low and our findings support the view that response to splenectomy is durable after a certain point in time.

0868**HB D PUNJAB [BETA 121 (GH4) GLU: GLN] IN WESTERN IRAN**Z. Rahimi¹, R. Akramipour², A. Shahriari Ahmadi³, R.L. Nagel⁴, A. Merat⁵, F. Bahrehmand⁶¹Kermanshah Univ. of Medical Sciences, KERMANSHAH, Iran²Department of Pediatrics, KERMANSHAH, Iran; ³Department of Hematology, KERMANSHAH, Iran; ⁴Albert Einstein College of Medicine, NEW YORK, Iran; ⁵Shiraz University of Medical Science, SHIRAZ, Iran; ⁶Medical Biology Research Center, KERMANSHAH, Iran

1. **Background.** While individuals carrying Hb D-Punjab are typically asymptomatic and even those homozygous for this hemoglobin variant have a relatively benign condition, diagnosis could be important for genetic counseling. 2. **Aims.** To study the clinical and hematological features of Hb D-Punjab as homozygote, single heterozygote and a combined heterozygote with α -thalassemia. 3. **Methods.** Hematological indices of 18 subjects from 9 unrelated families living in Western Iran (Kermanshah, Ilam and Kurdistan provinces) exhibiting HbD were measured by automated H1 blood counter, DAEA-52 column chromatography and electrophoresis. The Hb D-Punjab status of all individuals was confirmed by PCR followed by digestion

with EcoRI. 4. **Results.** Fifteen out of 18 individuals that were simple HbD-heterozygote had, in average, 39.8% HbD and 0.96% Hb F%. All were asymptomatic with normal hematological indices. In a 3 years old girl carrier for Hb D-Punjab, the Hb F% was high (11%). A 37 years old woman was found to be homozygous for Hb D-Punjab (91% HbD-Punjab), but free of clinical symptoms with normal hematological indices. Two patients were double heterozygote for Hb D-Punjab and α -thalassemia. They were two boys belong to a family in which the father was carrier for Hb D-Punjab and the mother was a α -thalassemic carrier. Hematological indices of these two patients with Hb D- α -thalassemia showed the presence of hypochromia and microcytosis along with elevation of Hb F (12% and 18%). 5. **Summary/conclusion:** The present study confirms the benign nature of homozygous Hb D-Punjab and indicates compound heterozygosity for Hb D-Punjab and α (0) thalassemia confers a thalassaemic-minor syndrome to individuals carrying this combined hemoglobinopathy.

0869**LOW-DOSE SUBCUTANEOUS ALEMTUZUMAB THERAPY FOR PATIENTS WITH REFRACTORY CHRONIC LYMPHOBLASTIC LEUKAEMIA (B-CLL)**A. Cortelezzi¹, M.C. Pasquini¹, N.N. Fantini¹, V.R. Zilioli¹, M. Colombi¹, B. Sarina², F. Somalvico¹, G. Lambertenghi Deliliers¹¹Ospedale Maggiore, University of Milan, MILAN, Italy; ²Humanitas Institute, MILAN, Italy

B-CLL patients have a poor prognosis after failing conventional chemotherapy with alkylators and purine analogues. We assessed the efficacy and safety of alemtuzumab as salvage therapy for patients with heavily pretreated and refractory CLL. As alemtuzumab may cause substantial 'first-dose' reactions when given i.v., the drug was delivered subcutaneously and, in order to further minimise untoward therapy-related effects and make the treatment more manageable, a low dose of 10mg was administered three times a week for a prolonged period of 18 weeks. Sixteen symptomatic patients entered this phase II study: 14 were refractory to both alkylators and fludarabine, of whom eight were also refractory to rituximab-containing regimens, and two were refractory to chlorambucil and had presented autoimmune haemolytic anemia (AIHA) that precluded fludarabine therapy. An overall response rate (OR) of 50% (95%CI, 24.6-75.3%) was achieved, including 25% complete responses (CR). CLL cells were cleared from blood in 87.5% of patients in a median time of 7.5 weeks. A 1-log depletion of peripheral blood lymphocytes, as assessed by a routine haematological analyser, occurred in 15 patients after a median of six weeks of therapy (range 2-17); this reduction in PBL was achieved by non-responding patients after a median of eight weeks of therapy and by responding patients after a median of three weeks (respectively 2.5 and 6 weeks in CR and PR patients). A 62.5% OR, including 31.3% CR and 25% nodular partial response was achieved in the bone marrow in a median time of 15 weeks. An objective response in lymphnodes and spleen was obtained in respectively 50% and 42.9% of the patients. The responses were substantial even in patients with unfavourable cytogenetics, rituximab refractoriness, Rai stage IV, previous infections, and age of over 65 years. Eleven patients, including seven out of 8 responders, are alive after a median follow-up of 15 months, giving an overall survival of 68.8%; the median time-to-treatment failure was 10 months (range 2-21). It is worth noting that there were no systemic side effects, except transient fever in 25% of the patients, while short-lived grade I-II injection site skin reactions were seen in 56%. Grade III-IV neutropenia developed in one-third of the patients, but severe infections were uncommon during Campath treatment (12.5%). During the follow-up 11 patients developed major infections (5 pneumonia, 2 FUO, 1 otitis, 1 perianal abscess, 2 septicemia), four of which were lethal. Asymptomatic CMV reactivation was documented in only 2/16 patients. No hepatic flares were seen in two HBsAg-positive patients treated with alemtuzumab in combination with reverse transcriptase

inhibitors. Reactivation of immune haemolysis was observed in one patient with previously documented AHIA, and *de novo* AHIA was documented in another patient, respectively five and fourteen months after therapy start. Subcutaneous low-dose alemtuzumab is effective in patients with poor prognosis B-CLL, and has a particularly favourable toxicity profile. This therapeutic approach appears to be more convenient for patients and physicians, enables the home administration of the antibody, and reduces health care costs in comparison with intravenous infusion or full-dose subcutaneous administration

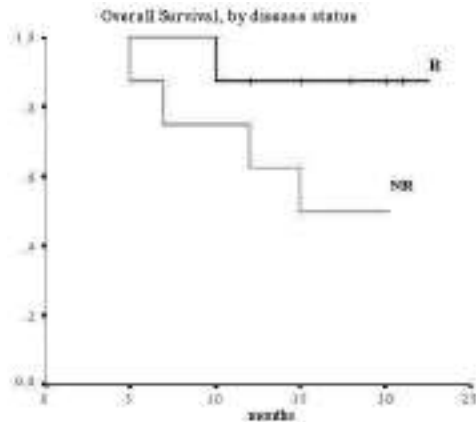


Figure. OS in responder (R) and non-responder (NR) patients.

0870

MULTIDRUG RESISTANCE 1 GENE EXPRESSION MIGHT DEFINE AN ASSOCIATION BETWEEN THE TYPE OF BCR-ABL TRANSCRIPTS AND PLATELET COUNT IN CHRONIC MYELOID LEUKEMIA

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Background. Currently it is generally accepted, that the type of the fusion BCR-ABL transcripts (b3a2 vs. b2a2) has no impact on the disease phenotype in chronic myeloid leukemia (CML) patients. Probably the only exception from this rule is the higher platelet count reported in b3a2 patients. Besides, data concerning the incidence of multi-drug resistance 1 (MDR1) gene over-expression in chronic phase CML patients are still scarce and controversial, and the association between the MDR1 levels and the type of BCR-ABL transcripts to our knowledge has not been studied yet. **AIM:** To analyze the correlation between the BCR-ABL mRNA type and CML patient characteristics at presentation, including MDR1 status. **Methods.** The type of BCR-ABL transcripts, detected by reverse transcription polymerase chain reaction (RT-PCR) in 97 untreated consecutive chronic phase CML patients (59 males and 38 females; mean age of 51±14 years), was correlated with the basic biological and haematological findings at diagnosis, as well as with MDR1 gene expression. MDR1 status was determined by a semi-quantitative RT-PCR with co-amplification of the mRNAs of MDR1 and b2-microglobulin, incorporated as an internal control. **Results.** B3a2 and b2a2 mRNAs were detected in 53 (55%) and 44 (45%) patients, respectively. Over-expression of MDR1 gene [MDR1 (+)] was found in 48 (49.5%) patients, including 25 with b3a2 (52%), and 23 with b2a2 (48%). Age, gender, hemoglobin, white blood cell (WBC) counts and MDR1 status showed no significant differences between the patients with b3a2 and b2a2 mRNAs, however, platelet counts of b3a2 patients (mean=791±

441G/L) were significantly higher than those of b2a2 patients (mean=440±283 G/L) (Mann Whitney U test, p=0.009). If b3a2 and b2a2 patients were additionally divided into subgroups, according to the MDR1 status, higher platelets and lower WBC count were found in the b3a2 patients, compared to b2a2 patients in the MDR1 (+) group: platelets 909±470 G/L vs. 473±356 G/L (p=0.006); and WBC 85±61 G/L vs. 130±94 G/L (p=0.004), respectively. In contrast, no differences were observed between b3a2 and b2a2 patients in the group with normal MDR1 expression: platelets 727±434 G/L vs. 404±189 G/L (p>0.05), WBC 151±87 G/L vs. 134±109 G/L (p>0.05), respectively. **Conclusions.** Our results indicate that the type of BCR-ABL transcripts might be associated with platelets and WBC count in the subgroup of CML patients characterized by over-expression of MDR1 gene. It seems possible that MDR1 over-expression reflects the preferential activation of a common signaling cascade in b3a2 positive cases, which is non-active in the patients with normal MDR1 expression, resulting in a particular CML phenotype, presented by thrombocytosis and relatively lower leukocytosis. If confirmed, these results may shed additional light on the mechanisms underlying the phenotypic and clinical heterogeneity of CML patients.

0871

CONSOLIDATION THERAPY OF MULTIPLE MYELOMA WITH THALIDOMIDE-DEXAMETHASONE AFTER AUTOLOGOUS STEM CELL TRANSPLANT

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Background. The optimum maintenance therapy for patients with multiple myeloma after autologous stem cell transplant remains unclear. **Aims.** The aim of this prospective study was to assess the tolerability of thalidomide-dexamethasone as maintenance treatment for multiple myeloma after autologous stem cell transplant. **Methods.** Between May 2003 and December 2004, eligibility criteria required administration of melphalan (200 mg/m²) with blood stem cell support, and initiation of maintenance treatment 90 days after stem cell infusion. All patients received thalidomide at a dose of 100 mg daily, during 5 months, and a monthly dose of dexamethasone (20 mg/m²/day for 4 days) during 3 months. The primary end point was the incidence of discontinuation or dose reduction due to treatment toxicity. **Results.** During the 20-month study period, 20 patients were enrolled [8 male, 12 female, median age 48 years (38-59 years)]. Only one patient (5%) required thalidomide discontinuation because of toxicity (neuropathy). **Conclusions.** Maintenance treatment with thalidomide (100 mg/day, during 5 months) and dexamethasone (20 mg/m²/day for 4 days, during 3 months) is well tolerated in patients with multiple myeloma after autologous stem cell transplant.

0872

LABORATORY DIAGNOSIS OF HIT BY IMMUNOLOGICAL ASSAYS

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Background. Heparin-induced thrombocytopenia (HIT) is an immune-mediated complication of heparine treatment. The formation of heparin-dependent antibodies can result in platelet activation. The most important antibody identified is one targeted against the heparin-platelet factor (PF) 4. Although the diagnosis of HIT remains essentially a clinical diagnosis, it is desirable to support the clinical suspicions with laboratory confirmation. Several *in vitro* tests are available to detect either the responsible antibodies by enzyme-linked immunosorbent assays (ELISA) or the platelet activation by functional tests, such

as platelet aggregation assay, serotonin release assay and flow cytometric assay. *Aims.* ELISA and functional testing are time-consuming and expensive and are reserved for specialised labs. A new, simple and rapid test based on the microtyping particle agglutination system, the ID-Micro Typing System (Diamed) which detects heparin-PF4 antibodies was compared with the classical ELISA. *Methods.* Samples from 75 clinically suspected HIT patients, 77 samples of non-HIT patients during heparin therapy, and 75 samples of healthy persons were analysed with two PF4/heparin antibody ELISA assays: Asserachrom HPIA (Diagnostica Stago, France) and GTI PF4-enhanced (Brookfield, USA), both performed according to the respective manufacturer's protocol. In parallel, all samples were tested with the ID-Heparin/PF4 antibody test (Diamed, Switzerland). The latter is a rapid screening test for the detection of heparin-PF4 antibodies in which the high density polymer particles coated with heparin-PF4 complex serve as a solid phase in the Particle Gel Immuno Assay (PaGIA). It is an immunological assay which provides results after 15 minutes. The PaGIA was, besides on serum as recommended by the manufacturer, also evaluated on citrated plasma and EDTA plasma. *Results.* Comparing the different sample types analysed with the ID-Heparin/PF4, correlation was best between citrated plasma and serum on citrated plasma correlated best with the Asserachrom HPIA ELISA. In comparison with the Asserachrom HPIA ELISA, the ID-Heparin/PF4 antibody test on citrated plasma scored 72% sensitivity and 94% specificity in the clinically suspected patients. In the heparinised patients and the normal population, sensitivity was 50% and 60%, respectively. Specificity was 98.5% and 97%, respectively. Sensitivity of the ID-Heparin/PF4 on citrated plasma in comparison with the GTI PF4-enhanced ELISA in the clinically suspected patient population was 65% and specificity 95%. In the heparinised patients and the normal population, sensitivity was 25% and 40% respectively, specificity was 100% and 96%, respectively. *Conclusions.* The ID-Heparin/PF4 antibody test (Diamed ID-PaGIA) is a quick, reliable and robust test to determine the presence of HIT antibodies and is much easier to perform than the ELISA assays. Although the ID-PaGIA shows in our study a lower sensitivity than described in literature, we believe it is a reliable tool for detection of HIT antibodies. Actually, there is no gold-standard assay for serological diagnosis of HIT. Clinical relevance of positive results by immunological assays only, is unknown. Therefore, in the diagnosis of HIT, it is recommended that this assay should be used in combination with a functional test.

0873

THE EPIDEMIOLOGY OF LYMPHOMA (HODGKIN'S AND NON-HODGKIN'S) IN VITEBSK REGION, REPUBLIC OF BELARUS

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Background. As part of a large investigation of cancer in Vitebsk region, we conducted a study of Hodgkin's lymphoma and Non-Hodgkin's lymphoma in adults presenting at hospitals in Vitebsk region. *Aims.* The purpose of this study was to evaluate age-specific cancer incidence rates in lymphoma among urban and rural population of Vitebsk region, differentiated by sex. *Methods.* In this retrospective analysis, patients with Hodgkin's lymphoma and Non-Hodgkin's lymphoma, treated in Vitebsk region between 1991 and 2003, were evaluated. Incidence rates were calculated using the Belarusian Cancer Registry, inpatient and outpatient medical cards. *Results.* In 1991-2003, a steady tendency to the increase of new Non-Hodgkin's lymphoma cases remained (the standardized incidence rate was 3,7 per 100,000 population in 1991 and 5,3 in 2003), especially among female population, as incidence rate of Hodgkin's lymphoma has a tendency to the decrease (the standardized incidence rate was 2,9 per 100,000 population in 1991 and 2,1 in 2003), especially among female population. The difference between the levels of Non-Hodgkin's lymphoma incidence among urban and rural population increased due to its more pronounced growth among urban. While comparing the dynamics of Non-Hodgkin's lymphoma incidence among urban and rural population, differentiated by sex, one can notice more pronounced growth among rural female population, as among male population this tendency does not remain. In Hodgkin's lymphoma patients one can notice more pronounced incidence rate among urban female population than among rural. Age-specific incidence rates of Hodgkin's lymphoma, differentiated by sex, has a tendency to the growth in the following age groups: 15-30 (total), 25-35 (males) and 20-24 (females), as incidence rates of Non-Hodgkin's lymphoma has a tendency to the growth in the following age groups: 60-80 (total), 45-49, 60-64 (males) and 60-80 (females). *Summary.* The detection of higher lymphoma incidence in female urban elderly (Non-Hodgkin's lymphoma) and young (Hodgkin's lymphoma) population should lead to a rational planning of population preventive examinations.

Table. Lymphoma incidence rates, per 100,000 population.

	HPIA		Stago ELISA	PaGIA	Sens.
	norm.	citrated plasma	EDTA plasma	citrated plasma	
Clinically suspected HIT					
number of patients	63	73	34	71	38
negative	41 (65.1%)	57 (78.1%)	28 (82.4%)	51 (71.8%)	20 (52.6%)
indeterminate	2 (3.2%)	0	1 (2.9%)	8	0
positive	20 (31.7%)	16 (21.9%)	5 (14.7%)	12 (16.9%)	20 (52.6%)
Equivalently positive					
number of patients	33	37	27	37	37
negative	21 (63.6%)	23 (62.2%)	18 (66.7%)	20 (54.1%)	27 (73%)
indeterminate	0	0	8	8	0
positive	12 (36.4%)	14 (37.8%)	7 (25.9%)	17 (45.9%)	10 (27%)
Healthy persons					
number of patients	73	73	25	25	73
negative	71 (97.3%)	71 (97.3%)	21 (84%)	20 (80%)	70 (95.9%)
positive	2 (2.7%)	2 (2.7%)	4 (16%)	5 (20%)	3 (4.1%)

		1991 - 1994	1995 - 1999	2000 - 2003
Hodgkin's lymphoma	Males	2,8	2,5	2,4
	Females	2,6	2,6	1,9
	Total	2,6	2,5	2,1
Non-Hodgkin's lymphoma	Males	4,5	4,8	5,3
	Females	3,3	3,7	4,8
	Total	3,8	4,1	5,1

0874**ACUTE MYELOID LEUKEMIAS WITH RECURRENT GENETIC ABNORMALITIES: FREQUENT ASSESSMENT OF MINIMAL RESIDUAL DISEASE AND TREATMENT OF MOLECULAR RELAPSE**

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Background. The majority of patients with acute myeloid leukemia (AML) achieve complete remission after induction and consolidation therapy. However, the significant proportion of patients eventually relapse. Early detection of minimal residual disease (MRD) may offer the possibility of implementing salvage therapies prior to overt clinical relapse. It is still not entirely clear, however, how to deal with the molecular relapse therapeutically nor how to monitor it. For technical reasons, MRD monitoring is considered especially for patients who suffer from AML with recurrent genetic abnormalities. Aim and methods. In an effort to answer the questions of MRD monitoring and treatment of molecular relapse, we have performed a pilot study in 13 patients (166 specimens, 13 in average in 1 patient) with the AML with t(8;21), inv (16) and 11q23 abnormalities. We regularly monitored the fusion transcripts AML1/ETO, CBFb/MYH11, and MLL gene using quantitative real-time polymerase chain reaction. The specimens of bone marrow and/or peripheral blood were collected before beginning therapy and later in monthly intervals. Molecular genetic examination was correlated with the results of cytogenetic and morphological studies. Molecular relapse (confirmed by repeated examination) was defined as the reappearance of positivity after a previous negative result or as an at least ten-fold rise in positivity. **Results.** In 7 patients, we observed isolated molecular relapse of the disease after a successful therapy. The patients who do not achieve stable complete molecular remission have a great probability of suffering from hematological relapse. We did not observe a spontaneous disappearance of the MRD positivity. The time period between the molecular relapse and hematological relapse is extremely variable. Molecular relapse can precede the hematological relapse by 1-6 months. In one patient, fulminate hematological relapse occurred despite being in molecular remission 1 month earlier. In 3 patients, we decided on chemotherapy (cytosine arabinoside plus daunorubicin or mitoxantrone) of the molecular relapse because of increasing dynamics of the MRD level. In 2 patients, only a decrease in the level of MRD was observed after chemotherapy. In 1 patient, however, the second complete molecular remission was achieved. Unfortunately, 4 months after final therapy this patient developed a second molecular relapse. The second relapse was treated using gemtuzumab ozogamicin. In 2 patients molecularly relapsed after allogeneic stem cells transplantation, we decided on interleukin-2 administration followed by donor lymphocyte infusion. These patients achieved a second complete molecular remission. **Conclusions.** According to the results of our MRD-monitoring study based on regular and frequent MRD analysis, we can hypothesize about the existence of two patterns of relapse of AML. In the first type, the molecular relapse precedes the hematological relapse in a few months and can be well monitored, whereas the second type is the fulminate relapse that is not revealed early enough by molecular methods. Furthermore, we can also hypothesize that after an administration of therapy for molecular relapse, three patterns of response can be seen: complete molecular remission, partial molecular response, and non-response.

0875**PREDICTION OF HAEMORRHAGE IN THE EARLY STAGE OF ACUTE MYELOID LEUKEMIA BY FLOW CYTOMETRIC ANALYSIS OF PLATELET FUNCTION**E.B. Leinoe¹, M.H. Hoffmann², E. Kjaersgaard², J.D. Nielsen³, O.J. Bergmann², T.W. Klausen², H.E. Johnsen²¹Hilleroed Hospital, HILLEROED, Denmark; ²Herlev Hospital, HERLEV, Denmark; ³Gentofte Hospital, GENTOFTE, Denmark

Background. Haemorrhage is often responsible for the lethal course of acute myeloid leukemia (AML). Platelet transfusion therapy has greatly reduced the risk of life threatening haemorrhage. However, haemorrhage in AML patients occurs regularly even with normal platelet counts. The value of flow cytometric analysis of platelet function for prediction of haemorrhage in AML patients has not previously been examined. **Aims.** To analyse the expression of platelet activation markers CD62P, CD42b, CD63, and PAC-1 by flow cytometry in 50 AML patients at diagnosis and to correlate the results with the clinical bleeding tendency of the patients in the first 28 days of the disease. **Methods.** For the flow cytometry analysis, an electronic gate enclosing platelets was set as defined by forward scatter and side scatter characteristics and CD61 platelet marker positivity. Expression of platelet activation markers CD62P, CD42b, CD63, and PAC-1 were analysed prior to and following *in vitro* platelet stimulation by thrombin receptor activating peptide (TRAP) 70 µM. Soluble P-selectin was analysed by ELISA. Activated partial thromboplastin time, thrombin time, prothrombin time, D-dimer, fibrinogen, and von Willebrand factor antigen were measured in plasma. Haemorrhage was evaluated by the common toxicity criteria (CTC) version 2.0 and was prospectively recorded for 28 days. Logistic regression analysis and Cox regression was used for the multivariate analyses. Continuous variables were dichotomized, using medians as cut-off values. **Results.** Twenty-six patients (52%) presented with minor (grade 1-2) bleeding tendency. None of the patients had major (grade 3-4) haemorrhage at diagnosis. Potential predictive variables of haemorrhage at diagnosis were entered into a multivariate logistic regression analysis. CD62P < 36 MESH x 10exp3 (OR 27.5; P<0.0015) and platelet count < 40 x 10exp9/L (OR 6.9; P = 0.01) were retained as significant predictors of haemorrhage at diagnosis. Major (grade 3-4) haemorrhage was observed in 17/49 assessable patients during the following 28 days. During this period, induction chemotherapy was initiated in 30 patients (60%), while 20 patients (40%) received symptomatic treatment only. The impact of potential variables was tested in a univariate Cox regression analysis. The subsequent multivariate analysis entered all variables with resulting P-values of the univariate analysis <0.2. Haemorrhage at diagnosis was found to be a clinical sign, predicting grade 3-4 haemorrhage during the following 28 days [relative risk (RR) = 3.6; P = 0.018]. To identify prognostic biological markers the multivariate analysis was repeated excluding haemorrhage at diagnosis, resulting in a significant prognostic value of CD62P (RR = 3.4; P = 0.015). **Conclusions.** Our current study identified CD62P as a putative biological marker of haemorrhage in AML. The arbitrarily defined CD62P cut-off value, as well as the predictive value of CD62P needs to be validated in future prospective trials.

0876**INFLUENCE OF THYROTROPINE RELEASING HORMONE AND IT'S SYNTHETIC ANALOG ON RAT PLATELET AGGREGATION IN EXPERIMENT IN VIVO**

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Background. It is known, that neuropeptide thyrotropine releasing hormone (TRH) has wide spectrum of biology activity. TRH improves vascular microcirculation, influences on red cell membrane and shows antidepressive and psychostimulate effects. Digypramine (DGP) is synthetic analog of TRH, that hasn't hormonal activity and is more stable for endopeptidase action. Now days huge attention is focused on the studying the

effects of TRH on haemostasis system. In our previous work we showed that TRH and DGP induce the increase in platelet aggregation in experiments *in vitro*. AIMS. The aim of this investigation was to study the effect of TRH and DGP on ADP-induced platelet aggregation (AIA) after various peptides administration to white rats. METHOTHS. TRH and DGP were used by intranasal and intravenous injection (5 and 10 mkg/kg in both cases). AIA were monitored during 60 min after peptides administration. *Results*. The results demonstrate that TRH dose-dependent influences on AIA by intravenous injection only. High TRH dose increases AIA on 20-25%. Lower dose of TRH doesn't change AIA level. The increase of AIA (on 20-35%) are observed both by intranasal and by intravenous injection of DGP. In the case of TRH the same effect is manifested after intranasal administration only. *Conclusions*. Thus, we conclude that TRH and DGP have polyfunctional influence on primary haemostasis, changing platelet aggregation. These effects can be both direct and indirect, that we showed in experiments *in vitro* and *in vivo*. However, DGP is more effective than TRH.

0877

HYPERHOMOCYSTEINEMIA ENHANCES STRESS-INDUCED THROMBOPHILIA IN RATS

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Stress induces the development of thrombophilia that increases cardiovascular morbidity and mortality. Hyperhomocysteinemia (HHcy) is an independent risk factor for cardiovascular disease. At the same time the effect of HHcy on stress-induced coagulation abnormalities is poorly studied. Thus the aim of this study was 1) to investigate platelet aggregation in response to thrombin and ADP, malondialdehyde (MDA) level, antithrombin III (AT III) and fibrinolytic activity (FL) of plasma in stressed rats with and without HHcy, 2) to evaluate the effect of methionine (Met) load (100 mg/kg) on the same parameters in stressed rats. Stress was induced by free swimming in water at 22 C for 40 min. Methionine was administered to all rats 2 h before swimming. Stress resulted in the increase in adrenalin and corticosterone concentrations in all groups. The rise in total plasma Hcy after stress was observed only in rats without HHcy but not in HHcy rats. In stressed rats with HHcy the increase in platelet aggregation in response to thrombin and ADP as well as in the thrombin-induced MDA synthesis and the decrease in AT III and FL activities were more significant than in stressed rats without HHcy. The Met load caused the increase in platelet aggregation and MDA level and the decrease in AT III and FL activities in stressed rats without HHcy up to the values observed in stressed HHcy rats. There were no significant changes in studied parameters in stressed rats with HHcy after the Met load. Our study suggests that HHcy promotes thrombophilia under stress condition increasing platelet activation and decreasing anticoagulant and fibrinolytic activities.

0878

VE-CADHERIN AND SCD146 SERUM LEVELS IN PATIENTS WITH MULTIPLE MYELOMA

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The role of angiogenesis in the multiple myeloma (MM) pathogenesis and progression is well established. Angiogenesis is linked to the functional state of endothelial junctions that are modulated by the growth and activation of endothelial cells (EC). New capillaries are formed by ECs derived from pre-existing vessels in the tumor microenvironment thus ECs are

indicative of angiogenic state. The increased number of circulating ECs was observed in some solid tumors, lymphomas and MM. CD146 and vascular endothelial-cadherin (VE-cadherin) are cell adhesion molecules localized at the endothelial junction. CD146 is a transmembrane glycoprotein that is constitutively expressed in the whole human endothelium, irrespective of its anatomical site or vessel caliber. VE-cadherin plays a key role in angiogenesis and in vascular permeability. The regulation of its biological activity may be a central mechanism in normal or pathological angiogenesis. Elevated VE-cadherin RNA levels were found in patients affected by lymphoproliferative disorders. The aim of the study was to assess VE-cadherin and sCD146 serum levels as markers of endothelial cells activation in patients with multiple myeloma. *Material and Methods*. 30 untreated and 36 in at least partial response multiple myeloma patients were included in this study (38 females and 28 males, median age 67 years). In addition 22 samples from healthy volunteers (14 females and 8 males, median age 65 years) were evaluated as the control. Serum levels of VE-cadherin and sCD146 were measured by ELISA kits (Bender Medsystem, Austria and CYQuant, France respectively) following the manufacturer instruction. The data were analyzed statistically using Anova rang Kruskal Wallis test ($p < 0,05$). *Results*. There was no significant difference in sCD146 level between MM patients and the normal control: $505,8 \pm 177,2$ and $469,4 \pm 156,9$ pg/mL respectively. In untreated MM patients VE-cadherin level was significantly higher than in healthy control ($0,84 \pm 0,55$ vs $0,64 \pm 0,56$ ng/mL respectively; $p < 0,05$). Similarly in untreated MM patients VE-cadherin level was significantly higher than in MM patients who achieved at least partial response ($0,84 \pm 0,55$ vs $0,5 \pm 0,33$ respectively; $p < 0,05$). *Conclusions*. VE-cadherin but not sCD146 serum level may reflect intensity of angiogenesis in multiple myeloma and may be useful in prognosis of response to treatment.

0879

LYMPHANGIOGENESIS MEASURED BY VEGF-C EXPRESSION IN LYMPH NODES OF PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Lymphangiogenesis is a formation of new lymphatic vessels from preexisting capillaries. VEGF-C stimulates lymphangiogenesis by binding VEGFR-2 and VEGFR-3 receptors on lymphatic endothelial cells. In many solid tumours increased VEGF-C expression correlate with increased risk of metastasis in regional lymph nodes. The role of lymphangiogenesis in the development of cancer and tumour metastasis is still controversial. Non-Hodgkin's lymphoma (nHL) may spread via blood vasculature or lymphatics. Lymphoma cells may infiltrate the lymph nodes, surrounding tissue or migrate within blood to other organs. Increased microvessel density (MVD) was demonstrated in NHL. However the relationship between MVD and histologic subtypes of lymphoma are not clear. Elevated serum level of proangiogenic cytokines (i.e. VEGF) is the independent prognostic factor in nHL. Data concerning the role of lymphangiogenesis in nHL are very limited. Salven et al. showed low VEGF-C mRNA in lymphoma cells. The aim of the study was the evaluation of intensity of lymphangiogenesis by VEGF-C expression in lymph nodes of patients with nHL. *Methods*. 60 paraffin-embedded lymph nodes from newly diagnosed patients with nHL were evaluated (23 aggressive and 37 indolent nHL). In addition 12 lymph nodes biopsy specimens from adult patients with reactive lymphonodulitis were used as controls Lymph nodes sections were stained immunohistochemically for VEGF-C (R&D Systems, USA). Intensity of immunocytochemical procedure was evaluated using the ImmunoReactive Score (IRS) according Remmele. The applied scale took into account both intensity of the colour reaction and percentage of cells which exhibited the positive reaction. Final result represented the product of the two parameters and its value ranged between 0 and 12. *Results*. Statistical analysis showed

that VEGF-C expression in lymph nodes of nHL patients was low and was not significantly higher than in the control: mean IRS 2 (range 0-6) and 1 (range 0-4) respectively. Moreover VEGF-C expression did not differ significantly between aggressive and indolent lymphomas. **Conclusions.** We demonstrated that expression of VEGF-C, the most potent cytokine stimulating lymphangiogenesis, was not increased in lymph nodes affected by lymphoma.

0880

BUSULFAN INDUCED MYELOABLATION IN THE MOUSE MODELS OF BONE MARROW TRANSPLANTATION WITH DIFFERENT DEGREES OF HISTOCOMPATIBILITY

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Bone marrow transplantation (BMT) is currently a widespread method used in a broad range of scientific research areas. One of the steps in classical models of BMT is the irradiation and/or chemotherapy preparative regimens. Chemotherapy protocols usually include busulfan, cyclophosphamide, rapamycin and other agents alone or in different combinations. It has been considered that busulfan cannot be used alone due to its profoundly myeloablative effect and lack of immunosuppressive properties. However, some literature data argue that busulfan may be successfully applied alone in cases where total suppression of the host immune system is not necessary: autologous, syngeneic and congenic BMT. Our aim was to examine whether it is possible to use single busulfan preconditioning regimen in experimental syngeneic and allogeneic MHC-matched BMT mouse models. BALB/c and DBA/2, 10-20 weeks old mice of both genders, were supplied from Animal Breeding Centre of the Institute of Immunology and Experimental Therapy (Wroclaw, Poland) and maintained in standard laboratory conditions. All transplantations were gender matched. The data from four experiments were pooled. BALB/c mice were administered 5 or 4, dependent on the experiment, injections of busulfan in a dose 25 mg/kg/day, i.p., on the daily basis, before BMT. Bone marrow (BM) was collected by flushing the shafts of bones of the donor DBA/2 or BALB/c mice. Following therapy with busulfan, recipient BALB/c animals in allogeneic BMT (allo-BMT) group have received i.v. injections of $1,1-3,2 \times 10^6$ allogeneic DBA/2 MHC-matched BM cells, whereas recipient mice in syngeneic BMT (syn-BMT) group have been injected with 3×10^6 syngeneic BALB/c BM cells. Animals were monitored daily for survival. The control myeloablated mice without any rescue BMT have died by the 16th day of experiment. The transplantation of allogeneic MHC-matched BM cells has resulted in statistically significant longer survival in experimental allo-BMT group over control mice ($p < 0,0001$). Nevertheless, all animals in allo-BMT group have died by the 88th day post-transplantation. The median survival time in control and allo-BMT groups was 10 ($n=24$) and 27 ($n=28$) days, respectively. The recipient mice that received syngeneic BM cells had significant advantage in survival over the both control ($p < 0,0001$) and allo-BMT ($p < 0,0001$) groups. Only one mouse in this group died at 67th day of experiment. The rest 7 out of 8 (88%) mice in syn-BMT group survived for more than 180 days post-transplantation. The data obtained in these experiments further confirm that busulfan may be successfully applied alone as a preparative chemotherapy agent in the mouse model of syngeneic BMT. All recipient mice received otherwise lethal dose of busulfan and rescued by syngeneic BMT, outlive mice in the control group and the difference in survival was statistically significant. The survival in the allo-BMT group was also significantly longer in comparison with control mice. Data received evidence that pre-treatment regimen with busulfan alone may also be successfully utilized as the model of allogeneic MHC-matched BMT with the GVHD development and post-transplantation mortality.

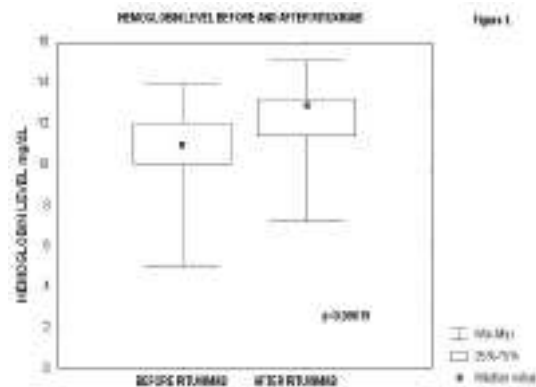
0881

RITUXIMAB AS RESCUE THERAPY IN AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA), SECONDARY TO MULTISYSTEMIC AUTOIMMUNE DISEASES (MAD) AND IDIOPATHIC AIHA

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Background. Rituximab (R), an anti-CD20 monoclonal antibody, has proved to be useful in the treatment of autoimmune mediated citopenias, including AIHA. Patients and **Methods.** Three patients with MAD (2 Primary Antiphospholipid syndrome, 1 with Systemic Lupus Erythematosus) and 1 idiopathic with AIHA were treated with R. All patients were female (median age: 35 years; range: 25-70). Coombs test scored from 0 (negative) to 4+ (very strong). Antinuclear, anti-DNA and anti- β -glycoprotein (A β G), lupic anticoagulant, and antiphospholipid (APL) antibodies were all evaluated before and after treatment. Two patients had warm /cold antibody type and 2 warm antibody type. Prior therapies included cyclosporine, azathioprin, danazol and steroids, that patients had received for a median 280 days (range, 103-570) with poor control (median number of relapse 2, range: 1-3). R was given at the usual dose and schedule ($375\text{mg}/\text{m}^2$ weekly for 4 doses). Number of all blood sample of four patients previous R were 39 and 16 after R. **Outcome:** The four treated patients achieved a complete response. Mild side effects were observed during R treatment. The Hemoglobin median level increased from 10.45 (range: 5.3-13.5) mg/dL before treatment to 13 (range: 7.2-15.1) mg/dL after treatment ($p < 0.00019$) and Coomb test became negative in 3 patients with a median follow-up time after R treatment of 129 days (range: 90-180), all patients continue being stable and in three of them all immunosuppressive therapy has been discontinued. In addition, A,G-IgM, A,G-IgG and APL-IgM titles diminished after R treatment [median (range): 224 (16.5-948) vs. 141 (22-279), 42 (2.2-432) vs. 18 (3.2-246) and 53.2 (24-114) vs. 40.6 (24-165), respectively]. **Conclusions.** These results confirm that Rituximab is effective and safe in patients with AIHA who fail to other therapies. The decrease in A β G-IgM, A β G-IgG and APL-IgM titles suggest a possible effect of Rituximab in MAD should be further investigated.



0882

CONFIRMING MARROW INVOLVEMENT WITH MULTIPLE MYELOMA USING BONE MARROW SECTIONS AND BIOPSY

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Background. At this centre bone marrow smears, trephines and granule sections are routinely examined at diagnosis and at relapse in all patients with multiple myeloma. **Aims.** The aim of this study was to see if examining granule sections in addition to bone marrow smears and trephines provided additional diag-

nostic information. **Methods.** Bone marrow samples from 96 patients at a single centre diagnosed with multiple myeloma were studied. Granule sections, marrow smears and trephine biopsies were examined to assess the contribution of each to the diagnosis. 'Granule sections' were obtained by processing aspirated material placed in 10% formalin, making 3-5µm sections and staining with H&E. A total of 219 smears, 196 granule sections and 184 trephines were examined. The authors personally reviewed 158 smears, 148 granule sections and 184 trephines. The remaining data was obtained from historical records if the study material was unavailable or inadequate for study. **Results.** Samples taken at diagnosis included 94 smears, 81 granule sections and 79 trephines. The remaining samples had been taken at follow-up. At first diagnosis smears were negative or inadequate in 7 cases, of these; diagnosis was confirmed on trephine alone in 1 patient and on both granule sections and trephine in 6 patients. Smears were inadequate or negative for disease in 21 patients at follow-up. In these cases disease was confirmed in 8 patients by trephine and granule sections, in 8 patients by trephine alone and in 5 patients by granule sections alone. **Conclusions.** These data show that additional and independent information for diagnosis can be obtained by examining granule sections. Additionally it is a quicker and less traumatic than trephine biopsy. Diagnostic sensitivity can be increased by employment of all of the above three methods.

No	Study Material	No. of patients at diagnosis (%)	No. of patients at follow-up (%)
1	Sm+Gr+Tr-	1 (1.04%)	2 (2.1%)
2	Sm-Gr+Tr+	6 (6.3%)	8 (8.3%)
3	Sm-Gr-Tr+	1 (1.04%)	8 (8.3%)
4	Sm-Gr+Tr+	0	5 (5.2%)

Sm = smears, Gr = granule sections, Tr = trephine.

0883

DAF (DAUNORUBICIN, ARAC, FLUDARABINE) AS THE INDUCTION REGIMEN OF RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA. PHASE II STUDY BY THE POLISH ADULT LEUKEMIA GROUP (PALG)

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In 1992-93 a synergistic interaction of ribonucleotide reductase inhibitors (fludarabine, cladribine) and cytarabine (AraC) resulting in increase of Ara-CTP concentration in myeloblasts was proved. In the previous study by the Polish Adult Leukemia Group (PALG 1999 Study) we demonstrated that addition of cladribine to standard daunorubicin + cytarabine induction potentiates antileukemic activity of the regimen in acute myeloid leukemia (AML). The goal of the current study was to assess the safety, tolerance and antileukemic activity of a new DAF regimen consisting of daunorubicin 60 mg/m²/d iv 1-3, AraC 200 mg/m² ci 1-7, and fludarabine 25 mg/m²/d 1-5. Twenty-five AML patients aged 40 (range 19-62) years with primary refractoriness 60% (n=15) or in relapse 40% (n=10) were included in the study between 2003-2004. DAF was administered as a second (n=24) or as a third line of induction treatment (n=1). Twelve patients (48%) achieved complete remission (CR), 1 (4%) had partial remission (PR), and 10 (40%) did not respond (NR, leukemia regrowth). Two patients died in aplasia from infectious complications (8%). All patients developed severe thrombocytopenia and granulocytopenia (WHO grade III or IV).

Median time to neutrophil >0.5 G/L recovery and platelet >50 G/L recovery equaled 21 d. (11-30) and 20 d. (11-27), respectively. Eleven (44%) patients experienced severe (WHO grade III or IV) neutropenic infections (bacterial n=9, fungal n=2). Other serious adverse events were infrequent: vomiting 24% (n=6), mucositis 24% (n=6). Severe hepatotoxicity, nephrotoxicity and cardiotoxicity was not observed. Median hospital stay lasted 28 (11-41) days. Patients required 5.5 (0-13) PRBC transfusions and 5.5 (1-14) platelet transfusions. Hematopoietic growth factors were not administered. **Results.** of this phase II study indicate that DAF is a relatively well-tolerated and safe regimen with high anti-leukemic potency in relapsed/refractory AML. Based on these findings a randomized, multicenter study comparing DAF to the standard DA induction has been designed for newly diagnosed AML patients.

0884

PROLIFERATION AND SURVIVAL OF NATURAL KILLER CELLS ARE SEVERELY ALTERED IN MYELODYSPLASTIC SYNDROMES (MDS)

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Natural Killer (NK) cells are critical in host defense against malignant transformation and are potent antileukemic cytotoxic effectors. We have previously shown that in the majority of MDS patients, NK cells displayed a very low expression of NKp46, one of the natural cytotoxicity activating receptors. Although activation with IL-2 and IL-15 resulted in up-regulation of NKp46 expression by MDS-NK cells, their cytolytic function remained deeply altered as compared to activated normal NK cells. In the present study we show that *in vitro* proliferation and survival of MDS-NK cells is also severely altered. Such defects are due to markedly elevated rates of spontaneous apoptosis. Apoptosis was measured by cytochrome c release by confocal microscopy, and annexin V expression by flow cytometry. Mean apoptosis rate of resting MDS-NK cells was 24%, significantly higher than in NK derived from healthy donors (5%, p=0.0053). This high rate of apoptosis was not reversed by IL-2 or IL-15 and could explain the failure of *in vitro* expansion of MDS-NK cells we constantly observed. Fas expression was not correlated with the level of apoptotic MDS-NK cells. The blocking of the Fas pathway using a neutralizing mAb did not reduce the rate of MDS-NK cells undergoing apoptosis, in contrast to donor NK cells. This is the first report of enhanced *ex vivo* apoptosis of NK cells in MDS, a disease characterized by enhanced apoptosis of myeloid bone marrow progenitors. All together, these results show that MDS-NK cells exhibit an important *ex vivo* rate of apoptosis and that their stimulation by cytokines results in the triggering of an incomplete activation process together with an apoptotic program.

0885

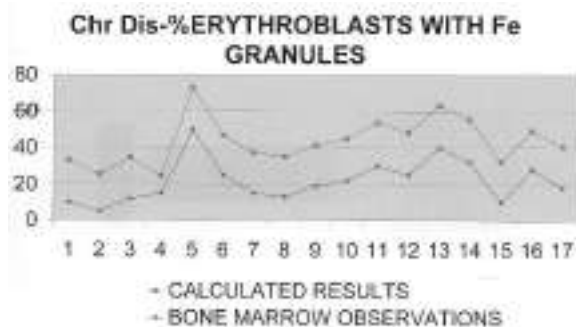
ESTIMATION OF BONE MARROW IRON STORES WITHOUT PERFORMING BONE MARROW ASPIRATION IN PATIENTS WITH ANEMIA

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Background. Bone marrow aspiration is a painful invasive method not easily acceptable from the patients, but it is the best method to provide information for macrophage storage iron and aggregated iron in the cytoplasm of the erythroid precursors. **Aims.** estimation of bone marrow iron stores without performing bone marrow aspiration. Patients and **Methods.** We studied 87 patients with anemia (hematocrit <36% and hemoglobin <12 mg/dL), 55 women and 32 men aged from 40 to 88 years old (M= 69,2). Of the patients studied 34 had infectious

disease, 10 had cancer, 17 had chronic disease and 26 had anemia without infection, cancer or chronic disease. We stained bone marrow from each patient using Prussian blue method and measured the percentage of erythroblasts containing iron granules in the cytoplasm (%Rbc). At the same time we measured iron granules within macrophages (Fe Dep) by oil immersion lens and graded as 0+ if no granules were observed, 1+ if scarce small granules were observed, 2+ if few small granules were observed, 3+ if numerous granules observed and 4+ if large granules in clumps were observed. Serum transferrin receptors (sTfr) and serum ferritin (fer) were measured by AIA pack method, with, AIA 600 II analyzer. We used non-parametric Spearman's correlation and statistical regression methods. **Results.** The correlation of %Rbc and stfr in patients with chronic disease is significant ($p=0,05$, Spearman's 2-tailed) and the regression analysis gives results equivalent with those obtained by bone marrow aspiration, pict.1. The correlation of Fe Dep and stfr in patients with chronic disease is highly significant ($p=0,01$, Spearman's 2-tailed) and the regression analysis gives results equivalent with those obtained by bone marrow aspiration, pict.2. The correlation of %Rbc and stfr in patients with cancer and the correlation of Fe Dep and sTfr, at the same group of patients are highly significant ($p=0,01$, Spearman's 2-tailed) and the regression analysis give results equivalent with those obtained by bone marrow aspiration pict.3 and 4. The correlation of %Rbc and Fer in patients with infectious disease is highly significant ($p=0,01$, Spearman's 2-tailed) and the regression analysis gives results equivalent with those obtained by bone marrow aspiration, pict.5. The correlation of Fe Dep and Fer in patients with infectious disease is significant ($p=0,05$, Spearman's 2-tailed) and the regression analysis gives results equivalent with those obtained by bone marrow aspiration, pict.6. The correlation of %Rbc and hemoglobin (Hb) in patients with anemia without chronic disease, cancer or infectious disease and the correlation of Fe Dep and sTfr at the same group of patients are highly significant ($p=0,01$, Spearman's 2-tailed), the regression analysis gives results equivalent with those obtained by bone marrow aspiration pict.7 and 8. **Conclusions.** We can calculate the bone marrow iron granules within macrophages as well as the bone marrow percentage of erythroblasts containing iron granules, without performing bone marrow aspiration, using the proper equation and the values of either ferritin, serum transferrin receptors, or hemoglobin for patients with anemia alone or together with other disorders as described above.



0886

CHRONIC LYMPHOBLASTIC LEUKAEMIA (CLL) WITH MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS (MPG): RESPONSE TO RITUXIMAB OF BOTH THE BLOOD DISEASE AND THE NEPHROTIC SYNDROME

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Background. CLL, the commonest form of indolent lymphoproliferative disorder, remains an incurable disease despite the

numbers of clinical trials over the last decades. Chlorambucil and fludarabine-based regimes, are still the mainstay in CLL treatment. The role of high-dose chemotherapy followed by stem cell transplantation is still controversial. Recent reports have described the anti-CLL activity of the anti-CD20 antibody Rituximab. However, the appropriate therapeutic schedule is not yet established. MPG associated with CLL is an autoimmune condition caused by immune complex deposition, but disordered immunoglobulin production and cellular immunity may be involved. Recent reports have shown the efficacy of Rituximab in patients with nephrotic syndrome due to chronic glomerulonephritis. AIMS. On account of the considerations mentioned above, we have used Rituximab in a patient with CLL and refractory MPG. **Methods.** The patient presented in 1991, aged 57, with stage 0 CD5/CD20 positive CLL and a lymphocyte count of $15 \times 10^9/L$. A year later he presented with ankle oedema, weight loss, lymphocyte count of $33.0 \times 10^9/L$, proteinuria of up to 15gr/24h and disease progression to stage 3 CLL. A renal biopsy was consistent with a diagnosis of MPG. From November 1992 the patient received several courses of chlorambucil and corticosteroids with very good response: the proteinuria gradually dropped to 0.25g/24h and the Hb stabilized above 12.0gr/dL within the first two years of treatment. In July 1998 kidney disease and his general condition worsened and urine protein rose to 10-17g/24h. Chlorambucil was thus replaced by cyclophosphamide. Kidney function and clinical condition improved again and the urine protein decreased to 1 gr/24h. By November 2002 a third relapse occurred, proteinuria increasing to 7 g/24h. Rituximab was then given at the dosage of 375mg/sqm as follows: three doses the first week, a repeat dose after four and eight weeks, then every 2 months as maintenance. **Results.** Following the third course of Rituximab the proteinuria became undetectable, while the plasma creatinine returned to near-normal values. The peripheral blood counts and the immunophenotype became normal within 12 months of beginning of Rituximab treatment, when the CD5/CD20 CLL clone was <5%. The patient experienced no drug-related untoward effects and had no infectious episodes. He is now 71 and in excellent physical condition with a performance status is 0 (WHO). He is currently on a bi-monthly maintenance treatment. **Summary/Conclusions.** This clinical case confirms the beneficial effect of Rituximab in the treatment of CLL. The schedule of administration was chosen empirically and proved effective on the haematological disease as well as on the nephrotic syndrome. The disappearance of the CD5/CD20 leukaemic clone from the peripheral blood was successfully achieved only after several months of maintenance. Whether Rituximab should become part of the treatment of CLL in induction and/or maintenance will be established by ongoing clinical trials. Finally, Rituximab may be specifically indicated in the presence of the autoimmune manifestations of CLL.

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0887

EFFICACY AND TOLERABILITY OF DANAZOL TREATMENT FOR THE ANEMIA OF MYELOFIBROSIS WITH MYELOID METAPLASIA: LONG-TERM RESULTS IN 30 PATIENTS

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Background. Androgen therapy is considered as the choice treatment for the anemia of myelofibrosis with myeloid metaplasia (MMM). In this regard, good results have been reported with the use of danazol, a synthetic attenuated androgen. However, the experience with such therapy is limited to a few patients with short follow-up. Aim. To evaluate the long-term efficacy and tolerability of danazol as a treatment for the anemia of MMM and to analyse the variables associated with a favorable response. **Methods.** Thirty-three patients with MMM

and anemia received danazol as an initial dose of 600 mg/day, which was maintained for six months and thereafter progressively tapered to the minimum effective dose in responding patients or stopped in non-responders. Complete response (CR) was defined as transfusion cessation with normalization of the hemoglobin levels (Hb) and partial response (PR) as an Hb increase > 1.5 g/dL with Hb values > 10 g/dL maintained for a minimum of 8 weeks. The pre-treatment variables associated with the response to danazol were analysed. *Results.* Due to early death, three patients received the treatment for less than two months and could not be evaluated for response. In the 30 assessable patients, median follow-up from treatment start was 19.6 months (range: 3.3-56). A favorable response was obtained in 11 patients (36.7%), including eight CRs (26.7%) and three PRs. Median time to obtain a response was 6 months (range: 3-9). Four patients lost the response at 6 to 24 months from achievement, in 2 responders danazol should be stopped due to toxicity, and 5 (among them, 4 of the complete responders) maintain the response at 2 to 39 months. Pre-treatment variables associated with a favourable response to danazol were lack of transfusion requirement ($p = 0.001$) and higher Hb at treatment start ($p = 0.02$). An increase in platelet counts was also observed in 2 of the 7 patients with thrombocytopenia. Treatment-related toxicity (usually consisting of a mild increase in the liver enzymes) was observed in 8 patients, but it led to treatment withdrawal in only 2 cases (toxic hepatitis and prostate adenocarcinoma, one case each). *Conclusions.* Danazol is an effective and usually well-tolerated therapy for a substantial proportion of MMM patients with anemia. Half of the responses are durable responses.

0888

HIGH MOLECULAR WEIGHT FIBRIN DEGRADATION PRODUCTS ARE MAIN CROSS-LINKED MATERIALS IN NORMAL AND PATHOLOGICAL PLASMAS

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Background. The elevated level of fibrin degradation products (FDP) is a marker of hypercoagulation or thrombus formation like in disseminated intravascular coagulation (DIC), thromboembolism, or deep venous thrombosis. For a long time, D-dimer was considered to be a final product of fibrinolytic process in blood, and a lot of kits for D-dimer determination were developed. Recently it was reported that high molecular weight FDP are main cross-linked materials in DIC patients. *Aims.* The aim of this work was to study plasmas from thrombophilia and DIC patients and from healthy volunteers. *Methods.* Three anti-D-dimer MAb pairs DD1-DD6, DD2-DD6, and DD3-DD4 (HyTest, Finland) were used for D-dimer analysis. FPLC gel filtration of purified D-dimer, fibrinogen, plasma samples, and their mixtures were performed on a Superdex 200 column. *Results.* The elution profile analysis of pure fibrinogen and D-dimer showed that antibodies do not react with fibrinogen but recognize D-dimer and high molecular weight material present in fibrinogen. FPLC gel filtrations of mixtures of fibrinogen with D-dimer and plasma with D-dimer showed that D-dimer, if present, does not form high molecular weight complexes with fibrinogen or with plasma components and eluted as pure D-dimer. It means that high molecular weight materials found in pure fibrinogen and in plasma are FDP reacting with anti-D-dimer MAbs. FPLC gel filtration of plasmas showed that only high molecular weight FDP are present in normal plasma. They were also predominant in plasmas of a DIC patient and a patient with hereditary thrombophilia, although D-dimer was also detected in pathological plasmas using the DD3-DD4 pair which is highly sensitive to D-dimer. *Conclusions.* High molecular weight cross-linked materials are the main component of normal and pathological plasma. No D-dimer was found in normal plasma.

0889

INTRA-ARTERIAL INJECTION OF IMMUNOSUPPRESSIVE THERAPY FOR SEVERE GASTROINTESTINAL AND LIVER GVHD

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Background. Graft versus host disease (GVHD) is a major complication in patients with allogeneic hematopoietic stem cell transplantation and steroid resistant GVHD often results in death. Therefore, new treatments are urgently needed. *Methods.* We evaluated the effect of immunosuppressive drug administration (methylprednisolone 40-60mg) into the mesenteric arteries in patients with severe gastrointestinal GVHD, and intra-arterial (hepatic artery) methylprednisolone (75mg/m²) with or without methotrexate (10mg/m²) administration in patients with liver GVHD. *Results.* 11 patients received in total 28 administrations. Each patient underwent from one up to four administrations. Intra-arterial infusion was administered into the mesenteric arteries alone in 3 patients, into the hepatic artery alone in 4 patients, and 4 patients received both treatments. Four out of 7 patients with gastrointestinal GVHD achieved response (57%); there were 3 CRs and 1 PR. Four out of 8 patients with liver GVHD responded (50%)-all PRs. Interestingly, 3 patients with very severe liver GVHD remaining without response to different systemic immunosuppressive drugs achieved a good response after intra-arterial infusion. Two patients with advanced severe gastrointestinal GVHD developed complicating GIT bleeding, both approximately four days after the intra-arterial administration. There were no complications directly linked with the puncture of artery or administration of the drug. However, in some patients there was a need for platelets transfusion before the procedure. Three patients died from the GVHD progression. *Summary.* Despite retrospective nature of this study and not easy estimation of responses due to many therapeutic interventions in patients with steroid-resistant GvHD, it can be stated that local intra-arterial steroid (\pm methotrexate) infusion seems to be effective and non-toxic treatment of patients with gastrointestinal and/or liver GVHD who failed to respond to other treatment options. It is possible to use it alone or in addition to other immunosuppressive drugs.

0890

MULTIDRUG RESISTANCE OF MYELOBLASTS FROM PATIENTS WITH AML DEPENDS ON ANTIGENIC PROFILE OF BLAST CELLS

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Background. Multidrug resistance (MDR) of leukemic cells is an important cause of treatment failure in acute myeloid leukemia (AML). Malignant cells from different patients with AML differ by the level of MDR. Malignant myeloblasts also vary by their antigenic properties. Antigenic variety of myeloblasts reflects different maturational potential of leukemic clones, and particularly their commitment to different branches of myelopoiesis. It remains, however, unclear whether the different levels of MDR in malignant cells are associated with different antigenic profiles of cells. The aim of this study was to investigate the level of MDR in normal and malignant myeloid cells with different antigenic properties, and to analyze the association between MDR and maturational grade of myeloid cells. *Methods.* Leukemic cells were isolated from bone marrow (BM) of patients with AML at the time of diagnosis, and normal cells were obtained from BM of patients with no hematological disease. Activity of proteins mediating MDR was measured by flow cytometry of cells labeled with rho-

damine123 (rho123) and incubated *in vitro* for 18 h to expel rho123 from the cell interior. Labeled cells were stained with different combinations of monoclonal antibodies before their analysis in flow cytometer. Residual fluorescence of rho123 was determined in gated cells with different antigenic profiles in order to characterize MDR-activity of different fractions of myeloid cells. **Results.** MDR-activity was studied in myeloblasts from 22 patients with different FAB types of AML. MDR of blasts in M0 or M1 subtype of AML was higher than MDR of blasts in M2, M3, and M4 or M5 types of AML. MDR of myeloblasts from patients with AML-M0 or AML-M1 did not, however, differ from MDR of CD34+CD33+ myeloid progenitors from normal BM. Level of MDR in M1, M2, and M3 blasts was similar to those in normal CD33+ granulocytes, and MDR of M4 and M5 blasts was similar to MDR of normal monocytes. Study of antigenic properties of myeloblasts revealed significant antigenic diversity of cells within leukemic clones in the majority of cases. Study of MDR in different subsets of myeloblasts derived from 13 leukemic clones (patients with AML) showed that CD34+CD33- blasts had very high MDR-activity, which was partially abrogated by verapamil, an inhibitor of p-glycoprotein. CD34+CD33+ blasts displayed slightly lower level of MDR, and the blasts with more mature phenotype (CD34-CD33+) had significantly lower level of MDR than CD34+CD33- blasts. Study of normal BM cells showed high level of MDR in CD34+CD33-CD19- non-committed progenitors and in CD34+CD33+ normal myeloid progenitors. More mature (CD34-CD33+) normal myeloid cells displayed significantly lower level of MDR-activity. **Conclusions.** Obtained data showed that normal and malignant myeloid cells with antigenic properties of immature cells had higher level of MDR than myeloid cells with mature antigenic profile. The level of MDR in different subsets of myeloid cells may, therefore, reflect their maturational grade, while the overall MDR of myeloblasts in particular case of AML may reflect the maturational potential of leukemic clone.

0891

CROSS ARSENIC TRIOXIDE TO CENTRAL NERVOUS SYSTEM? EXTRAMEDULLARY RELAPSED IN ACUTE PROMYELOCYTIC LEUKAEMIA POST-ARSENIC TRIOXIDE TREATMENT

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Background. Extramedullary relapse (EMR) of acute promyelocytic leukaemia (APL) is rare. It is not clear whether a true increment of EMR has occurred since the ATRA era. An explanation for EMR may be that ATRA by up-regulating of adhesion molecules on blast cells. ATRA facilitate the entry of malignant promyelocytes into the cerebrospinal fluid (CSF). Arsenic trioxide (ATO) approved for treatment of relapsed or refractory APL. However, this up-regulation of adhesion molecules, seen with ATRA, occurred also with the use of ATO. **Aims.** We report the EMR in an APL patient treated with ATO, in second relapsed molecular. **Pass the TAO to the CNS?** **Case Report:** A 24-year-old woman was diagnosed with APL (M3 variant, bcr1 type PML/RAR- transcript, and intermediate risk) in January 2002. Her past history was diagnosed of hepatic cirrhosis (C-Hepatitis Virus, Child A), and she had not HLA-identical donors. A molecular complete remission (CR) was achieved within the first month from starting ATRA and idarubicin (APL 99 PETHEMA protocol). In July 2003, RT-PCR revealed molecular relapsed in the bone marrow. She received re-induction therapy with TAO and ATRA. She had a molecular remission after 2 months of therapy. She received consolidation therapy with the same regimen. In October 2004, RT-PCR revealed second molecular relapsed. She received re-treatment with ATRA and idarubicin. At ten days, she came to emergency unit because of fever, diarrhoea, nausea and disorientation. Laboratory data showed haemoglobin 7.2 g/L, platelet count $5 \times 10^9/L$, leukocytes $0.5 \times 10^9/L$ with 0% neutrophils, fibrinogen 1.28 g/L, TTPA 56 (32

sec), PT 53%. Brain CT and chest radiography: normal. She received support therapy (antibiotics, fluids, hems). She had a septic shock (bacteraemia by E. coli). On 10 day, she was in comatose status. Brain CT showed a meningoencephalitis and massive hydrocephaly. 12 hours later, she is dead. CSF examination showed the presence of atypical promyelocytes. Necropsy: cerebral, meningeal and renal parenchyma was infiltrated by malignant promyelocytes. Bone marrow was hypo-plastic with isolate promyelocytes. **Conclusions.** 1) EMR in patients with APL should be regarded as a systemic disease. 2) The clinical activity of ATO in extramedullary involvement is limited, even controvert. 3) The actual incidence of EMR and its relationship to new therapies (ATO, gemtuzumab ozogamicin) should be prospectively assessed.

0892

PLATELET HYPERAGGREGABILITY AND RELATIONSHIP WITH ENDOTHELIAL DAMAGE IN POLYCYTHAEMIA VERA AND ESSENTIAL THROMBOCYTHAEMIA PATIENTS

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INTRODUCTION: Activation of blood coagulation occurred primary through interaction of platelets a vessel wall. In chronic myeloproliferative disorders (cMPD) there are platelet-leukocyte aggregates that damage the endothelial. It has a direct risk of thrombotic complications. Correlation between platelet-endothelial activation and thrombo-haemorrhagic episode in MPD patients is unclear. **OBJECTIVES:** 1) To establish a correlation between the platelet activation and endothelial dysfunction in MPD patients. 2) To evaluate the thrombotic events in these patients.

Id Patient	MPD	vWF (%)	PFA Col:ADP (71-110 sec.)	PFA Col:Epi (94-192 sec.)	Event
13	ET	209	62	108	Non
15	ET	66	121	87	Non
21	ET	259	67	100	VTD
27	ET	146	70	200	Non
29	ET	167	70	300	Gastroic bleeding
38	ET	188	70	300	Heart ischaemic
32	ET	196	70	300	Stroke
33	PV	116	70	300	Heart ischaemic
48	ET	155	70	300	Non
42	ET	146	64	136	Stroke
44	ET	141	300	74	Non
47	PV	75	67	112	VTD
58	ET	166	167	78	VTD
56	ET	130	154	69	Non
68	ET	143	167	78	VTD
44	PV	100	70	300	Non

MPD: Myeloproliferative Disorders; Col:ADP: Collagen; Col:Epi: Collagen/Epinephrine; ET: Essential thrombocythemia; PV: Polycythemia vera; vWF: von Willebrand Factor; VTD: vascular thrombotic disease.

Material and Methods. Patients: We studied 65 patients with ET (41) and PV. 39 females, mean age 56 years (range age 25-89). Evolution time from diagnosis was 2-17 years (mean 8). **Methods.** Platelet activation was measured by PFA-100 device (Dade Behring). Platelet hyperaggregability status was defined to

Col/ADP and Col/Epi with a closure time lower 71 and 94 seconds, respectively. Plasma marker of endothelial activation was the plasma concentration of von Willebrand factor (vWF). Plasma vWF antigen (vWF:Ag) was determined by STA-R clot analyser (Diagnostica STAGO, Asnières, France) using STA LIATEST vWF reagents from Roche (Roche Diagnostics, GmbH). The results were expressed as percentage vWF, by comparison with a standard curve constructed with known dilutions of pooled normal plasma. The undiluted plasma pool consisted of 100% vWF; vWF of control group ranged from 66-149% (mean 103±6.7%). Statistical Analysis: The statistical significance was analyzed by the ordinary ANOVA test of variance using SPSS v 11.0, Inc., Chicago, IL. It was realized a multiple logistic regression analysis of expression of vWF in patients versus control group, according to thrombotic events, expression of endothelial damage, and closure time by PFA-100. Normally distributed data are expressed as means±standard deviation (SD). **Results.** 1) 16 patients (11 ET, 5 PV) presented platelet hyperaggregability. In this group, mean vWF was higher than in non-hyperaggregation patients (142±35 vs 122.6±12%). 2) 50% of the patients with platelet hyperaggregability had a thrombotic event, in contrast with the 30.8% of total group. 3) Patients with platelet hyperaggregation had a good correlation between endothelial damage and thrombotic complication (R2 0.786). 4) Only one patient had a non-fatal haemorrhagic episode (acute gastric bleeding). **Conclusions.** 1) Platelet hyperaggregation is associated with endothelial damage in MPD patients. 2) Patients with platelet hyperaggregation had more thrombotic complications. 3) In this group, we have not found more haemorrhagic event.

0893

GENDER DIFFERENCES IN THE RISK OF RECURRENT VENOUS THROMBOEMBOLISM

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Background. Recent studies had determined than patient's sex is associated with the risk of recurrent venous thromboembolism, and men had higher risk than women. To try to determine if men had more risk of recurrence in our hospital. **Methods.** We studied patients for an average of 21 months after a first episode of spontaneous venous thromboembolism who were admitted in our hospital from January 1998 to December 2004. We excluded pregnant patients, patients with a neoplastic disease or patients with an antecedent of immobilization or surgery. Patients with a deficiency of antithrombin, protein C, or protein S; the lupus anticoagulant were included in the study. The end point was objective evidence of a recurrence of symptomatic venous thromboembolism. **Results.** We studied 306 patients (156 men and 150 women). The median ages of these men and women were 64.8 SD 18.2 years and 73.8 SD 14.6 years respectively ($p<0.001$). The median duration of follow-up was 642.3 SD 42.3 days among the men and 544.1 SD 38.0 days among the women ($p=0.087$). A thrombophilic status were diagnosed in 56 in 56 patients (183. %), with a similar rate in men and women (16.4% vs 14.3%). Venous thromboembolism recurred in 30 patients, 17 men and 13 women (10.9 percent vs. 8.7 percent; relative risk of recurrence, 0.7; 95 percent confidence interval, 0.3 to 1.6). The risk remained unchanged after adjustment for age or thrombophilic status. **Conclusions.** We are not able to demonstrate a sex difference in the rate of recurrence in patients who had had a first episode of spontaneous venous thromboembolism. Advance age is an important risk factor for venous thrombosis and our women are significantly older than our men, and it could explain the differences.

0894

MULTIPLE LYMPHOMATOUS POLYPOSIS OF THE ENTIRE GI TRACT: TWO CASES REPORT

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Background. Multiple lymphomatous polyposis (MLP) is an uncommon type of primary non-Hodgkin gastrointestinal (GI) B cell lymphoma characterized by the presence of multiple polyps along the entire GI tract. Its tumor cells may be regarded as originating from the mantle zone of the lymphoid follicle. This entity is actually considered the counterpart of the mantle cell lymphoma (MCL) in the GI tract. MLP is rare. Since 1961, when it was first described by Cornes, no more than 70 well-documented cases have been published. We report two cases diagnosed as having MLP. Report case 1 A 52-year-old woman presented with a several-month history of diffuse abdominal pain, acute diarrhoea and recurrent night fever. Endoscopy revealed lymphomatous polyps involving several gastrointestinal segments (stomach, duodenum, colon and rectum). Figure 1 Histologically the lesions were all composed of a monotonous infiltrate of small mature lymphocytes, whose diffuse growth was somewhat reminiscent of a Mucosa Associates Lymphoma.



Figure. Colonoscopy: multiple polypoid lesions.

Tissue (MALT), type low grade. No bone marrow infiltration. Immunophenotypic studies showed a cell phenotype typical of MCL (pan-B+, CD5+, CD22+, CD25- and CD10-). The patient was treated with a combined cyclophosphamide, epirubicine, vincristine and prednisone chemotherapy regimen (CEOP), resulting in a complete response. Seven months later, some superficial lymph nodes were palpable; a Fine Needle Aspiration (FNAB) showed disease relapse. Then the patient was treated by high dose chemotherapy and stem cell autotransplantation and by now she's still in complete remission of the disease 28 months after diagnosis. case2 The second case is a 70-year-old man presented with the same symptoms. Colonoscopy revealed multiple polypoid tumors, the first histological diagnosis, performed on an inguinal lymph node, was MALT Lymphoma, compatible with stage IV of the Ann Arbor staging system. No bone marrow infiltration, but the performed cytometry on the marrow sample underlined a small population of CD19+, CD5+, CD20+, CD22+, smIg -, lambda + cells. The patient was submitted to treatment with oral Fludarabine, with no results. A second colonoscopy confirmed the presence of multiple lymphomatous polyps involving the entire colon and the rectum until the sigma; a diagnosis of MCL was set. A combined

chemotherapy with CEOP followed by stem cell autotransplantation is going to be given the patient. **Conclusions.** MLP is a distinct entity among GI lymphomas, accounted about 9% of primary GI lymphomas. It occurs more commonly in men between 55 and 64 years and rarely involves the entire GI tract; that makes these two cases quite remarkable. Immunophenotypic studies are very helpful for the correct diagnosis. The prognosis of MLP is very poor due to its accelerated proliferation; most of the patients have an advanced disease at the diagnosis. The published cases of MLP had poor prognosis with an overall mean survival of 20-30 months. We directed our patients to stem cell autotransplantation, a very intensive therapy seems to be effective in this disease.

0895

NON-MYELOABLATIVE STEM CELL TRANSPLANTATION IN CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE. RESULTS OF THE LATINAMERICAN COOPERATIVE ONCOHEMATOLOGY GROUP (LACOHG) STUDY

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Aims. To analyze the usefulness of non-ablative stem cell transplantation (NST) in patients with chronic myelogenous leukemia (CML). **Method:** Using an NST schedule employing fludarabine, cyclophosphamide and busulfan, 24 patients with Ph1 (+) CML in chronic phase were prospectively allografted in six institutions in four latinamerican countries: México, Brasil, Colombia and Venezuela. Median age of the patients was 41years (range 10 to 71); there were 8 females. **Results.** Median time from diagnosis to the allograft was 344 days (range 46-10 280). Patients received a median of 4.4×10^6 /Kg CD34 (+) cells. Median time to achieve above 0.5×10^9 /L granulocytes was 12 days, range 0-41, whereas median time to achieve above 20×10^9 /L platelets was also 12 days, range 0-45. Twenty patients are alive 81 to 830 (median 497) days after the NST. The 830-day survival is 92%, whereas median survival has not been reached, being above 830 days. Eleven patients (46%) developed acute graft versus-host disease (GVHD) (5 cases grade I and 6 grade II) whereas 5 of 11 have developed chronic GVHD. Two patients died 43 to 201 days after the NST, one as a result of sepsis and the other one of chronic GVHD. **Conclusions.** The preparative regimen, which is affordable and feasible for developing countries, is adequate for individuals with CML. Further studies and a longer-follow up to define the role of this approach are needed.

0896

STUDY OF IMPEDANCE AND FLUORESCENCE (OPTICAL) PLATELET COUNT ON SYSMEX XT-2000I

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Background. In the instrument XT-2000I, platelets are detected in RET (reticulocyte) scattergram clearly separated from erythroid cells. Using a logarithmic scale, an enlarged platelet cluster is visible in the PLT-O scattergram. This is the basis of the fluorescence (optical) platelet count. The instrument always performs an impedance (PLT-I) platelet count. Under some circumstances where an abnormal cell distribution (platelets or erythrocytes) is present, a flag is generated suggesting the need for an optical platelet count(PLT-O). The XT optical count is reported to have an excellent agreement with the flow cytome-

ter reference count($r=0.97$). **Aims.** The aim of this study is the comparison between the impedance and the optical platelet count in samples with normal platelet count(impedance). **Methods.** 304 samples analysed on the XT-2000i with normal impedance platelet count($150-450 \times 10^3/\mu\text{l}$) were included in the study. All samples were rechecked by the fluorescent count method on the RET channel of the XT-2000i. Microscope analysis of blood smears showed no platelets clumps. All samples were divided into three groups according to the MCV and the presence or not of a flag suggesting the need for a PLT-O count: group A (MCV: 80-97 fl, no flag, n=164), group B (MCV \leq 79 fl, no flag, n=91), group C (MCV \leq 79 fl, flag, n=49). The correlation of PLT-I / PLT-O count and the difference-PLT between the two counts were investigated. **Results.** (mean \pm SD, correlation r, statistically significant $p<0.05$). MCV (fl): Group A: 87.7 \pm 3.9, group B: 70.1 \pm 6.9, group C: 63.1 \pm 6.5. PLT-I ($\times 10^3/\mu\text{l}$): Group A: 241 \pm 73, group B: 308 \pm 106, group C: 252 \pm 99. Correlation PLT-I / PLT-O (r): Group A: 0.95, group B: 0.92, group C: 0.88. Difference-PLT (PLT-I / PLT-O) ($\times 10^3/\mu\text{l}$): Group A: 17.1 \pm 14.2, group B: 42.4 \pm 33.4, group C: 52 \pm 45. The mean value of MCV of group B is higher than that of group C ($p=0.001$). There is a significant positive correlation between the two methods in all groups. The agreement between impedance and optical count of group C is less good than those of A and B. The differences-PLT (PLT-I / PLT-O) are statistically significant in all groups (A: $p=0.001$, B: $p=0.001$, C: $p=0.001$). Group A presents a smaller difference than groups B and C ($p=0.001$). The differences-PLT (PLT-I / PLT-O) of groups B and C are statistically equal ($p>0.05$, NS). **Conclusions.** The difference between the two methods of platelet count(impedance / optical) is statistically significant in samples with normal platelet count, normocytic as well as microcytic. The groups B and C behave similarly according to difference-PLT (PLT-I / PLT-O). The samples with the suggested flag should be rechecked by the fluorescence (optical) count method. It should be evaluated in which additional cases the PLT-O count is useful in the assessment of patients' clinical courses. It should be established cut off values for the repetition of platelet count in RET channel in samples with normal PLT-I count.

0897

TREATMENT AND PROGNOSIS OF IDIOPATHIC PULMONARY HEMOSIDEROSIS IN CHILDHOOD - OUR EXPERIENCE

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Idiopathic pulmonary hemosiderosis (IPH) is a rare disease of unknown etiology whose diagnostic, prognostic and therapeutic approach is still open to discussion. It usually occurs during infancy and childhood, characterized by numerous and repeated intra-alveolar bleedings, interstitial iron build-up with consequent progressive fibrosis and severe anemia. The purpose of the present study was to evaluate our long-term experience (36 years period) in diagnosis, evolution and treatment of this disease. **Materials and Methods.** Forty-one patient with IPH diagnosed from 1969 to 2004 at the Hematology-Oncology Department - Pediatric Clinic in Skopje. Mean age at diagnosis was 6 \pm 3,6 years (range 0,25 to 15), sex incidence is almost equal (m:f = 19:22). At diagnosis all patients had anemia and pulmonary infiltrates, 26 patients (63,4%) had dyspnea, 10 patients (24,3%) had tachycardia, 4 patients (9,75%) had hemoptysis and 16 patients (39%) had icterus. The diagnosis was made by detection of hemosiderin-laden macrophages in gastric aspirate in 40 patients (97,5%) and by clinical presentation alone in 1 patient. Open lung biopsy was made just in 1 patient. The mean duration of follow-up was 61,95 \pm 83,73 (range 1 to 355 months). Initial treatment consisted of prednisone in 38 patients (92,6%). Ten patients (24,3%) required long-term corticosteroids because of recurrent attacks; 17 patients (41,4%) required other immunosuppressants (immuran or leuceran) in addition to prednisone to control their hemoptysis. Eight patients (19,5%) died of acute massive pulmonary hemorrhage (5 within 1 year, 2 within 3 years and 1 after 12 years post diagnosis). 3 patients

have been cured (with long-term follow-up of 22,25 and 30 years) and being without treatment of 18, 11 and 19 years respectively. Five-year survival for IPH patients in our study was 80% (by Kaplan-Meier method). *Conclusions.* The prognosis of IPH is difficult to establish because of its rarity and variability. One early series showed an average of 2,5 years between diagnosis and death. A more recent studies describes a much better prognosis in patients treated early with immunosuppressive therapy. We also speculate that early and long term immunosuppressive therapy may improve the prognosis of IPH.

0898

EVALUATION OF RETICULATED AND ACTIVATED PLATELETS IN SICKLE CELL DISEASE

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Sickle cell disease (SCD) is considered a 'hypercoagulable state'. Several components of haemostasis are altered, and an increase in the adherence of sickle erythrocytes to vascular endothelium, mediated by adhesion receptor and other cellular elements of the blood, contribute to the development of vaso-occlusive crisis (VOC). Reticulated platelets (RP) are newly formed platelets that contain some rough endoplasmic reticulum and mRNA. RP measurement has had considerable clinical utility for monitoring thrombopoiesis and platelet turnover. The present study was performed to evaluate RP and their degree of activation in patients with SCD. Sixty-one adults with SCD were studied: 22 in steady phase, 21 in hemolytic crisis (HC) and 18 in VOC. Platelet evaluation: platelet count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet Larger Cell Ratio (P-LCR) were determined by the Sysmex XE-2100. RP were determined by flow cytometry in platelet-rich plasma. Platelets were identified by anti CD41-PeCy5 monoclonal antibody and RP were stained for RNA with thiazole orange (TO). Platelet activation was monitored using anti CD62P- PE. The action of interleukin on thrombopoiesis was verified by determination of serum IL-6 levels. Control group: thirty healthy subjects. *Results.* In relation to control group it was observed: the number of platelets was higher in SCD patients; MPV and % of P-LCR were lower in steady SCD patients. Those more numerous and small size platelets in steady phase probably are consequent to the lack of splenic sequestration due to functional asplenia. The absolute number of RP (TO+) and activated platelets (CDP62+) were significantly higher ($p < 0.005$) in the three phases of SCD when compared with control group, but not among patient subgroups. Using double fluorescence (anti-CD 62P-PE and TO) it was possible to determine the absolute number of activated RP. SCD patients presented higher number of activated RP than controls ($p < 0.0002$). Serum IL-6 levels were significantly higher in SCD patients (HC and VOC subgroups higher than steady phase) than in control group. It was not observed correlation between IL-6 levels and platelets parameters, suggesting that IL-6, although significantly increased, does not have a direct action on thrombopoiesis activity in SCD. It was shown a significant correlation between CD62P+ platelets and RP in percentage and absolute numbers, suggesting that those youngest platelets exhibit some activation degree. The increase of RP in SCD indicates an elevated activation-dependent turnover of platelets. Those activated platelets release thrombospondin and fibronectin, leading to further red cell adherence, potentially enhancing the microvascular occlusion. Patients with SCD present two factors, among others, that contribute to adhesion of sickle cells to endothelial cells: high number of reticulocytes, particularly active in adhesion mechanism, and young activated platelets. Our data confirm that platelet activation, potentially increased by activation from youngest platelets, is present in SCD and suggest that elevated number of activated RP participate in the cellular adhesion process and in the occurrence of vasoocclusive episodes and of thrombosis (Supported by FAPE-SP n0. 02/13801-7).

0899

ETIOLOGY AND TREATMENT OF IRON DEFICIENCY ANEMIA IN PRE-SCHOOL AGE

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Anemia resulting from lack of sufficient iron for synthesis of hemoglobin is by far the most frequent hematological disease of infancy and childhood. Purpose: To evaluate the etiological, clinical, hematological and evolutive features of iron deficiency anemia in infants and in pre-school age. Materials and *Methods.* The subject of this study were 284 children with iron deficiency anemia (IDA) aged from 1 month to 6 years diagnosed between January 1998 to December 2002 year at the Hematology-Oncology department-Pediatric clinic in Skopje. *Results.* Most of the patients - 186 (65,5%) are 1-3 years old. Male to female ratio was 63%:37%. The most common etiological factors were: poor dietary practices-206 patients (72,5%), recurring infections-45 patients (15,8%), GIT disease- 30 patients (10,5%), prematurity and low birth weight- 20 patients (7,0%) and blood loss- 10 patients (3,5%). Pallor, anorexia and prolonged fever (detected in 100%, 63% and 29% respectively) were the most important clinical signs at the admission. The level of the Hb was usually between 50-80 gr/L in 206 children (72,5%). The severe anemia correlates with extremely low levels of serum iron in 209 children (74,4%) and low levels of serum ferritin in 130 children (50%). The treatment of the patients was with oral administration of iron, only 13 children (4,5%) required transfusion of eritocytes. With the oral iron administration an optimal response was attained in a period of two months with a daily increase of Hb of approximately 1 gr/L. *Conclusions.* In the evaluated group of 284 patients aged from 1 month to 6 years dominates the risk aged group between 1-3 years with the level of Hb <70gr/L. Oral iron therapy is the treatment of choice, as it is cheap, safe, effective and well tolerated. Transfusion of eritocytes are necessary when the anemia is very severe, when infection may interfere with the response and if heart failure is imminent.

0900

TELOMERE LENGTH REDUCTION IN FOLLICULAR LYMPHOMA (FL) AND DIFFUSE LARGE B CELL LYMPHOMA ARISING FROM FL

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Background. Telomeres are essential structures for maintaining chromosomal integrity and stability and cell's replicative capability. Telomere length (TL) reduction increases the occurrence of errors which could generate important changes for promoting neoplastic transformation, determining genetic imbalances. The hallmark of follicular lymphoma (FL) is t(14;18) (q32;q21), involving BCL-2 gene rearrangements, which is observed in more than 75% of cases. FL transforms to a more aggressive lymphoma in 25%-60% of patients, an event that represents the outgrowth of a more malignant subclone. *Aims.* The aim of this work was to evaluate the possible TL modifications in bone marrow (BM) and/or lymph nodes (LN) from patients with FL and diffuse large B cell lymphoma (DLBCL) arising from FL, and its correlation with the BCL-2 gene rearrangements, in order to analyze the participation of telomere shortening in tumour progression process. *Methods.* We have studied TL in samples from 40 non-Hodgkin's lymphoma patients: 33 cases with FL at diagnosis (mean age: 53.2 years; range 29-77 years, 18 male) and 7 with DLBCL (mean age: 61 years; range 53-69 years, 5 males). TL was evaluated using terminal restriction fragments (TRF) assay. As no differences between BM and LN tissues were observed, both type of samples were analyzed together. For BCL-2 gene rearrangements, both nested and long distance PCR were used. *Results.* Mean

TRF values showed significant telomere shortening in FL (4.14 ± 0.16 Kb) and DLBCL (3.31 ± 0.25 Kb) compared to controls (8.50 ± 0.50 Kb) ($p < 0.001$). Differences between both histological subtypes (FL and DLBCL) were also detected ($p = 0.033$). BCL-2 gene rearrangements were found in 85.7% of FL and 71.4% of DLBCL patients. BCL-2 positive patients with FL showed longer TL (4.23 ± 0.17 Kb) than the negative ones (3.38 ± 0.25 Kb) ($p = 0.05$). The analysis of TRF values in relation to BCL-2 breakpoints showed a trend to telomere shortening when MBR-JH, mcr-JH and BCL-2 negative patients were compared (4.35 ± 0.21 Kb; 3.84 ± 0.45 Kb and 3.38 ± 0.25 Kb, respectively). **Conclusions.** Our results show a TL reduction in FL and DLBCL, with significant short TRFs in the last group, suggesting the participation of telomere shortening in tumour progression. Moreover, it is important to emphasize that DLBCL showed similar TRF values compared to BCL-2 negative FL that could be related with a higher probability to high grade lymphoma evolution. Furthermore, the differences detected between BCL-2 positive and negative FL would support the existence of diverse pathogenic mechanisms involved in the origin of these different FL.

0901

OVEREXPRESSION OF TOLL-LIKE RECEPTORS BY BONE MARROW CD14+ CELLS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background. Bone marrow (BM) macrophages are activated in patients with myelodysplastic syndromes (MDS) and produce increased amounts of tumor necrosis factor- α (TNF α). This cytokine is implicated in the pathophysiology of ineffective hemopoiesis by inducing apoptosis of hemopoietic progenitor/precursor cells in the affected subjects. **Aim.** The cause of activation of BM macrophages in MDS patients is largely unknown. The aim of the current study was to investigate the possible involvement of microbial products in this activation acting via the Toll-like receptors (TLRs). **Patients and Methods.** Sixteen patients with various types of MDS and 15 age- and sex-matched normal volunteers were studied. Informed consent according to the Helsinki protocol was obtained from all subjects. Studies were performed by two-color flow cytometry using the respective mouse anti-human monoclonal antibodies (eBioscience, San Diego, CA). Data were expressed as mean percentages of positive cells within the CD14+ BM mononuclear cell population. An estimation of the TLR density on cell surface was performed by evaluating the mean channel fluorescence value (MCFV). Statistical analysis was performed by means of the nonparametric Mann-Whitney U test. **Results.** We found that the proportion of CD14+ cells were significantly higher in MDS patients compared to controls ($7.12 \pm 5.03\%$ vs $3.82 \pm 1.20\%$, $p = 0.0257$) suggesting increased monocyte/macrophage numbers in MDS BM. The proportion of TLR1+ and, more importantly, TLR2+ cells within the CD14+ cell subpopulations was significantly increased in patients compared to controls ($p = 0.0027$ and $p = 0.0002$, respectively). No statistically significant differences were demonstrated between patients and controls in the proportion of TLR4+ or TLR9+ cells detected in the CD14+ cell compartment. The MCFV of TLR2+ cells, but not of cells expressing other types of TLRs, was significantly increased in patients compared to controls (3.18 ± 1.45 vs 2.02 ± 0.97 arbitrarily units, $p = 0.373$). Furthermore, the proportions of CD54 and CD80 expressing CD14+ cells were significantly higher in the patients than in the normal controls ($84.4 \pm 5.8\%$ vs $56.7 \pm 17.9\%$; $p = 0.0195$ and $9.94 \pm 4.95\%$ vs $4.60 \pm 2.25\%$; $p = 0.0142$, respectively) suggesting a degree of monocyte/macrophage activation in MDS patients. **Summary.** Triacyl-lipopeptides and diacyl-lipopeptides are two microbial products of Gram (+) bacteria representing the main ligands of TLR-1 and TLR-2, respectively. In our patients, overexpression of these TLRs is suggestive of macrophage activation via the

above mentioned microbial products. Such an activation might explain, to some extent, the increased amounts of TNF α produced in patients' BM microenvironment. Whether these products are causatively related with the development of myelodysplasia remains to be elucidated.

0902

MYELODYSPLASTIC SYNDROMES: CLINICAL FEATURES AND ETHNIC DIFFERENCES AMONG 109 PATIENTS IN THE TEL-AVIV AREA-A MULTI CENTER EXPERIENCE

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Background and Aims. Myelodysplastic syndromes (MDS) comprise a heterogeneous group of disorders, characterized by ineffective and dysplastic hematopoiesis, peripheral cytopenias and a predisposition to leukemic transformation. The demographically diverse population in Israel allows us to analyze various clinical and laboratory features of the disease, focusing on ethnic differences. **Materials & Methods.** We reviewed the medical records of MDS patients treated in three medical centers in the Tel-Aviv area, over the last decade, and allocated 109 patient files with complete demographic, clinical and laboratory data. A retrospective analysis regarding the FAB classification, risk assessment, clinical course, and treatment outcome is presented and discussed. **Results.** The median age of patients at diagnosis was 72.7 years, with equal gender distribution. The majority (64%) of patients were of Ashkenazi origin while only 36% were Sephardic. Physical findings at diagnosis were rare and included hepatomegaly (8 patients, 7.5%), splenomegaly (8 patients, 7.5%) and cutaneous bleeding (4 patients, 3.7%). FAB classification at diagnosis showed 61% RA, 18% RARS, 12% RAEB, 3% RAEBt, 6% CMML. Using the IPSS scoring system, 55% of the patients were classified as low risk group, 31% as intermediate risk group 1, 10% as intermediate risk group 2, and 4% as high risk. Cytogenetic abnormalities were detected in 40.8% of the patients. The common abnormalities were, as expected, chromosome 5 q aberrations (5.5% as a single defect, 16.7% as a combined abnormality, 42.1% of all cytogenetics defects) and del 7 (9.3% of the patients, 22.7% of the abnormal cytogenetics). Abnormal karyotype was observed in 33% of the patients with RA/RARS but in 87% in RAEB/t. Clinical course and treatment: Twenty-three (21%) patients transformed into acute leukemia, with a median time from diagnosis to leukemia of 9 months (range: 1-95). Thirty nine patients had documented infections during the follow up period (14 grade I, 18 grade II, 8 grade III). Thirty-five patients were treated with erythropoietin (Epo), with 45.7% (16 patients) minor or major erythroid response. Serum endogenous pretreatment Epo level (performed in 26 patients) was found to predict response: of 12 patients with serum Epo < 100 mu/mL, 6 experienced a major response, and 3 a minor one. Of 14 patients with serum Epo > 100 mu/mL, only 2 patients achieved a major response and 2 minor response. Three of the 5 patients with low risk MDS (or RA) treated with G-CSF, evolved into acute leukemia. **Conclusions.** Our retrospective data on a non-consecutive patient population show that MDS is more common among Ashkenazi Jews, in contrast with the distribution in the non-MDS population. Moreover, the median age at presentation in our patient population appears to be older than previously published, while the fraction of low risk MDS patients appears to be greater. Our small series support previous observation that serum pretreatment Epo levels may serve as a good predictor for response.

0903**COMBINED IMMUNOSUPPRESSIVE TREATMENT IN PATIENTS WITH EARLY MYELODYSPLASIA**

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Background. and **Aims.** An abnormal immune response is considered an important factor promoting clonal progression and increased rate of apoptosis leading to cytopenia in early myelodysplastic syndrome (MDS). In 1998, we first published a beneficial effect of Cyclosporin A (CS-A) on cytopenia in a part of MDS patients. In present study we evaluate an effect of combined immunosuppressive treatment in early MDS. **Patients and Methods.** 20 patients with refractory cytopenia with multilineage dysplasia (RCMD) and 3 MDS patients with isolated granulocytopenia or thrombocytopenia (RA unclassifiable-RAU according to WHO criteria) were treated with Prednisone (0,4-0,5 mg/kg/day) in combination with CS-A (3 mg/kg/day and adjusted according to serum CS-A level). After achievement of response, the treatment was continued with minimal doses of both the drugs necessary for maintaining stable hemoglobin (Hb), neutrophil (NS) and platelet(PLT) values. Criteria for complete response (CR) were: Hb > 110 g/L, NS > 1,5x10⁹/L, PLT > 100x10⁹/L, partial response (PR) was evaluated as an increase in Hb > 20 g/L, NS > 0,5x10⁹/L, PLT > 30x10⁹/L. **Results.** 6 out of 23 patients (5 with RCMD,1 with RAU-26%) achieved CR, 4 patients with RCMD (17%) achieved PR. Five out of 10 responding patients had hypoplastic MDS, 5 patients had normo- or hypercellular bone marrow, 1 of them had 'PNH' like MDS. All responders had 'good' karyotype according to IPSS, 4 patients (2 with CR,2 with PR) exhibited areas of non-clonal lymphoid cells in bone marrow biopsy in contrast to none of non-responding patients. Clonal patterns of hematopoiesis were assessed by XCI assay in 8 out of 12 treated females, clonal CD3+ lymphocyte subpopulation in peripheral blood and bone marrow were found in 1 responder and in 1 non-responding female. Three non-responding patients developed advanced MDS within 8,9 and 57 months, respectively. Four non-responders with hypoplastic MDS were consequently treated with antithymocyte globulin (ATG), 2 of them achieved PR, however, all but one patient who received ATG developed acute leukemia 25 to 89 months after ATG administration. **Conclusions.** combined immunosuppressive treatment with Prednisone + CS-A may represent an effective treatment modality in patients with early MDS without excess of blasts, CR or PR were observed in 41% of patients. Nevertheless, more detailed studies using different laboratory techniques for characterization of patients who may benefit from this treatment are needed. The study was supported by scientific grant NK 5937/3 obtained from Czech Ministry of Health.

0904**METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM DOES NOT HAVE ANY EFFECT IN HODGKIN'S LYMPHOMA, DIFFUSE LARGE B CELL LYMPHOMA, SMALL LYMPHOBLASTIC LYMPHOMA**

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Background. Methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme in the regulation of folate and methionine metabolism. Genetic polymorphisms in this pathway may change the susceptibility of cancer development and several studies have been demonstrated that the variations of MTHFR gene polymorphism change the lymphoma risk. **Aims.** The aim of our study was to detect MTHFR C677T polymorphism in patients with lymphoproliferative disease and, analyse the response rate of these patients to first-line chemotherapy in order to show if polymorphism alters the susceptibility of chemotherapy. **Method:** 99 patients with lymphoproliferative disease [15 Hodgkin's lymphoma, 84 Non-Hodgkin's lymphoma (41 diffuse large B-cell, 24 small lymphocytic, 5

Burkitt's/Burkitt's like, 4 Lymphoblastic, 3 follicular, 2 mantle-cell, 3 marginal zone, 2 peripheral T cell lymphoma), 15 Hodgkin's Lymphoma] and 51 healthy people were included in the study. MTHFR gene polymorphism was studied by using lightcycler. Allel frequencies and genotype frequencies of overall group and subgroups were calculated. **Results.** We couldn't find statistically significant association between MTHFR polymorphism and development of lymphoma neither overall group nor subgroups [Hodgkin's lymphoma (HL), diffuse large B cell lymphoma (DLBCL), small lymphocytic lymphoma (SLL)]. In addition we couldn't demonstrate any effect of gene polymorphism of MTHFR in chemotherapy response. **Conclusions.** As a result our data suggests that MTHFR gene C677T polymorphism does not contribute to pathogenesis of DLBCL, SLL and HL and it does not alter the patients' response rate to chemotherapy in these groups.

0905**EXPRESSION OF NATURAL KILLER CELL INHIBITORY RECEPTORS ON CYTOTOXIC CELLS IN B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA (B-CLL)**

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Background. Apart from humoral immune abnormalities, such as high prevalence of autoantibodies and hypogammaglobulinemia, a number of studies have shown defects and alterations in the T cell population in patients with B-CLL. Indeed, this T cell dysfunction in B-CLL patients is believed to contribute both to the etiology and pathogenesis of the disease. Furthermore, control of tumours by the immune system is partly mediated by cytotoxic T cells (CTL) that recognise tumour antigen. CTL recognise antigens presented on MHC class I via their T cell receptor. But MHC class I also interact with receptors that control the activation of both T and NK cells. Such inhibitory NK cell receptors inhibit CTL and NK cell immune responses by preventing lysis of target cells. They belong mainly to two families: killer cell immunoglobulin-like receptors (KIR) and lectin-like receptors (CD94). It has been reported that tumour infiltrating CTL in renal carcinoma expresses KIR, which inhibit the lysis of renal tumour cells. Also, ligation of KIR causes a partial inhibition of CTL cytotoxic activity in patients with melanoma and hepatic malignancy. Together, this could imply that an induced expression of inhibitory NK cell receptors on CTL could possibly result in lost control of tumour cell growth. **Aims.** This study was performed to evaluate the expression KIR and CD94 on CTL in B-CLL patients in different disease stages. **Methods.** Twenty-four patients with B-CLL (14 with Binet stage A and 10 with Binet stage C) and from 10 healthy controls were included in the study. CD8+ cells from heparinised peripheral blood were examined by flow cytometry analysis using the following antibodies: CD158a (KIR2DL1), CD158b (KIR2DL2/3), CD158e (KIR3DL1) and CD94. **Results.** B-CLL patients in Binet stage C had significantly higher percentage of CD8+ cells expressing CD158b, CD158e and CD94 compared both to patients in Binet A and healthy controls (7.84±1.83% versus 3.06±1.02% and 3.00±0.63%, p<0.05; 2.57±0.90% versus 1.09±0.44% and 0.42±0.11%, p<0.05; 37.65±4.81% versus 15.68±3.94% and 16.19±3.74%, p<0.01). No statistically significant differences were seen regarding CD8+ cells expressing CD158a. **Summary/conclusions:** In this study we found that B-CLL patients with Binet C had significantly higher percentage of CTL expressing several inhibitory NK cell receptors compared to patients with Binet A. High expression of KIR and CD94 on CTL in B-CLL patients may result in inhibition of cytolytic function and lost tumour control. Conversely, downregulation of KIR and CD94 expression may be a mechanism by which CTL can maintain uninhibited cytotoxic activity and thus clearance of B-CLL cells. Further studies aimed at modelling the expression and function of NK cell inhibitory receptor expression on CTL could give a better understanding of the role of CTL in B-CLL and possibly provide new potential immunotherapeutic strategies.

0906

MYCOPHENOLATE MOFETIL IN THE TREATMENT OF GRAFT VERSUS HOST DISEASE (GVHD): A SINGLE CENTER EXPERIENCE

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Background. GVHD is the most common complication after allogeneic hematopoietic stem cell transplantation (HSCT) and afflicts mortality morbidity and quality of life. The associated graft versus tumor effect is able to reduce the relapse rate. Cyclosporin A and steroids are the standard treatment for both acute and chronic GVHD. The role of MMF is under investigation but the results of small series of patients are encouraging. The aim of this study is to evaluate the efficacy, tolerability and adverse events of MMF in a setting of allogeneic HSC transplanted patients treated for GVHD at our center. Patients and **Methods.** Fourteen patients were treated with MMF between June 2002 and January 2005, 7 within 100 days after the HSCT and 7 after 100 days. The rationale of the treatment was: 2nd or 3rd line in the treatment of refractory acute GVHD, substitution of a calcineurin inhibitor in the prophylaxis of aGVHD, first treatment line in the chronic GVHD in patients with medical contraindications to methylprednisolone (MP), cyclosporin A or tacrolimus. **Results.** One patient out of four receiving MMF for aGVHD responded partially but died for progression of his myeloma. The other 3 patients of this group received a further line of treatment and only in one patient with benefit. Four patients were treated with MMF in order to prevent GVHD: 2 developed chronic GVHD and 2 are currently without signs of cGVHD. Seven patients were treated with MMF for a histological documented cGVHD. Cutaneous GVHD was proven in all the patients, 6 with mucosal, 4 gastrointestinal and hepatic, 3 ocular and 1 joint involvement. cGVHD resolved completely in 5 patients. Two patients had a partial response. Three patients with a GVHD flare-up were treated successfully with MMF and MP (and photopheresis in one). Response rate (partial and complete response, prevention of GVHD) was 71% (10/14). Adverse events were anemia (<10 g/dL) in 8 cases and abdominal pain in 1 case. Seven patients with anemia responded to erythropoietin. Discontinuation of MMF was necessary in the patient with abdominal pain. **Conclusions.** and comment Despite the low number of patients reported, MMF seems to be a promising immune-suppressant agent with a low toxicity profile. It may be considered as a valid alternative to calcineurin inhibitors or MP in case of severe adverse events (neurotoxicity, nephrotoxicity or osteoporosis). The activity in the treatment of aGVHD needs further studies. In the setting of cGVHD MMF seems to be effective but randomized studies are needed.

0907

IMAGE DNA ANALYSIS OF BONE MARROW MEGAKARYOCYTES CAN DIFFERENTIATE CLONAL FROM REACTIVE THROMBOCYTOSIS

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Background. It is of practical clinical importance to make a quick distinction between reactive thrombocytosis (RT) and clonal thrombocytosis of chronic myeloproliferative disorders (CMPD). However, in spite of numerous available laboratory tests and clinical procedures, the answer sometimes depends on watching the platelet count over a period of time. Morphological criteria for diagnosis of CMPD are centered around changes in the megakaryocytes (Mk) number, distribution, size, and ploidy, which is difficult to assess in a reproducible manner. Objective measurement of Mk ploidy could be a useful tool for differentiating clonal from reactive states. However, the analysis is not widely employed owing to the lack of a simple and standardized technique. **Aims.** The aim of research is to perform an objective measurement of Mk ploidy by the image DNA analysis and to set its value in the differential diagnosis of

thrombocytosis. **Methods.** In order to differentiate RT from CMPD, Mk nuclear DNA content was measured by image analysis using the new S-Form software (Vamstec, Zagreb). Feulgen stained bone marrow aspirates from 34 patients with platelet count $>800 \times 10^9$ were analyzed as a part of the initial evaluation. During the follow up in 14 patients diagnosis of RT and in 20 patients of CMPD (15 ET, 3 PRV, 2 CMPD-unclassified) was confirmed. Ploidy of 100 Mk was measured for each patient and the fractions of hyperploidy cells $>96n$ and $>128n$ were compared using the SPSS software. Nuclei of normal granulocytes were used as 2n standard. **Results.** In 19 out of 20 patients with CMPD there were $\geq 5\%$ of Mk with nuclear DNA content $>96n$ or at least 2% of Mk with nuclear DNA content $>128n$. Among the patients with RT only four had occasional hyperploidy cells (1%, 1%, 1%, 3%) in the region $>96n < 128n$, and no hyperploidy cells $>128n$ were observed. **Conclusions.** The presence of at least 5% hyperploidy megakaryocytes with DNA content $>96n$ or at least 2% of megakaryocytes with DNA content $>128n$ in the bone marrow aspirates of patient with thrombocytosis strongly suggests CMPD. Reactive thrombocytosis is characterized by the absence of hyperploidy cells, although in some cases up to 3% of cells in the region $>96n < 128n$ are encountered. We believe the method of image DNA analysis has the potential for a widespread clinical use and can be a useful aid to cytological analysis of bone marrow in the differential diagnosis of megakaryocyte disorders. Personal details Josko Vuckovic, Rok Pavlic Department of Internal Medicine, General Hospital, Oblakova 5, Celje, Slovenia.

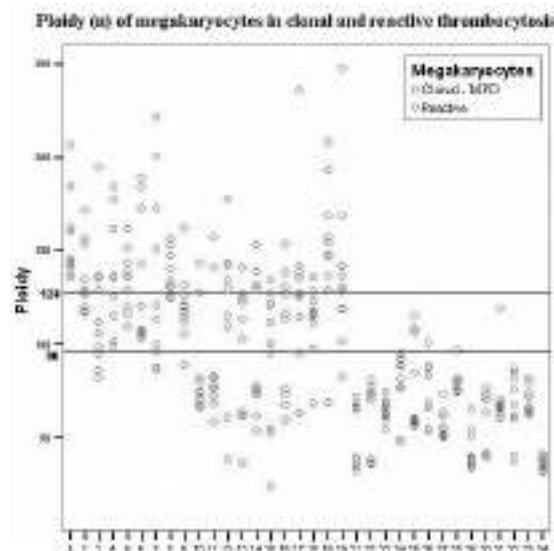


Figure. Ploidy of megakaryocytes - comparison.

0908

MYELODYSPLASTIC SYNDROMES (MDS) WITH ISOLATED DELETION OF LONG ARM OF CHROMOSOME X - A NEW CYTOGENETIC SUBTYPE?

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Clonal chromosome aberrations detected in 50-70% of MDS cases, prove monoclonal nature of disease. Being disease-specific marker cytogenetic abnormality defines biology, morphological features and clinical outcome of MDS subtypes. For some abnormalities corresponding genes involved in pathogenesis of MDS are known. In spite of the existence of certain well-characterized MDS subtypes, some new recurrent chromosome

aberrations and their special clinical and genetic features proceed to be detected. Structural rearrangements of long arm of chromosome X are infrequent but non-random event in myeloid malignancies. Thus, deletion of (X) (q13) as sole chromosome abnormality was previously described just in two MDS cases. MDS patients with isolated deletion of Xq24 were not reported in literature. Isolated deletion of Xq was rather frequent in our study, it was observed in 3 of 127 MDS cases with clonal chromosome changes seen at the Karyology laboratory of National Center for Hematology, Moscow, between 1994 - 2003 years. We report for the first time two unique cases of isolated del (X) (q24) and one new case of del (X) (q13) in MDS patients. Patients in our study were female with median age 58 years (range 46-65). All of them had RAEB subtype of MDS and morphologically were characterized by two/trilineage dysplasia with prominent dysplastic features in granulocytic and megakaryocytic lineage. Leukemic transformation had place at 9-20 months since initial diagnosis. AML (M2 variant) was diagnosed in all patients. Median survival was 17,7 month (range 9-25). Detailed analysis of clinical and morphological data of presented and previously published cases indicates that: 1) del (X) (q24) and del (X) (q13) are non-random chromosome abnormalities in MDS; 2) MDS with deletions of Xq affects exclusively female of elderly age; 3) deletions of Xq are associated with aggressive form of MDS and indicates adverse prognosis. Thus, nonrandom appearance and defined clinico-morphological characteristics of MDS cases with del (Xq13) and del (Xq24) allow to suppose that it represent a separate MDS subtype and that loss of certain genes, located in Xq13-Xq24 might be important in the development of disease.

0909

HLA ASSOCIATIONS WITH CHRONIC MYELOID LEUKEMIA

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Some MHC (HLA in humans) genes provide resistance to certain diseases via mechanisms that remain unclear. Chronic myeloid leukemia (CML) is a serious neoplastic disorder of the hematopoietic stem cell. The BCR-ABL oncogene is central in the pathogenesis of CML. The novel amino acid sequence may be recognized in complex with some HLA molecules by T cells and induce the development of specific immune response. The HLA molecules which can bind BCR-ABL junctional peptides have to be in negative associations with CML. The aim of our investigation was to find the associations between HLA-specificities and predisposition/resistance to CML development. *Methods.* HLA-A, -B, -Cw, -DRB1 in 82 CML patients and 332 healthy donors were typed. The HLA frequencies were counted and compared by exact Fisher test. Relative risk (RR), etiological fraction (EF) or preventive fraction (PF) were determined. *Results:* Positive associations with the CML development have been found for B51 (23.2% in patients vs. 8.7% in donors, $p < 0.01$, $RR = 3.2$, $EF = 0.06$), $DRB1^*11$ (39.2% vs. 17.8%, $p < 0.01$, $RR = 2.9$, $EF = 0.12$), $DRB1^*14$ (9.7% vs. 1.5%, $p < 0.01$, $RR = 7.1$, $EF = 0.01$) and $DRB1^*16$ (9.7% vs. 2.4%, $p < 0.05$, $RR = 4.4$, $EF = 0.02$). The negative associations with the CML development have been determined for HLA-A2 (30.5% vs. 51.8% in controls, $p < 0.01$, $RR = 0.41$, $PF = 0.42$), $DRB1^*01$ (9.8% vs. 25%, $p < 0.01$, $RR = 0.36$, $PF = 0.32$), $DRB1^*04$ (12.2% vs. 25%, $p < 0.05$, $RR = 0.44$, $PF = 0.24$). *Conclusions.* The HLA-specificities in positive and negative associations with CML have been established. The latter ones are the HLA-A2, -DRB1*01 and -DRB1*04. It's known that the HLA-DR1 and -DR4 can bind BCR-ABL (b3a2) junctional peptides. The data about the HLA-A2 ability of binding of b3a2 peptides are ambiguous. So, there are connections between the HLA class II molecules (DR) capability to bind b3a2 peptides and resistance to the CML development.

0910

IMMATURE T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA RELAPSING AS ACUTE MYELOGENOUS LEUKEMIA WITHOUT DIFFERENTIATION

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T-cell acute lymphoblastic leukemia (ALL) comprises approximately 15% of childhood ALL. According to the Reinherz model of T-cell classification, three types of T-cell ALL exist: stage I with CD7+, CD2+, CD5+, CD1-, CD4-, CD8-, cyCD3-, stage II with CD1+, CD5+, CD7+ and CD2+ with variable expression of cyCD3, CD4 and CD8, and stage III mature leukemias expressing cyCD3+, CD7+, CD2+, CD5+, and either CD4+ or CD8+. We present an unusual case of stage II, immature (CD7+, CD2+, CD5+, CD4-, CD8-) T-cell ALL in a 16-year-old girl who relapsed as acute myelogenous leukemia (AML) with minimal differentiation (M0). The patient was presented with superior vena cava syndrome due to a large mediastinal mass. Peripheral blood smear revealed 11% blasts and a bone marrow aspirate showed >90% infiltration. Bone marrow immunophenotype was HLA-DR+, cyCD3+, CD2+, CD5+, CD7+, CD19+, CD34+, CD3-, CD4-, CD8-, CD10-, CD11b-, CD11c-, CD20-, CD33-, and CD79a-, MPO-, cyCD22-, TdT-. Serologic testing for HTLV-I was negative. Conventional marrow cytogenetics revealed a complex karyotype: 46XX[2]/8288XX, del(1)(q31), 12p+X2, +mar2, +mar3, +mar4, +mar5 [18]. She received induction chemotherapy according to the dexamethasone high-risk (HR) arm of ALL BFM-2000 protocol. The bone marrow examination the 33rd day of induction showed induction failure. Because of the lack of a suitable donor for allogeneic transplantation she received multiagent conventional chemotherapy. 13 months later, a routine hemogram showed leukocytes 4,700/mm³ with 48% blasts, hemoglobin 11 g/dL and platelets 72,000/mm³. A bone marrow aspiration showed >98% infiltration with blasts. Marrow immunophenotype was CD19+, CD33+, CD34+/CD56+, CD71+, CD117+, CD2-, CD4-, CD7-, CD10-, CD13-, CD14-, CD15-, CD16-, CD20-, CD36-, CD38-, CD41-, CD64-, HLA-DR-, CD116-, CD123-, MPO-, TdT-, KOR-SA 3544-, CD3-, CD13-, CD68-, and CD79a-. Marrow cytogenetics revealed the following complex karyotype: 46-62, X..., del(3)(p11), 12p+, der(14), +mar1, +mar2, +mar3, +mar4, +mar5 [15]/46-62, X..., del(3)(p11), del(5)(q21q23), 12p+, der(14), +mar1, +mar2, +mar3, +mar4, +mar5 [5]. An interphase fluorescence in situ hybridization (FISH) study showed three copies of the non-rearranged MLL and five copies of the AML1 gene in >95% of the marrow cells. The patient received chemotherapy according to the AML-BFM 98 protocol, but she never achieved remission and died approximately three months after relapse. Kurtzberg et al were the first to describe the *in vivo* conversion of CD7+, CD4-, CD8- lymphoid cells to cells of the myeloid lineage. Ferrara et al studied prospectively 44 cases of T-cell ALL, 20 of whom displayed an immature phenotype classified as T-stem cell leukemia (T-SCL) with poor prognosis. Several cases of lineage switch from a T-cell to a myeloid phenotype have been described and vice versa. Our patient at diagnosis had an immature phenotype consistent with T-SCL. Our report adds further support to the available literature suggesting that some forms of T-cell ALL arise from very immature cells belonging to the common myeloid-T lymphoid progenitors. Finally, given the worse prognosis of patients with T-SCL, more intensive chemotherapy regimens are likely needed for these patients in order to improve the chances for survival.

0911**EFFECT OF LEUKAPHERESIS IN CIRCULATING PROGENITOR CELLS**M. Juliana Majado¹, A. Minguela², C.M. González³, L. Marín², A. Fernández³, M.V. Sánchez³, E. Sanz³, A. Morales³¹Hospital Virgen de la Arrixaca, MURCIA, Spain; ²S Immunología. HV Arrixaca, MURCIA, Spain; ³S Hematología. HV Arrixaca, MURCIA, Spain

Introduction: Large volume apheresis have been demonstrated to be more useful for obtaining progenitor cells in autologous peripheral blood cells transplantation than short volume. A stimulating effect of apheresis in mobilising CD34 cells has been argued as an explanation for that success. The aim in this study is to evaluate how apheresis affects blood CD34 levels in our patients *Material and Methods.* 338 apheresis were performed to 161 patients (CS3000, Baxter), through a central venous access. Blood volume was calculated according to patients height and weight, twice blood volume was processed in every apheresis. Peripheral blood CD34 were measured in the patients, before and after the apheresis, as well as in the mononuclear cells obtained, on a FACScan (Becton-Dickinson): anti-CD34 (clone HPCA-2), anti-CD45 (perCP) and anti-CD14 FITC monoclonal antibodies were utilised; a 'live gate' was established to select CD45+ cells and 50000 events were collected as list mode data. Total peripheral blood content of CD34 was calculated, as well as the percentage of CD34 of it obtained in the apheresis product. According to these results, two groups were performed: group A, when percentage of CD34 obtained was equal or higher to 100%, and group B, when lower to 100%. Two tailed Student t-test and Pearson correlation index were used for statistical analysis. *Results.* In group A there were 222 (66%) apheresis with 163 (100-2296)% of total peripheral blood CD34 obtained, and in group B there were 116 (34%), with 67 (2-99)% of total CD34 obtained. No difference was found between both groups, neither in peripheral blood leukocyte count before (16.13+18.63 x10⁹/L vs. 17.60+12.77 x10⁹/L) or after (15.06+16.85 x10⁹/L vs. 17.34+12.61 x10⁹/L) the apheresis procedure, nor in the blood CD34 level. In group A, pre-apheresis CD34 were 26.00+74.10x10⁹/L (mean+std deviation), and post-apheresis they were 17.09+45.14.10⁹/L (p<0.001). In group B, pre-apheresis CD34 were 27.80+48.10x10⁹/L, and post-apheresis they were 21.59+32.11x10⁹/L (p=0.03). No correlation was found between peripheral blood pre-apheresis CD34 and the amount of this cells obtained in apheresis in group B, but it was significant in group A (p<0.001). *Conclusions.* After two fold total blood volume apheresis CD34 harvest is very variable, and in 66% of our patients more than 100% were obtained. Nonetheless CD34 cells do not disappear from circulation, although some decrease is observed (more significant in higher yield); so it seems that, in fact, as other authors suspect, apheresis might play a role in mobilising stem from bone marrow. This would explain the efficiency of large volumes apheresis in obtaining higher amount of CD34.

0912**CHARACTERIZATION OF MESENCHYMAL STEM CELLS IN SYSTEMIC SCLEROSIS**S. Urbani¹, S. Guiducci², M. Cinelli², I. Miniati², V. Rogai², M. Matucci Cerinic², A. Bosi¹, R. Saccardi¹¹Careggi Hospital, FLORENCE, Italy; ²University of Florence, FLORENCE, Italy

Background. Systemic sclerosis (SSc) is a chronic disorder of unknown etiology. The pathology of SSc shows an involvement of three major systems: endothelial, immune and connective. Excessive deposition of collagen and other extracellular components (fibrosis) in the skin and in visceral organs is one of the hallmark of the disease. The pathology of fibrosis in SSc demonstrates excessive fibroproliferation and deposition of various extracellular matrix proteins by mesenchymal cells.

Haematopoietic Stem Cells Transplantation (HSCT) has been proposed for patients not responding to conventional treatments. Mesenchymal stem cells (MSCs) are progenitors of multiple mesenchymal lineages and can generate a wide range of fully differentiated connective tissues such as cartilage, bone, fat, fibrous tissue and myelosupportive stroma. *Aims.* We investigated some basic biological properties of MSCs collected from the Bone Marrow (BM) of SSc patients diagnosed with the diffuse subset of SSc and BM cells from healthy donors were used as controls. *Methods.* and *Results.* CFU-F (Colony Forming Units-Fibroblastoid) mean number (±SEM) /106 nucleated seeded cells in SSc patients (n=10) and healthy donors (n=30) were 119±47 and 47±8 respectively (p<0.001). MSCs were grown in plastic flasks at standard conditions. At the fourth passage the mean fold expansion in patients and donors was 1824±738 and 55±37 respectively (p<0.05). The phenotypic expression of some haematopoietic and mesenchymal markers (CD34, CD14, CD45, HLA-A B C, HLA-DP-DQ-DR, CD29, CD44, CD73, CD90, CD105, CD166) was analysed by flow cytometry at the first passage (n=8). All cells showed a typical mesenchymal phenotype and antigenic expression was not significantly different in patients and healthy donors. Induction of osteogenic and adipogenic differentiation was performed in standard conditions at first passage and was not different from healthy donors. *Conclusions.* Pathophysiology of SSc is still unclear and involves different tissues. The finding of a normal pattern of biological markers in MSCs, collected from patients affected by a rapidly aggressive subset of the disease, suggests that MSCs are not modified in respect to controls. This observation may indicate that, in SSc, mesenchymal progenitors of the bone marrow compartment are not modified. Monitoring of biological properties of MSCs in SSc might play a role in the evaluation of the efficacy of HSCT in these patients.

0913**TEN YEAR FOLLOW UP IN HEREDITARY SIDEROBLASTIC ANEMIA (HSA) AFTER BONE MARROW TRASPLANTATION (BMT): HOW A NET NEGATIVE IRON BALANCE HAS BEEN REACHED**

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Bone marrow transplantation (BMT) provides a permanent cure for hereditary sideroblastic anemia (HSA). Among the issues requiring long term management are iron overload, chronic hepatitis and liver fibrosis. In this retrospective study, we evaluate, after BMT ten year follow-up, how a negative iron balance and a normal liver function have been reached. Phlebotomy program, phlebotomy and sc deferoxamine, phlebotomy and combined chelation therapy (DFX and DFP) have been approached. We report a case of HSA diagnosed in a 14 year old boy treated with blood transfusions and iron chelation treatment to remove iron load by using sc DFX. The patient, HCV positive for blood-borne infection, was affected by chronic active hepatitis (CAH) with marked fibrosis (Ishak staging score 4). In 1994, when he was 21 year old, he underwent allogeneic BMT and he was assigned to the third Pesaro risk class. Although successful BMT provided permanent cure for HSA, there was a persistence of iron overload associated to organ damage. Two years after BMT, he started phlebotomy schedule to remove iron (6 ml/Kg of blood withdrawn at 14-day intervals, Pesaro protocol). Because of a very low decrease in iron overload, in 1998, phlebotomy and sc DFX (50 mg/kg, 5 times weekly), and in 2001, phlebotomy plus combined iron chelation (DFP 75 mg/kg, 7 days/week orally and sc DFX 40 mg/kg 2 times weekly), respectively, was approached, with written consent. Body iron assessment was performed by laboratory testing, liver biopsies, SQUID and MRI T2* evaluation. Laboratory testing included complete blood count before each phlebotomy, liver and kidney function testing at baseline and every three months, serum ferritin (SF) at baseline and every two months. After receiving DFX and DFP, alone and in combination, urinary iron

excretion (UIE) over a 24 h period was measured. Two years after phlebotomy schedule was started, no decrease in iron stores occurred, documented by laboratory data and LIC by SQUID (6344 ± 300 microg Fe/g liver w.w.). (Tab1) Three years after phlebotomy and sc DFX program, only a very limited iron decrease has been observed. MRI T2* showed severe myocardial iron loading with a cardiac T2* measuring 8.9 ms (n.v. 20-82 ms) and mild to moderate liver iron deposition 8.7 mg/g liver d.w. (n.v. < 2 mg/g liver d.w.) Two years after phlebotomy and combined therapy with DFP and DFX, a dramatic drop of iron burden until a negative iron balance was recorded. LIC by SQUID evidenced 520 microg Fe/g liver w.w. and 1.7 mgFe/g liver d.w. Liver biopsy disclosed fibrosis in most portal areas (Ishak staging score 2) MRI T2* showed normal signal intensity and contractility with a cardiac T2* measuring 58 ms and liver iron deposition 1.9 mg/g liver d.w. All treatments have been stopped and the patient maintains a negative iron balance with normal active life. Phlebotomy and combined iron chelation therapy with DFP and DFX, may be the best method for achieving negative iron balance in iron overload.

Table 1. Patient's characteristics at the annual follow-up.

Parameter	1	2	3	4	5	6	7	8	9	10	11	12
Age (years)	63.5	64.2	65.1	66.0	67.0	68.0	69.0	70.0	71.0	72.0	73.0	74.0
Sex (M/F)	10/2	11/1	12/0	13/0	14/0	15/0	16/0	17/0	18/0	19/0	20/0	21/0
Time (years)	1	2	3	4	5	6	7	8	9	10	11	12
LDH (U/L)	110	115	120	125	130	135	140	145	150	155	160	165
ALT (U/L)	15	16	17	18	19	20	21	22	23	24	25	26
AST (U/L)	18	19	20	21	22	23	24	25	26	27	28	29
Gamma-GT (U/L)	20	21	22	23	24	25	26	27	28	29	30	31
Alkaline Phosphatase (U/L)	30	31	32	33	34	35	36	37	38	39	40	41
Iron (µg/g liver w.w.)	6344	6300	6250	6200	6150	6100	6050	6000	5950	5900	5850	5800
Liver iron deposition (mg/g liver d.w.)	8.7	8.5	8.3	8.1	7.9	7.7	7.5	7.3	7.1	6.9	6.7	6.5
Cardiac T2* (ms)	8.9	8.8	8.7	8.6	8.5	8.4	8.3	8.2	8.1	8.0	7.9	7.8

* Multiple of upper limit of reference range

0914

RECOMBINANT URATE OXIDASE (RASBURICASE) IN THE PREVENTION AND TREATMENT OF HYPERURICAEMIA IN CHILDREN WITH HAEMATOLOGICAL MALIGNANCIES

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Recombinant urate oxidase (rasburicase) catalyzes the enzymatic oxidation of uric acid into allantoin. Rasburicase has been defined as a potent urolitic agent for the management of malignancy-associated hyperuricaemia and tumour lysis syndrome (TLS). TLS is a rare, but potentially fatal, metabolic complication that can arise from the treatment of rapidly proliferating and drug-sensitive neoplasms. We retrospectively analyzed 29 children (median age 6,8), 20 with Acute Lymphoblastic Leukaemia (ALL), 3 with Acute Myelogenous Leukaemia (AML) and 6 with non-Hodgkin's lymphoma (NHL) who received rasburicase (0,20mg/Kg/day for 5-7 days) for prophylaxis (24 children, group A) or treatment (5 children, group B). At presentation the mean uric acid levels (UAL) was $7,2 \pm 1,05$ mg/dL for children group A and $13,9 \pm 2,8$ for children of group B, whereas serum creatinine was $3,8$ mg/dL in 3 children of group B. All patients showed a significant reduction of UAL 48h after treatment, $0,43 \pm 0,68$ mg/dL (group A) and $1,6 \pm 1,02$ mg/dL (group B) ($p < 0,001$). None of the 29 children experienced any adverse event. In all cases the treatment with rasburicase was associated with hyperhydration and urine alkalinization. From our experience we conclude that rasburicase is safe and effective in preventing and treating hyperuricaemia in children with haematological malignancies.

0915

MOLECULAR ANALYSIS OF IGVH GENE MUTATIONAL STATUS AS A PROGNOSTIC FACTOR IN B-CLL WITHIN CZECH PATIENTS

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Introduction: B-cell chronic lymphocytic leukemia (B-CLL) is currently considered as an incurable disease and follow very heterogeneous clinical presentation. It has been shown that the mutational status of the variable region of the immunoglobulin heavy chain gene (IgVH) could be the best biological prognostic factor for predicting of the prognosis and for dividing CLL into two subgroups. The aim of our study was to identify spectrum of the malignant leukemic clones, quantify homology to the germinal lines used in our CLL cohort and identify patients with an increasingly uncontrolled proliferative capacity, leading to a more aggressive disease. *Methods.* and *Results.* We have used PCR method and direct sequencing to analyse the IgVH rearrangements and mutational status in 146 Czech CLL patients. 30 various VH sequences were detected. The hierarchy of preferentially used gene families was following: VH3 (48.5%), VH1 (31.5%), VH4 (14.4%). The most frequent rearranged VH genes were VH1-69 (20%), 3-23 (9.6%), 3-30 (6%). Applying the 98% germline homology cut-off, level of mutated B-cell clones was 37%. The most commonly rearranged D genes were 3-03 and 3-22 and they represent 26% of the identified genes. Rearranged JH genes were JH4 > JH6 > JH5 accounting for 93%. The most prevalent unmutated VH subgene was 1-69 cohered mostly D3-03 and JH6. VH 3-23 rearrangement represents typically mutated subgene and was almost solely associated with JH4 subgene. The prototypic mutated rearrangement VH 3-11 showed absolute homology to germline sequence in all cases examined. Determination of the CDR3 region showed 6-24 length of aminoacids. In the presence of bi/polyclonal sequence it proves to be favorable using of non-degenerate family specific leader region primers. It provides information about distinctive yield of PCR product of six VH gene families and following sequencing of obtained VH gene clone may results in rearrangements confirmation. For evaluation of abundance level of malignant cells after therapy we have used SYBR Green based Real time PCR assay, IgVH specific PCR with clone specific primers. IgVH copy number was quantified with the cloned IgVH sequence as an external standard. This method seems to be convenient option for minimal residual disease detection. *Conclusions.* Our results indicate broad spectrum of leukemic clones preferentially with unmutated rearrangement. It appears the connection of B-cell chronic lymphocytis leukemia receptors represented by VH subgenes 1-69, 3-23 and 3-11 with characteristic mutational status and D or JH rearrangement. The CDR3 length doesn't differ significantly in relation to the VH family and VH subgene except for VH 1-69. Within our patient cohort, the CDR3 average length is 19aa among the 1-69 expressing B-CLL cells. The entire obtained results follow up clinical disease progress. Whereas further studies are necessary to determine the clinical value of the sequential genetic analysis. This study was supported by grant no. NR/8443-3 IGA MZ.

0916

EXPRESSION OF ADHESION MOLECULES IN BONE MARROW FIBROBLASTS OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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The myelodysplastic syndromes (MDS) are clonal stem cell disorders associated with a variety of abnormalities of mature and maturing cells including surface antigen abnormalities. Cell-cell and cell-extracellular matrix interactions through adhesion

receptors are required for the residence of stem cells and progenitors in bone marrow. We analyzed the expression of genes and proteins essential in hematopoietic cell adhesion and homing to stroma in MDS. The expression of cell adhesion molecules, such as alpha5beta1, alpha2beta1, VCAM-1, CD44 was evaluated by immunoprecipitation and RT-PCR in bone marrow fibroblasts of patients with MDS (n=5), MDS-AML (n=2), polycythemia vera (n=4) and 4 healthy donors. There were no differences in expression levels of alpha5beta1, alpha2beta1 and CD44 in bone marrow fibroblasts between patients and donors. The expression level of VCAM-1 (both protein and mRNA) in bone marrow fibroblasts of MDS and MDS-AML patients was lower than in healthy donors with exception for a single patient with high VCAM-1 expression. The expression level of VCAM-1 in patients with polycythemia vera was no differ from healthy donors. It seems that the change of VCAM-1 expression in MDS bone marrow fibroblasts is one of the reasons in disturbance the interactions between hematopoietic cells and stroma. These findings suggest that changes in adhesion molecule expression by fibroblasts are the important in the pathology of MDS.

0917

USEFULNESS OF HEMATOPOIETIC CHIMERISM MONITORING USING STANDARD PCR METHOD IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES TREATED ALLOGENEIC TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING

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Background. To evaluate the clinical outcome of the disease of patients (pts) with hematological malignancies treated with non-myeloablative allogeneic stem cell transplantation, the hematopoietic chimerism screening is necessary. *Aims.* We have analyzed the usefulness some genetic markers in hematopoietic chimerism monitoring in patients undergoing non-myeloablative allogeneic stem cell transplantation. *Material and Methods.* Gradisol L was used to separate lymphocytes from peripheral blood. DNA was isolated using standard method. Molecular assessment using PCR method involved analysis of short tandem repeats F13A1, TOPIP2, 1P32 (MYCL), D11S533, IL2-R beta) and variable number of tandem repeats genetic markers {SRY, Apo-B2, D4S95, pMCT118 (D1S80), YNZ22 (D17S5)}.

Table 1.

Genetic marker	Patients group (%)	Donors group (%)
F13A1	20	20
TOPIP2	14,3	36,8
1P32 (MYCL)	60	56,5
D11S533	74,6	81,8
IL2-R beta	50	43,8
SRY	33	41
Apo-B2	77,6	73,7
Apo-B1	0	28,6
D4S95	61,8	59,2
YNZ22 (D17S5)	20	5,5

The schedule of hematopoietic chimerism monitoring involved initially evaluation of the SRY, Apo-B2, D4S95 and D11S533. If evaluated markers do not allowed to discriminate between donor and recipients, the second line genetic markers

were used: F13A1, TOPIP2, 1P32 (MYCL) and IL2Rbeta. The study group (45 males and 33 females; mean age 52, range 22-67 years) consisted of patients with chronic myeloid leukemia (n=19), acute myeloid leukemia (n=20), ALL (n=2) chronic lymphocytic leukemia (n=10), multiple myeloma (n=8), non-Hodgkin lymphomas (n=6), myelofibrosis with agnogenic myeloid metaplasia (n=5), myelodysplastic syndromes (n=5), chronic eosinophilic leukemia (n=2) and paroxysmal nocturnal haemoglobinuria (n=1). *Results.* Genetic test was also performed in a group of 78 hematopoietic cells donors (48 males and 30 females; mean age 52, range 24-76 years). The first line primers set allowed to discriminate between donors and patients in 49/78 (62,8%) cases. The second lines primer set was used in 29 pts (37,1%). In 5 cases our procedure applied do not allowed to distinguished between patient and donor genotype. On the basis of EMBT 2004 criteria full donor and mixed chimerism was documented on day +30 in 88,1% of patients. Autologous recovery was found in 11,9% of pts. *Conclusions.* our strategy is a useful method of chimerism monitoring in patients after allogeneic transplantation with reduced intensity conditioning. However, in most of the patients mixed chimerism was found, even 90 days after transplantation. Therefore, quantitative chimerism status analysis should be performed to proper qualification of pts to treatment with donor lymphocyte infusion procedure. Table 1. The frequency of genetic markers in patients and donor group.

0918

LAPAROSCOPIC CHOLECYSTECTOMY IN CHILDREN WITH BETA-THALASSEMIA MAJOR AND ASYMPTOMATIC CHOLELITHIASIS

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Background. Our study aimed to evaluate the role of elective laparoscopic cholecystectomy in children with beta-thalassemia and asymptomatic cholelithiasis. *Methods.* From June 1995 to February 2005 17 children with beta-thalassemia were referred to our Division of Surgery for cholelithiasis. All 17 children were asymptomatic at the time of the first visit. The mean age was 18 years (range, 12 to 25). We proposed an elective LC to all children before the onset of symptoms. Splenectomy was previously performed in 4 children. The operation was accepted by 10 children (Group A) and refused by 7 (Group B). In Group B 4 children decided to undergo laparoscopic cholecystectomy after the first or second episode of biliary colic and 3 children were admitted in emergency for acute cholecystitis (n=2) or choledocholithiasis (n=1). We correlated the outcome in the 2 groups of children with the treatment chosen and the operation timing. *Results.* No significant difference were found in morbidity rate and postoperative stay between children operated on before the onset of symptoms and children operated on after the onset of symptoms. Operative time was longer in symptomatic children than asymptomatic due to the presence of an inflamed gallbladder (chronic cholecystitis) which makes tissue dissection more difficult. The correlation between cholecystectomy performed electively and urgently showed significant difference in the outcome. Morbidity rate and postoperative stay increased when children with beta-thalassemia underwent emergency laparoscopic cholecystectomy. *Conclusions.* Elective LC should be the gold standard in children with beta-thalassemia and asymptomatic cholelithiasis in order to avoid the potential complications of cholecystitis and choledocholithiasis which lead to major risks, discomfort and longer hospital stay.

0919

MOLECULAR ANALYSIS OF β -THALASSEMIA CASES IN POLANDB. Burzynska¹, M. Maciag¹, E. Zdebska², E. Mendek-Czajkowska², A. Adamowicz-Salach³¹Institute of Biochemistry and Biophysics, WARSAW, Poland; ²Institute of Haematology, WARSAW, Poland; ³Medical University of Warsaw, WARSAW, Poland

Background. β -thalassemia is a heterogeneous, inherited disease resulting from reduced or absent synthesis of the β -globin chain of haemoglobin. This disorder is very common in Mediterranean, Middle Eastern, African and South East Asian populations. In Poland β -thalassemia syndrome has been observed very infrequently. **Aims.** The aim of our study was to look for the mutations in the β -globin gene in the group of unrelated Polish patients with the β -thalassemia trait. We also discuss the effect of these mutations on the expression level of the β -globin gene. **Methods.** DNA Isolation Kit for Blood/Bone Marrow/Tissue (Roche Diagnostics GmbH, Germany) was used to isolate DNA from leukocytes. Polymerase chain reaction (PCR) was used to amplify the fragments of the β -globin gene. Total RNA was isolated from peripheral blood using PAXgene Blood RNA Kit (QIAGEN, Germany). Reverse transcription (RT) was performed using 0.2-1.0 μ g RNA and oligo (dT) primers (BD Biosciences Clontech). Real-time PCR was carried out using a LightCycler (Roche Diagnostics GmbH, Germany). Reactions contains 9 μ l of SYBR Green master mix and 10 ng of cDNA, were set up in microcapillary. Data were analyzed using LightCycler analysis software. **Results.** DNA analysis revealed different mutations in β -thalassemic subjects of 17 Polish families. The IVS-1-6 (T>C) mutation was found in 5 families and IVS-2-745 (C>G) was also established in five other families. The mutations: IVS-2-1 (G>A), CD 39 (C>T) were also found in the studied group, but less frequently. We also detected two novel mutations: IVS-1-1 (G>A) G^AGTTGGT>AGATTGGT and 5'UTR; +33 (C>T). **Conclusions.** The first novel mutation significantly decreased the mRNA level of the β -globin gene. The second mutation seems to be silent and does not affect β -globin synthesis. In this case, this mutation occurs with IVS-1-6 (T>C) mutation in compound the heterozygotes stage.

0920

COMPLETE RESPONSE IN MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE

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Background. Thalidomide as monotherapy is active in 25-50% of patients with relapsed and refractory myeloma. Usually the response is partial, a 10-15% achieve a >90% reduction in component monoclonal (CM) or even a complete response (CR) in electrophoresis but with a immunofixation (IF) positive. The true CR (IF negative) is unusually reported. Two reports used IF to evaluate the response: Singhal S (N Engl J Med 1999;341) found 2 cases in 84 patients and Mileschkin (Blood 2003;102) one in 75 cases. **Aims.** We report our experience with thalidomide and 2 cases reports that reached a CR IF -. **Methods.** patients with advanced multiple myeloma (n=24) were treated with oral thalidomide between July 2000 and July 2004, 9 with a refractory myeloma and 15 who relapsed following previous therapies. In 13 the thalidomide was given as monotherapy, in the others (n=11) was associated with dexamethasone and/or chemotherapy. **Results.** there was 3 CR IF-, 5 PR and 3 with a minimal response (OR 45%). We report two cases with a CR IF-treated with thalidomide in monotherapy: Case 1: Male 41 ys diagnosed of IgG MM in September 98 presented with multiple bone lesions. He was treated with chemotherapy VCAP (6 cycles) with a partial response. In August 2000 the MM pro-

gressed and 9 cycles of VBCMP were given with a minor response. In June 2001 thalidomide (400 mg/d) was started and a decreased of IgG was seen two months later, but the CR by IF was documented in July 2002. After this data the thalidomide dose is 200 mg/d, and the patient have a continuous CR IF- Case 2. Male 61 ys diagnosed of IgA MM in July 2001, presented with anemia, bone lesions, renal failure and spinal cord compression. He was treated with radiotherapy and chemotherapy (VBCMP/VBAD) and reached an initial very good partial response. However the IgA level showed a rapid increased 2 months after the last cycle and thalidomide 200 mg/d was started in June 2002. The remission by routine electrophoresis was seen after 6 months, but the CR IF- was documented in May 2003. In October 2003 (IF-) the patient presented a increase in the numbness and paresthesia and the thalidomide was stopped during 2 months. In December 03 (IF-) the thalidomide was restarted but again was stopped in March 04 (IF- unknown). In June 04 a rapid progression of the myeloma occurred, the thalidomide was restarted associated with dexamethasone, and a partial response was reached. Nevertheless in December 04 the patient presented a cranial plasmacytoma that was treated with radiotherapy. Now the patient is in treatment with bortezomib. **Conclusions.** this study confirms that thalidomide is an effective agent in patients with advanced myeloma and is possible to reach a complete remission of the CM. However a stop in the treatment is followed by a relapse even in patients that reach a CR IF-

0921

NEONATAL SCREENING OF HEMOGLOBINOPATHIES AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN CATALONIAM. Mañú Pereira¹, A. Maya¹, V. Cararach¹, J. Sabrià², J. Boixadere³, R. Teixidor⁴, I. Martín⁵, M. López⁵, J.L. Vives Corrons¹¹Hospital Clínic i Provincial, BARCELONA, Spain; ²Hospital Josep Trueta, GIRONA, Spain; ³Hospital Joan XXIII, TARRAGONA, Spain; ⁴Hospital Sant Jaume d'Olot, OLOT, Spain; ⁵Hospital del Mar, BARCELONA, Spain

Background. AND OBJECTIVES: Migratory flows in our country have driven to the emergency of sickle cell disease (SCD) as well as of other congenital red cell defects. The main objectives of this project are: 1. To determine the prevalence of SCD, other hemoglobinopathies (homozygote and heterozygote conditions) and G6PD deficiency in the population of Catalonia. 2. Early detection of these genetic defects and the performance of family studies. 3. Genetic characterization of the identified variants. **Methods.** An anonymous not related study for haemoglobinopathies and G6PD deficiency has been carried out in 3189 samples including 1620 from immigrant population, using whole-blood spots on filter papers from the Catalan Neonatal Screening Program for Metabolic Diseases (CNSP-MD). Simultaneously, with the collaboration of several Hospitals from different geographical areas of Catalonia we have also designed a second descriptive study by using 1573 umbilical cord whole-blood spots on filter papers, including 1080 from immigrant population. The high performance liquid chromatography (HPLC) method has been used for the screening of hemoglobinopathies and the fluorescent spot test (ICSH) for the screening of G6PD deficiency. Confirmation and molecular characterization of haemoglobin variants found and G6PD deficiency cases have been performed by amplification refractory mutation system (ARMS), enzyme restriction polymerase chain reaction (ER-PCR) or gene sequencing. **Results.** Anonymous not related study: 1- Hemoglobinopathy was found in 47 samples from the immigrant population, 2 cases associated with SCD (phenotypes FS and FSC) and 45 cases with heterozygous haemoglobinopathy condition (HbS, HbC, HbD and HbE) 2-G6PD deficiency was found in 29 samples, 3 of them were from non-immigrated population. Descriptive study: 1- Haemoglobinopathy was found in 48 samples from immigrated population, 3 of them associated with SCD (phenotype FS) and 45 with heterozygous haemoglobinopathy condition (HbS, HbC,

and HbD). SCD cases have been confirmed by ARMS and in one of them, a compound heterozygous HbSS/ alpha-thalassaemia was identified. In all these cases family study was also performed. 2- G6PD deficiency was found in 23 samples, one from non-immigrated population. The family studies increased the number of cases with G6PD deficiency. *Conclusions.* The results of anonymous not related study show an incidence of SCD in the risk population of Catalonia of 1 case out of 810 samples. This value is significantly higher than the incidence reported for any of the metabolic diseases included in the CNSP-MD. The results obtained in the present study give further support to the convenience of incorporate the neonatal screening for haemoglobinopathies into the already existing official neonatal screening programmes, at least for risk populations. Moreover, due to its feasibility and low cost, a similar criterion may also be adopted for the neonatal screening of G6PD deficiency. Finally, the results of descriptive study with an incidence of 1 SCD case out of 360 samples from immigrated population show a marked heterogeneity in the distribution of this population in Catalonia and the importance of also performing systematic family studies.

0922

BONE MARROW B CELLS FROM MYELODYSPLASTIC SYNDROME DISPLAY MATURATION-ASSOCIATED IMMUNOPHENOTYPIC ABNORMALITIES

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Background and Objective: Myelodysplastic syndromes (MDS) are considered to be clonal disorders of hematopoietic myeloid stem cells. However, accumulating evidence supports an origin of the disease at a more immature stem cell with ability to differentiate into both myeloid and lymphoid cells. In this study we analyse the distribution and phenotypic characteristics of the different maturation-associated compartments of bone marrow (BM) B-cells in MDS. *Methods.* A total of 41 newly-diagnosed MDS patients including both low risk (LR; n=22) and high risk (HR; n=19) cases were studied. Specific enumeration and characterization of CD34+ and CD34- B-cell precursors and mature B-lymphocytes was performed in all cases using multiparameter flow cytometry. BM samples from 14 individuals undergoing orthopedic surgery were studied as normal controls. *Results.* Our results show the existence of both quantitative and qualitative immunophenotypic abnormalities involving BM B-cells in MDS patients. Numerical abnormalities were more pronounced in the CD34+/CD45lo and CD34-/CD45int compartments of B-cell precursors and varied between LR-MDS and HR-MDS cases where they consisted of abnormally high and low numbers of immature B-cells, respectively. Additionally, most of LR-MDS and HR-MDS studied showed abnormal patterns of expression for at least one of the markers analyzed. Interestingly, in LR-MDS patients phenotypic abnormalities were more frequently observed in the early CD34+/CD45lo B-cell precursors while in HR-MDS cases phenotypic aberrations were more frequently found in the two more mature CD34- B-cell compartments. Overall, antigen underexpression was the most frequent abnormality followed by antigen overexpression, both abnormalities involving the TdT, HLADR, CD45, and CD34 antigens. In turn, asynchronous antigen expression was restricted to the CD19+/cCD79a- phenotype on CD34+ B-cell precursors, although this phenotypic abnormality was constantly present in all patients analyzed who showed detectable levels of B-cell precursors in the BM. In contrast, no aberrant expression of the CD3, CD4 and CD16 T/NK-cell associated markers was detected on B-cells from none of the MDS patients. *Conclusions.* We show phenotypic evidence about the existence of an abnormal B-cell maturation in MDS patients, the understanding of its nature deserving further investigation.

0923

THE ROLE OF LEPTIN IN THE PATHOGENESIS OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background. Leptin is a pleiotropic hormone whose multiple effects include regulation of endocrine function, reproduction, and immunity. The role of leptin in the modulation of immune response and inflammation has recently become increasingly evident. Leptin can be considered as a pro-inflammatory cytokine that has structural similarity with several interleukins. Leptin plays an important role in inflammatory processes involving T cells and has been reported to modulate T-helper cells activity in the cellular immune response. Several studies have implicated leptin in the pathogenesis of autoimmune inflammatory conditions, such as type 1 diabetes, rheumatoid arthritis, and intestinal inflammation. Nevertheless, there is a paucity of data about the role of leptin in the pathogenesis of idiopathic thrombocytopenic purpura (ITP). Recently, serum leptin concentration was found to be increased in untreated ITP patients when compared with control subjects, suggesting a modulatory role for leptin in ITP. *Aims.* The purpose of this study was to evaluate serum leptin levels in ITP, in order to determine the influence of leptin on the pathogenesis of ITP. *Methods.* 16 Greek adult patients with ITP [3 males (18.8%) and 13 females (81.2%); mean age 47.7±20.4 years] with a mean duration of ITP of 102±81 months were studied. ITP was diagnosed on the basis of the presence of isolated thrombocytopenia (platelets <100×10⁹/L) and megakaryocytic bone marrow hyperplasia. Other causes of thrombocytopenia (eg. drugs, HCV, HIV, autoimmune disorders, pseudothrombocytopenia) were excluded. All patients had been treated previously with corticosteroids, while at the time of the evaluation none of them was receiving any treatment. The mean platelet count at ITP onset was 27.4×10⁹/L±21.2×10⁹/L. At the time of the study the mean platelet count was 232×10⁹/L±178×10⁹/L; six patients (37.5%) had less than 150×10⁹/L platelets and two patients (12.5%) had less than 100×10⁹/L platelets. 11 patients (68.8%) had undergone splenectomy. Mean body mass index (BMI), which is the body weight in kg per square surface area (kg/m²), was 26.2±6.3 kg/m². Duplicate serum samples were assayed for leptin using a radioimmunoassay. Coefficient of variance of the assay was <5%. *Results.* Mean serum leptin levels were 33.5±24.4 ng/mL. Leptin levels showed no correlation with platelet count (r=0.038, p=0.888). Separate analyses according to BMI (<25 or <30) and platelet count (<100×10⁹/L or <150×10⁹/L) also failed to identify any association between leptin levels and platelet count. As expected, leptin levels were significantly positively related to BMI (r=0.696, p<0.005). *Conclusions.* In our group of patients we could not demonstrate any significant association between leptin levels and platelet counts. All our patients had received immunomodulatory treatment in the past and most of them had almost normal platelet counts at the time of evaluation; both these parameters might have affected our results. Further studies are required to elucidate the role of leptin in the pathogenesis of ITP.

0924

MONOCLONAL CRYOGLOBULINEMIA: THE FIRST MANIFESTATION OF EXTRANODAL MARGINAL B-CELL LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT LYMPHOMA)

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Background. Cryoglobulins are complexes of immunoglobulins that precipitate on exposure to cold. Three different classes have been recognized. Type I cryoglobulins are monoclonal Ig M, IgG or IgA molecules, usually associated with multiple

myeloma and Waldenström's macroglobulinemia. PATIENT A 69-year-old woman was admitted to our Hospital on December 2004 for evaluation of peripheral edema and bilateral purpura on lower legs. She had a past history of disseminated hydatidosis with liver, kidney and bone involvement. On physical examination, the splenic tip was palpable. In lower legs, hyperpigmented areas and peripheral edema were observed. Initial laboratory tests revealed: serum total protein 5.3 g/dL, albumin 2.8 g/dL, LDH 344 (150-450) UI/L, creatinine 0.86 mg/dL, hemoglobin 87 g/L, white cell count $6.2 \times 10^9/L$, platelets $703 \times 10^9/L$. In a random specimen of urine, the creatinine level was 67 mg per deciliter, and the protein level was 3.05 g/L. Antibodies for HCV, HBV and HIV type 1 and 2 were negatives. Complement levels were under normal values. Immunoserological analyses were normal, and immunofixation on specific antisera revealed the expression of cryoglobulins identified as monoclonal IgM-kappa. In order to rule out a lymphoproliferative disorder, a computed tomographic scan of the thorax and abdomen was performed. It revealed a thickening of gastric wall. Upper endoscopy and ultrasound endoscopy showed a thickened gastric wall with 2.6 cm of maximum diameter, superficial ulcers and locoregional lymph nodes. Gastric biopsies showed lymphoid infiltrates consistent with extranodal marginal B-cell lymphoma of MALT with the presence of rods suggestive of *Helicobacter pylori*. The bone marrow biopsy was not infiltrated. The diagnosis of extranodal marginal B-cell lymphoma of MALT associated with *Helicobacter pylori* infection was performed with stage II1 (Lugano), T3N1M0 (adapted TNM) and modified IPI for localized lymphomas 2/5. Triple therapy for the eradication of *Helicobacter pylori* and chemotherapy with endovenous fludarabine was started. CONCLUSIONS We report a case of gastric MALT lymphoma presenting as monoclonal type I cryoglobulinemia. We have reviewed the literature from 1962 to 2005, and to our knowledge, this is the second case of monoclonal cryoglobulinemia reported to be associated to MALT lymphoma. Type II cryoglobulinemia and lymphoma associated with HCV infection is well known. However, monoclonal cryoglobulinemia type I has been described in association with multiple myeloma but it is rare in other lymphoproliferative diseases. In our patient, there were not clinical or biological findings suggesting the presence of a lymphoproliferative disorder. Therefore, if abnormal serum proteins with monoclonal immunoglobulins are identified, without a plasma cell disease, a clinical investigation should be performed in order to rule out the presence of a lymphoproliferative disorder. It is well known the association between infectious agents (*Helicobacter pylori*, *Campylobacter jejuni*, *Borrelia burgdorferi*) with MALT lymphoma development. Our patient had a long history of *Echinococcus granulosus* infection without clinical or serologic activity at the moment of lymphoma's diagnosis. However, in our knowledge, there is not any report linking a pathogenic relationship between *Equinococcus granulosus* infection with MALT lymphoma or monoclonal cryoglobulinemia

0925

HIGH RESPONSE AND COMPLETE REMISSION RATES IN ASIAN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA TREATED WITH BORTEZOMIB

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Background. Recent reports suggest that Asian patients may respond differently from non-Asian patients to certain drugs. Studies conducted on non-Asian patients have demonstrated that the novel proteasome inhibitor, bortezomib (VelcadeTM), has significant therapeutic activity in patients with relapsed/refractory multiple myeloma (MM). Whether Asian patients respond differently to bortezomib is currently unknown. Aim The aim of this study was to determine the efficacy of bortezomib in Asian patients with relapsed/refractory MM. The primary end-points were response rate (RR) or com-

plete remission (CR); and the secondary end points were intolerance to bortezomib or death. The results of this study were compared to published data from clinical trials conducted on predominantly non-Asian patients. **Methods.** Patients with relapsed/refractory MM were treated for 2 to 6 cycles of bortezomib using the widely published 3-weekly regimen (1.3 mg/m² on days 1, 4, 8 and 11, followed by rest for 10 days). Concomitant oral dexamethasone 20 mg OM was administered on days 1 to 4, 8 to 11, and 15 to 18. A single intravenous dose of zoledronic acid (4 mg) was given on the first day of each cycle. Patients also received oral thalidomide 50 mg ON. The definition of CR required 2 serial negative immunofixation (IF) readings performed 6 weeks apart. **Results.** A total of 8 patients (2 male and 6 female; median age = 61 years, range 51 to 92 years) were studied. All patients had complex karyotypes at diagnosis, including 3 patients with deletion chromosome 13 (del(13)). Three patients had plasma cell leukaemia (PCL) at the time of starting bortezomib. The overall RR was 87.5%, i.e. 7 out of 8 patients responded to bortezomib. Four patients out of 8 (50%) achieved IF-negative CR, including one patient who had relapsed with PCL after receiving conventional vincristine, adriamycin, dexamethasone (VAD) chemotherapy, followed by autografting, followed by a mini allogeneic transplant, and finally followed by 2 donor lymphocyte infusions. In addition, 2 patients achieving IF-negative CR had del(13). The 3 (37.5%) patients who achieved partial response (PR) all demonstrated >90% reduction of paraprotein levels. One patient with far advanced PCL failed to respond to bortezomib and died after 2 cycles of therapy. The average number of cycles of bortezomib administered per patient was 2.8. Full dose bortezomib was well tolerated by all patients. Transient grade 3 thrombocytopenia occurred in 3 patients (37.5%). No patient developed peripheral neuropathy. **Conclusions.** Our preliminary data demonstrates that bortezomib is extremely effective and safe in Asian MM patients. Both the high CR and good PR rates were evident even after relatively few cycles of bortezomib. We therefore conclude that bortezomib is potentially an exceptionally important agent for the treatment of Asian patients with relapsed/refractory MM.

0926

ASSOCIATION OF CYTARABINE AND MITOXANTRONE FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA RESISTANT TO IMATINIB

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Background. Imatinib is currently the treatment of choice for chronic myeloid leukaemia (CML). In patients in accelerated phase (AP) and blast crisis (BC), it has been observed a high rate of primary and secondary resistance to imatinib. In vitro studies have demonstrated that cytarabine and imatinib interact synergistically to induce apoptosis in CML leukaemia cells resistant to imatinib. **Aims.** The aim of this study was to evaluate a combined treatment for imatinib resistant CML patients in advanced phases. Patients and **Methods.** Patients with CML in AP or BC with hematological resistance to imatinib were included. Patients received a daily oral dose of imatinib 600mg, Ara-C sc 10mg/m² per day and mitoxantrone 10 mg/m² iv every 15 days, during two months as induction and then once a month. Patients were followed once a week for clinical and laboratory evaluation or less if necessary. Toxicity and response were evaluated. **Results.** From June through December 2004, five patients were included in this protocol, age 29-63 (median 57 years); 4 patients in BC and 1 patient in AP. One patient had primary resistance and the others secondary resistance to imatinib. The median duration of treatment with the combined drugs was 85 days (65-189 days). Adverse events included neutropenia G IV, thrombocytopenia G IV, anemia G III, with transfusion and G-CSF requirement in 4 patients; febrile neutropenia in 2 patients. Neutropenia led to discontinuation of Ara-C and mitoxantrone in 2 patients. All patients had an initial haematological response of short duration. Four are alive: one

em BC, one in AP, one in chronic phase and one with aplasia. One patient with haematological progression and clonal evolution was treated with conventional induction chemotherapy and died 75 days after the beginning of treatment. The protocol has been interrupted in one of the patients who developed neutropenia. *Conclusions.* We concluded that this protocol might be performed for outpatients with close monitoring of cytopenias and its complications. However, the association of imatinib, Ara-C and mitoxantrone was not enough to avoid disease progression. Development of new drugs will be necessary and clinical trials will demonstrated the best treatment options.

0927**FACTORS INFLUENCING CYTOGENETIC RESPONSE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB**

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Background. Imatinib mesilate has been available for the treatment of chronic myeloid leukemia (CML) in the last 5 years. Until recently, it has been used in accelerated phase (AP) and blast crisis (BC) as well as for patients in chronic phase (CP) resistant or intolerant to interferon, presenting a high rate of hematologic as well as cytogenetic response. *Aims.* and *Methods.* We analyzed the factors predicting cytogenetic response and those related to acquisition of resistance in patients with CML treated at our Institution according to the above mentioned indications. Diagnosis was made by peripheral blood counts, bone marrow histology, karyotype and nested PCR. After normalization of peripheral blood counts, karyotype and PCR were repeated every 3 months. Parameters influencing cytogenetic response and resistance were analysed by the Cox model. *Results.* 52 patients entered the study. Median age: 41 years (15-74). Among them, 20 patients were in CP, 17 were in AP and 15 in BC. Major cytogenetic response was achieved by 13/20 patients in CP, 10/16 in AP and 1/15 in BC in a median of 8.1 (3-29), 12.7 (3-19) and 7.0 months respectively. Percentage of bone marrow blasts at diagnosis, phase of the disease and the interval between diagnosis and the day of start of Imatinib treatment(interval) influenced the occurrence of cytogenetic response. Only blast percentage and the interval were significant in the multivariate analysis. Loss of cytogenetic response was predicted by the spleen size, peripheral blood percentage of basophils and bone marrow blasts before start of Imatinib treatment as well as the time to achieve the primary response. *Conclusions.* in our study, recognized prognostic parameters in CML were also useful to predict cytogenetic response after Imatinib. Start of Imatinib treatment early in the course of CML is important to optimize the response.

0928**CHROMATIN TEXTURE ANALYSIS IN ACUTE LEUKEMIAS AND ITS RELATION TO IMMUNOPHENOTYPE FEATURES**

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Background. the chromatin structure of blasts is a key feature in morphologic diagnosis of acute leukemias. On the other hand, the study of expression of several lineage- or maturation-linked antigens by flow cytometry give important information for the final diagnosis. Changes of chromatin arrangement as well as the expression of cytoplasmic or membrane proteins reflect the degree of cellular differentiation. *Aims.* to examine the relation between chromatin texture features and the expression of membrane and cytoplasmatic proteins in blasts of patients with acute leukemia. *Methods.* diagnosis was based on cytologic features, immunophenotyping (by flow cytometry) and cytogenetic analysis. In each patient, texture analysis was done on gray-scale transformed digitalized images of 100 cells of routinely stained

May-Grünwald-Giemsa preparations. We measured granulometric features (using the gray level height of the basins as filter parameter) as well as Shannon's entropy and the fractal dimension in images after application of thick and thin contour detection. Antigen expression was quantified by mean fluorescence intensity (10 000 cells acquired, analysis in the Paint-a-gate software). *Results.* 32 patients with acute lymphoblastic (ALL) and 31 patients with acute myeloid leukemia (AML) entered the study. In ALL there was a negative correlation between the expression of immunoglobulins (light chains) and the number of granulometric residues at lower height of the basins ($r = -0,54$; $p = 0,005$). Furthermore, we found a positive correlation between the CD20 expression and the fractal dimension after morphologic gradient filtering ($r = 0,39$; $p = 0,036$). In AML, the number of granulometric residues at lower height of basins were inversely correlated with CD7 ($r = -0,50$; $p = 0,004$) and myeloperoxidase expression ($r = -0,42$; $p = 0,036$). A positive correlation between CD45 expression and the number of granulometric residues at higher basins ($r = 0,45$; $p = 0,038$) was also observed. *Conclusions.* the analysis of the chromatin structure revealed differences in blast maturation stage and were related to the intensity of expression of antigens routinely examined in diagnostic procedures in acute leukemias. supported by FAPESP, FAEP, CNPq.

0929**EFFICIENCY OF DIFFERENT HEMOTHERAPY REGIMENS, INCLUDING FLUDARABINE, IN THE TREATMENT OF CHRONIC LYMPHOBLASTIC LEUCAEMIA**

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Background. traditional chemotherapy of chronic lymphocytic leukaemia (CLL), including monochemotherapy with alkilating agents and with different monochemotherapy regimens, such as COP, CHOP, which includes these agents, in very rare cases induce overall responses with very short duration. With the aim of inducing the effectiveness of chemotherapy results in progressive disease, it is reasonable to find out new therapeutic modalities. In this research work we introduce the results of treatment 172 CLL patients, with different chemotherapy regimens with purine analogs and monoclonal antibodies anti-CD20: FCM - fludarabine, cyclophosphamide, mitoxantrone (63 patients); RFC - rituximab, fludarabine, cyclophosphamide (17 patients); FC - fludarabine, cyclophosphamide (65 patients), F fludarabine (27 patients). The age of patients was varied from 32 to 76 years (the average age was 58 years). 18 patients were in stage A, 99 in stage B, 55 patients were in stage C. Among all patients, 98 were previously untreated, 74 were pre-treated with 1-3 and more chemotherapy regimens (chlorambucil, COP, CHOP). Treatment results showed that in CLL, chemotherapy including fludarabine has a high level of effectiveness in comparison with conventional therapeutic approaches. The most effective programmes are RFC and FCM combinations that permit to receive overall response in 100% and 94% of cases, respectively. On FC treatment, the overall response was achieved in 79% patients, with F-monotherapy - in 64%. Therefore, all these regimens are well tolerated with acceptable haematology toxicity, which was less expressive, if these programmes were used as a first line therapy. The FCM and RFC combinations increase the number of complete remissions significantly, especially in previously untreated patients.

0930**UNUSUAL ISOLATED RETINAL INFILTRATES AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION IN AN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENT, WHICH REGRESSED AFTER UNRELATED - DONOR BONE MARROW TRANSPLANTATION**

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We report for the first time an unusual case of isolated bilat-

eral infiltrates of retina in an adult patient with T-cell acute lymphoblastic leukemia (ALL) following autologous bone marrow transplantation (ABMT), which regressed after unrelated-donor bone marrow transplantation (URD-BMT). The 27-year-old woman was diagnosed in December 2001 with T-cell ALL (CD1, CD2, CD3, CD5, CD7 positive, CD4, CD8 negative). There was no evidence of primary central nervous system (CNS) or other extramedullary involvement. A short-lived complete remission (CR) of 4 months was obtained with combination induction - consolidation chemotherapy consisted of daunorubicine, vincristine, prednisone, L-Asp, cyclophosphamide and cytarabine. The patient received CNS prophylaxis including 8 lumbar punctions with the intrathecal administration of methotrexate, but without cerebrospinal irradiation. After 4 months an early relapse in bone marrow was diagnosed and the patient was successfully re-induced with the use of mitoxantron and cytarabine. She was assigned for URD-BMT, but to prevent next relapse ABMT was performed after CAV conditioning regimen in September 2002. 9 months later the patient developed impaired vision in both eyes, that was not accompanied by any signs of infection. Serologic tests for hepatotropic viruses (HBV, HCV), EBV and toxoplasmosis, as well as biomolecular evaluation (RT-PCR) of CMV were negative. Funduscopic examination documented by fundus photographs and echographic studies revealed bilateral retinal yellowish white tumoral infiltrates localized in the posterior poles of both eyes. In the left one pathologic masses were also observed in vitreous. At this time bone marrow aspirate showed no evidence of leukemia confirmed by immunophenotypic (CD7/CD1a+, CD3/Tdt+) analysis. No pathologic lesions could also be demonstrated by magnetic resonance imaging within CNS including optic nerves. Cerebrospinal fluid was clear. We were unable to determine the origin of the retinal infiltrates by histological biopsy, but taking the risk of imminent systemic relapse into consideration we performed BMT from unrelated mismatched in HLA-C donor in November 2003. Preparative regimen included cyclophosphamide, ATG and total body irradiation. The patient experienced moderate intestine and skin form of GVHD. Repeated funduscopic and echographic examination performed in March 2004 confirmed complete disappearance of the pathologic masses in both eyes. Bone marrow of the patient showed 100% donor chimerism evaluated by PCR-STR method. Recurrent episodes of severe haemorrhagic cystitis with impairment of the renal function caused by adenoviral infection were observed and the patient unfortunately died due to additional septic complications in May 2004. In this case clinical course and ophthalmological picture strongly suggest a diagnosis of unusual isolated leukemic infiltrates that developed in spite of high-dose conditioning regimen for ABMT. **Conclusions.** In case of ocular involvement URD-BMT should be taken into consideration after careful evaluation of the risk/benefit ratio in each individual patient.

0931

FACTOR V LEIDEN G1691A, METHYLENETETRAHYDROFOLATE REDUCTASE C677T AND PROTHROMBIN G20210A MUTATIONS WITH LIGHTCYCLER IN CEREBRAL ISCHEMIC INFARCTS

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Factor V Leiden (FVL) G1691A, prothrombin (P) G20210A and methylenetetrahydrofolate reductase (MTHFR) C677T mutations are most common hereditary thrombophilic causes. The objective of this study was to determine the role of the FVL G1691A, prothrombin PG20210A, and MTHFR C677T mutations in the pathogenesis of stroke and particularly in certain pathogenic subtypes of infarct. Peripheral blood specimens from 24 patients with cerebral infarcts and 53 controls were obtained by venepuncture of the antecubital vein. Whole blood was collected in EDTA and rapidly frozen at -80 C until the time of

DNA extraction. For factor V Leiden G1691A, prothrombin G20210A and MTHFR C677T genes polymorphism, a hybridization probe technique (sequence-specific fluorescence detection with oligonucleotide hybridization probes that are coupled to suitable fluorophores) was used. Polymerase chain reactions were performed in the LightCycler glass capillaries. The clinical data were expressed as means±SD where appropriate. The differences between the groups were assessed by using the student's t test and Yates chi-square tests. Among 24 cerebral infarct patients we found 9 (%15) heterozygote prothrombin G20210A point mutations, 2 (%8.3) heterozygote FVL G1691A mutations, 8 (%33.3) heterozygote MTHFR C677T mutations, and 2 (%8.3) homozygote MTHFR C677T mutations. In the control group 2 (%3.7) heterozygote FV Leiden mutations G1691A, 13 (%24.5) heterozygote, 2 (%3.7) homozygote MTHFR C677T mutations were detected. There was neither homozygote nor heterozygote P G20210A mutations in both groups (p<0.001).

0932

PROGNOSTIC IMPACT OF GENOTYPIC AND PHENOTYPIC MARKERS IN PLASMA CELL DISORDERS

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The diagnosis and staging of multiple myeloma (MM) has been based on morphological, clinical and radiological features. Recently, immunophenotypic and genetic analysis proved to be useful for prognostic evaluation and disease monitoring. Yet, the influence of each of the newly identified prognostic markers is still under investigation. In order to evaluate the relative impact of the 13q14 deletions, aneuploidy, percentage of S-phase cells and immunophenotype upon the clinical outcome of plasma cell disorders, we studied sixty two patients (24 men and 38 women), with a median age of 63 (39 to 83) years old, who were diagnosed with monoclonal gammopathy of undetermined significance (MGUS, 14), MM (47) and plasma cell leukemia (1). The Durie-Salmon stage, serum albumin, C-reactive protein, beta2 microglobulin and lactic dehydrogenase were assessed. To evaluate the nuclear ploidy, the DNA index was calculated in CD138 positive bone marrow cells by flow cytometry, using propidium iodide as a DNA stain; a DNA index higher than 1.10 was considered hyperdiploid and an index lower than 0.95 hypodiploid. The percentage of cells in S-phase was also evaluated by flow cytometry, with the ModFit software. The plasma cell immunophenotype was studied using monoclonal antibodies against CD38, CD138, CD19, CD56, CD45 and CD117 evaluated by flow cytometry). The 13q14 deletions (13q-) were identified by FISH in bone marrow samples enriched in plasma cells by immunomagnetic selection, with the D13S319 fluorescent probe. Finally, the clinical outcome was evaluated by the time to first treatment, time to progression and overall survival. The laboratorial parameters studied were similar in MGUS and MM, except for higher beta2 microglobulin levels in the last disorder (mean 2.0 and 3.7 mg/L respectively, p=0.002). MGUS cases showed systematically more than 3% of plasma cells with a normal phenotype (mean 19%); the immunophenotype of the plasma cells was characteristically aberrant in MM cases. The percentage of cells in S-phase was higher in MM as compared to MGUS (mean 2.4% and 1.0% respectively, p=0.001). The incidence of aneuploidy was analogous, with 79% of the MGUS and 71% of the MM samples being hyperdiploid (p=0.87). Hyperdiploid MM cases were clinically indolent when compared to diploid cases (time to first treatment 5.6 versus 2.2 months respectively, p=0.03). 13q14 deletions were identified in 17 of 29 tested patients (59%); none of the 4 MGUS patients presented the deletion (p=0.04). We found no statistical relationship between the 13q14- and other prognostic factors tested, including the DNA index. Patients with a documented 13q14- had a higher chance of needing treatment (100% versus 65% without the deletion,

p=0.05) and were treated earlier (time to first treatment 1.2 and 7.4 months respectively, p=0.02). These results show that 1) plasma cells phenotype might help to distinguish MGUS from MM; 2) hyperdiploid cases have an indolent clinical behavior, when compared with diploid ones; 3) the 13q14 deletion is the most important prognostic marker in this series and should be included in the initial evaluation of all MM patients.

0933

THE CLINICAL APPROACH TO PRIMARY MEDIASTINAL B-CELL LYMPHOMA

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Primary large cell lymphoma of the mediastinum (PMLCL), defined in the WHO classification, has been recognized as relatively heterogeneous regarding histological subtypes, clinical presentation and outcome. PMLCL is defined as a diffuse proliferation of large neoplastic lymphoid cell with primary involvement of the mediastinum, and of putative thymus B-cell origin. A retrospective review of all patients (pts) with PLCLM diagnosed in daily hospital during the 1998-2004, with help of Roche Lymphoma Data-Rolyda revealed 11 consecutive previously untreated pts. Most of pts 6/11 were males; average age of 33 years (22-55); all of the pts had B-symptoms and superior v.cava syndrome; the majority had disease in clinically advanced stage, according Ann Arbor staging system 9/11 were in IV st., 2/11 in III st.; performance status was 3 (confined to bed, chair for >50% of working hours); average value of LDH was 738U/L (148-1708); average diameter of mediastinal mass was 13,3 cm (8-16cm), Bone marrow examination showed no signs of involvement in all pts. Histology findings in tumor like necrosis were present in 7/11pts. Immunophenotype of neoplastic cells were consistent with B-cell i.e. CD19, CD20, CD79alfa, there was not expression of bcl-2 and bcl-6 protein. According to Age adjusted International Prognostic Index-I.P.I-Age all pts had 3 unfavorable parameters. The front-line therapy was Rituximab-CHOP regiment -9/11pts, CHOP 2/11pts, Radiotherapy 5/11pts, 1/11pt ARA-C/MtX/i.t. (after dissemination in CNS), 1/11pts autologous peripheral stem cell transplantation + Maintenance with Rituximab (375mg/m² at 4 monthly intervals during 12 month period), 2pts died because of aggressive lymphoma. Average overall survival (OS) is 24,1 months (m) (5-81). Average event free survival (EFS) is 23,5m. Average time to treatment failure (TTF) is 23,5m. Average duration of response (DoR) (9pts) is 25,8m. Average progression free survival (PFS) is 25,5m. **Conclusions.** The presence of adverse biological prognostic factors in PMBL is not specifically established in this lymphoma category. Our results confirm that PMLCL is an aggressive disease and that intensive chemotherapy regimens are therapeutic choice.

0934

ORAL LICHEN PLANUS-LIKE CHRONIC GRAFT VERSUS HOST DISEASE IN PATIENTS TREATED WITH REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION: SUCCESSFUL TREATMENT WITH DEXAMETHASONE ELIXIR AND TOPICAL TACROLIMUS

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Background. Nowadays an increasing number of patients are treated with a reduced intensity conditioning (RIC) regimen before allogeneic stem cell transplantation as compared with the 'classical' myeloablative conditioning. In this particular group of patients a specific type of lichen planus-like chronic graft versus host disease (GvHD) of the oral mucosa is seen. Furthermore, these patients present with painful oral mucosal lesions, changes in taste and appetite and painful swallowing, sometimes leading to significant weight loss. Also, lichen

planus-like lesions and complaints may arise in the genital area as well, as seen in the classical lichen planus syndrome. In most cases, no other organ systems are affected by GvHD. Therefore we treat these lesions locally with dexamethasone elixir and topical tacrolimus, avoiding systemically immunosuppressive medication. **Aim** Assessment of the effectiveness of treatment with dexamethasone elixir and topical tacrolimus of lichen planus-like GvHD (both orally and genital) in patients treated with RIC allogeneic stem cell transplantation. **Methods** - Data analysis of 7 patients, dealing with oral and/or genital lichen planus-like chronic GvHD, for the following factors: disease and type of conditioning regimen, earlier treatment (especially radiotherapy), time of onset, other treatments, changes in body-weight. - Histopathological review of biopsies. - Standardized interview for nutritional status, side effects of treatment and quality of life. **Treatment** - Dexamethasone elixir (0.11 mg/mL, 6 dd 10 cc) + Fluconazole 1dd 50mg. - Tacrolimus 0,1% in 20% hypromellose, 3 dd application + Valaciclovir 2dd 500mg. **Results.** Five patients with multiple myeloma, one patient with acute myeloid leukemia and one patient with Non-Hodgkin lymphoma were evaluated. 5/7 received low dose total body irradiation (2 Gy) as part of their conditioning regimen. 4/7 patients were treated with an autologous stem cell transplantation in the past. 5/7 patients had mycophenolate mofetil and 2/7 methotrexate as GvHD prophylaxis after transplantation. After a mean of 148 (82-294) days the clinical diagnose of lichen planus-like GvHD was made, which was histopathological proven with biopsies in 5 patients (in two patients no biopsies were taken during start of treatment). Viral cultures were all negative. All patients started with dexamethasone elixir, 6/7 used topical tacrolimus as well. During treatment 3/7 received immunosuppressive therapy as well because of progressive, extended GvHD (skin 2/7 and lung 1/7). In three tested patients, there were no detectable blood levels of tacrolimus. All patients showed moderate to good response to the initial treatment, with remarkable fewer complaints, better taste and appetite. Bodyweight increased in four patients with several kilos after starting treatment. Treatment had to be continued for several months. No serious side effects were seen, except in two patients developing oral candidiasis. **Summary/conclusions** Lichen planus-like chronic GvHD is increasingly encountered after RIC allogeneic stem cell transplantation. Treatment with both dexamethasone elixir and topical tacrolimus is successful without serious side effects. Attention to patient's complaints about oral and/or genital changes is important. We are now prospectively investigating these results and experiences.

0935

PRELIMINARY EFFICACY ANALYSIS IN A GROUP OF RELAPSED REFRACTORY MYELOMA RECEIVING BORTEZOMIB ALONE

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Background. Bortezomib, a boronic acid dipeptide is a proteasome inhibitor that has been shown to be effective in the therapy of refractory multiple myeloma. **Aims.** To evaluate the efficacy of Bortezomib in a group of refractory relapsed myeloma treated in our Center from December 03 to February 05 Patients and **Methods.** We enrolled 13 patients with relapsed multiple myeloma that were refractory to the last therapy, all of them had received previously 2-4 different schedules (VBCMP/VBAD, VAD, PBSCT, TACYDEX, radiotherapy). They received standard dose of Bortezomib (1,3 mg/m² twice weekly two weeks on days 1,4,8,11 in a 21-day cycle). The response was evaluated according to the criteria of the Consensus Report Scientific Advisor International Myeloma Foundation 2003. CR: without symptoms, no monoclonal component (MC) detected by immunofixation electrophoresis (IFE) (Sebia standardized procedure), PR: reduction of MC >50%, Minimal Response (MR): reduction of MC 25-50%, Clinical response (Clin R): no clinical symptoms, and Non-response (NR). Adverse effects were registered. **Results.** 13 patients (5 females, 8 males), mean age 60

years (35-74), only four patients were over 65 years. Type IgG: 3 II-A, 1 III-A; IgA: 3 II-A, 2 III-A; BJ: 2 II-A, 2 III-A. Different schedules administrated previously: 2 schedules (4), 3 (8), 4 (1). For the analysis patients have been classified in two groups. Group A (8): minimal follow up available since 1st dose of Bortezomib 6 months 8 (see Table). Group B (5): minimal follow up available since 1st dose of Bortezomib less than 6 months. Response: Clin R 2 patients, MR 2, non-available 1; 2 cycles: 2 patients, 1 cycle: 3; 2 months of follow-up in all of them; overall survival 45,47,102,66,121 months. Adverse events: Thrombocytopenia (grade 3: 5, grade 1: 1) 46.1%, fatigue 5 (38.5%), peripheral neuropathy 4 (30.8%), constipation 3 (23%), diarrhea 2 (15.4%), ZHV 2 (15.4%), pneumonia 2 (15.4%), pyrexia 1 (7.8%), grade 3 leucopenia 1 (7.8%). In 2 patients (15.4%) the therapy was disrupted by toxicity, in 8 patients (61.5%) the cycles were delayed. **Conclusions.** A high rate of response (69%) was obtained in refractory multiple myeloma patients treated with Bortezomib, CR negative IFE 62.5%. No severe toxicity has been observed with an incidence of reversible haematological side effects in 46.1% and mild non-haematological side effects in 92%.

Table.

A. group	Response	Cycles	Consolidation	Follow-up since 1 st dose (mo)	Actual status	OS (mo)
1	CR	**7	Yes	14	Alive, CR	120
2	CR	3	Not#	8	Alive, CR	64
3	CR	3	Not#	8	Alive, CR	47
4	CR	2	Yes	8	Alive, CR	18
5	MR	5	-	5	Alive, MR	92
6	CR	5	Yes	4	Alive, CR	68
7	NA	1	-	1	Died	80
8	NA	1	-	1	Died	42

Table. * IFE negative. **3 cycles 50% dose. # Side events

0936**CHRONIC LYMPHOBLASTIC LEUKEMIAS IN UZBEKISTAN: 10 YEAR RETROSPECTIVE STUDY**

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Background. B-cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia in Western countries and accounting about 30% of all cases. In common structure of main type of leukemia in Uzbekistan CLL consists about 10-11%. The aim of study was to analyze epidemiological and initial clinical characteristics of 154 patients with CLL, primary diagnosed and treated at our Institute of Hematology since Jan 1990 through Jan 2000. **Results.** There were 100 (60,31%) males with a median age of 63,0±1,42 yrs (range 41-85) and 54 (39,69%) females with a median age of 64,8±1,99 yrs (33-91). CLL were revealed frequently among men, female: male ratio was 1:1,6. The urban population was affected twice more than rural, probably due to environmental exposure that act as risk factors. Result of our studies has shown that among native inhabitants of the republic (the Uzbeks, Tadjiks, and Kazakhs) CLL met in 11%, in Slavonic population-30%, in Jews-54% in common structure of all cases. There were revealed 4 cases of inherited predisposition to CLL. Clinical features were characterized in

139 (90%) cases by moderate lymphadenopathy, in 69 (45%) cases by splenomegaly and hepatomegaly, in 65 (42%) by asthenia, infections complication in 67 (43%) cases. Hemorrhagic syndrome was revealed in 29 (19%) patients, moderate anemia in 55 (36%) patients, median hemoglobin value was 94,16±2,48 g/L, median RBC count-3,49±0,08x10¹²/L, median WBC count-112,14±10,57x10⁹/L and median platelet count-113,89±7,39x10⁹/L. **Conclusions.** This retrospective study revealed correlation between incidence CLL and age, sex, ethnic distribution and will be useful in the creation of the national register of haemoblastosis. Further studies in potential therapeutic strategy in CLL are warranted.

This work was supported by research grant of Ministry of Health, of Uzbekistan P.14.3.22

0937**DRUG SENSITIVITY TESTING EX VIVO IN PROGNOSIS OF 5 YEARS DFS SURVIVAL IN CHILDHOOD ALL**

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Background. The measurement of cellular drug resistance may provide clinically relevant information for prognosis the efficacy of chemotherapy. **Aims.** of this study was to evaluate the correlation between blast cells sensitivity profile to chemotherapy ex vivo (according to range scale) and the probabilities of 5-year disease-free survival. **Methods.** The blast cells sensitivity to chemotherapy was examined in MTT-assay. The material of the study-bone marrow from 136 children with newly diagnosed ALL. **Results.** For each drug (Pred, Vcr, L-Asp - PVA) LC50 was determined. The degree of cytotoxicity was estimated in ranks scale: each step of a drug dilution (from greatest to lowest) was corresponded to rank (from 1 up to 7). The high sensitivity to a drug - LC50 value from 1 to 4 rank; low sensitivity - LC50 value from 5 to 7 rank. The probability of 5-year disease-free survival was significantly higher in patients with high sensitivity (1-4 ranks) to Vcr (p=0.043), Pred (p=0.036), L-Asp (p=0.042), in comparison with the patients with low sensitivity (5-7 ranks). The probability of 5-year disease-free survival in patients with high simultaneous sensitivity to 2 of 3 drugs (PVA) was 0,86 versus 0,52 in patients with low simultaneous sensitivity to three of these drugs (p=0.037). The leukocytosis at initial diagnosis doesn't influence the disease-free survival in groups with different sensitivity to chemotherapy in MTT-assay (p=0.22). **Conclusions.** the initial sensitivity of leukemic cells to Prednisolone, Vincristine, and L-Asparaginase is very significant in prognosis of 5 years disease-free survival in pediatric ALL. High simultaneous sensitivity to at least 2 of 3 above mentioned drugs appears to be good prognostic factor.

0938**CLINICAL SIGNIFICANCE OF SPONTANEOUS AND INDUCED BLAST CELLS APOPTOSIS IN THE PROGNOSIS OF EFFICACY OF THERAPY IN CHILDHOOD ALL**

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Aims. to estimate the value of the correlation of the blast cells ability to spontaneous apoptosis and their sensitivity to induced apoptosis ex vivo in the prognosis of the probabilities of 5-year disease-free survival. **Methods.** the material of the study-bone marrow from 136 children with newly diagnosed ALL. The level of spontaneous apoptosis was estimated by flow cytometry using Apo-Direct kit. The blast cells sensitivity to induced apoptosis was examined in MTT-assay. **Results.** For each drug (Vcr, Pred, L-asp - PVA) LC50 was determined. The degree of cyto-

toxicity was estimated in ranks scale. Blast cells sensitivity to apoptosis, induced by PVA has the most clinical value in the prognosis of efficacy of inductive therapy. The blast cells from resistant (on 33/36 day) patients had low sensitivity to these drugs. There is positive correlation between the ability of blast cells to spontaneous and induced apoptosis. The level of spontaneous apoptosis of blast cells from patients in remission on 33/36 day of therapy was higher in comparison with the resistant patients (4.7% and 2.1% accordingly). The probability of 5-year disease-free survival in patients with high simultaneous sensitivity to 2 of 3 drugs (PVA) was 0.86 versus 0.52 in patients with low simultaneous sensitivity to three of these drugs (p=0.037). The level of spontaneous apoptosis of blast cells before treatment from patients with high sensitivity to PVA was 4.8% versus 1.2% in patients with low sensitivity. The blast cells sensitivity to apoptosis, induced by PVA, correlates with their ability to spontaneous apoptosis ex vivo. In the prognosis of 5-year disease-free survival the level of blast cells spontaneous apoptosis before treatment has the clinical value. The probability of 5-year disease-free survival in patients with the level of spontaneous apoptosis >2% was 0.88 versus 0.58 in patients with the level of spontaneous apoptosis <2% (p=0.008). **Conclusions.** the initial ability of leukemic cells to spontaneous apoptosis and their sensitivity to Pred, Vcr and L-asf ex vivo is significant in prognosis of 5 years disease-free survival in pediatric ALL. Spontaneous apoptosis level of blast cells >2% and high simultaneous initial sensitivity to at least 2 of 3 drugs appears to be good prognostic factor.

0939

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MACIEL HOSPITAL, URUGUAY. SINGLE CENTER STUDY

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Allogeneic stem cell transplantation (AlloSCT) is a well established procedure for the treatment of hematological and non-hematological diseases. In Uruguay, AlloSCT is a regulated procedure and fully financed by a government agency ('Fondo Nacional de Recursos'). We describe the activity in AlloSCT in the 'Servicio de Hematología del Hospital Maciel', a public hospital in Montevideo, Uruguay. The first autotransplant was performed in 1995 and the first AlloSCT in 1997. Since 2001 reduced intensity conditioning was incorporated; the unrelated donor AlloSCT program started in 2003. The aim of this study was to describe the experience with AlloSCT from 1997 to 2004 and to evaluate the feasibility of this procedure in a Public Hospital of an emergent country. Thirty-seven patients [20 females, 17 males, median age of 28 years (15 - 47)] underwent AlloSCT; 30 conventional (28 Busulfan/Cyclophosphamide, 2 Cyclophosphamide/antithymocyte globuline) and 7 after reduced-intensity conditioning (Busulfan/Fludarabine/antithymocyte globuline). Donors were HLA identical siblings in 33 and HLA matched unrelated volunteers in 4 cases. Diagnoses were: acute myeloid leukemia (n=12), myelodysplastic syndrome (n=4), acute lymphoblastic leukemia (n=6), chronic myeloid leukemia (n=9), chronic lymphocytic leukemia (n=1), Hodgkin's lymphoma (n=2) and aplastic anemia (n=3). Stem cell source was peripheral blood (n=33), bone marrow (n=2) or both (n=2). Graft-versus-host disease (GVHD) prophylaxis consisted in the association of Cyclosporine and short-course methotrexate. Date of last follow-up was February 7th, 2005. With a median follow up of 5.6 months (0.2-87.2) from transplantation, 16 (43%) patients are alive (median follow up for patients alive was 21.1 months). Ten patients (27%) developed acute GVHD and 8 (35% of 23 evaluable patients) developed chronic GVHD (6 limited and 2 extensive). Overall survival at 6 months from

transplantation was 58% (95%CI, 42-74) and at 2 years was 38% (95%CI, 22-54). Cumulative incidence of relapse at 6 months and 2 years was 18% (95%CI, 9-37) and 28% (95%CI, 16-48) respectively. Cumulative incidence of chronic GVHD (from day 100 post-transplantation) at 1 year was 31% (95%CI, 17-57). Cumulative incidence of non-relapse mortality at 100 days and 1 year was 28% (95%CI, 16-47) and 34% (95%CI, 22-55) respectively. Stem cell transplants were performed in the context of a program of increasing complexity during a ten year period. The data supports the feasibility of performing AlloSCT in a public hospital in Uruguay. We highlight the operation of the Uruguayan financial system for stem cell transplants. This system allows unrestricted access to this technique by Uruguayan citizens who may benefit from a standard prescription of an AlloSCT.

0940

A COMPARISON OF 3 STAGING SYSTEMS FOR THE OUTCOME FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN PATIENTS WITH MULTIPLE MYELOMA (MM)

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Background. In 1975, Durie and Salmon (DS) developed a staging system that has remained, for over 25 years, the gold standard for stratification of MM patients. However, the system does not contain beta 2 microglobulin (B2M) widely recognized as the most useful prognostic factor. Recently SWOG and ISS staging systems utilizing B2M have been proposed. **Aims.** We aimed to evaluate the predictability of the stages assessed at the time of ASCT by DS, SWOG, or ISS for the outcome of ASCT in patients with MM. **Methods.** Between November 1996 and July 2004, a total of 55 patients with MM who were treated by ASCT at Asan Medical Center were available for analysis. **Results.** The distribution of patients' stage at ASCT by 3 staging systems was as Table 1. There was a strong correlation between SWOG and ISS stages at ASCT (r=0.72, P<0.01). DS stage at ASCT showed no significant correlation with either SWOG or ISS at ASCT. With a median follow-up of 13 months, median overall survival (OS) and event-free survival (EFS) were 36 months and 11 months, respectively. Stages at diagnosis by the 3 systems were not predictive for either OS or EFS following ASCT. The median survival of each stage group according to the 3 staging systems at ASCT was as Table 2. **Summary/conclusion:** In this small-sized single center study from Korea, the SWOG stage at ASCT is the most significant predictor of survival following ASCT in patients with MM.

Table 1, 2

Stage	I	II	III	IV
DS	4	3	49	-
SWOG	15	32	7	1
ISS	30	17	8	-

Stage	EFS (median, months)					OS (median, months)				
	I	II	III	IV	P	I	II	III	IV	P
DS	NR	12	10	-	0.44	NR	25	30	-	0.27
SWOG	15	9	10	2	0.06	NR	26	16	8	0.01
ISS	9	11	8	-	0.62	NR	26	14	-	0.18

NR: not reached

0941**LONG TERM FOLLOW UP AFTER TREATMENT OF HAIRY CELL LEUKEMIA WITH 2-CDA**M. Ramzi¹, M. Hagshenas²¹Shiraz University of Medical Sciences, SHIRAZ, Iran; ²Shiraz University, SHIRAZ, Iran

Background. Hairy cell leukemia (HCL) is a chronic B cell disorder that follows an indolent but progressive course. The ability of new purine analogue deoxyadenosine to induce long lasting complete remission in patients with hairy cell leukemia has revolutionized the treatment of this disease. **Aims.** We report the long term outcomes of patients with HCL treated in Shiraz, in south of Iran, with this drug. **Method:** Between October 1993 till April 2004, 79 patients with classic symptomatic hairy cell leukemia were treated with 2-cholorodeoxy adenosine (2-CdA) with dose of 0.1 mg/kg of body weight per day by continuous intravenous infusion for 7 days. **Results.** 69 (87.3) patients achieved an initial complete response and 10 (12.6) a partial response with an overall median duration of response follow up of 78 months. Five patients had relapsed at a median of 43 months. All of 5 patients after relapse treated with second courses of 2-CdA, 4 (80%) achieved second complete responses and one (20%) partial response. In our study we had not any case of second malignancy after treatment with 2-CdA with median follow up of 82.5 months. **Conclusions.** This study confirms single course of 2-CdA induced long lasting complete response in the vast majority of patients. Relapse rate for complete responders were low. Patients who relapse can be successfully retreated with this drug, so we conclude 2-CdA had high efficacy and a favorable acute and long term toxicity profile in our patients in south of Iran, without any increase in risk of second malignancy.

0942**CYTOGENETIC CHARACTERIZATION OF 'DE NOVO' ACUTE LEUKEMIAS - 8 YEARS OF EXPERIENCE-A RETROSPECTIVE ANALYSIS (1997-2004)**

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Background. It is today of unquestionable importance the cytogenetic characterization of *de novo* acute leukemias- either myeloid or lymphoblastic-, having a well defined prognostic value that allows patients stratification on different risk groups and therapeutic approaches. **Aims/Methods.** A retrospective analysis was made, concerning all patients admitted on our Unit, from January 1st 1997 to December 31st 2004, with diagnosis of '*de novo*' acute leukemia. Important parameters were registered: sex, age, histological subtype, previous hematological disease, conventional karyotype and molecular biology (RT-PCR or FISH). **Results.** 245 were (118 males, 117 females); the annual average incidence of acute leukemia was of 29,93 cases per year. The average age was of 44,17 years (between 15 and 88) for men and 45,83 years (between 12 and 92) for women. 192 patients had acute myelogenous leukemia (AML) with the following FAB distribution: M0=1; M1=7; M2= 28; M3=29; M4=45; M4Eo=28; M5=34; M6=9; M7=5; M0/M1=1; M1/M7=1; M2/M3=3; M2/M4=1; 51 patients had acute lymphoblastic leukemia (ALL): B lineage=36; T lineage=13; biphenotypic=2; 1 acute leukemia was not characterized (patient died on the day of admittance) and another was classified as a granulocytic sarcoma (involvement of central nervous system without bone marrow infiltration). 19,59% (n=48, predominantly AML) had previous hematological disease: myelodysplasia (32), myeloproliferative disease (10), mixed syndrome (2), aplastic anemia (1), non-Hodgkin lymphoma (1). 151 of 192 AML had cytogenetical characterization: normal 56 (+PML/RARalfa=3; +AML/ETO=1; +inv (16)=1; +FLT3=1); complex 23; without growth 21 (+PML/RARalfa=4; +AML/ETO=1; +BCR/ABL=1); t(15;17) 15 (PML/RARalfa=

BCR1=3; BCR2=1; BCR3=6); t(8;21) =6; t(9;22)=5; +8 =4; del 11=3; del7=3; inv (16)=2; other alterations 13. 45 of 51 ALL had cytogenetical characterization: ALL-B (30): complex=11; no growth=4; t(9;22)=9; normal=5 (+BCR/ABL=1; + t(8;14)=1); t(8;14), t(8;22)=1. ALL-T (13): normal=8; complex=3; no growth=2; biphenotypic ALL (2): normal=1; t(9;22)=1. **Conclusions.** According to literature data, on our group, 52,94% of ALL (27/51) and 52,6% of AML (101/192) presented as intermediate or poor prognostic leukemias. 60% of secondary leukemias presented with complex and/or poor prognosis alterations.

0943**FIP1L1-PDGFRFA + EOSINOPHILIC LEUKEMIA - A NEW ENTITY WITHIN HYPEREOSINOPHILIC SYNDROME**W. Prejzner, D. Szatkowski, A. Piekarska, A. Hellmann
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Idiopathic hypereosinophilic syndrome (HES) is a heterogeneous group of hematological disorders, characterized by eosinophilia (more than 1,5 G/L), exclusion of reactive eosinophilia and evidence of end organ damage. 'Nonreactive' eosinophilia is considered clonal in the presence of either cytogenetic or molecular abnormalities or morphologic evidence of myeloid neoplasm. Recently a novel tyrosine kinase generated from fusion of FIP1L1 gene and PDGFRFA was identified in about 14% patients with HES. The patients with this fusion gene response to kinase tyrosine inhibitor-imatinib. We present 20 patients (male 12 males, 8 females) with diagnosed HES currently under observation at the Department of Hematology Medical University in Gdansk. All patients at the diagnosis had eosinophilia more than 1,5 G/L and reactive causes of eosinophilia were excluded. In three of them the evidence of cardiac injury (valvular insufficiency, endomyocardial fibrosis) was observed. The FIP1L1 PDGFRFA fusion gene - performed at Medical University in Gdansk - was found in 2 patients (10%), but in all who had cardiac injury. One patient with FIP1L1 PDGFRFA fusion gene presented neurological changes and because of aggressive clinical course and resistance to corticosteroids and chemotherapy (it was before imatinib era) sibling bone marrow transplantation was performed. The second FIP1L1-PDGFRFA + patient was treated successfully with imatinib 100 mg a day and 100mg a week as maintenance therapy and he achieved molecular remission. Evaluation of clinical and laboratory parameters showed that patients with FIP1L1 PDGFRFA gene have higher number of eosinophils at diagnosis and are resistant to first line therapy

0944**MATRIX METALLOPROTEINASE-9, TISSUE INHIBITOR OF METALLOPROTEINASE-1, AND TRANSFORMING GROWTH FACTOR-BETA IN CHRONIC MYELOID LEUKEMIA. CLINICAL AND PROGNOSTIC SIGNIFICANCE**S. Muawia¹, S. Muawia¹, M. El Sorady², H. Nunu³¹Genetic Engineering & Biotechnology, MINUFIYA, Egypt; ²Haematology Unit, ALEXANDRIA, Egypt; ³Biochemistry Department, ALEXANDRIA, Egypt

Background. Matrix metalloproteinase-9 (MMP-9) is an enzyme which plays an important role in Tissue remodeling and tumor invasion by digestion of extracellular matrix barriers. In Leukemia, there is an excessive egress of the leukemic cells from the bone marrow into The peripheral blood, followed by infiltration of organs, skin and mucous membranes. These movement include the necessity of the cells to cross matrix barriers which is catalyze By the matrix digesting properties of MMPs. Aim of the present work was to study Serum levels of MMP-9 and its naturally occurring inhibitor, TIMP-1 in chronic myeloid Leukemia. We also measured serum TGF-Beta in an attempt to determine any possible Correlations with clinical or hematological findings, phase of the disease or survival. **Methods.** We Conducted this study on 30 patients with CML (16 in the chronic phase, 3 in the accelerated Phase and 11 in the

blast crises phase). All were Philadelphia chromosome positive. Determination of the total MMP-9 and TIMP-1 was carried out using the quantitative Sandwich enzyme immunoassay. Also the serum TGF- β enzyme linked immunosorbant Assay. *Results.* We found that the mean serum MMP-9, TIMP-1 and TGF- β levels in our CML Patients (2406.3+244.3 ng/mL and 2015.26+862.94 pg/mL respectively) significantly Exceeded those of the normal controls. (346.1+196.96 ng/mL, 279.6+139.44 ng/mL and 249.4+49.92 pg/mL respectively) Serum TIMP-1 levels were significantly higher in blast crises and accelerated phase patients than in chronic phase patients ($P<0.01$). They were higher in thrombocytopenia patients ($P<0.01$) and in patients with lower hemoglobin levels and presenting with lymphadenopathy ($P<0.05$). Also, higher values correlated with poor one year survival ($P0.05$) *Conclusions.* we found that serum TGF- β levels correlated with TIMP-1 levels in our patients and with lower serum TGF- β levels had a better one year Survival ($P<0.05$).

0945

THE RETROSPECTIVE ANALYSIS OF MULTIPLE MYELOMA: EXPERIENCE FROM A SINGLE CENTER

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Background. Multiple myeloma (MM) is characterized by plasma cell infiltration in the bone marrow, suppression of normal haematopoiesis, destruction of bone and renal failure. *Aims.* the aims of this study is to assess the use of melphalan/prednisone (MP), VMCP and VAD regimen as the first-line therapy in patients (pts) with MM. Our study evaluated 268 pts who were treated for MM in the period 1989-2004 in Oran. The age range was 31 to 85 years (Me 60). 65% of pts had a monoclonal IgG component, 20% had IgA, 14% had only light chains in urine and 1% was non secretory myeloma. 98% of pts were in stage III, 1,5% in stage II, and 1% in stage I. 31% of pts with renal failure were in stage III. 41% pts received MP, 40% VMCP, and 19% VAD combination as first-line chemotherapy. *Results.* MP regimen: plateau-phase was achieved in 45% of pts and the overall survival was 37 months, VMCP regimen: plateau-phase was achieved in 54% of pts and overall survival was 41,5 months, VAD regimen: plateau-phase was achieved in 64% of pts and overall survival was 48 months. *Conclusions.* Our results confirm that MP AND VMCP regimen presents standard initial chemotherapy of the pts with MM in stage I and II. The VAD regimen is the treatment of choice as a first-line therapy for MM in stage III and are the rapidity of response, and the lack of myelotoxicity. Therefore, this regimen is superior to VMCP chemotherapy in pts with pancytopenia and renal failure.

0946

SUCCESSFUL MOBILIZATION OF HEMATOPOETIC PROGENITOR CELLS FOR AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION WITH ADMINISTRATION OF G-CSF AND CHEMOTHERAPY IN PATIENTS WITH LYMPHOMA AND MYELOMA

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The administration of a combination of chemotherapy and cytokines G-CSF is associated with a significantly increased efficacy of stem cell mobilization compared with either modality alone. The aim of this study was to evaluate the efficacy of G-CSF preceded by chemotherapy (cyclophosphamide 4g/m² for 1 dose) for hematopoietic progenitor cell mobilization for lymphoma and myeloma patients. We started G-CSF as a fixed dose 480MU SQ every day as soon as the leukocyte counts began to rise after chemotherapy induced myelosuppression.

Leukapheresis was commenced at the time when leukocyte count rose up to 1000/uL, and repeated for 2-4 consecutive days until target number of CD34+ cell, at least 2x10⁶/kg was collected. Sixteen patients (male to female, 7:9, age range 21-65, lymphoma 9, myeloma 7) underwent a total of 33 courses of leukapheresis for hematopoietic progenitor cell collection prior to autologous transplantation from April 2002 through May 2004. The target amount of marrow was harvest in all patients. All the patients achieved good engraftment after autologous transplantation. The mean days required for WBC count to be over 1000/uL was 8-16 days. Patient's age, sex, underlying malignancy, exposure to chemotherapy before mobilization did not show any statistically significant correlation. We can conclude that chemotherapy followed by G-CSF administration is an effective way for mobilization of hematopoietic progenitor cell and verified itself as a good mobilization method.

0947

RENAL FAILURE IN MULTIPLE MYELOMA-REVERSIBILITY AND PROGNOSTIC SIGNIFICANCE: A 20 -YEAR STUDY IN A SINGLE HEMATOLOGICAL UNIVERSITY CENTRE

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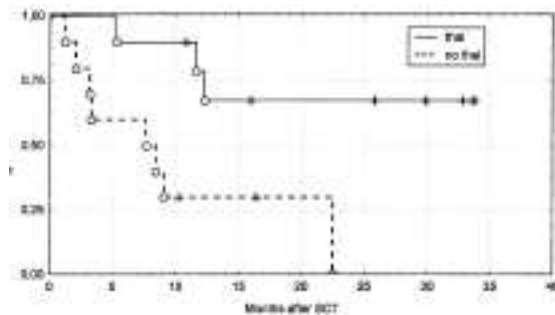
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Background. Renal Failure (RF) in Multiple Myeloma (MM) can alter the clinical course, influence the therapeutic response and determine the prognosis and survival of the patients. *AIM:* The study analyses the frequency and characteristic features of RF in MM, the prognostic factors for the response to therapy and the survival of the patients. *Methods.* For the period 1984-2004, 341 previously untreated myeloma patients were followed. RF was found in 131 (38,4%) of them. RF was divided according to the degree-initial, marked and advanced; and according to the therapeutic response-reversible, partially reversible, non-reversible or progressive. The statistical methods consisted of Kaplan-Meier survival curves, log rank test, univariate and Cox step-down multivariate analyses. The basic parameters of the disease and the renal function were analyzed in a prognostic aspect. *Results.* Patients with RF had median survival (MS) of 21 months and those without RF - 40 months (log rank = 48,89; $P<0,001$). In 70,2% RF was initial, low-grade and they had MS of 26 months. In 22,9% RF was marked with MS 16 months and in 6,9% - advanced with MS of 9 months. In 41,2% RF was reversible with MS 31 months, in 32,1% partially reversible with MS 21 months and in 26,7% it was non-reversible or progressive with MS 7 months. A statistically significant association was found between the degree of RF and its reversibility ($\chi^2=29,36$, $P<0,001$). A significant positive correlation between the hypercalcaemia and RF was found ($r=+0,58$) and a parallel dynamics depending on the treatment. In 22,9% of the patients with RF the presenting symptom were isolated renal disturbances and a cause for making the diagnosis. Patients with RF had lower therapeutic response (33% vs 62%) and in the group with non-reversible RF there was no response to therapy. In the univariate analysis the parameters that proved to be statistically significant were marked anaemia; low platelet count; low albumin level; hypercalcaemia; moderate proteinuria (1-4g/24h) which was tubular or tubular non selective immunoelectrophoretic type; age >65; male sex; light chain immunological variant, previous renal diseases. In the step-down multivariate Cox analysis independent prognostic factors for the outcome of the RF and survival of the patients were the creatinine level > 353 μ mol/L, response to therapy, light chain immunological variant and LDH twice above the normal level. *Conclusions.* RF in MM is predominantly initial, low grade and reversible in outcome in about half of the patients. The contemporary treatment of the patients with MM, combined with the specific schedules of prophylaxis and treatment of RF can normalize renal function and neutralize the difference in survival.

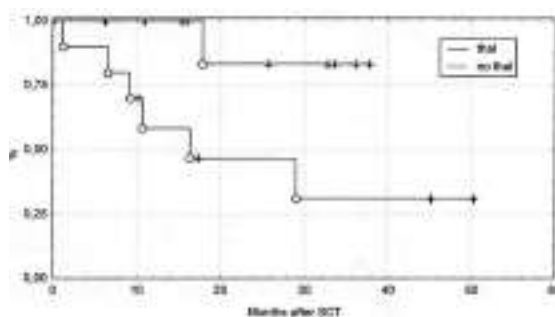
0948

MAINTENANCE WITH LOW DOSE THALIDOMIDE AFTER AUTO-SCT IN MULTIPLE MYELOMAV. Mettievier¹, L. Pezzullo², S. Rocco², O. Finizio², L. Bene², C. De Rosa²¹A.O.R.N. 'A. Cardarelli', NAPLES, Italy; ²A.O.R.N. 'A. Cardarelli', NAPLES, Italy

High dose therapy with single or double transplantation (auto-SCT) has improved prognosis of multiple myeloma (MM). New drugs are promising in upfront therapy while the role of maintenance is still debated. Thalidomide (thal) is an active drug in the treatment of myeloma, and is been investigated as first line therapy. It could be useful in the control of minimal residual disease. We used thal as maintenance after autologous transplantation (single or double) and compare the outcome with other maintenance or none.



Grafic 1. Progression free survival (PFS) (Kaplan-Meier).
○ Relapse + No relapse (p:0.01).



Grafic 2. Overall Survival (OS) (Kaplan-Meier).
○ Dead + Alive (p:0.6).

From January 2001 to January 2005 20 patients (10 males and 10 females) with MM have been treated in our institution. Median age was 58 years (range 51-72). 9 were IgG, 6 IgA, 2 light chains and 2 plasma-cell leukaemia. Treatment was 4 cycles of VAD regimen followed by auto-SCT. 10/20 performed double auto-SCT. 3 months after SCT, 10 patients (5 single and 5 double SCT) began thal 50 mg/die as maintenance therapy. 10 patients (5 single and 5 double SCT) received IFN-g (3/10), dexamethasone (3/10) or no therapy (4/10). The 2 groups were regarding the type of myeloma: 5 IgG, 2 IgA, 2 light chains and 1 plasma-cell leukaemia in the thal group; 4 IgG, 4 IgA, 1 IgD and 1 plasma-cell leukaemia in the other. Response to SCT: 2 CR and 8 PR in the thal group; 5 CR, 4 PR and 1 NR in the other. In the thal group 3/10 patient relapsed. Median follow up from the beginning of maintenance therapy was 21 months (range 7-35) with 7/10 patients in CR or stable disease, with a progression free survival (PFS) and overall survival (OS) projected at 35 months of 70% and 83% respectively. In the other group, 8/10 patients relapsed. Median follow up was 30 months (range 5-50) with a median PFS of 8 months and OS projected at 50 months of 30%. The difference between the 2 groups is statistically significant

for PFS (p: 0,01), and not significant for OS (p: 0,6) even if difference (83% vs. 30%) appears clear. (Graph. 1-2) Thal was administered for a median period of 6 months, being neurological toxicity the main reason of suspension (3/10 patients). Neurological toxicity grade I-III was present in 65% of patients, while haematological toxicity grade I occur in 55% of patients. In conclusion, in a small number of patients low dose thal as maintenance after auto-SCT resulted in an improved PFS and OS when compared with other or none maintenance, with acceptable toxicity. Further studies in larger cohorts and randomized trials are needed to confirm this experience.

0949

MAY FLT3 AND TELOMERASE (hTERT) GENES BE USED AS ADDITIONAL MOLECULAR MARKERS IN ACUTE PROMYELOCYTIC LEUKEMIA (APL) WITH MULTIPLE RELAPSES?

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FLT3 internal tandem duplication (ITD) is detected in 20-40% of APL patients with poor clinical outcome. hTERT, the catalytic subunit of telomerase, serves to maintain telomere length. Its role in hematologic malignancies is still controversial. The present study, performed in three APL patients with multiple relapses, was aimed at detecting any possible correlation between FLT3 ITD and FLT3 expression and at establishing whether FLT3 and hTERT genes might be used as additional prognostic molecular markers in these patients. Relative quantifications of FLT3 and hTERT transcripts were performed by real-time PCR using SybrGreen I as double-stranded DNA binding fluorescent dye. For FLT3 we performed a quantitative RT-PCR assay using total RNA from a normal subject for calibration and the DDCT method for quantification. For hTERT serial dilution of total RNA from K562 cells was used to construct a standard curve for real-time quantification. hTERT expression levels were normalized to ABL mRNA expression and calibrated on K562. Specificity of hTERT amplification was demonstrated by dissociation curve analysis. Two of the three presented with hyper-leucocytosis and on RT-PCR showed a PML-RARA fusion, an ITD of FLT3, and a high expression of both FLT3 and hTERT genes. The first patient achieved a complete haematological remission (CHR) but not a molecular remission (MR). During CHR he showed the disappearance of FLT3 ITD, a low-medium FLT3 and a high hTERT expression. He experienced two relapses marked by the reappearance of FLT3 ITD and a high expression of either the FLT3 gene (first relapse) or the hTERT gene (second relapse). The second patient achieved a CHR and a MR, during which he showed FLT3 ITD disappearance, a normal FLT3 and a high hTERT expression. The first relapse was marked by an increase in the expression of FLT3 which did not carry any ITD and by a high hTERT expression. Since then she remained PML-RARA positive with high FLT3 and hTERT levels. The third patient was treated in another Centre and reached our observation in CHR and in MR, which lasted three months. He experienced two relapses both of which were associated with the development of a FLT3 ITD and by a high FLT3 expression, always preceded by a progressive rise in hTERT expression. In conclusion, the worth of monitoring FLT3 expression by real-time PCR in APL patients is suggested by the following data: i) FLT3 ITD, which identifies APL patients with a high relapse risk, is not the only mechanism through which the leukemic cell increases its FLT3 expression, ii) there is no correlation between the levels of FLT3 expression and FLT3 ITD development, iii) the increase of FLT3 expression always marks clinical relapse. In addition, hTERT might be a very effective marker to predict relapse since its expression was constantly elevated either during MR as occurred in our second patient despite her negative PML-RARA results and FLT3 ITD negativity or prior to clinical relapse when FLT3 expression was not yet increased. These data should be confirmed with additional patients.

0950**EXPANSION OF MESENCHYMAL STEM CELLS (MSCS) AND CO-TRANSPLANTATION OF AUTOLOGOUS STEM CELLS AND EXPANDED MSCS IN HEMATOLOGIC MALIGNANCIES**

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Background. Mesenchymal stem cells (MSC) are multipotent, high potential to proliferate and self-renew and believed to facilitate the engraftment of hematopoietic stem cells (HSC) when transplanted simultaneously in animal studies and recently even in human trials. **Aims.** To explore the clinical scale expansion of the feasibility, safety and hematopoietic reconstitution effects of culture-expanded MSCs in patients with hematologic malignancies receiving co-transplantation of autologous peripheral blood hematopoietic stem cells (APBHSCs) and expanded MSCs. **Methods.** BM mononucleated cells (MNCs) were obtained from 12 healthy donors or patients with hematologic malignancies and MSCs were expanded *in vitro* in Mesencult serum free media. Four patients including three non-Hodgkin's lymphoma and one acute promyelocytic leukemia received high-dose chemoradiotherapy and supported by co-infusion of APBSCs and culture-expanded MSCs. **Results.** Human MSCs were isolated from a mean±SD of 26.3±8.8 ml of BM aspirates from all subjects. Ten of the 12 samples have been successfully expanded. All four patients were successfully transplanted expanded autologous MSCs as well as APBSCs. There were no toxicities related to the infusion of MSCs. Median time to achieve neutrophil counts greater than 0.5x10⁹/L and platelet counts greater than 20x10⁹/L were 9 days (range, 8 to 11 days) and 8.3 days (range, 7 to 10days), respectively. **Conclusions.** Mesencult serum free media could generate sufficient MSCs for clinical purpose and cotransplantation of MSCs and APBSCs was feasible and safe. Rapid hematopoietic recovery suggested that MSCs infusion after myeloablative therapy might have a positive impact on hematopoiesis.

0951**ARTERIAL STENOSIS AND THROMBOTIC EVENTS IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA**A. Cucuianu¹, M. Stoia², A. Farcas², A. Bojan¹, L. Urian¹, A. Vasilache¹, D. Dima¹, M. Patiu¹, C. Basarab¹, T. Torok¹, M. Zdrengea¹, D. Olinic², L. Petrov¹¹*Ion Chiricuta Cancer Institute, CLUJ-NAPOCA, Romania;* ²*Medical Clinic I, CLUJ-NAPOCA, Romania*

Background. Patients with polycythemia vera (PV) and essential thrombocythemia (ET) are at risk of developing arterial and venous thromboembolic complications. **Aims.** We used Doppler arterial ultrasound to assess the presence of arterial stenosis in a sample of our PV and ET patients. **Methods.** A total of 19 patients with PV and ET (13 PV and 6 ET) were investigated. There were 14 men and 5 women, with an average age of 56.6 years (32-75). Eleven patients (57%) had a history of atherothrombotic events including cerebral ischemic attacks (CIA) in 6 patients, ischemic heart disease (IHD) in 5 patients and peripheral occlusive arterial disease (POAD) in 7 patients; four patients had both CIA and POAD, one had both IHD and POAD and one had CIA, IHD and POAD. Five patients were heavy smokers, in 6 patients hypertension was documented and 4 patients were dyslipidemic. Arterial Doppler ultrasonography was performed in all patients. **Results.** Significant arterial stenosis was found in 13 patients (68%) including 9 patients with carotid plaques, 2 with peripheral arterial stenosis, one with aortic plaque and one with common iliac artery stenosis. The degree of arterial stenosis and the occurrence of clinical events was not correlated with the severity of polyglobulia or thrombocytosis, being more pronounced in older patients and

in those with additional risk factors such as smoking, hypertension and dyslipidemia. Summary and conclusions. The high incidence of arterial thrombotic events in our PV and ET patients was reflected in a high rate of detection of stenosis by arterial ultrasound. Hyperviscosity, endothelial damage due to leukocyte activation with subsequent thrombus formation, hyperhomocysteinemia and hyperexpression of activating genes such as STAT3 are all features characteristic of PV and ET that may contribute, along with other risk factors, to the development and progression of atherothrombosis.

0952**CLINICAL RESISTANCE TO BORTEZOMIB IS NOT DUE TO MUTATIONS OF THE BETA-5 SUBUNIT OF PROTEASOME**

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Background. Bortezomib is a proteasome inhibitor used in the treatment of refractory/relapsed multiple myeloma. Its anti-myeloma effect is ascribed to the inhibition of nuclear factor kappa-B (NF-kappa B) due to the reduced proteolytic degradation of its inhibitor (I-kappa B). The 20S proteasome is a barrel-shaped core proteolytic complex consisting of four rings with seven subunits each. Subunits of the inner two rings are of the beta-type and bear proteolytically active centres. The outer two rings are of alpha-type and control access to the inner chambers of the proteasome and association with regulatory complexes. The proteolytic activity is thought to reside in three of the seven beta subunits. These subunits are classified, according to the nature of the P1 residue of the peptide they hydrolyse, as follows: PSMB5 for chymotrypsin-like activity, PSMB6 for petidyl-glutamyl-peptide hydrolytic (PGPH) activity and PSMB7 for trypsin-like activity. Bortezomib binds to PSMB5 and inhibits the chymotryptic activity but its effect on the two other proteolytic activities of the proteasome has not been characterized. **Aims.** The aim of this study was to investigate whether mutations in the beta-5 subunit of the proteasome are responsible for clinical resistance to bortezomib in a patient with multiple myeloma. **Patients-Methods/Results.** A 65-year-old female with relapsed light-chain myeloma was treated with bortezomib after having received 4 lines of treatment (including autologous transplantation). Bortezomib was given at a dose of 1.3 mg/m², iv, in four 3-week cycles, on days 1, 4, 8, and 11 of each cycle. At the end of the 3rd cycle the patient had achieved a minimal response according to EBMT criteria, and plasma cell infiltration in the bone marrow had a reduction of 35% from baseline. Impressively, before bortezomib administration the patient had a Karnofsky Performance Status score of 40, while after 3 cycles of treatment her performance status improved to 90. However, at the end of 4th cycle, the patient experienced sudden and fast progression of the disease and died within three weeks. The initial response and the fast re-emergence of the disease, while on treatment, were very suggestive of clinical resistance to bortezomib. To investigate the molecular basis of this phenomenon we extracted DNA from plasma cells as identified by CD138 staining and purified by laser capture microdissection on a trephine biopsy performed shortly after patient's relapse. Each of the 3 exons of the PSMB5 gene (OMIM 600306) were amplified by PCR and subsequently sequenced either directly or following cloning (TOP-TA, Invitrogen). No mutations were detected by sequencing either the direct or the cloned PCR products. **Conclusions.** We describe here a patient with myeloma who developed clinical resistance to bortezomib. The molecular basis of this phenomenon in this case was not due to mutations of the beta-5 subunit of the proteasome. However, it remains to be seen whether resistance to bortezomib is conferred at the level of proteasome inhibition (i.e its other proteolytic activities) or down stream pathways (i.e NF-kappa B activation).

0953**THE FAVORABLE RESPONSE TO IMATINIB MESILATE 400 MG/DAY AND INTERFERON ALPHA 3 X10⁶ UI/DAY COMBINATION IN SECONDARY PHASE OF CHRONIC MYELOID LEUKEMIA AFTER CHEMOTHERAPY OF BLAST CRISIS**

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We had in study a number of 11 patients with chronic myeloid leukemia (4 female and 7 male with ages between 22-54 years) treated with Imatinib mesilate in dose by 400mg/day. After 12-21 months, all 11 patients were in blast crisis of disease with or without accelerated intermediary phase (Kantarjian criterions). The metamorphosis was AML2-2 patients, AML4-6 patients, AML7-3 patients. The cytogenetic abnormalities were: isochromosome 17, an additional Philadelphia chromosome, 9q deletion chromosome. Before blast crisis only 7 patients were in complete cytogenetic response. Though it was tried to increase the imatinib dose to 600-800 mg/day, the patients status was not considerable improved, so it was started chemotherapy in all 11 patients (cytosar 200mg/day, 7 days and adriablastine 45 mg/mp, 3 days -7/3 regimen in 7 cases and cytosar 200 mg/day, 5 days and mitoxantrone 20 mg/day, 5 days in 4 cases). All patients was obtained the secondary chronic phase). After that it was started the treatment with imatinib mesilate 400 mg/day and interferon alpha 3 x10⁶ ui/day. All patients are alive after 3-14 months start therapy period. After 6 months, 5 patients were in complete molecular response, 4 patients in complete cytogenetic response and one patient in partial cytogenetic response. Though the number of patients was low, the imatinib mesilate and interferon alpha combination seems to be effective to the patients in secondary chronic phase after blast crisis or accelerated phase. Following studies must confirm if this combination or imatinib mesilate and intermittent administration of cytosar combination may conduct to a superior survival in secondary chronic phase comparatively with accelerated or blast phase.

0954**HEMATOPOIETIC CHIMERISM EVALUATION IN PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION BY REAL TIME QUANTITATIVE PCR TECHNIQUE**

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for many patients suffering from malignant and non-malignant hematological disorders. Successful transplantation is a process that requires the engraftment of transplanted pluripotent hematopoietic stem cells which re-establish normal hematological and immunological systems. Distinguishing between host and donor origin of bone marrow and blood cells is vitally important for monitoring of the engraftment process. One of the most useful tool for engraftment monitoring is the assessment of hematopoietic chimerism which occurs after alloHSCT and describes the percentage of donor hematopoietic and lymphoid cells in a transplant recipient. The aim of this study was to develop the molecular method based on Real Time quantitative PCR technique and to compare obtained results with semiquantitative molecular STR-PCR method performed on a routine basis in our department. *Methods.* The evaluation of hematopoietic chimerism was based on amplification of biallelic non-coding DNA sequences - short tandem repeats (STR). The main tool was a quantitative Real Time PCR technique (STR-RQ-PCR) based on SYBRgreen chemistry. For each sample 3 different PCR reactions were performed (recipient specific marker, donor specific marker and GAPDH as a reference gene). Thirty eight adult patients, after alloHSCT entered the study and the total number of transplantation were 43 (22 non-myeloablative alloNMSCT). The distribution of hematologic disorders was as follows: acute myeloid leukemia - 45%, acute lymphoblastic leukemia-16%, chronic

myeloid leukemia 13%, severe aplastic anemia-13%, chronic lymphocytic leukemia - 5%, myeloma multiple 3%, dendritic sarcoma-3%, myelofibrosis-2%. Hematopoietic chimerism evaluations were performed on +30, +60, +90, +120, +150, +180, +270 and +360 day post alloHSCT on peripheral blood or bone marrow samples. *Results.* To hematopoietic chimerism assessment 11 human biallelic STR loci (described by Alizadeh et al Leukemia 2001) were selected. That panel allowed for easy discrimination between donor and recipient in 100% of all analyzed pairs. The sensitivity was tested in serial DNA dilutions experiment which showed that it was possible to detect as low as 1 residual cell among 10000 cells (minor genotype 0,0125%). In comparison the sensitivity of STR-PCR method where the product of amplification appeared and was analyzed by means of the sequencer was about 3 per 100 cells (minor genotype 3%). The differences between two analyzed methods showing higher sensitivity of STR-RQ-PCR were in a range±5% and the biggest discrepancy was observed when the stutter peak was the same size as allele informative for donor or for recipient. The clinical implication of hematopoietic chimerism monitoring by STR-RQ-PCR method was modification of immunosuppressive therapy after detection of decreased percentage of donor hematopoiesis (3 patients after alloNMSCT). **SUMMARY** The STR-RQ-PCR is a sensitive and accurate method for hematopoietic chimerism assessment after sex-matched and sex-mismatched donor/recipient alloHSCT. The STR-RQ-PCR is very useful diagnostic tool especially in patients after alloNMSCT where precise chimerism assessment is crucial and allows for therapeutic modifications regarding immunosuppression or DLI delivery.

0955**RITUXIMAB FOR STEROID-REFRACTORY AUTOIMMUNE HEMOLYTIC ANEMIA ASSOCIATED WITH CHRONIC LYMPHOBLASTIC LEUKEMIA**G. D'Arena¹, L. Laurenti², F. Ferrara³, S. Capalbo⁴, S. Storti², G. Marcacci⁵, M. Tarnani², C. Copia³, M.L. Vigliotti⁵, M.P. Petrilli², R. De Filippi⁵, F. Russo⁵, A. Pinto⁵

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Background. Autoimmune hemolytic anemia (AHA) is a well-known complication of chronic lymphocytic leukemia (CLL). Despite the origin of autoantibodies in CLL-associated AHA is controversial (malignant B-cell clone or residual normal B-lymphocytes), steroids represent the first-line treatment option. Different immunosuppressive agents are also used for steroid-refractory diseases. However, the prognosis of AHA in CLL patients still remains poor. In the recent years the anti-CD20 monoclonal antibody Rituximab has been used in the therapy of AHA and autoimmune thrombocytopenia in association with CLL. *Aims.* We report the results of Rituximab treatment in 9 patients suffering from AHA and CLL seen at our Institutions between March 2001 and September 2004. Patients and *Methods.* The mean age of patients was 69 years (range 48-87 years) and all were affected by typical CLL (CD19+CD5+CD23+slgIow). They developed direct Coombs positive AHA at a mean time of 26,8 months (range 0-108 months) from diagnosis of CLL. In 3 cases AHA was concurrently diagnosed with CLL (pts. no. 1, 7, and 9). All patients received as first-line treatment steroid therapy (prednisone 1-2 mg/Kg body weight) and all were considered non responders. Some patients were given cyclophosphamide (pts. No. 6, 7 and 8) and in 1 case (pt. no. 8) 2 mg of vincristine were intravenously infused. At a mean time from the diagnosis of AHA of 63 days (range 0-155 days) all patients received Rituximab 375 mg/m²/weekly for 4 weeks. *Results.* Table shows Hb (g/dL) levels (transfusion-free more than 8 days), platelets (PLT) x10E⁹/L and absolute lymphocyte count(LC) x 10E⁹/L, before and after Rituximab treatment. All but two patients (pts. no. 6 and 8, who died because of cardiac failure and sepsis soon after the 3rd cycle) received the sched-

uled 4 courses. The first infusion side effects of Rituximab were irrelevant (fever only in 2 cases). All but one patient (pt. no. 8) showed an increase in Hb levels in response to Rituximab with a mean value of 3,5 gr/dL (range 0,8-10 g/dL) and a reduction in absolute LC and in nodes and spleen sizes also. Six pts. required packed red cell transfusions before starting Rituximab and 4 of them became transfusions-free after the 3rd cycle. Only 3 pts. underwent to a maintenance program with Rituximab administration with different schedules. At a mean follow-up of 17 months, 4 pts. were still alive and transfusion-free. **Conclusions.** Our results clearly demonstrate that anti-CD20 monoclonal antibody is an effective and well tolerated alternative treatment option for CLL-associated steroid-refractory AHA.

Table. Response to Rituximab treatment

Pt No	Age/ Gender	Pre Hb	Pre LC	Pre PLT	Post Hb	Post LC	Post PLT	No. of courses received	Follow-up (laste dgr)
1	57M	11,7	82	79	13,6	1,4	123	4	49
2	66F	8,6	94	192	9,8	1,8	293	4	27+
3	72F	8,3	1,3	297	10,2	1,1	200	4	14
4	76F	7,3	166	237	10,8	0,8	199	4	24
5	69M	7,8	78,4	15	10,4	8,7	12	4	22+
6	82F	7,2	38	8	8,1	1,8	15	3	5+
7	87M	4,2	3,3	148	5,5	8,2	139	4	44
8	60M	8,8	82,5	41	4,8	5,2	7	3	5+
9	48M	5,0	86	154	15	4,1	179	4	12

0956

AUTOIMMUNE MANIFESTATIONS IN CASTLEMAN'S DISEASE

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Castleman's disease was previously described as a localized and clinical asymptomatic angiofollicular hyperplasia. In the last 3 decades was described a multicentric and plasmablast forms of disease. On a 13 years period we had in observation a number of 16 patients with Castleman's disease (localized form of disease with vascular hyaline deposit 3 cases, multicentric form 8 cases and 5 cases with multicentric plasmablast variant form of disease). A number of 9 patients have at diagnosis or developed in evolution autoimmune manifestations. We tried to demonstrate if the autoimmune clinical or biological manifestations are related with morphologic form of disease, age or viral infectious presence at these patients. The characteristics of patients were: For localized form of disease- 3 cases, age between 32-41 years, 2 male and 1 female, mediastinal (2 cases) and retroperitoneal (1 case) localization with superior cave syndromes symptoms (1 case) and without symptoms (2 cases), hypergammaglobulinaemia (2 cases), decreases of C3 (1 case) and anti Epstein Barr virus antibodies (1 case) For multicentric form of disease-8 cases, age between 46-72 years, 2 male and 6 female, with over diaphragmatic lymph nodes and splenomegaly and hepatomegaly (6 cases) and under diaphragmatic lymph nodes and splenomegaly and hepatomegaly (2 cases) localization, symptoms of rheumatoid arthritis (4 cases), vascular purpura (1 case) and autoimmune thrombocytopenic purpura (3 cases); hypergammaglobulinaemia (7 cases), decreases of C3 (4 cases), rheumatoid factor positive (4 cases) Dixon test positive (3 cases) and Coombs test positive (3 cases). For multicentric plasmablast variant form of disease- 5 cases, age between 45-56 years, 3 male and 2 female with over diaphragmatic lymph nodes, splenomegaly and cutaneous manifestations (3 cases), under diaphragmatic lymph nodes and cutaneous manifestations (1 case) and over and under diaphragmatic lymph nodes, splenomegaly and cutaneous manifestations (1

case), symptoms by B type (5 cases), Kasposi tumor (2 cases), plasmablast cutaneous tumor (2 cases) chrioglobulinaemic purpura (1 case); anti HTLV1 antibodies (1 case), anti HHV8 antibodies (2 cases) anti HTLV1-HTLV2-HTLV3 antibodies (2 cases) positive. **Conclusions.** 1.The vascular hyaline localized form of disease are rarely symptomatic (by mediastinal localization in our case); though clinical manifestations of autoimmune disease are missing, are presented biological hypergammaglobulinemia and decreased C3 value signs. 2.The multicentric forms of disease are clinical manifestation of autoimmune disease, most frequent with rheumatoid arthritis manifestations (75%) or cutaneous manifestations (autoimmune thrombocytopenic purpura, vascular autoimmune purpura) (50%). This form of disease seems to be most frequently associated with autoimmune manifestations. 3.The plasmablast forms have an aggressive evolution, being associated most frequently with viral infection (HHV8, HTLV1, HTLV2, HTLV3), cutaneous manifestations and Kasposi tumor or plasmablast cutaneous tumor in the end. It was not presented the autoimmune clinical manifestations excepted 2 patients who developed aggressive secondary non-Hodgkin's lymphomas (HIV infection).

0957

DOES EXIST AN INITIAL NUMBER OF PLATELETS COUNT PREDICTIVE 'LIMIT' FOR AN EFFECTIVE RESPONSE TO DEXAMETHASONE HIGH DOSE IN IMMUNE THROMBOCYTOPENIC PURPURA (ITP)?

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The therapy with dexamethasone high dose in ITP was started in 1995. As a first line of therapy, this therapeutic procedure was extremely effective demonstrated on large groups of patients. Started from this, we evaluated the superior evolution in the cases in which the initial number of platelets at diagnosis was higher than 50 000/mm³. In 1988-2004 period we treated with dexamethasone high dose (40-50mg/day, 4 days consecutively, 6-8 regimens at 21 days) a number of 69 patients with ITP (17 male and 52 female with ages between 26-69 years, sex ratio female/male= 3/1). We mention that in 18 cases, the patients were unresponsive to the treatment with prednisone 1mg/Kg weight, a 4-6 weeks period (the patients were refractory to prednisone). The other 51 patients was treated with dexamethasone as first line of treatment (all the patients was female with ages under 45 years). The results are: 80% complete response (from this, only 2% have a number of platelets under 50000/mm³ at diagnosis time), 15% partial response and 5% minor response (these patients have a number of platelets between 5000-36000/mm³ at diagnosis time). On large groups of patients is possible to study the predictive value of 50000/mm³ platelets limit for the start of treatment with dexamethasone high dose especially in countries in which the immunoglobulin high dose infusion administration isn't always a possible alternative in ITP who are refractory to the first line of therapy with prednisone.

0958

LONG TERM ADVERSE EFFECTS WERE SEEN AFTER ACUTE LYMPHOBLASTIC LEUKEMIA TREATMENT

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During the period of January 1990-December 1999, 35 cases of Acute Lymphoblastic Leukemia (ALL) who were diagnosed in the Department of Pediatric Hematology and Oncology in our Hospital were evaluated prospectively. Seventeen cases (48.5%) were female and 18 patients (51.5%) were male. All the patients were treated according to the Berlin- Frankfurt-Münster 90 (BFM 90) treatment protocol. The patients were followed up 1-9 years after the treatment. The ages were between 1-13 years (average 6.55 years). Height and weight of the patients were measured at diagnosis and after treatment. Body mass

index (BMI) was calculated. Thyroid hormones, cortisole, prolactine, LH, FSH, estradiol, progesterone, ACTH, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, triglyceride values were measured during follow up period after treatment. Hypophyseal magnetic resonans (MRI) was carried out to evaluate the hypophyseal secretory capacity of the cases to whom profilactic cranial irradiation was applied. All the parameters were evaluated according to reference interval of sex and age. 20% of females were overweight, 39% females were obese, 29.4% of males were overweight and 29% of males were obese. Height measures were normal according to value of standard deviation. Meaningful increase were observed in BMI value. It was observed that 5 of cases had hypertriglyceridemia and serum cholesterol levels were elevated in three of the patients. There was no pathology in their hypophyse MRIs. T3 levels were decreased in 3 of the patients and T4 levels were decreased in one of the patients, however no clinical finding which is well adjusted data was observed. While hyperprolactinemia was determined at 3 of the patients there wasn't a decrease at estradiol and free testosterone values. ACTH and basal cortisole values were normal. No secondary malignity was seen at patients during the follow up period. In our study, the adverse effects which can come out were searched after leukemia treatment and it was aimed to prevent them.

0959

CELL LINEAGE SPECIFIC CHIMERISM IN PATIENTS IN EARLY PHASE AFTER MYELOABLATIVE AND REDUCED-INTENSITY STEM CELL TRANSPLANTATION

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Molecular techniques (PCR for STR/VNTR) are preferable methods for assessment of donor- or recipient hematopoiesis after hematopoietic stem cell transplantation (HSCT). The sensitivity and specificity of this method may be improved using lineage-specific analysis. Separation of cell populations allows to detect the kinetics of lineage engraftment and to connect the data with such events as graft-versus host disease (GVHD) and relapse. The usage of reduced-intensity HSCT (RD-HSCT) states the question of possible different kinetics and contribution of donor cells to the different cell lineages if compared to conventional myeloablative HSCT (M-HSCT). *Aims.* To compare the engraftment of T, B and myeloid cell lineages in patients (pts) with high-risk hematological malignancies up to day +90 after RD-HSCT and M-HSCT and to reveal it's influence on GVHD or relapse. *Methods.* We performed chimerism analysis in 12 pts with high risk hematological malignancies underwent alloHSCT. 7 pts were transplanted after conventional conditioning (Busulfan 16 mg/kg and Cytoxan 120 mg/kg). 4 pts - ALL, 2 - AML and 1 - CML, middle age 24 (19-43). 5-after reduced-intensity conditioning (Busulfan 8 mg/kg, Fludarabine 150 mg/m² and ATG 40 mg/kg or Cytoxan 900 mg/m²) 3 of them with AML, 2 - MDS, middle age 40 (22-51). GVHD was prophylacted by CSA and MTX in 5 pts after RD-HSCT and CSA+MTX+Prednisone in 7 pts after M-HSCT. The evaluation of chimerism was performed on subpopulations of peripheral blood (PB): CD3+, CD19+ and CD15+ on +30, +60,+90 days after HSCT using PCR-based method for VNTR/STR analysis. The analysed subpopulations have been obtained using immunomagnetic separation technique (Dyna). *Results.* We revealed striking differences in engraftment of cell lineages in patients after RD- HSCT and M-HSCT in first 90 days post transplantation. In group after RD-HSCT 4 pts demonstrated complete donor chimerism (CC) in all cell fractions. Only one pt has got mixed chimerism (MC) in all cell lineages up to day +60, which was converted to CC after cessation of immunosuppression to day +90. All of this pts have got acute GVHD > II grade. In the group of pts after M-HSCT 6 of 7 pts demonstrated MC on day +30 in CD3+ fraction, on day +60 and +90 2 pts showed MC (immunosuppression was stopped in 2 pts). In CD19+ fraction 2 pts had got MC on days +30 and

+60 and in CD 15+ one pt showed MC during all time of follow-up. 3 pts (all with MC in CD3+) died of relapse. No one have got acute GVHD. *Conclusions.* We suppose that our data refelects the differences in biology of malignancies (the prevalence of ALL pts in M-HSCT group) and the specificity of GVHD prophylaxis (Prednisone addition in M-HSCT pts). We revealed that CC in CD3+ cells was connected with aGVHD and MC-with high risk of relapse. The early detection of MC in cell lineages can be used for cessation of immunosuppression which lead to CC in majority of cases.

0960

GAUCHER DISEASE IN CHILDREN: DIAGNOSIS, TREATMENT AND MANAGEMENT

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Gaucher disease is an autosomal recessive inherited lysosomal storage disorder. There are three types of Gaucher disease (GD). Each of them is a progressive disorder and is a result of glucocerebrosidase (GC) deficiency. This deficiency leads to the accumulation of glucocerebroside in the macrophages, producing the characteristic Gaucher cells, that can be found in the liver, spleen and bone marrow. Mutation analysis is used in combination with GC enzyme assay results to diagnose GD and for identification of carriers. *Aims.* To present demographic, clinical, analytical, genetic and follow-up data of children with GD in Macedonia. *Methods and Results.* By January 2005, at Department of Hematology in Skopje, 11 children from 10 families had been diagnosed as GD. Type 1 was verified in 7 patients (pts) and type 2 GD in 4 pts. 60% of patients were from Albanian ethnic population. The age of onset(4-18 years) and severity of symptoms varied widely in the type 1 patients. Enlargement of the spleen and liver was the earliest symptom in 7 pts, three of them developed hypersplenism with pancytopenia and bleeding diathesis. One patient with type 1 was heterozygous for Hemoglobin Lepore. 73% of patients had bone lesions. The skeletal abnormalities in patients were osteopenia in 3 pts, osteonecrosis in 2 pts and the characteristic 'Erlenmeyer flask' in 4 pts. Gaucher cells were found in bone marrow of all patients. Biochemical and hematological abnormalities were attributable to skeletal or visceral involvement. Four patients manifested type 2 GD as the acute neuronopathic or infantile form. Three of them had fatal prognosis at age of 6 weeks to 11 months. Lysosomal enzyme diagnostics were made in 4 pts. (3 pts type 1 and one type 2 GD). The results showed a deficiency of Beta-D- glucosidase in leukocytes (0.3-0.4 nmol/mg.h) and strongly increased level of chitotriosidase in plasma from all of analyzed pts (4891-31714 nmol/mL.h). This data fit with the diagnosis GD. The molecular diagnosis was confirmed in 2 pts. with type 1, one homozygous for N370S and one heterozygous for N370S. These patients had slightly progressive evolution. The treatment procedures of patients were: symptomatic treatment; transfusions with fresh frozen plasma or platelets and splenectomy in patients with hypersplenism, severe anemia and thrombocytopenia; orthopedic intervention and analgesics in patients with skeletal problems. The enzyme replacement therapy (ERT) was used in one patient and has greatly improved his quality of live. *Conclusions.* The diagnosis of GD should be considered in any case of unexplained splenomegaly, with or without bleeding diathesis; in other disease manifestations in the liver or the skeleton; and especially in infants with hepatosplenomegaly and neurodegenerative course. The presence of Gaucher cells in bone marrow aspirates was documented in all patients in this study as highly indicative of the disease. Definitive diagnosis of GD was confirmed by assay of GC activity in leukocytes and mutation analysis in 4 pts. ERT is save and effective, but expensive treatment, especially for developing countries. The symptomatic treatment, transfusions and splenectomy are still necessary.

0961**FLT3 MUTATION IN ACUTE PROMYELOCYTIC LEUKEMIA AND ITS CLINICAL SIGNIFICANCE**

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Background: FLT3 is a class III receptor tyrosine kinase which is widely expressed on hematopoietic stem/progenitor cells and its mutations are of growing importance for classification, risk assessment and therapeutic targeting in acute myeloid leukemia (AML), however, FLT3 mutation in acute promyelocytic leukemia (APL) has not been extensively studied. **Aims:** To explore the incidence of FLT3 mutation and its clinical significance in APL patients. **Methods:** From Feb 2002 to Mar 2004, 115 adults with previously untreated APL (age 18-49, median 24; male/female=1.26:1) which was confirmed by t(15;17) or PML-RARa fusion gene were included. Internal tandem duplication (ITD) of the juxtamembrane domain-coding sequence and Asp835 point mutations within the activation loop of FLT3 were screened by genomic PCR, RT-PCR, PCR-RFLP and sequencing. **Results:** Among 115 patients with APL, 10 (8.7%) were found to have FLT3-ITD mutation and 11 (9.6%) were found to have Asp835 point mutation. FLT3-ITD in APL patients was associated with higher leukocyte counts, higher percentage of leukemic cells in bone marrow and poor prognosis. Asp835 point mutation was not associated with leukocyte counts, percentage of leukemic cells in bone marrow and prognosis. **Conclusions:** These results suggested that FLT3-ITD but not Asp835 was an adverse prognostic risk factor in previously untreated APL.

0962**ELEVATED PLASMA LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IS ASSOCIATED WITH MARKED SPLENOMEGALY IN CHRONIC MYELOID LEUKEMIA**P. Liu¹, J. Li¹, H. Lu¹, Y. Wang², B. Xu¹, Z. Peng², W. Shen¹, R. Lin¹, Z.C. Han²¹*st Affiliated Hosp. Nanjing Med. Univ., NANJING, China;* ²*Institute of Hematology, CAMS, TIANJIN, China*

Background. Recent investigations support the idea that angiogenesis is involved in the pathophysiology of hematologic malignancies, including chronic myeloid leukemia (CML). Many studies have tried to evaluate angiogenesis through measurement of circulating angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Several studies have shown that elevated plasma levels of these two angiogenic growth factors are measurable in CML patients. However, the study groups always comprised heterogeneous patients treated with various modalities according to their stage. Furthermore, the reported circulating levels of the cytokines have also been conflicting. **Aims.** The aim of the present study was to evaluate plasma levels of VEGF and bFGF in a cohort of 51 chronic-phase CML patients at the time of diagnosis, as well as to determine relationship between circulating levels of these two cytokines and clinical features of CML. **Methods.** VEGF and bFGF concentrations in plasma samples obtained from 51 chronic-phase CML patients at the time of diagnosis were measured using a commercial quantitative sandwich enzyme immunoassay technique. Control plasma samples from 20 healthy volunteers were also evaluated. **Results.** Plasma VEGF levels were significantly higher in CML patients as compared with the healthy subjects ($p=0.001$), the median plasma VEGF level detected in patients analyzed and healthy controls was 433.4 pg/mL (range, 65.2-2452.7 pg/mL) and 81.6 pg/mL (range, 44.2-338.7 pg/mL), respectively. On the other hand, no difference in bFGF plasma levels could be found between chronic-phase CML patients (median, 11.9 pg/mL; range, 5.4-66.7 pg/mL) and the control group (median, 12.6 pg/mL; range, 6.0-25.8 pg/mL) ($p=0.54$). There were significant associations between plasma VEGF levels and some character-

istics of patients evaluated, with trends for higher VEGF values in patients with enlarged spleens ($p=0.02$) and those with higher platelet count ($p=0.001$). There was no correlation between VEGF levels and age, hemoglobin, white blood cell count, percentage of peripheral or marrow blasts. **Conclusions.** VEGF may play an important pathophysiological role in CML. Further studies aiming to explore the detailed angiogenic profile of CML may help in developing new therapeutic strategies for this myeloproliferative disorder.

0963**USE OF DEFIBROTIDE IN THE PROPHYLAXIS OF VOD IN SIX PATIENTS AFFECTED BY RELAPSING OR CHEMOREFRACTORY AML TREATED WITH GEMTUZUMAB OZOGAMICYN (MYLOTARG)**

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Gemtuzumab ozogamicin (GO, Mylotarg) is a recombinant humanized anti-CD33 monoclonal IgG4 antibody that delivers the potent cytotoxin, Calicheamicyn, into CD33 expressing cells. The CD33 antigen is expressed on blast cells in 80-90% of AML but it is not expressed on pluripotent hematopoietic stem cells or on nonhematologic cells. Mylotarg is today a novel and effective biotechnological drug for refractory and relapsed AML but is associated with an incidence of approximately 20% of Grade 3 or 4 hyperbilirubinemia and liver hypertensinemia and it can induce the development of potentially fatal hepatic veno-occlusive disease (VOD) that occurs when GO is used either as a single agent or when it is given with other cytotoxic agents. VOD, diagnosed by Seattle and Baltimore standard criteria, include hyperbilirubinemia, painful hepatomegaly, fluid retention or sudden weight gain. Generally, the median time of occurrence of VOD is 25 days (range 10-35) after the first dose of Mylotarg. Recently, promising results in the treatment of VOD with Defibrotide were reported suggesting the use of Defibrotide as a prophylactic regimen for VOD. Defibrotide induces the release of the plasminogen activator and reduces the blood levels on the plasmin inhibitors. Moreover, it increases the synthesis of PGI₂, that is a strong inhibitor of platelet aggregation and an inducer of vessel dilatation, without affecting the coagulation. Therefore, it prevents ischemic and thrombotic events that are both present in VOD. We report our experience on 6 patients treated with Mylotarg regimen and Defibrotide prophylaxis. Six consecutive patients (4 females and 2 male, median age: 55 years, range 38-70 years) with CD33+ AML were admitted between October 2003 and December 2004. They were affected by relapsed (older patients) or refractory (younger patients) AMLs. They received Mylotarg at the dose of 6 milligrams/m², day 1 and 14 and Defibrotide prophylaxis, 10 milligrams/kilo/day intravenously, from day -1 to day +30. Before GO therapy Bilirubin, AST and ALT values were in normal range. VOD was never observed in our series, but 3 patients have developed grade 2 hypertransaminasemia/hyperbilirubinemia. Another patients showed on day +13 abdominal pain and transient hypertransaminasemia/hyperbilirubinemia that disappeared after 5 days. An abdomen US demonstrated the presence of gallbladder stones. The remaining patient showed no toxicity signs. Although GO induced severe thrombocytopenia it was never observed an increase of the incidence of haemorrhage signs. On the other hand, Defibrotide administration was well tolerated and it was never observed any toxicity sign during the long-lasting administration. GO induced a PR in the three resistant patients. Moreover, the other three patients achieved a CR. Two patients died four months after therapy and two after six months, respectively. We have not found death due to therapy toxicity. **Conclusions.** Defibrotide is a well tolerated agent that could be useful for the prophylaxis of VOD in patients treated with GO. Further studies are needed to confirm the effectiveness to prevent VOD.

0964

DRUG TRANSPORTERS AND APOPTOSIS-RELATED PROTEINS EXPRESSION ANALYSIS IN ACUTE MYELOID LEUKEMIA PATIENTS EXPOSED TO IONIZING RADIATION DUE TO THE CHERNOBYL ACCIDENT

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Background. Sensitivity or resistance of malignant cell to chemotherapy may correlate with its apoptotic potential. While the apoptotic machinery is the final determinant of drug-induced death, leukemic cells call into action multiple factors from the cell surface to the genome that confer an ability to survive in an adverse environment. For example, drug transporters promote efflux of exogenous toxins and prevent intracellular accumulation at high-enough concentrations or for long-enough periods of time to inflict lethal damage. **Aim.** The aim of this study was to determine whether drug transporters (P-glycoprotein, MRP, LRP, Bcl-2, Bax, Bcl-XI and Fas) expression and apoptosis-related proteins (Bcl-2, Bax, Bcl-XI and Fas) expression in acute myeloid leukemia (AML) patients with the history of radiation exposure differed from those in spontaneous AML cases. **Methods.** P-glycoprotein, MRP, LRP, Bcl-2, Bax, Bcl-XI and Fas expression was evaluated in 49 AML patients by flow cytometry at presentation. Of these patients, there were 19 persons exposed to ionizing radiation due to the Chernobyl accident with radiation-associated AML and 30 patients with spontaneous disease. **Results.** Leukemic cells in patients with radiation-associated AML compared to spontaneous cases more often expressed anti-apoptotic protein Bcl-2 (10/19 vs 6/30, $p=0.04$) and less often demonstrated expression of Fas receptor (8/19 vs 4/30, $p=0.04$). Moreover, leukemic cells were simultaneously Fas negative and Bcl-2 positive in 4 out of 19 patients exposed to ionizing radiation but none of spontaneous cases had similar phenotype ($p=0.02$). We assume that malignant cells overexpressing anti-apoptotic protein Bcl-2 and demonstrating low Fas expression are doubly protected, in that they are refractory to apoptosis and are more likely to quiescent. For the whole cohort of patients it was direct correlation between age over 60 years and FAS negativity ($r=0.41$, $p<0.005$) and between antecedent myelodysplasia and Bcl-2 positivity ($r=0.3$, $p=0.04$). It was noticed that transformation of myelodysplastic syndrome into AML could be accompanied by changeover of Bcl-2 expression from negative to positive. There were no differences between groups in terms of Bax and Bcl-XI expression. In our series of AML patients, because of limited size we could not determine the relationship between apoptotic markers and treatment outcome. Leukemic cells in patients with radiation-associated AML compared to spontaneous cases more often were P-glycoprotein positive (12/19 vs 8/30, $p=0.02$). P-glycoprotein overexpression significantly correlated with resistant disease in patients with radiation-associated AML ($r=0.47$, $p<0.05$), but was not a prognostic variable for the treatment outcome in terms of overall survival. MRP and LRP expression did not differ significantly between radiation-associated and spontaneous AML cases. **Conclusions.** Defects in pathways of drug-induced apoptosis and function of transport proteins, that actively effluxes drugs could contribute significantly to developing chemotherapy resistance in radiation-associated AML. The additional studies are needed to further elucidate the role of apoptotic-related proteins and drug transporters in rendering tumor cells resistant to chemotherapeutic agents in radiation-associated AML.

0965

CD133/2 (AC141) EXPRESSION ANALYSIS IN ACUTE LEUKAEMIA IMMUNOPHENOTYPING

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Background. Acute leukaemias are clonal disorders involving the malignant transformation of early haematopoietic progenitors. CD133 (AC141) is a recently identified marker expressed on haematopoietic stem cells, which is restricted to a subset of CD34^{bright} stem and progenitor cells in human fetal liver, bone marrow, cord blood and peripheral blood, as well as to a small proportion of CD34-negative cells in these tissues. As a novel marker with a potential to distinguish uncommitted multipotent stem cells from lineage restricted progenitors it is of particular interest in the immunophenotyping of acute leukaemias, hence data reported are still scarce and contradictory. **AIM** of the present study was to assess the diagnostic value of CD133/2 (clone AC141) by flow cytometry in acute leukaemia blast cells. **MATERIALS AND Methods.** A total of 43 bone marrow and/or peripheral blood samples of newly diagnosed patients with acute myeloid leukaemia (AML) [$n=22$]; acute lymphoblastic leukaemia (ALL) [$n=17$: B-ALL $n=10$ and T-ALL $n=7$], and acute biphenotypic leukaemia (ABL) [$n=4$] were investigated by flow cytometry for CD133/2 (AC141) reactivity. CD133/2 expression was further compared to CD34 and MDR1 expression [P-glycoprotein and MDR1 mRNA], immunophenotypic features, morphology and cytogenetic/molecular data of the examined patients. **Results.** Flow cytometry allowed for the detection of CD133 expression in 18/43 patients [41,9%] with AML [$n=15$], ABL [$n=2$] and My+B-ALL [$n=1$]. Neither of the T-cell leukaemic blasts showed positive staining. Not only a strong correlation with myeloid affiliation was observed [Pearson Chi-Square, $p=0,002$], but all CD133/2-positive cases were of immature myeloid morphology, corresponding to M0 and M1 subtypes or AML with trilineage myeloid dysplasia, while CD133/2-negative AMLs bore features of maturation, corresponding to M2, M4Eo, M5 and M7 subtypes [$p=0,001$]. Furthermore, a strong concordance with CD34 positivity was also observed [$p<0.000$], a discordant pattern of expression found in only 2 samples: CD133pos/CD34neg [$n=1$] and CD133neg/CD34pos [$n=1$]. Besides, CD133/2 expression correlated with CD7 [$p=0,04$] and with the absence of mature immunophenotypic markers such as CD14 [$p=0,04$], CD15 [$p=0,03$] and CD64 [$p=0,009$]. Similarly to CD34, P-glycoprotein levels were also significantly higher in CD133-positive AML cases [mean CD133pos = $0,62\pm0,19$ vs. mean CD133neg = $0,35\pm0,26$; $p=0,018$]. No correlation was found with cytogenetic/molecular data of the examined patients. **Conclusions.** CD133/2 (AC141) immunoreactivity allowed for the identification of a subgroup of acute leukaemias, which showed myeloid affiliation, including biphenotypic cases with immature or dysplastic morphology. Further studies are warranted to clarify the actual incidence of the stem cell marker in lymphoid malignancies and the potential of CD133 as an alternative for stem cell selection in patients with discordant CD133/CD34 pattern. Despite the low number of examined cases, the observed correlation with immature phenotype and MDR1 over-expression might define clinical resistance to therapy and needs additional investigations.

0966

USEFULNESS OF FLUORESCENCE IN SITU HYBRIDIZATION ON PARAFFIN-EMBEDDED TISSUE SECTIONS IN THE RETROSPECTIVE DIAGNOSIS OF NON-HODGKIN'S LYMPHOMA: CASE REPORT

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Background. Cytogenetic analysis can contribute valuable information to the diagnosis and classification of non-Hodgkin

lymphomas. Interphase fluorescence in situ hybridization (FISH) studies offer the ability to assess the presence of characteristic cytogenetic abnormalities even when material for standard metaphase cytogenetic analysis is not available. Case description: We report a rare case of an intestinal follicular lymphoma presenting as multiple lymphomatoid lesions along the small intestine mimicking mantle cell lymphoma (multiple lymphomatous polyposis). During follow-up the indolent nature of the disease was questioned and therefore the diagnosis reconsidered. As follicular lymphoma is characterized by t(14;18) (q32;q21) resulting in the bcl2/IgH fusion gene, we performed dual-color FISH using probes for bcl2 and IgH genes on tissue sections as well as on nuclei extracted from the original paraffin-embedded intestinal biopsy, which was the only available material. We could identify bcl2/IgH fusion positive cells in the neoplastic follicles as well as on slides prepared from the extracted nuclei suspension, thereby confirming the diagnosis of follicular lymphoma. Discussion/conclusion: This case illustrates the usefulness of FISH in the diagnosis of lymphoma, especially in those instances where only paraffin-embedded material is available. It might also represent a valuable alternative when PCR analysis is hampered by fragmentation of the DNA due to fixation, in addition to scattering of breakpoints.

0967

SEQUENTIAL THERAPY RASBURICASE®->ALLOPURINOL IN THE TREATMENT OF HYPERURICEMIA IN LEUKEMIAS AND LYMPHOMAS

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Hyperuricemia can be caused by the 'Tumor lysis Syndrome' found in patients affected by hyperleukocytotic leukemias or other hematological malignancies with a large tumour burden characterized by a rapid cell turn-over. It is due to the shedding of purinic basis from the tumour following spontaneous or chemotherapy-induced cell tumour lysis. Recent studies have reported that uric acid > 8 mg/dL and white blood cell count > 50 x 10³/ul are correlated to a high early death risk (Estey. Semin Hematol 2001; 38 (suppl.10): 32-37). About 25% of patients affected by acute hyperleukocytotic leukemias and by Burkitt Lymphomas in advanced stages develops acute renal failure during chemotherapy, even if these patients are correctly treated for hyperuricemia. The therapeutic weapons that are used until to-day are the hydratant therapy, allopurinol and rasburicase. Allopurinol acts through the inhibition of Xanthine-Oxydase that catalyzes the formation of uric acid from xanthine inhibiting the synthesis of uric acid, but not eliminating the already accumulated uric acid. Allopurinol has a slow action, is deliverable only per os and can determine a nephropathy with urolithiasis due to high blood concentrations of xanthine. Rasburicase®, urate-oxydase from recombinant DNA, an enzyme able to catalyze the oxydation of uric acid in allantoin and to allow its elimination. Rasburicase is i.v. administered and has a prompt action, good tolerability and poor toxicity (Goldman et al. Blood 2001; 97:2998-3003). The sequential administration of the two drugs could be an effective strategy for the treatment of hyperuricemia and the prevention of uratic nephropathy. Thirteen patients, 8 males and 5 females, mean age 56 years (range: 17-84), affected by hematologic malignancies, were treated with Rasburicase®. All the patients have elevated levels of uric acid and two of them have low renal indexes with conserved renal functions. After the beginning of the treatment with Rasburicase® all the patients have a constant and gradual decrease of blood uric acid from the day 2 even in presence of chemotherapy administration. Plateau was reached at the third day of therapy with 1-2 mg/dL uric acid levels. From day 4 Rasburicase® was suspended and 300mg/die/os Allopurinol was begun. Rasburicase® administration in 1 h 250 ml NaCl i.v. was well tolerated and did not cause important side effects and renal functions were at high limits but normal. Blood uric acid determination was performed maintaining the vial at 4°C to block

Rasburicase® *in vitro* activity. The sequential therapy with Rasburicase®. Allopurinol has allowed the optimal treatment of hyperuricemia and the maintainance of blood uric acid in a normal range for all the period in which chemotherapy was administered. Moreover, chronic renal failure was solved in the 2 patients who have alteration of renal functions at the presentation. The prevention of uratic nephropathy with this treatment modality has given promising results for the long-term management of these subsets of patients.

0968

MULTIPLE MYELOMA WITH IGA SECRETION (CHARACTERISTICS OF IMMUNOCHEMICAL DIAGNOSIS, CLINICAL COURSE AND RESPONSE TO THERAPY)

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Incidence of IgA MM in population occupies the second place after IgG MM (21-25%). We dealt a lot with IgA MM patients in our clinic and came to the opinion that: 1) IgA MM could have slow and favorable clinical course or it could be completely different and have fulminant progression and aggressive course; 2) IgA MM less predictable and it is more often primarily resistant to chemotherapy than other MM variants. *Results.* of high-dose therapy followed by bone marrow transplantation in IgA MM patients are less favorable than in IgG MM patients. 63 patients with IgA MM and the same quantity of patients with IgG MM were enrolled in comparative study that lasted since 2001 till 2004. We selected IgG patients by stages of disease and presence or absence of BJ protein. Clinical course of IgA MM at the onset of the disease was more aggressive than IgG MM. Patients with IgA MM more often demonstrated B-symptoms (52,4% vs. 36,5%), specific fever (3,2% vs. 1,6%), soft tissue masses with skull base involvement (15,9% vs. 11,1%), neurological symptoms with lower paraparesis (4,8% vs. 3,2%), disturbance of nitrogen excretion and renal concentration (49,2% vs. 23,8%), hypercalcemia (20,6% vs. 12,7%), elevation of b2-m in patients without renal insufficiency (39,7% vs. 19%), deletion or monosomy of the 13th chromosome (43,8%). It is typical of IgA MM to produce multiple, wide and 'stepped' M-components (79,4%) not dependent of IgA subclasses. Serum analysis of 22 IgA MM patients shown that in 100% of cases IgA belonged to the IgA1 subclass and in 72,7% of these cases M-component was wide. Maximal concentration of polyclonal IgA falls at b2-zone and at the same place most of monoclonal IgA M-components are localized. Maximal concentration of polyclonal IgG falls at g2-zone. Electrophoretic migration of IgA coincides with IgG in g1-zone. That is why in cases small IgA-components migration in g1-zone with normal level of IgG it is often impossible to reveal IgA monoclonality masked by NIgG. In such cases IgA MM monitoring by dynamic assessment of IgA level practically impossible. In this category of patients suspicion of IgA MM progression arouses if we see dynamic rise of «polyclonal» IgA in serum determined by RID without M-component identification. With contemporary standard chemotherapy based on programs selection by objective criteria of effectiveness and change of treatment if response to previous treatment is insufficient, statistically significant differences in overall and recurrence-free survival between IgA and IgG MM patients were not found. Both median overall survival from the initiation of treatment (45,6 months) and overall survival from the initial diagnosis (48,2 months) were approximately 7 months shorter in IgA MM patients and came to 46,6 months. 5-year survival was 54,5% (±10%). Primarily polyresistance was registered 2 times more often in IgA MM group (15,8% vs. 9,5%). At the moment of initial diagnosis MM had smoldering clinical course 1,5 times more often (14,3% vs. 9,5%). Fulminant, aggressive course of MM was found 2 times more often in IgA MM patients (11% vs. 6,3%).

0969

DEFERIPRONE IS USEFUL IN BLOOD TRANSFUSION-DEPENDENT PATIENTS AFFECTED BY MYELODISPLASTIC SYNDROME (MDS)

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Blood transfusion is the only supportive therapeutic chance in MDS patients refractory to other treatments ('maturing' therapy, growth factors, low dose chemotherapy, biological response modifiers etc.). Repeated transfusions always cause an iron overload with an elevated associated comorbidity and mortality risk independently from their primitive hematological disease. Several studies (Rose C. et al. *Transfus. Clin. Biol.*, 2001, 8 (5), 422-432) have demonstrated that patients with 'good prognosis' (Refractory Anemia, Acquired Idiopathic Sideroblastic Anemia, and 5q- Syndrome) have an elevated morbidity and mortality risk after the transfusion of more than 100 units of blood red cells. On the basis of these results the use of iron chelators could reduce or prevent the iron overload damage. Until few years ago desferoxamine has been the only drug registered for clinical use. However, the chronic use of desferoxamine (s.c. administered) causes, sometimes irreversible, ototoxicity and nephrotoxicity in polytransfused thalassemic patients. Deferiprone is a new iron chelator administered per os approved for clinical use. We have treated 6 patients affected by MDS with deferiprone (4 AISA, 2 RA) refractory to any treatment modality and blood transfusion dependent form at least 1 year. All the patients showed before the beginning of the iron chelator treatment more than 2000 ng/mL of ferritinemia and a mean blood transfusion request of 1 unit of red blood cells every week in order to maintain Hgb levels higher than 8g/dL. After one month from the beginning of the therapy with deferiprone all the patients showed a reduction of ferritinemia (an about 15% decrease, r: 8-25%). Five out of six patients have shown a transitory increase of the transfusion request, however, after 3 months from the beginning of deferiprone therapy, a reduction of the transfusion request was recorded in all the patients (at the present, one unit every 14 days). Until to-day (8 months after the beginning of the therapy) we have not recorded either toxicity or adverse events. Further studies are warranted to define the role of deferiprone in reducing the blood transfusion request in patients affected by MDS refractory to any other kind of conventional therapeutic strategy. mrvilla@aliceposta.it

0970

FEASIBILITY OF A SIMPLE METHOD TO DETERMINE THE IG-VH MUTATIONAL STATUS IN ROUTINE DIAGNOSTICS OF B-CLL BASED ON THE BIOMED-2 PROTOCOL

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Background. The Ig-VH usage and mutational status are important characteristics that are used in research aiming at unravelling B-cell ontogeny and the origin of different B-cell non-Hodgkin's lymphoma (NHL) subtypes. Recently, Ig-VH mutation analysis is also introduced in routine diagnostics of B-CLL as it has been shown to be highly predictive for clinical outcome in B-CLL. **Aims.** As current VH mutation analysis methods are laborious, expensive and lacking standardisation, we aimed at evaluating the direct sequencing of the multiplex VH-FR1 PCR product derived from the standardized BIOMED-2 protocol as a suitable tool in routine diagnostics. **Methods.** Namalwa cell line DNA as well as DNA from patients diagnosed with a variety of B-cell NHL, including B-CLL, MCL, FCCL and SLVL, was used in the study. Each DNA sample was amplified in a single multiplex PCR reaction combining 6 different VH-family primers (VH 1 to 6) and one JH primer as designed for the BIOMED-2 protocol. The resulting multiplex product was directly sequenced on a Beckman Coulter CEQ

8000 Genetic Analysis System. Namalwa cell line DNA was also amplified in 6 single PCRs using the 6 VH-FR1/JH primer combinations separately followed by sequencing of the resulting products. Uniplex PCR was also performed on patient samples using the specific VH-FR1 family primer as determined by multiplex PCR and sequencing. VH-gene usage and mutations were identified by comparison with the closest germline VH sequence using the Ig Blast database available on the internet. Cases were divided into 'unmutated' and 'mutated' cases defined as cases with respectively more or less than 97% homology. **Results.** For all multiplex VH-FR1 PCR products, good quality nucleotide sequences were obtained, all showing homology with known VH germline genes ranging from 87% to 100%. There was complete concordance between results derived from multiplex and uniplex PCR products. Namalwa cell line VH sequences showed 100% homology to the published sequence. Analysis of both VH mutational status and VH gene usage in the B-NHL cases was in line with published findings with 'mutated' cases comprising 56% of B-CLL and all FCCL cases whereas 'unmutated' cases were 44% of B-CLL and most of the MCL cases and with overrepresentation of certain VH genes in the different B-NHL subtypes. **Conclusions.** We have demonstrated the feasibility of using the single VH-FR1 multiplex PCR reaction as described by the standardized BIOMED-2 protocol, followed by a single sequencing reaction for the analysis of the Ig-VH usage and mutational status. This approach is faster, less expensive, less prone to inter-laboratory variation and requires less handling than all commonly used VH mutational analysis methods and is thus highly suitable for routine diagnostics in B-CLL.

0971

CASE REPORT: PATIENT WITH MYCOSIS FUNGOIDES TREATED WITH SIX CYCLES OF CHOP AND CONTINUOUS CYCLOSPORINE ACHIEVED LONG-TERM REMISSION

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Mycosis fungoides is most common subtype of cutaneous T-cell lymphoma. The etiology of disease remains unknown. Patients usually present with cutaneous lesions (patch, plaque, tumor stage) and pruritus. Involvement of lymph nodes and visceral organs in early stage of disease is rare. The malignant cells are typically CD3+, CD4+, CD45RO+, CD8-, CD30-, and T-cell receptor genes are clonally rearranged. The diagnosis is based on histopathology of the skin lesions. Clinical staging system identified several independent factors: extent of skin lesions at diagnosis, presence or absence of peripheral blood, lymph node or visceral involvement. Therapy is divided in two approaches. Topical therapy (PUVA, UVB, total skin electron beam radiation, topical chemotherapy, topical retinoids) and systemic therapy (photophoresis, interferon-alpha, single agent chemotherapy, combination chemotherapy, oral retinoids). In spite of various treatment options, the disease remains not curable with standard therapies, therefore new therapies with different mechanisms of action are under investigation. Cyclosporine, fungal peptide, is potent immunosuppressive drug with preferential effect on early activation of helper T4+ lymphocytes. Such as the majority of cases of mycosis fungoides are of the T-helper subset of lymphocytes with a CD4+ phenotype, cyclosporine may have role in treatment of this disease. Our patient was admitted at hospital in July 2001, with intensive skin lesions on the whole body in form of the diffuse nonspecific erythematous patches, one plaque lesion on the lower abdomen, and with symptoms of intensely pruritus and fever. The diagnosis of mycosis fungoides was confirmed by histopathology of the skin lesions. Smears of peripheral blood and bone marrow revealed absence of infiltration with malignant cells, and there were no lymph node or visceral involvement. The patient was classified in clinical stage III (T4, N0, PB0, M0) by TNM classification of the cutaneous T-cell lymphoma. He received six courses of CHOP, after that skin lesions were in regression, but still present on the lower back and whole legs.

In that time, because of objective reasons, it was impossible to apply topical therapy or interferon-alpha. The treatment was continued with oral cyclosporine in doses of 100-200 mg/die, with weekly checking of the serum level of the drug. There were not any side effects related to use of cyclosporine. After six months of continuous therapy with oral cyclosporine, patient achieved complete remission, with total regression of all skin lesions. In further three years long follow-up, patient received only oral cyclosporine and remained in complete remission. Our case report shows that cyclosporine can be effective in treatment of mycosis fungoides. It is already known that cyclosporine influences activation of the normal helper T4+ lymphocytes, and it is possible that has a similar effect on malignant helper T4+ lymphocytes in mycosis fungoides.

0972

CIRCULATING METALLOPROTEINASE-9 IN PATIENTS WITH MULTIPLE MYELOMA IS A MARKER OF BONE DISEASE

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Background. Matrix metalloproteinase-9 (MMP-9) is a proteolytic enzyme that plays a major role in bone resorption as well as in cancer growth, invasiveness and angiogenesis. Multiple myeloma, a B-cell malignancy localized primarily in the bone marrow, produces MMP-9. Multiple myeloma (MM) is associated with accelerated bone turnover leading to formation/resorption imbalance and osteopenia/osteoporosis. Aim of the present work was to correlate the severity of multiple myeloma with the systemic levels of MMP-9 and the rate of bone turnover. **Methods.** Thirty-seven newly diagnosed MM patients and 12 controls were included: 9 patients were of stage I, 12 of stage II and 16 of stage III (Durie-Slamon classification). Bone lesions were scored grade 0 to 3 according to x-ray findings. Serum MMP-9 and interleukin-6 (IL-6) levels and free urine pyridinolines (Pyd) and deoxy-pyridinolines (Dpd), free urine Dpds and the urine N telopeptide fragment (Ntx) were measured by Elisa. **Results.** MMP-9 levels were significantly higher at stage II compared to stage I (188.78±91.27 vs 59.25±33.09, p<0.004). Bone resorption markers free urine Pyd +Dpd, Dpds and Ntx were increased in advancing disease stage (p<0.001, p<0.003, p<0.001 respectively). Bone marrow infiltration and IL-6 were also increased in advancing disease stage (p<0.0001, p<0.01 respectively). MMP-9 levels were lower in patients in comparison to controls (p<0.001), while IL-6, free urine Pyd + Dpd, free urine Dpd and Ntx levels were significantly higher in patients in comparison to controls (p<0.001, p<0.001, p<0.001 respectively). Furthermore, statistically significant differences were found between infiltration, MMP-9, free urine Pyd + Dpd, free urine Dpd, Ntx according to bone disease grade (p<0.03, p<0.03, p<0.002, p<0.003 and p<0.001 respectively). **Conclusions.** We found that MMP-9 correlates with the severity of multiple myeloma and the rate of bone turnover indicating that MMP-9 may be used as a marker of bone disease in MM.

0973

STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKAEMIA (AML). AN ASSESSMENT OF FACTORS AFFECTING THE RESULTS OF AUTO AND ALLOSCT. SINGLE CENTRE EXPERIENCE

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Background. HSCT remains the best therapy for the control of AML in adults patients. It is a strategy associated with lowest relapse incidence. Available data indicates that autoHSCT entails more favourable outcome if the harvest is performed

after the 2nd or 3rd course of CT. The disadvantage compared to alloHSCT is that the relapse rate is much higher probably due to the lack of GvL effect. The aim of this study was to assess the results of auto and alloHSCT in patients with AML including factors affecting the results obtained and those influencing the risk of relapse after SCT. **Materials and Methods.** SCT was conducted in 77 patients with AML; 43 with alloSCT and 34 with autoSCT. The average age was 39 (16-61). Patients were treated according to the 3+7 or 3+7+Cladribine protocols. In patients treated with autoSCT progenitor cells collection was conducted following the 2nd consolidation cycle. Conditioning regimen according to the BEA (Bu+VP16+ARA-C) protocol was employed. In patients treated with alloSCT transplantation was conducted after the 2nd consolidation cycle. Conditioning regimen according to the BuCy120 protocol was employed. PB was a source of progenitor cells in 70 patients. The potential influence of recognized prognostic factors (age, WBC, cytogenetics), the number of cells transfused in transplanted material (MNC, CD34+, CFU-GM), time elapsed from diagnosis and CR to SCT and haematopoietic recovery following transplantation, on the risk of relapse of the disease, was assessed. **Results.** 75 of 77 patients achieved a haematopoietic recovery. 2 patients treated with autoSCT did not achieve engraftment. Initial WBC, age, immunophenotype and morphological subtype had been shown, in the examined group, to have no influence on the risk of relapse. Cytogenetics was conducted only in 55% of the patients, for the reason that the influence of this factor on the risk of relapse had not been chosen to be assessed. Neither the number of CD34+, MNC, CFU-GM cells, nor the recovery rate after transplantation (ANC>0.5G/L, PLT>50G/L) affected the risk of relapse. Time elapsed from diagnosis and CR to autoHSCT had also proven not to affect the risk of relapse, but was the only factor affecting the relapse rate after alloSCT. **Conclusions.** Auto and alloHSCT results obtained are comparable to those achieved in other centres. Results achieved do not allow an unequivocal indication of factors affecting the risk of relapse in patients with AML besides the fact that time elapsed from remission to transplantation has been shown to be of importance in alloSCT. New prognostic factors, that would allow the isolation of a group of patients with AML, for whom autoHSCT would be the therapy of choice, should be investigated.

0974

RECURRENT HISTIOCYTOSIS EVOLVING TO MYELODENDRITIC LEUKEMIA

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Background. Langerhans cells histiocytosis (LCH) is a rare disorder caused by proliferation of activated Langerhans cells. Although current therapies have shown to be very effective treatments to induce remission, multiple recurrences are usually still quite frequent. **Aims.** To describe our experience in the use of cladribine as a treatment for myelodendritic leukemia. **Methods.** In 1999 a 20-year-old female was admitted to the hospital for study of amenorrhoea for 5 months. In the body computed tomography (CT) scan a tumour located was identified in the cranial base and a nodular infiltration was observed in the lung. Histological diagnosis concluded with eosinophilic granuloma. Clinical manifestations were: hypothalamic dysfunction, pituitary failure and diabetes insipidus (DI). Treatment: Telecobaltherapy in CNS and chemotherapy (schedule DAL HX-83/90). After therapy CNS, magnetic resonance imaging became normal, and CT scan showed minimal residual lung disease. The patient became asymptomatic following hormonal substitutive therapy. Three years later, the patient remained asymptomatic with ECOG 0, but examination of peripheral blood showed 3% of blast cells (hemoglobin 8.2 g/dL, WBC 3.2x10⁹/L neutrophils 3%, monocytes 8%, lymphocytes 86%, blasts 3%, platelets 213x10⁹/L). Bone marrow study: 0.5% blasts: CD45+, CD117+, CD33+, CD13+ weak, CD64++, CD15++, CD3-, CD2-, CD7-, CD79a-, CD19-, MPO-, CD34-, CD56++, HLADR+++,

CD36+, CD11b-, CD7.1-/+, CD117+, CD14 and 77.5% of cells with the same immunophenotype except for CD117-, 7.1- and CD45-. One week later the bone marrow exam was repeated and 88.8% blasts cells with immunophenotype similar to blast as first exam were found. FISH: 88% translocation in 11q23. CT scan showed small and few nodules in lung. She was diagnosed of leukemia involving progenitors of dendritic Langerhans/monocytic differentiation cells: FAB AML-M0/M4. The patient had no sibling and she received treatment with 2-Chlorodeoxyadenosine (CDA) (0.12 mg/kg, on days 1 to 5 in a 28-day cycle up to 4 cycles) associated to epotin-a. After two cycles the patient had normalized the peripheral blood (hemoglobin 12.5 g/dL, WBC 6.7x10⁹/L with normal distribution and platelets 485 x10⁹/L). After treatment she achieved complete remission in the bone marrow exam with < 0.002% of leukemic cells. Nowadays she is being prepared to carry out PBSCT. Tolerance to therapy has been excellent without any adverse effects and she has a good quality of life. **Conclusions.** The use of CDA in LCH is based in their antiproliferative and immunomodulatory effects. CDA has been used in LCH with good results and minimal toxicity. Some cases published have showed a durable complete response in patients with LCH in skin, gastrointestinal, lungs, bone, diabetes insipidus and parenchymal CNS. In our knowledge, any case of myelodendritic leukemia treated successfully with CDA has been described previously. Further studies are needed to determine the role of CDA in this disease, the optimal and schedule of therapy, but we have proved it may be effective even in high-risk patients with LCH. More therapies based on the pathogenesis of LCH are needed to investigate.

0975

THYMIDINE KINASE LEVELS IN SERUM IN THE ACUTE MYELOID LEUKAEMIA PATIENTS AS PROGNOSIS FACTOR OF COURSE OF THE DISEASE

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Studying changes of serum thymidine kinase levels in 70 patients with different variants of acute myeloid leukaemia in time of induction remission show the prognosis value of this method. Serum thymidine kinase levels lower than 10 units/L at the time of diagnosis of acute myeloid leukaemia predicts good and rapid results in patients who were treated with standard chemotherapy and also is a factor of favorable course of the disease. Reducing the serum thymidine kinase levels after completion of the standard chemotherapy up to the normal range (lower than 6 units/L) proves reaching the remission rate, and serum thymidine kinase level after completion of the standard chemotherapy program above 6 units/L is an evidence of incomplete positive clinico-hematological results on the given chemotherapy. Having analyzed the research data, we can confirm that serum thymidine kinase levels above 30 units/L at the time of diagnosis of acute myeloid leukaemia indicates a high risk of resistant course of disease and probably requires individual treatment regime. Lack of changes in thymidine kinase levels before and after chemotherapy is an unfavorable prognosis factor.

0976

ANAPLASTIC LARGE CELL LYMPHOMA - ATYPICAL FORM, WITH A PARTICULAR CLINICAL PRESENTATION

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Background. Primitive pulmonary malignancies, including lymphomas, are extremely rare in children and may therefore

not be recognized as the cause of a respiratory distress. **Aims.** Analysis of a case with severe progressive respiratory distress that raised numerous differential diagnosis and therapeutic problems, proved to be an initially unrecognized malignancy - a rare type of lymphoma in children, anaplastic large cell lymphoma (ALCL). **Methods.** An 8 years old male patient admitted in the intensive care unit with severe respiratory distress was investigated for the suspicion of an infectious illness (blood and sputum analysis, x-Ray, CT scan, bronchoscopy with bronchial lavage). The cytological examination of the sputum and bronchial fluid raised the suspicion of a malignant disease, confirmed after the histological examination of a supraclavicular lymph node. Complex investigations were performed (serum and tissue tumor markers, tissue immunocytochemistry, and immunophenotyping) before establishing the final diagnosis. **Results.** The patient was admitted in our clinic one month after the onset of an illness characterized by loss of weight, asthenia, irregular fever, cough, and wheezing, progressive respiratory distress. Antibiotics, bronchodilators, and corticosteroids were not effective, and the child's state has continuously aggravated. Malignancy was suspected after finding of a supraclavicular lymphadenopathy, and the presence of large atypical cells in sputum. The bronchoscopy showed diffuse infiltration of the mucosa, and large atypical cells were found in the bronchial lavage fluid. No bone marrow infiltration was found. The evolution was complicated by a pericarditis with massive exudate and impending pericardial tamponade that imposed an emergency pericardectomy; no atypical cells were found in the pericardial fluid. Finally a supraclavicular lymph node biopsy was performed, but the histological examination couldn't identify the type of malignancy. The diagnosis of lymphoma was established after complex investigations. The anaplastic non-lymphoid diffuse malignancies with large cells, and Hodgkin's disease were excluded (carcinoma-citokeratin, pancreatine - KL1, TTF1; germ cell tumor-AFP, β -hCG; sarcoma-vimentin, keratin; melanoma-HMB45; Hodgkin's disease-CD15, EMA). The final diagnosis was ALCL null immunophenotype (CD45, CD20, CD79a, CD3 negative; cytoplasmic CD30 intensely positive, perinuclear CD30 slightly positive); the NPM-ALK was negative. The specific treatment (NHL-BFM 95 protocol) induced a rapid and spectacular improvement, and complete remission maintained at 26 months from diagnosis. Certain particular features of the case must be noted: primitive pulmonary malignancies (including lymphomas) are extremely rare in children; ALCL represent 20-30% of children's lymphoma with large cells; ALCL-ALK negative are more characteristic for older patients, and have a much worse prognosis. **Conclusions.** The continuously increasing incidence of malignant diseases in the last years, frequently with atypical forms and locations, should raise the rate of suspicion, beginning with the clinical examination.

0977

PROLIFERATION OF ACUTE MYELOID LEUKEMIC CELLS

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Autoradiographic studies in 80's revealed that myeloid leukemia cells have different proliferation rate, and in general it is low, with most cells in G0 phase of cell cycle. Introduction of flow cytometry with DNA/RNA measurement confirmed those data. As these measurements have certain technical obstacles, proliferative markers were introduced in several human neoplasms to evaluate cell proliferation and therefore behaviour of tumour. Objective. We analyzed proliferative fraction of leukemic cells by the application of monoclonal antibodies to Ki-67 and PCNA proliferative markers, and compared these findings with clinical features and initial treatment outcome (CR/NR). Materials and methods. In order to avoid permeabilization of cells for nuclear antigens, we analyzed proliferation on bone marrow samples (trephines) in a cohort of 42 patients suffering from *de novo* AML. Mean age was 46 yrs. (19-66 yrs),

and all were treated with similar treatment (ADE/MAE 30 pts, and DA/MC schedule 12 pts). After obtaining informed consent, bone marrow biopsy samples were taken at diagnosis and fixed in B5, embedded in paraffin. After deparaffinization, antigen retrieval and immunohistochemistry was performed by use of commercial Ki-67 (MIB-1) and PCNA (PC-10) antibodies and imaging kits (LSAB2, Dako Denmark) according to manufacturer prescription. Statistical analysis included parametric and nonparametric tests. **Results.** According to FAB classification 3 patients had M1 and 18 M2. Seventeen patients had AML M4 and 3 patients M5. One patient had AML M0. Fifteen patients had trilineage dysplasia. Twenty eight patients achieved CR (67%) and 14 were non responders. Mean Ki-67 positivity was $9.05 \pm 17.7\%$ (0-82%). In 15/42 patients (35.7%) Ki-67 was absent, and in 38.1% was <10% of leukemic cells. In 11/42 patients (26.2%) Ki-67 was positive in >10% of leukemic cells. Mean PCNA positivity was higher than Ki-67, $34.7 \pm 25.1\%$ (0-90%). In only 4/42 patients (9.5%) PCNA was absent and in other 4 (9.5%) was <10%. In 13/42 patients (30.9%) PCNA was positive in >40% of leukemic cells. There was good linear and rank correlation between these two proliferation markers ($p < 0.05$). Patients with trilineage dysplasia had higher Ki-67 positivity (14 vs. 6.3%, MWU test $p < 0.05$) but not PCNA positivity. There were no correlation between percentage of Ki-67 or PCNA positive leukemic cells according to morphology type or cytogenetic features. Patients responded to induction chemotherapy (achieved CR) had significantly lower proliferation measured through Ki-67 positivity (CR 4.6 vs. 17.9% for NR, MWU $p < 0.05$), but not by PCNA determination (31.2 vs. 41%, $p > 0.05$). Patients with lower proliferation, Ki-67 and PCNA labeled leukemic cells <10%, had better survival than others (log rank test for Ki-67 $p = 0.06$, for PCNA $p > 0.05$). **Conclusions.** Our results in a small cohort of patients confirmed that leukemic cells in AML are dormant, in most cases are non proliferative, which is similar to other findings. There is a good correlation between Ki-67 and PCNA proliferation markers. Also, patients with lower proliferation have better treatment outcome (CR achievement) and better survival.

0978

APOPTOTIC RESPONSE TO ANTILEUKEMIC TREATMENT MAY PREDICT OUTCOME OF INDUCTION CHEMOTHERAPY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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It has been known that apoptosis is one of the major mechanisms in action of chemotherapy. In *in vitro* conditions myeloid leukemic cells undergo apoptosis after exposure to cytotoxic drugs but less is known about its in *in vivo* effects. Objective. We analyzed morphologically recognized apoptosis measured as apoptotic index (AI) during induction antileukemic treatment and relationship between clinical features and influence to initial treatment outcome (CR/NR). Materials and methods. We histologically analyzed bone marrow samples (particles) from a cohort of 32 patients suffering from *de novo* AML, aged 45 yrs. (19-67 yrs), treated with similar treatment (ADE/MAE 26 pts, and DA/MC schedule 6 pts). After obtaining informed consent, samples were taken at diagnosis and also at 3rd day (D3) from start of therapy. Analysis was performed on morphological level by counting cells with morphologically recognized apoptosis on at least 1000 cells and expressed as AI (%). Also bcl-2 evaluation was performed by immunohistochemistry with use of commercial Bcl-2 antibody and imaging kits (LSAB2, CSA, Dako Denmark). Statistical analysis included parametric and non-parametric tests. **Results.** According to FAB classification 2 patients had M1 and 14 M2. Twelve patients had AML M4 and 3 patients M5. Only one patient had AML M0. Eleven patients had trilineage dysplasia. Twenty one patient achieved CR (66%) and 11 were non responders. Initial mean AI was $3.0 \pm 1.59\%$. There is no difference in initial AI between patients according

to FAB and leukemia type (myeloid/monocyte), trilineage dysplasia or karyotype (ANOVA & Kruskal Wallis $p > 0.05$). AI was increased to $7.77 \pm 4.5\%$ at D3 (t-test $p < 0.01$). We have not found differences in increase of AI concerning FAB, dysplasia and karyotype. When we analyzed increase of AI and outcome of induction therapy we have found that patients in CR have significantly higher therapeutic increase in AI at D3 than nonresponders (DAI $6.1 \pm 4.9\%$ vs. $2.3 \pm 1.9\%$, t-test and Mann Whitney $p < 0.05$). Bcl-2 negative patients in CR had higher increase of DAI ($5.8 \pm 1.3\%$) than patients with NR ($1.7 \pm 1.3\%$). That difference was much lower in group of bcl-2 positive patients. **Conclusions.** Our results of a small cohort of patients in our model showed that antileukemic treatment induce apoptosis measured *in vivo*, during induction treatment of AML. That increase in apoptosis have impact on treatment outcome and have prognostic significance, but further studies are needed.

0979

RECOMBINANT HUMAN ERYTHROPOIETIN ALFA IN PATIENTS WITH LOW-RISK MYELOYDYSPLASTIC SYNDROMES, SELECTED ON THE BASIS OF A LOW PRE-TREATMENT SERUM ERYTHROPOIETIN CONCENTRATION

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Background. Recombinant human erythropoietin (rHuEPO), given daily or 3 times a week, subcutaneously, at a dosage of 30-70.000 U/week, has proven effective in relieving symptomatic anemia in 20-30% of patients (pts) with myelodysplastic syndromes (MDS). A low or intermediate-low risk score, according to International Prognostic Score System (IPSS) (Greenberg et al, 1997) and low pre-treatment serum EPO levels are both associated with a higher probability of response. **Aims.** As rHuEPO is expensive, and the percentage of responder MDS pts is low, an attempt to select MDS pts on the basis of basal serum EPO levels and IPSS score, is justified. **Methods.** From September 2001, 29 pts (16 males, median age: 76, range 34-92 yrs) with low-or-intermediate risk MDS were treated with rHuEPO alfa because of symptomatic anemia (Hb < 10 g/dL). The pts received a high doses rHuEPO regimen: 40.000 U twice weekly, for the first month, followed by a single weekly dose of 40.000 as maintenance treatment, for at least 8 weeks. Only pts with a pre-treatment serum EPO < 200 U/L were selected for rHuEPO treatment. **Results.** 27/29 pts showed a favourable response. 8 pts were transfusion-dependent. Among them, 6 showed a $\geq 50\%$ decrease of their transfusion need. All the remaining 19 pts, with less severe anemia, not requiring transfusions, showed a clinical significant response (i.e. a > 1g/dL increase of Hb). The median duration of response was of 13 (2-41) months, and 23 pts are still maintaining response under maintenance treatment. **Summary/Conclusions.** Although only 20-30% of MDS pts show a clinical significant response to rHuEPO, if pts are carefully selected on the basis of a low risk score (following IPSS) and a low (< 200 U/L) pre-treatment serum EPO level, the percentage of responses is higher.

0980

ANALYSIS OF X-CHROMOSOME INACTIVATION PATTERNS IN IRANIAN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) USING RNA POLYMORPHISM OF P55, IDS AND G6PD GENES

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Background. Analysis of x-chromosome inactivation patterns in females has been used to assess clonality of various tumours

and X-linked disorders. Conflicting results have been published on the frequency of clonal patterns of X chromosome inactivation in female patients with Acute Myeloid Leukemia. Previous studies have used DNA methylation to measure X inactivation, but aberrant methylation is known to occur in some situations. **Aims & Methods.** We have developed a non-radioactive reverse-transcription polymerase chain reaction (RT-PCR) method to study expression of the polymorphism at nt.1311 of the G6PD gene at the RNA level. Using this, and a similar method for the iduronate 2 sulfatase (IDS) and Palmitoylated membrane protein (P55) genes, we have re-evaluated X inactivation in AML patients. **Results.** 92/100 normal female individuals (92%) showed polyclonal haemopoiesis. Patients with presumed clonal diseases showed both monoclonal and polyclonal patterns, consistent with previous reports. Overall, clonal patterns were observed in granulocytes of 44/45 *de novo* AML patients (98%), a significantly higher proportion than in controls ($p < 0.01$). Three cases showed discordance between lymphocytes and granulocytes, indicating clonality arising within the myeloid lineage. Forty one cases showed clonal patterns in both myeloid and lymphoid cells, indicating the involvement of a pluripotent stem cell. Clonal patterns did not correlate with age, but there appeared to be an association with duration of disease.

0981

ENDOGENOUS ERYTHROPOIETIN PRODUCTION AND ERYTHROPOIETIC ACTIVITY IN ANEMIC CANCER PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background. Cancer anemia is multifactorial: blunted erythropoietin (Epo) response has also been encountered. As it is particularly well documented only in some types of anemia of chronic disease (ACD), we investigated the Epo deficiency in anemic cancer patients with hematologic malignancies. **Patients and Methods.** 42 patients (pts) with multiple myeloma (MM), 27 pts with malignant lymphoma (ML) and 19 pts with chronic lymphocytic leukemia (CLL) were included in the study. 25 pts with iron deficiency anemia represented the control group. Serum Epo and serum transferrin receptors (sTfR) were measured with commercially available assays. O/PEpo and O/PsTfR ratios (O-observed value, P-predicted value) were derived for each patient in order to assess if Epo response and erythropoietic activity are appropriate for a given degree of anemia. Predicted values were calculated from the regression equation of serum Epo, respectively sTfR versus hemoglobin (Hb) determined in the control group. A correlation between O/PEpo and O/PsTfR was searched in order to assess the impact of Epo deficiency on erythropoietic activity and therefore the anemia. **Results.** All pts, except for the MM pts with renal failure showed Epo response to anemia: a significant inverse correlation between serum Epo and Hb was found. Epo production and erythropoietic activity as determined from the control group were inappropriate if the values for O/PEpo and O/PsTfR were ≤ 0.8 and ≤ 0.9 , respectively. 43% pts with MM and no renal failure, 33% pts with ML and 11% pts with CLL had inappropriate Epo response to anemia. Erythropoietic activity was inappropriate in 76% pts with MM, 48% pts with ML and 37% with CLL. The inappropriate erythropoietic activity and therefore the anemia were significantly influenced from the inappropriate erythropoietin production: this could be shown by the positive correlation between O/PEpo and O/PsTfR in all three patient groups. **Conclusions.** Significant number anemic pts with hematologic malignancies have blunted Epo response to anemia. The adequacy of serum Epo levels could be convincingly assessed by O/PEpo and O/PsTfR ratio. The relationship between Epo deficiency and therapy response to rHuEpo in anemic cancer pts remains to be determined.

0982

FLOW CYTOMETRIC EVALUATION OF THE BONE MARROW IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Introduction: Many studies and effective immunosuppressive therapy suggest that the immune system plays a role in the pathophysiology of MDS. **Material and Methods.** Using flow cytometry immunophenotyping we studied lymphoid population in the bone marrow specimens from 41 patients with MDS (RA-2, RARS-1, 5q-3, RCMD-14, RCMD-RS-4, MDS-U-2, RAEB1-11, RAEB2-2 and MDS/MPS-2) and 19 healthy donors served as controls. These samples were analyzed for percentages following subtypes of lymphocytes: CD3+, CD3+/CD4+, CD3+/CD8+, CD3-,16+,56+, CD19+ and granulocytes. Cell staining was performed on whole bone marrow during routine phenotyping of lymphocytes and analyzed in FACS Calibur (BDIS) flow cytometer. All of the results have been statistically tested by using T-student test for the independent groups and Pearson's correlation. For statistically significant results were $p < 0.05$. **Results.** Our analysis showed significantly increased BM lymphocytes (20,5% vs. 13,1%, $p = 0,006$) and their following subtypes: CD3+ (73% vs. 56%, $p = 0,000$), CD3+4+ (36% vs. 28,9%, $p = 0,009$), CD3+8+ (38,3% vs. 26,6%, $p = 0,001$) in MDS patients as compared the controls, but there was a smaller percentage of CD19+ (9% vs. 25%, $p = 0,000$) and granulocytes (54,3% vs. 69%, $p = 0,046$). NK cells and CD4:CD8 ratios demonstrated no significant differences between these groups. The statistically significant differences were between the subtype of RCMD+RCMD/RS and RAEB1 in proportion to normal BM in: CD3+ (73,7% vs 56,2% $p = 0,001$ and 75,1% vs 56,2% $p = 0,001$) and CD19+ (9,1% vs 25,2% $p = 0,0000$ and 8,5 vs 25,2 $p = 0,002$). The statistically significant differences in lymphocytes CD3+4+ and CD3+8+ were only between RAEB1 and healthy donors (39,2% vs 28,9% $p = 0,003$ and 39,1% vs 26,6% $p = 0,004$). NK cells and CD4:CD8 ratios demonstrated no significant differences between these groups, too. There weren't significant differences between these 2 (RCMD+RCMD-RS vs RAEB1) subtypes of MDS. Remaining subtypes of MDS were too small to be analyzed statistically. **Summary.** We found out increased percentage of BM lymphocytes and their subtypes (CD3+, CD3+/4+ and CD 3+/8+) in MDS patients comparing healthy donors, but B-cells proportion was clearly decreased.

0983

DECREASED SERUM LEVEL OF PRO-HEPCIDIN IN MULTIPLE MYELOMA PATIENTS WITH ANAEMIA

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Background. Heparin is an antimicrobial protein mainly synthesized in the liver, which has been isolated from human plasma and urine. It is a central regulator of intestinal iron absorption and iron recycling by macrophages. It has been proposed that hepcidin is a mediator of the common clinical syndrome-anaemia of chronic disease (ACD). Multiple myeloma (MM) is a neoplastic disease of the hematopoietic system. Anaemia is a common symptom of MM especially in patients with advanced disease and correlates with the clinical stage of myeloma. **Aims.** The aim of this study was to investigate the serum level of pro-hepcidin in multiple myeloma patients. **Methods.** We report our experience in 40 patients, 25 females and 15 males. The median age of patients was 60,5 yrs. 3 patients were in I stage of MM, 17 in II stage and 20 in III stage acc Durie and Salmon. The healthy control group included 22 persons. Clinical data are showed in table 1. We compared pro-hepcidin levels in 3

groups: the first group: patients with MM and anaemia (haemoglobin <11g%), the second group: patients with MM without anaemia and the third group: control with normal level of haemoglobin. Pro-hepcidin levels were detected by a sensitive enzyme linked immunosorbent assay ELISA (DRG Instruments GmbH, Germany). For statistical analysis U-Mann-Whitney test and ANOVA-rang Kruskal-Wallis test were used. **Results.** In patients with MM with anaemia mean serum concentration of pro hepcidin was 67,24 ng/mL (range:23,7-353,6 ng/mL). In group of patients with MM without anaemia mean serum concentrations of pro hepcidin was 120,98 ng/mLs (range 24,2-548,8 ng/mL). Using the sensitive pro-hepcidin Elisa, pro-hepcidin in the rang 44,0-1953,2 ng/mL in serum was detected in healthy control group (mean: 1081,2 ng/mL). These were lower compared with those in control (p<0,001). Mean serum level of pro hepcidin in MM patients with anaemia was lower compared with those in MM patients with anaemia and with control (p<0,001). **Conclusions.** Decreased serum level of pro-hepcidin in MM patients may suggest one of the reason of anaemia in MM.

Table 1. Clinical data.

	MM	Control
Mean age	60,5 years	42,6 years
Sex	25 Female 15 Male	14 Female 8 Male
Stage sec Durie and Salmon	I-3 II-17 III-20	

0984**GENETIC ABERRATIONS AND RESPONSE TO FLUDARABINE AS FIRST LINE TREATMENT IN A SERIE OF B-CLL PATIENTS**

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Background. Genetic factors have been established in the recent years as the most important independent predictors of disease progression and survival in patients with B-chronic lymphocytic leukaemia (B-CLL). Fludarabine is an approved first-line treatment for B-CLL, which achieves superior remission rates than traditional therapies. The drug is equally effective in early and advanced disease and in younger and elderly patients with B-CLL but different results have been found in patients with cytogenetic aberrations as methylation in the TP53 promoter. **Aims.** To evaluate the response to Fludarabine as first-line therapy in B-CLL patients with advanced stages. **Patients and Methods.** In the study 55 B-CLL patients were enrolled. The diagnosis was established based on standard morphologic and immunophenotypic criteria, and classified on high clinical risk category Rai stage III/IV treated with Fludarabine (25 mg/m² daily for 5 days IV in a 28-day cycle) as first-line therapy from January 00 to December 03. Response was evaluated after 4 cycles of therapy and in those patients which had not achieved complete remission (CR) two additional cycles were administered. Criteria for response were established using the revised 1996 National Cancer Institute-sponsored Working group Guidelines. FISH studies were performed using the probes LSI p53 (17p13), LSI D13S25 (13q14), CEP 12 and ATM gene (11q22). The sequences of the IGVH genes were determined in cDNA of the patients. Responses to therapy, time to progression disease and overall survival were available to analyse in 41 patients. Association was studied using contingency tables fol-

lowed by Fisher exact test and to evaluate survival by acturial method of Kaplan Meier and Cox regression. **Results.** Mean age 64 (36.9-83.9), female 53.7%. According to the advanced stage of the disease in this serie, 75% of patients presented genetic aberrations associated with worse prognosis: del (17p) and/or del (11q) (45%), and no-mutated IGVH genes (57%). 50% of the patients achieved CR and in 50%. 83.5% of patients achieved response (CR 47.8%). Related to genetic aberrations the responses were: del (17p) 55.5%, del (11q) 75.0%, +12 100%, del (13q) 88.8% and 100% of patients without genetic alterations showed response to Fludarabine. The 48.8% of patients developed progression of disease with mean duration of the response of 15.1 months. Related to genetic aberrations the progression was over 81% of patients with genetic aberrations versus 55.5% without genetic alterations (p=0.044). The overall survival was 68.0 months. 34.1% of patients are alive in CR, 36.6% are alive with stable disease 7.3% are alive with disease in progression and 22.0% have died. **Conclusions.** A high response to Fludarabine in first-line therapy was observed, however only 55.5% of patients with del (17p) showed response. Patients with genetic aberrations had higher significant rate of progression in comparison with those containing normal genotypes.

This study has been supported by a grant from MMA Foundation

0985**MYELOMA-SPECIFIC T CELLS FOR TARGETED IMMUNOTHERAPY: IDENTIFICATION AND IN VITRO EXPANSION**

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Background and Aims. Multiple myeloma has been considered as low immunogenic incurable disease. The attempts has been made to invert the immune status to recognize myeloma cells by T cells or other cells of the immune system. Here we studied the possibility of identification of myeloma-specific T cells *in vitro*, their clinical-grade expansion, and specific cytotoxicity of expanded T cells to myeloma cells. **Methods.** Irradiated myeloma cell line ARH 77 has been used as tumor antigen to stimulate peripheral blood mononuclear cells (PBMC) of 8 healthy volunteers. Activated responder T cells has been immunomagnetically separated based on surface expression of interferon gamma (Miltenyi Biotech) and expanded in 5 cases by phytohemagglutinin and repeated high-doses of interleukin 2. Cytotoxicity against the original myeloma cell line has been tested after the T cell expansion using propidium iodide. Tested expanded T cells were labeled by CFSE to distinguish them from myeloma cells. Third-party PBMC and non-expanded interferon gamma negative fraction served as controls. **Results.** The percentage of interferon gamma positive cells has been enriched from 2.8±0.9 and 2.6±0.8 to 48.6±23.4 and 73.2±25.9 of CD3+CD4+ and CD3+CD8+ T cells, respectively, by immunomagnetic separation. Interferon gamma positive T cells has been further expanded *in vitro* from 0.54x10⁶±0.05x10⁶ to 214.00x10⁶±103.46x10⁶ within 4 weeks. The cytotoxicity has been tested after expansion. The killing of myeloma cells reached 68.1±14.2%. Interferon gamma negative fraction killed only 0.8±0.3% of myeloma cells. As a control, killing of third-party PBMC by expanded interferon gamma positive T cells was 6.9±2.5%. **Conclusions.** These data demonstrate a specific cytotoxicity effect of expanded interferon gamma positive T cells against myeloma cell line ARH 77 and open the possibility for clinical use of tumor-specific T cells in cancer immunotherapy.

0986**INDUCTION THERAPY AND HIGH DOSE SEQUENTIAL THERAPY FOR THE TREATMENT OF HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA**

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Introduction: Patients (pts) with diagnosis of DLBCL and IPI age adjusted 2 or 3 present a poor prognosis. The results with high dose sequential therapy (HDST) showed a significant improvement of Overall Survival (OS) and Event Free Survival (EFS) in comparison with a standard chemotherapy. **Methods.** We decided to use HDST adding an intensified scheme as debulking phase. The phase I was characterized by an intensified CHOP (MegaCHOP): cyclophosphamide 3 g/sqm; doxorubicin 75 mg/sqm; oncovin 1,4 mg/sqm on day 1 and prednisone 100 mg for 5 days. Stem cells collection was planned after the third cycle. The phase II was the classical HDST. The phase III was the peripheral blood stem cell transplantation (PBSCT) with melphalan 180 mg/sqm and mitoxantrone 60 mg/sqm as conditioning regimen. From March 2002 until February 2004 we enrolled 11 pts. Median age was 39 years (range 25-49), 8 pts were stage III-IV (73%), 7 presented bulky disease (64%), all but one had an abnormal LDH value. All pts were IPI 2 or 3. Phase one and two were performed on outpatient basis. **Results.** Six pts obtained a very good partial remission (PR) after first phase, six were in CR unconfirmed after phase II and 4 were in PR. After PBSCT 8 pts were in CR and 2 were in PR. At the end of planned therapy 9 pts obtained a complete remission (CR) (82%), a patient after radiotherapy was alive with disease and one died of progressive disease during phase I. After a median follow-up of 22 months (range 5-36) OS was 81%. With a median follow-up of 19 months 88% of complete remission pts were free from disease and only one patient relapsed after 2 months from CR. We did not find grade III or IV WHO extra-haematological toxicity. **Conclusions.** We can conclude that this protocol is feasible as outpatient basis in phases I and II. Moreover, this therapy was highly effective (81% OS) in the subset of pts with very high risk characteristics at diagnosis.

0987**ANTIAPOPTOTIC EFFECT OF G-CSF UPON CD34+ CELLS IN BONE MARROW AND BLOOD**

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The purpose: to estimate the effect of granulocyte colony stimulating factor (G-CSF) on the level of spontaneous apoptosis of bone marrow and blood cells. **Methods.** the level of spontaneous apoptosis was estimated by flow cytometry as the number of hypodiploid cells after propidium iodide staining and bipositive cells (CD34 PE + / Annexin V FITC + and CD15 PE + / Annexin V FITC +) after Annexin V staining in 28 children's with different hematological malignancies, receiving G-CSF for CD34+cells mobilization (14 patients-group 1) and treatment of cytostatic cytopenia (14 patients-group 2). **Results.** the level of spontaneous apoptosis of CD34+cells before G-CSF therapy in patients from group 2 was significantly higher in comparison with the patients from group 1 in bone marrow (14,82±1,15% and 4,86±0,27% accordingly, p < 0.001) and blood (12,84±0,89% and 5,94±0,69% accordingly, p = 0.001). The level of spontaneous apoptosis of CD15+cells (mature granulocytes) before G-CSF therapy in bone marrow and peripheral blood demonstrated no differences in both groups. The level of spontaneous apoptosis of CD34+cells after G-CSF therapy was significantly lower in bone marrow and blood in comparison with their pretreatment level in both groups. The level of spontaneous apoptosis of CD15+cells after G-CSF therapy

did not change in blood and bone marrow in patients of group 1 and was significantly lower in bone marrow and blood in group 2. **Conclusions.** The ground level of spontaneous apoptosis CD34+cells was much higher after chemotherapy in bone marrow and blood. G-CSF demonstrates antiapoptotic effect upon CD34+cells in blood and bone marrow and did not change it significantly in CD15+cells. The intensity of G-CSF-induced antiapoptotic effect was higher in CD34+cells in comparison with CD15+cells in bone marrow as well as blood.

0988**SUCCESSFUL PREGNANCIES ON DEFERIPRONE: REPORT OF TWO CASES**

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Introduction. In Europe, the product monograph for deferiprone (Ferriprox) advises against the use of this iron chelator in pregnant women, based on studies conducted in pregnant animals (rats and rabbits). However, sometimes patients become pregnant without realizing it and there is a need to understand the risk in humans. **Aims.** To report two (2) cases of non-scheduled pregnancy in patients with homozygous α -thalassaemia who received deferiprone treatment in the first trimester. **Patients:** Two transfusion-dependent α -thalassaemic women, 23 and 26 years old, with regular menstruation without hormonal substitution, conceived spontaneously while receiving deferiprone chelation therapy, 75 mg/kg body weight. The serum ferritin concentrations for the 23 and 26 year old patients closest to the time of pregnancy revealed values of 1,900 and 5,500ng/mL and 1,400 and 6,300ng/mL post delivery respectively. Treatment was discontinued during week 5 after the last menstrual cycle in one patient and week 6 in the other. This was the second pregnancy for both patients. Both pregnancies were carried to term normally and two healthy male infants were delivered without any complications following Caesarian section during the 38th week of pregnancy. Male infants were born with an Apgar Score >8 and 9 in the first minute and 10 for both in the fifth minute. There were no neonatal complications and the infants are developing normally to date at 1 year and five months old respectively. **Conclusions.** Increased quality of life might possibly result in a larger number of unexpected pregnancies during deferiprone use, warranting close monitoring, based on the product monograph. Whether iron loading protects against deferiprone-related teratogenesis in thalassaemia patients, or whether animal models are not predictive of the human situation remains to be determined. Despite the positive outcome of these 2 cases, discontinuation of deferiprone therapy should be recommended in planning a pregnancy in a transfusion-dependent, α -thalassaemic patient and the experience of other clinicians needs to be reported.

0989**EFFECT OF ERYTHROPOIETIN IN REFRACTORY ANEMIA**

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Introduction: Supportive care with packed red cells transfusions is the primary support strategy for the majority of patients with MDS. Human recombinant erythropoietin (rhuEPO) is an effective therapy for the anemia in about 30-35% of selected patients with MDS. Some patients who are red cell transfusion dependent can benefit from rhuEPO with a significant reduction of transfusion need. The aim of this report is show the effect of rhuEPO therapy in Hb levels and transfusion requirements in our MDS (RA) patients, as well as the assessment of the corre-

lation between serum EPO (s-EPO) level and Hb increase. *Material and methods.* 48 patients (21 male and 27 female) were reviewed, with a mean age of 69 (58-82) years, all of them suffering of refractory anemia RA. Alpha rhuEPO 10.000 IU was given subcutaneously three times a week. Hb and s-EPO were measured monthly, from the third month before the treatment to the fifth after the start of treatment. Hb increase was the parameter considered for response. Good response was considered when Hb increase was equal or higher than 1 gr/dL. Correlation between Hb increase and s-EPO levels was studied. Blood transfusion requirements were taken into account. Student-t test and Pearson correlation test were used for statistical analysis. *Results.* In the three months before rhuEPO administration, median Hb level was 8.25±/0.12 gr/dL, 16 of the 48 patients (33.33%) had a good response and get an increase of Hb level equal or more than 1 gr/dL. In the group of patients that respond to the treatment the average of s-EPO in the three months before treatment was 250±/97 U/L, and in the following five months it was 426±/45 U/L. In the unresponsive group of patients s-EPO was 195±/41 U/L and 223±/54, before and after treatment respectively; statistical difference was found between two groups in s-EPO after treatment (p<0.01). We found good correlation between increase of Hb and s-EPO (p<0.001). In good responders the transfusion frequency reduces 50%. Good responses were obtained as soon as at the first month of rhuEPO therapy. Transient rash was present in 18 patients and blood hypertension in 6 during treatment. *Conclusions.* Erythropoietin administration may increase hemoglobin concentration and, consequently, decreases transfusion frequency in 33% of our patients, as soon as in one month. Besides, we observe a significant difference in the s-EPO post-treatment between both groups of patients so, we think s-EPO measure could be a good parameter for detecting rhuEPO responders.

0990

PROGNOSIS OF THE PATIENTS WITH NON-HODGKIN LYMPHOMA BASED ON THE CLINICAL CHARACTERISTICS OF THE DISEASE: SINGLE CENTER EXPERIENCE

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Background. International Prognostic Index (IPI) represents clinical indicator of prognosis of patients with Non-Hodgkin's lymphoma (NHL). It takes into account parameters such as patient characteristics and disease stage and helps to identify NHL patients with high risk disease eligible for risk-adjusted treatment. *Aims.* The aim of our study was to assess the applicability of the IPI in our group of patients. *Methods.* Our study was conducted as retrospective one. We have evaluated 137 patients with NHL. The histo-pathological diagnosis was made according the REAL classification and 71 patients were diagnosed as diffuse large cell lymphoma, 28 as follicular lymphoma, 24 as small lymphocytic lymphoma and 14 as marginal cell lymphoma. Pretreatment patient characteristics, as age, patient performance status, serum lactate dehydrogenase level, tumor stage and the number of extra nodal disease sites were taken into consideration and the IPI was assessed. *Results.* According to the number of 'poor risks' factors, our patients were divided in to the four IPI risk groups: 57 in low risk, 25 in low-intermediate, 28 to intermediate-high and 27 in high risk group. Five years survival rates in each group were 88%, 82%, 18% and 0% respectively. *Summary/conclusion:* Our results showed that the stratifications of the HNL patients based on IPI correlates with the response rate to the standard treatment and with overall survival of the patients. In the future, routine IPI application in HNL patients will enables us to use the most appropriate therapeutic approach to each individual NHL patient.

0991

ACUTE LEUKEMIA, CO-EXISTING WITH A CHRONIC LYMPHOPROLIFERATIVE DISORDER, 20 YEARS AFTER A PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDER

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Introduction. A patient with a Philadelphia-negative Chronic Myeloproliferative Disorder (Ph-CMD) of 20 years of evolution, ending in a Monoblastic Acute Leukemia (AL) is presented. Two years before a simultaneous Indolent Lymphoproliferative Disorder (ILD) was observed. *Patient and methods.* A 64 years old female was diagnosed in 1984 of a Philadelphia-negative chronic myeloproliferative disorder. She was in good general conditions with no B symptoms. The abnormalities in the peripheral blood (PB) tests were: Hb 18.5 g/dL, hematocrit 60.6%, leukocytes 25.2x10⁹/L, platelets 1153x10⁹/L, granulocytic alkaline phosphatase score 80% (normal value 20%-40%). Bone marrow (BM) aspirate showed marrow hypercellularity with megakaryocytic hyperplasia. BM karyotype was normal: 46XX. She was treated with Busulfan until 1996, and with Hydroxyurea from 1996 to 2002, with good control of her PB picture, she remained stable along 18 years. In December of 2002 she, suddenly, presented anemia of Hb 8,6gr/dL. Leukocyte count was normal with 64% lymphocytes, 40% of them villous, and spherocytes were seen on the blood smears. Reticulocytes 3.5% and LDH level at 1.5 fold normal value, but direct antiglobulin test was negative. BM showed a normal distribution and morphology of lymphoid, myelocytic and thrombocytic series, but erythroid had important maturation changes. Immunophenotypic markers expressed in about 35% of PB cells and 6,2% in BM: CD19+, CD5-, CD10-, CD20+, CD22++, CD23-, CD25-, CD11C-/+, CD43+low, CD79b-/+, FMC7+++, with surface IgG lambda medium/low, it was consistent with WHO type immunocytoma B-cell malignancy. BM karyotype showed 60% of normal metaphases, 20% with monosomy 7 and 20% with del20q. A translocation t(14;18) was detected by PCR. Serum immunoglobulins level was: IgG 1130, IgA 58, IgM 22 mg/dL, with polyclonal increase of IgG. On physical exam and CT Scan hepatic and splenic visceromegalies, but no adenopathies, were detected. Hydroxyurea was stopped and Hb rises to 10 gr/dL, and from May 2003 to September 2004 Anagrelide was used with good tolerance, in order to control moderate thrombocytosis. From September of 2004 a transformation into AL is observed in PB with 24.5x10⁹/L leukocytes and the presence of 29% of circulating immature blastic myelomonocytic cells. BM shows 27% of monoblastic atypical cells, immunophenotypic findings are similar both, in BM and PB: a 11% of immature myeloid cells in myeloblastic phase of differentiation (CD34-, CD117+, CD13+, CD33+, DR+, MPO-/+, and 29% with phenotype of a monocytic AL (CD34-, DR+, CD11b+, CD13-, CD14+, CD15-, CD33++, CD36+, CD64+, MPO++). In 30% of interphases a fusion of chromosome 16 was observed by FISH. The clinical picture of a monoblastic acute leukaemia (M5) was quickly established and she died two months after. *Conclusion.* The treatment with alkylating agents might, probably, contribute to the final leukemic onset, but the leukemogenicity of Hydroxyurea is still debated. Nevertheless the bare probability of these Ph-CMD of ending in AL after 20 years will reach the 20%. The co-existence of a ILD could not be as rare as it seems, when using modern genetic and immunophenotypic techniques nowadays available, because the diagnosis of this ILD was based on morphologic and phenotypic criteria.

0992**MOLECULAR AND BIOCHEMICAL ANALYSIS OF POLISH PATIENTS WITH HEREDITARY SPHEROCYTOSIS**

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Background. Hereditary spherocytosis (HS), the most frequent hereditary red blood cell disorder in Poland, is associated with partial deficiency of erythrocyte membrane proteins. It has been estimated that hereditary spherocytosis occurs with the prevalence rate of 1:2000. Clinical manifestations of the disease range from mild cases of stabilized haemolytic form to severe ones requiring regular blood transfusions. The inheritance follows an autosomal dominant pattern, rarely, an autosomal recessive one. **Aims.** The aim of our study was to elucidate the molecular basis of the erythrocyte membrane proteins deficiency. **Methods.** The level of red cell membrane proteins was measured by flow cytometry and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). DNA Isolation Kit for Blood/Bone Marrow/Tissue (Roche Diagnostics GmbH, Germany) was used to isolate DNA from leukocytes. Total RNA was isolated from peripheral blood using PAXgene Blood RNA Kit(QIAGEN, Germany). Reverse transcription (RT) was performed using 0.2-1.0 µg RNA and oligo (dT) primers (BD Biosciences Clontech). Polymerase chain reaction (PCR) was used to amplify the entire coding sequence of the AE1 gene, and also the promoter sequence of the ANK1 gene. DNA fragments generated by PCR amplification were directly sequenced. The appropriate restriction enzyme analysis was used to verify the presence of found mutations. **Results.** All the probands revealed a decreased level of band 3 measured by two methods. Direct sequencing of band 3 gene showed the presence of mutations in the C-terminal domain of band 3 in four unrelated families: R490H (Pinhal), R490C (Bicette I), R808H (Nara) and R870W (Prague III). R490C mutation of our hereditary spherocytosis subject appeared *de novo*. In the same family we found an ankyrin promoter mutation, -108T>C. R808H and R870W substitutions of band 3 in our patients were connected with typical HS. Families of the probands were also tested for the presence of band 3 mutations. **Conclusions.** Molecular analysis showed four different substitutions of three highly conserved arginine residues. In two cases with different R490 substitutions, we observed a mild picture of HS with normal range in standard haematological tests, which indicates that R490 mutations of the AE1 gene do not severely destabilize the lipid bilayer. It correlates with the hypothesis of extracellular localization of R490 and suggest that it is localized outside of the membrane motif.

0993**RITUXIMAB PLUS CHOP IS SUPERIOR TO STANDARD CHOP AS INITIAL TREATMENT FOR PATIENTS WITH PREVIOUSLY UNTREATED AGGRESSIVE NON-HODGKIN'S LYMPHOMA: A SINGLE CENTRE EXPERIENCE**

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Background. Treatment of aggressive Non-Hodgkin lymphoma (NHL) with a standard CHOP regimen has yielded overall response rates of 80-90% with complete response rates 45-55%, and long-term (5-year) survival is seen in only 30-40% of patients. Attempts to improve these results by using more dose-intensive regimens have not resulted in a significant increase improvement in outcome. It is therefore essential to develop improved therapeutic approaches for patients with aggressive NHL. Rituximab is a chimeric human/murine monoclonal antibody that reacts specifically with B-cell antigen

CD20 and results in both complement-mediated and antibody-dependent cell-mediated lysis of CD20+ cells, induces apoptosis *in vitro* and sensitizes drug-resistant human B-cell lymphoma cell lines to the cytotoxic effect of some chemotherapeutic agents. Randomised phase III studies have indicated that the addition of rituximab to CHOP (R-CHOP) improves response rates and prolongs event-free and overall survival in patients with aggressive NHL. **Aims.** In this study we evaluate results of treatment with R-CHOP in comparison with standard CHOP as initial treatment for patients with aggressive NHL in a single centre in Macedonia. **Results.** Patients (n=123) with previously untreated, histologically confirmed aggressive B-cell NHL were treated at the Department of Hematology from 1989-2003 with 6-8 21-day cycles of standard CHOP chemotherapy (n=95) or R-CHOP (n=28). There were no significant differences in sex, age or prognostic factors between treatment groups. Most patients were in the advanced clinical stage at the disease on initial presentation (24% stage 3 and 43% stage 4). B symptoms were present in 44% of patients and bone marrow infiltration in 29%. Following treatment overall and complete responses were higher in patients treated with R-CHOP (ORR 86% vs. 64%; CR 75% vs. 60%). There were four early deaths (4%) in the CHOP-treated patients and none in the R-CHOP group. Although the follow-up in the group of patients treated with R-CHOP is still ongoing, there is significant improvement in event-free and overall survival (p<0.05). **Conclusions.** Our study confirms that the addition of monoclonal antibody rituximab to CHOP represents a major advance in the treatment of aggressive lymphoma and should be standard initial treatment for previously untreated diffuse large B-cell lymphoma.

0994**INTRAVASCULAR LYMPHOMA: LONG SURVIVAL AFTER HIGH-DOSE CHEMOTHERAPY**

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Background. Intravascular lymphoma is an uncommon form of non-Hodgkin lymphoma characterized by clonal proliferation of neoplastic lymphoid cells within the lumina of small and medium-sized vessels. The signs and symptoms of the disorder are attributed to vascular occlusion and are often nonspecific, mostly fever, skin lesions and mental disorders. The organs most commonly involved are the central nervous system, skin, lung and kidney. Furthermore the hematopoietic organs are usually spared. Diagnosis is very difficult due to variety of clinical manifestations and aggressive course and is made usually through biopsy of an affected organ. About 30-50% of cases are diagnosed post-mortem. The prognosis is dismal with a short reported median survival; nevertheless, complete remission can be achieved with combination chemotherapy. **CASE REPORT:** We report the case of a 51-year-old woman who started with chills and fever of unknown origin four months before admission. Progressively her condition worsened with drenching sweats despite corticosteroid and antibiotic therapy. Additionally she developed pain and numbness on right leg on admission. Generalized oedema and renal failure subsequently appeared. The only abnormal laboratory findings were an increased LDH (1655 U/L) and ferritin (1388 ng/mL). These symptoms progressed into paresia of both legs as well as urinary and fecal incontinence. Magnetic resonance imaging, thoracoabdominal computed tomographic scan and laboratory analyses of cerebrospinal fluid showed no abnormal findings. In functional nerve studies sensorimotor polyneuropathy and S1 radiculopathy was detected. Biopsy of nerve and muscle was performed in the end, leading to the diagnosis of intravascular lymphoma when the patient status was of a coma with a Glasgow score of 7. Subsequent CHOP chemotherapy was administered in the intensive care unit. Initially multiorgan failure and respiratory distress complicated the clinical picture, as well as disseminated intravascular coagulation. After intensive supportive

measures her condition began to improve slowly. CHOP chemotherapy was continued to a total of six cycles, and the patient recovered. Complete remission was assessed by a negative muscle and sural nerve biopsy. Only a paresia and hyposthesia on right leg persisted. Finally high-dose chemotherapy with autologous stem cell support was administered without significant complications nor variations on the patient status. Four years after diagnosis the patient is alive and well, except for the neurologic sequelae above mentioned. *Conclusions.* The diagnosis of intravascular lymphoma requires a high index of suspicion when facing a patient with fever of unknown origin. The presence of respiratory distress, focal neurologic symptoms and high serum LDH level may lead to such diagnosis. High-dose chemotherapy may provide an opportunity of curing this aggressive disease.

0995

OPEN-LABEL TRIAL OF AN INITIAL 15-MINUTE INFUSION OF INTRAVENOUS IBANDRONATE FOLLOWED BY DAILY ORAL DOSING IN PATIENTS WITH BREAST CANCER AND MULTIPLE MYELOMA

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Background. In phase III trials, intravenous ibandronate 6mg infused over 1–2 hours was well-tolerated and had a renal safety profile comparable to placebo. *Aims.* In this open-label study, we assessed the safety and effects on bone turnover of a single infusion of intravenous ibandronate given over 15 minutes, followed by daily oral ibandronate. Here we report the safety results of this 3-month trial. *Methods.* Patients with breast cancer (n=31) or multiple myeloma (n=8) received a single intravenous dose of ibandronate (6mg infused over 15 minutes) immediately followed by oral ibandronate 50mg/day for 12 weeks. All patients were included in the safety analyses. Adverse events (AEs) and laboratory assessments (including serum creatinine levels) were monitored. *Results.* Three patients reported non-serious AEs on day 1 considered possibly or probably related to ibandronate infusion (flu-like syndrome, asthenia, headache and cervical pain). During the 12-week treatment period, 25 patients experienced at least one AE. The most common AEs reported, but not necessarily related to ibandronate, were nausea (n=6), asthenia (n=5), raised temperature (n=3) and bone pain (n=3). Four patients were withdrawn due to AEs prior to completion of the 12-week study (flu-like syndrome, nausea, epigastric pain, stomach ulcer). Two patients experienced serious AEs, of which only one was considered related to ibandronate (stomach ulcer, in a patient with a medical history of gastritis). No renal AEs were reported, and calculated creatinine clearance levels were similar at baseline and week 12 (mean 77.9±22.4mL/min vs 79.4±25.8mL/min). *Summary/conclusions:* A single dose of intravenous ibandronate 6mg infused over 15 minutes, followed by oral ibandronate 50mg/day for 12 weeks, was well-tolerated in patients with metastatic bone disease from breast cancer and multiple myeloma. Importantly, no renal adverse events were reported.

0996

RENAL SAFETY OF IBANDRONATE IN AN OPEN-LABEL STUDY OF PATIENTS WITH RENAL DETERIORATION AND MULTIPLE MYELOMA

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Background. In phase III trials of patients with breast cancer and bone metastases, the single nitrogen-containing bisphosphonate, ibandronate, had a renal safety profile comparable to

placebo. Because of this renal safety profile, ibandronate can be used in patients with mild-to-moderate renal impairment without dose adjustment (6mg), and is suitable for those with severe renal impairment (at a reduced dose of 2mg). *Aims.* Here we present the results of a study that assessed the safety of intravenous ibandronate 6mg in patients with multiple myeloma and pre-existing renal insufficiency (1). *Methods.* In an open-label study of patients with multiple myeloma (n=21, creatinine clearance 8–120mL/min), ibandronate 6mg was administered over 30 minutes. Ibandronate excretion and serum levels were measured over 24 hours. AUC of serum ibandronate levels were calculated. Renal function deterioration was graded depending on creatinine clearance (grade 0: >= 80, 1: 50–79, 2: 30–49, 3: < 30mL/min). Markers of tubular damage, alpha-glutamyl-S-transferase [alpha GST] and beta-N-acetyl-glucosaminidase [beta NAG], were measured at baseline and at 24 and 72 hours after ibandronate infusion. *Results.* Four patients had normal renal function and the rest had varying degrees of renal insufficiency at baseline. Mean proteinuria was 1483±1588mg/24 hours. There was a statistically significant positive correlation between ibandronate elimination and creatinine clearance (r=0.81, p<0.001). The AUC for ibandronate was not significantly different over the four grades of creatinine clearance. Serum creatinine and urinary enzymes did not change significantly within 72 hours of ibandronate infusion. Beta NAG showed a significant positive correlation to proteinuria (r=0.49, p=0.01). *Summary/conclusions:* Ibandronate elimination correlated with renal function, however ibandronate AUC levels did not increase significantly. This may indicate that the amount of ibandronate bound to the bone increases with renal insufficiency. In patients with varying degrees of renal insufficiency, intravenous ibandronate was well tolerated and there was no evidence of acute renal toxicity with ibandronate in these high-risk patients. These data suggest that ibandronate may be suitable for use in multiple myeloma patients with pre-existing renal impairment.

1. Bergner R, Henrich DM, Hoffmann M, et al. Renal safety of ibandronate in multiple myeloma patients with renal deterioration. Focus on Myeloma and Plasma Cell Disorders, Las Vegas, USA, January 21–22, 2005.

0997

CELLULAR IMMUNITY IN DIALYSIS PATIENTS TREATED WITH IBANDRONATE FOR 48 WEEKS

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Background. Acute phase reactions are common after intravenous infusion of bisphosphonates. Changes in the cellular immune system, particularly in lymphocyte subtypes, may be one possible mechanism for these reactions. Similar to the acute phase reaction, these immune changes normally disappear after 48 hours. Previous studies have shown that kidney transplant recipients treated with immunosuppressive therapy and ibandronate for bone protection have a lower rejection rate than those treated with immunosuppressive therapy and placebo (1). This suggests that there may be long-term changes in the immune system occurring with ibandronate treatment. *Aims.* To study the long term changes in the cellular immune system during ibandronate treatment in dialysis patients. As these patients are not normally treated with immunosuppressive agents or cytotoxic agents, which can cause changes in the cellular immune system, they are an eligible population for such a study. *Methods.* In this open-label trial, 16 patients with end-stage renal disease receiving regular hemodialysis were recruited. All patients were treated with ibandronate 2mg every 4 weeks for renal osteopathy, which correlates to ibandronate 4–5mg in patients with normal renal function. The cellular immune system was investigated before first ibandronate application and the measurement was repeated at weeks 2, 4 and 48. The following parameters were measured by blood count dif-

ferentiation: leucocytes, granulocytes lymphocytes, monocytes. Lymphocyte subtypes were measured by flow cytometry: B-lymphocytes (CD3+/CD19+), T-helper cells (CD3+/CD4+), T-suppressor cells (CD3+/CD8+), natural killer (NK)-cells (CD3+/CD16+56+), helper-inducer cells (CD4+/CD29+), activated T-cells (CD3+/HLA-DR+), activated T-lymphocytes (CD3+/CD25+), naive T-cells (CD3+/CD45RA+) and memory-T-lymphocytes (CD3+/CD45RO+). **Results.** Twelve patients completed the study and were evaluated. One patient dropped out because of flu-like symptoms with muscle pain after the first ibandronate infusion; however this was well controlled with paracetamol. Three patients died due to concomitant diseases (diabetes and cardiovascular events). There were no statistically significant differences in cellular immunity over time as measured in weeks 0, 2, 4 and 48 (see Figure). **Summary/conclusions:** In this small-pilot study of dialysis patients receiving ibandronate, no changes in the cellular immune system were observed over time. Changes in different lymphocyte subtypes, which occur in the acute phase reaction after first infusion, were not seen. Reduced rejection rate in transplant recipients after ibandronate infusion cannot be explained by changes in the cellular immune system, and must therefore occur by another mechanism.

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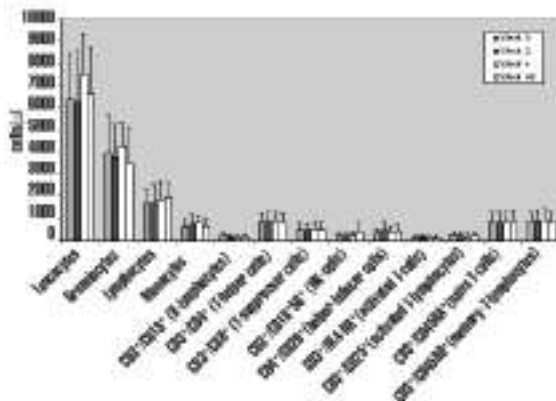


Figure. Cellular immunity over time.

0998

IBANDRONATE IN THE TREATMENT OF HYPERCALCEMIA OR NEPHROCALCI-NOSIS IN PATIENTS WITH MULTIPLE MYELOMA AND ACUTE RENAL FAILURE

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Background. Bone lesions occur in a high proportion of multiple myeloma patients. Metastatic cells often have osteolytic characteristics due to the release of cytokines that increase osteoclast-mediated bone resorption, leading to hypercalcemia and severe pain. Serious complications of hypercalcemia include nephrocalcinosis and acute renal failure. Bisphosphonates are indicated for the treatment of hypercalcemia. These drugs are pyrophosphate analogs that bind to active sites of bone remodeling and inhibit osteoclast-mediated bone destruction. However, renal toxicity is a known adverse event associated with the use of some bisphosphonates, which may preclude their use in patients with renal insufficiency. Ibandronate, a third-generation bisphosphonate that has potent bone-stabilizing and calcium-lowering properties, has had no published reports of treatment-induced renal failure in clinical practice since its introduction in 1996. **Aims.** To investigate the renal safety of ibandronate in

multiple myeloma patients with pre-existing renal failure due to hypercalcemia or nephrocalcinosis. **Methods.** The study group comprised seven multiple myeloma patients with acute renal failure caused by hypercalcemia (n=6) or nephrocalcinosis (n=3). Ibandronate was infused over 30 minutes prior to administration of cytoreductive therapy. Patients received ibandronate 6mg except for one patient whose dose was reduced to 2mg due to concurrent hemodialysis. In two patients a second dose of ibandronate was administered (6mg or 4mg). Renal function parameters, calcium levels and diuresis were measured and kidney biopsies were performed in four patients. **Results.** In all patients, ibandronate treatment returned renal function to normal or almost normal levels and caused elevated calcium levels to decrease to within a normal range. Improved renal function was also seen in patients who received two consecutive doses of ibandronate, and no hemodialysis was required due to ibandronate use. Ibandronate treatment was well tolerated and no adverse events were reported. **Summary/Conclusions.** This study illustrates the potential for ibandronate use in multiple myeloma patients with renal failure or nephrocalcinosis due to hypercalcemia. The lack of renal toxicity following ibandronate treatment is in agreement with a previous study performed by our working group of multiple myeloma patients with reduced renal function (1). This concurs with a phase III trial of intravenous ibandronate in metastatic breast cancer patients, which found that ibandronate had a renal safety profile comparable to placebo (2). Our study found that ibandronate was effective at normalizing serum calcium levels in multiple myeloma patients with hypercalcemia. Larger studies are required in this patient group.

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THE COMBINATION OF RITUXIMAB, ETOPOSIDE, IFOSFAMIDE AND CARBOPLATIN (RICE) IS SAFE AND EFFECTIVE SALVAGE THERAPY FOR ELDERLY PATIENTS WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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Background. In the elderly, aggressive chemotherapy is frequently underused or modified because of accompanying comorbid diseases, higher therapy-related toxicity and bias regarding tolerance of therapy in this age group. However in recent years, more patients appear to be eligible for potentially curative aggressive chemotherapy in this age group. **Aims.** to present our early initial experience using the combination regimen RICE as salvage chemotherapy for elderly patients with relapsed/refractory aggressive non-Hodgkin's lymphoma (NHL) and as primary therapy in a few patients with diffuse large B-cell NHL who were unable to receive anthracyclines because of reduced left ventricular cardiac function. **Methods.** RICE was given to 11 elderly patients (median age-70.5 years, range 66-74 years), in 9 patients it was given as salvage therapy and in 2 as primary therapy. RICE included rituximab (375 mg/m²) on day 1, etoposide (100mg/m²) on day 2-4, carboplatin (dose=5x [25+creatinine clearance], capped at 800mg) and ifosfamide (5000mg/m², 24 hours continuous infusion) on day 3. All received G-CSF (5mcg/Kg) from day 6 until ANC recovery. Response was evaluated by PET-CT after two cycles. Eight patients received 2 cycles of RICE, 2 others had 3 and 4 cycles, respectively, while another patient is currently completing the first cycle. **Results.** The overall response rate was 88.9% (CR-66.7% and PR-22.2%). In another 2 patients evaluation by PET-CT has not been completed yet. Four patients received full dose rituximab but had 25% dose reduction of the ICE (2 for all

cycles and another 2 in their second and third cycles respectively, due to thrombocytopenia or infections and renal failure). Grade 3/4 neutropenia occurred in 5 patients and grade 3/4 thrombocytopenia occurred in nine. Three patients had overall 5 episodes of neutropenic fever and one died from *Escherichia coli* sepsis. The median time between cycles was 28 days (15 to 32 days). After RICE, 2 patients underwent peripheral stem cell harvest (mean 4.985×10^6 CD34 cells/Kg). Three patients underwent successful autologous peripheral stem cell transplantation (SCT), while another patient died during the transplant. Two of the oldest patients (74 yrs old), who were unable to undergo autologous SCT, received involved field radiotherapy to sites of residual disease. **Conclusions.** Despite the small sample size it seems that RICE is a relatively safe and effective regimen in elderly patients, yielding high response rates. It is probably best used as primary salvage therapy for the elderly with relapsed/refractory disease. Dose reduction is suggested for patients with a significant baseline renal impairment or following a major infectious complication.

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OCCURRENCE OF STRONGYLOIDES STERCORALIS INFECTION IN A PATIENT TREATED FOR CHRONIC LYMPHOBLASTIC LEUKEMIA

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A 72-year-old male patient, suffering from CLL, hypertension, cholelithiasis, and Bechterew disease, presented with a ten months history of persistent abdominal pain, diarrhoea, and a weight loss of 20 kgs. The patient has been on prednisolon therapy, but it was discontinued three months before his admittance. He has never traveled outside Hungary. The significant laboratory parameters are as follows: WBC count 21.800 μ L (normal range 4000-10.000 μ L) with 1,8 (8,6%) neutrophils, 14,2 (65,1%) lymphocytes, and 4,8 (22%) eosinophils; hemoglobin 100 G/L (normal range: 120-180 G/L); MCV 103 fL (normal range 80-95 fL); protein, albumin, platelet count, and biochemical tests of liver and kidney were within normal ranges. HIV-1, HIV-2 virus antibody titers were negative. There were no hepatosplenomegaly or enlargement of the lymph nodes. Gastroduodenoscopy had showed a non-specific white speckled appearance was noted throughout the entire duodenal mucosa, but no biopsy was taken. The patient was admitted to hospital three times since the onset of his symptoms, but the performed gastroenterologic examinations did not reveal the specific cause of diarrhoea and weight loss. Examination of stool specimens for ova and parasites confirmed the diagnosis of *Strongyloides stercoralis* infection. The patient was treated with oral thiabendazole, 25 mg/kg, for 3 days. Diarrhoea gradually improved. On follow-up five month later, his hemoglobin and iron levels were found to be in the normal range, hypereosinophilia disappeared, quality of life improved and the patient gained 15 kgs weight. *Strongyloides stercoralis* potentially fatal infection has been described not only in patients with AIDS but also in immunocompetent patients and subjects being treated with corticosteroids and/or other immunosuppressive drugs. Thus, *Strongyloides stercoralis* infection should also be considered in the differential diagnosis of immunocompromised patients with gastrointestinal symptoms.

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THALIDOMIDE USE IN MULTIPLE MYELOMA - CURRENT CLINICAL PRACTICE IN THE WEST OF SCOTLAND

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Background. Thalidomide is established as having a role in the treatment of multiple myeloma and although its dose in clinical

trials has been predetermined its dose in clinical practice remains uncertain. **Aims.** We undertook a study to investigate consultant haematologists' prescribing practice with respect to thalidomide administration within the West of Scotland (population circa 3 million). **Methods.** Using a Haematology Regional Audit Network an audit form was sent to all haematology departments in the West of Scotland. **Results.** 13 of 15 audit forms were completed. 84.6% of respondents could tell us the numbers of patients with multiple myeloma within their catchment population (368 patients, prevalence 143×10^6). The calculated incidence of myeloma was 42×10^6 /annum. Of all patients, 16.6% were being treated with thalidomide. 38.5% of haematologists used thalidomide outwith the Pharmion Risk Management Programme. 84.6% perceived the target dose of thalidomide to be 200 mg, 7.7% suggested 100mg and 7.7% 300mg. 31% commenced thalidomide at a dose of 50mg, 54% used 100mg and 15% used 50-100 mg. 15.5% increased the thalidomide dose weekly, 69% increased fortnightly and 15.5% increased monthly. 92% used thalidomide in combination with other agents and 38.5% used thalidomide in a pulsed fashion. 46% of the haematologists used neurophysiological studies (NPS) as a pre-treatment investigation. In the event of a neuropathy, 61.5% either stopped/reduced thalidomide, 46% repeated NPS and 38.5% continued thalidomide unless the patient was incapacitated. If venous thromboembolism occurred all would anticoagulate the patient and 92% continued with thalidomide therapy. 77% used 3 months as an evaluation point to assess thalidomide's clinical effectiveness, 15.5% used 2-3 months and 7.5% used 1-2 months. **Summary and Conclusions.** The calculated incidence of 4.2×10^6 /annum is consistent with published Scottish data indicating the population under study was truly representative. 61 patients (population circa 3 million) were on thalidomide therapy and surprisingly over one third used thalidomide products outwith Pharmion Risk Management Programme, a strategy with financial cost benefits (Pharmion £14/100mg, Allan £4.50/100mg). Thalidomide target doses of 200mg were used and the greater target doses described in the literature were not used. Although neuropathy is a recognised complication of thalidomide use neurophysiological studies (NPS) are not mandatory. In this group 54% did not use NPS as a pre-treatment investigation. In the event of venous thromboembolism, 92% of the group would continue thalidomide with concurrent anticoagulation, a view concurrent with published guidelines. An early response pattern with thalidomide administration in patients has been described, with responses noted as early as 3 weeks and continued for up to 3 months. In this audit, all groups appropriately evaluated thalidomide's effectiveness within a 3-month period. There is no information in the literature regarding the duration of therapy, assuming clinical response to thalidomide, but from this audit it would appear that once thalidomide is started it is rarely stopped.

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REPORT OF THE FIRST CASE OF PRIMARY CUTANEOUS LARGE B CELL LYMPHOMA OF THE LEG WITH SOLE SITE OF RELAPSE IN THE BRAIN PARENCHYMA

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Primary cutaneous large B cell lymphoma (PCLBCL) of the leg has a relatively good prognosis. Localised recurrence and extra cutaneous spread are well recognised, but CNS involvement was reported in only 4 cases. These reports were of cases with other sites of involvement. We report a patient with PCLBCL-leg who relapsed in brain parenchyma without any evidence of extra cutaneous disease. We believe that this is the first case in the literature that developed brain involvement without other extra-cutaneous relapse. 63 year old male presented with a non-pruritic skin lesion in the right leg. Skin biopsy showed lymphocytic infiltrate with cells positive for CD45, CD20 with high labelling index with MIB-1 staining. The cells were negative for CD3 and CD30. An expert lymphoma panel made the diagnosis of DLBCL. Clinical staging including bone marrow exami-

nation with immunophenotyping and CT scan of the body showed no other site of involvement. Four months prior to presentation, an MRI scan of the brain as part of investigation of VII nerve palsy was normal and the recovery from the Bell's palsy was complete. The skin lesion was treated with radiotherapy with excellent response. He had further lesions on the leg that again responded well to radiotherapy. Fourteen months after the original presentation, the patient developed weakness in the left upper limb. MRI scan revealed a 2cm mass in the right parietal lobe with another lesion in the posterior fossa. A biopsy of the parietal lesion showed histological and immunological features identical to the original skin lesions at presentation. Repeat clinical staging including bone marrow and CT studies showed no other site of involvement with lymphoma. Although the patient initially responded to treatment with radiotherapy, his condition deteriorated and he died due to progression of his CNS disease 27 months after original presentation. Although cutaneous recurrence and extra cutaneous spread are well recognised in PLBCL-leg, CNS involvement is reported in only 4 cases in the English literature. These reports were in patients with other sites of involvement. The relapse in our patient was confined to the brain with no other site of involvement.

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INCIDENCE OF INFECTIOUS COMPLICATIONS DURING CYCLOSPORINE (CSA) FORMULATIONS ADMINISTRATION POST TRANSPLANTATION

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Background. Prolonged immunosuppressive administration may be associated with negative effects including infectious complications, relapse of basic diseases and secondary malignancies. **Aims.** We therefore performed a multi-center survey in 174 pts. receiving Deximune® (Dexcel Ltd.) or Sandimmun Neoral® (Novartis Inc.) after stem cells (SCT) and solid organ transplantation (SOT). **Methods.** Toxicity profile and bioavailability were assessed. Fifty percent of the pts. underwent SCT and 50% SOT. Transplants were performed between 1987 and 2004 (50% between 2003-2004). **Results.** SOT includes: kidney (41%), combined kidney and pancreas (7%) and liver (2%) transplants. Sixty one percent of the patients were in the 40-65 years age category, 21% were in the 18-40 category, while 13% were over 65 years. Five percent of the pts. were below 18 years. Sixty-six percent of pts. were males and 34% females. Sixty six percent of the pts. were males and 34% females. SCT were performed for various hematological indications including: AML-29, ALL-10; NHL-11; MM-8; CML-5; HD-2; CLL-1 and others -15. Seventy three percent were transplanted from matched sibling donors, while 24% and 3% from matched unrelated or mismatched related donors, respectively. Ninety six percent were transplanted with mobilized peripheral blood stem cells, while 4% with bone marrow grafts. The main indications for post transplantation immunosuppressive therapy were treatment (51%) or prevention (38%) of rejection, while 11% received CSA for prevention or treatment of GVHD. Most of the side effects were mild and transient (hypertension-43%, tremor -16%, headache-16%, hepatotoxicity-16% and hypomagnesemia -11%) while, only 23% of the patients reported serious adverse effects (mainly hypertension-15%). No significant differences were observed in systolic or diastolic BP, creatinine, BUN, K+, Na+, Mg+, Bil, SGPT and cholesterol pre- and during CSA formulation administration while, Hgb, SGOT and uric acid increased from 11.5±0.17 to 12.1±0.16 gr% (p=0.01), 27.5±1.8 to 38.4±4.8 IU/L (p=0.02) and 6.0±0.29 to 6.7±0.2 mg/dL (p=0.003) and triglycerides decreased from 267.9±17.4 to 210.3 ±14.5 mg/dL (p=0.002) pre- and during CSA therapy, respectively. Twenty percent of the pts. developed infectious complications, while on therapy: UTI including pyelonephritis-36%, CMV-11%, pneumonia-8%, aspergillosis-7%, herpes zoster-7%, abdominal wall abscess -7%, bacteremia, intrabdominal abscess, perianal abscess, peritonitis, septic foot and encephalitis-4% each. Interestingly, 4 pts. were diagnosed with

malignancy while on CSA therapy (SCC-2, BCC-1, Kaposi sarcoma-1). **Conclusions.** We conclude that Deximune® administration is safe, with similar toxicity profile, side effects and bioavailability as Sandimmun Neoral®. Finally, CSA based immunosuppressive therapy post transplantation may be associated with increase risk of infectious complications (20%) and malignant transformation.

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BONE MARROW BIOPSY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: PROLIFERATIVE ACTIVITY AND MORPHOLOGICAL CHARACTERISTICS

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We examined bone marrow (BM) sections in 60 untreated patients (pts) with newly diagnosed acute lymphoblastic leukemia (ALL) in 5-year period. BM biopsy was performed in order to assess the degree and prognostic significance of the histological data of the disease. BM features studied were reticular fibrosis, total cellularity, blastic infiltration, residual hematopoiesis, and leukemic cells proliferative activity expressed as percent (%) of Ki-67 positive cells. Median age of the entire cohort was 35.5 yrs (range, 18-60). T-cell lineage ALL were diagnosed in 28% of the cases, while B-cell lineage ALL represented 72% of the cases. The majority of the pts (60%) had ≥90% blastic infiltration, also 83.3% of the pts had BM cellularity ≥90%. Mean Ki-67 expression was 14.55% (range, 1-35). The course reticulin fibrosis (gr. II-III) was found in 20 pts (33.3%), while null or mild was found in the majority of the pts (66.7%). Among all pts, 50% had ≥2 cell lines in BM sections. The percentage of BM leukemic cells was related to cellularity (p<0.01), while it was related to the disappearance of normal cell lines (p<0.001). The degree of fibrosis was inversely related to BM cellularity (p<0.01). Also, the degree of fibrosis was inversely related to the persistence of residual hematopoiesis (p<0.05). Proliferative activity presented as % of Ki-67 positive leukemic cells was strongly correlated to residual hematopoiesis (p<0.001). All pts received standard ALL induction therapy according to protocol LALA-94. Complete remission rate (CR) was 75%. Median leukemia free survival (LFS) and median overall survival (OS) were 11.45 and 18.45 months, respectively. Our findings did not confirm that among BM features, fibrosis has any prognostic value. A multivariate analysis of both BM histological data, proliferative Ki-67 expression, and some clinical data (age, immunophenotype, and % of circulating blasts) was performed to test their prognostic significance. In this model residual hematopoiesis and % of Ki-67 positive blasts were of prognostic value for OS and LFS. We conclude that some characteristics of BM biopsy afford not only descriptive, but also prognostic information for predicting the outcome. The persistence of residual hematopoiesis (≥2 cell lines) and high Ki-67 expression were both factors of favourable outcome, while reticular fibrosis, total cellularity, and blastic infiltration did not display any prognostic value.

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RITUXIMAB PLUS FLUDARABINE AND CYCLOPHOSPHAMIDE AS SALVAGE TREATMENT FOR PATIENTS WITH CHRONIC LYMPHOBLASTIC LEUKAEMIA

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Background. Fludarabine and fludarabine-containing regimens are the most commonly used chemotherapies in CLL and immunochemotherapy studies in CLL have focused on combination of rituximab with these chemotherapies. The rationale for combining chemotherapy with rituximab in CLL is that rituximab has single-agent efficacy and will not compromise the tolerability of chemotherapy and also that there is evidence of synergy between rituximab and fludarabine *in vitro*. **Methods.** Since January 2003, nine CLL patients with disease progression

have been treated with FCR regimen at the Clinic of Hematology, Clinical Center-Skopje. The median age of the patients was 58.5 (range 43–68 years). Two patients were in Binet stage B and seven in stage C. Patients received rituximab 375–500mg/m² on day 1, fludarabine 25mg/m²/day plus cyclophosphamide 250mg/m²/day IV for 3 consecutive days, repeated every 28 days for a total of 6 cycles. **Results.** The overall response rate was 77% (7 patients) with 33% (3 patients) achieving complete remission. Three patients had a partial response, one patient had a minor response and in two patients disease progressed during the treatment. The patients achieving complete remission had no detectable minimal residual disease (MRD) by flow cytometry, but polymerase chain reaction for MRD was positive. The regimen was well tolerated and no serious side effects were observed. Only one patient had significant and prolonged myelosuppression followed by fever and severe infections, which were well resolved. The follow up of our patients is 8–24 months. **Conclusions.** Our initial results support the thesis that rituximab has synergistic activity with fludarabine and cyclophosphamide, confirming FCR as one of the most effective treatments for relapsed and refractory CLL.

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FREQUENCY DISTRIBUTION OF THE HPA-4 AND HPA-15 (GOV) PLATELET ALLOANTIGEN SYSTEMS IN GREEK BLOOD DONORS POPULATION

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Background. Antibodies to human platelet antigens (HPA) have been implicated in clinically important thrombocytopenias. The HPA-15 (Gov a/Gov b) is a biallelic system located on the CD 109 glycoprotein and reveals an immunogenicity equal to this of the HPA-5 system, while the incidence of HPA-4 antibodies is extremely low. **Aims.** To calculate the gene frequencies of HPA-4 and HPA-15 systems in Greeks and to compare them with those observed in other Caucasian populations. **Methods.** Genomic DNA was extracted from leucocytes of 179 random, healthy Greek blood donors by QIAamp DNA Blood Mini kit. The genotyping analysis for HPA-15 was performed by PCR with sequence-specific primers (PCR-SSP) according to Schuh et al. 2002, while for the HPA-4 the method described by Cavanagh et al. 1997 was slightly modified, so that a combined PCR-SSP tested the samples simultaneously for both systems. **Results.** The genotype frequencies that were derived from the results of PCR-SSP by the counting method, as well as the allele frequencies obtained are summarized in the table below. **Conclusions.** The phenotype frequency of the HPA-4a allele among Greeks is similar to this in other Caucasian populations. The HPA-15 genotype frequencies are quite similar to the distribution already observed in other Caucasoids and the HPA-15b (Gov-a) allele (54.75) seems to be more common than HPA-15a (Gov-b).

System	Genotype	n	%	Allele	n	%
HPA-4	aa	179	100	4a 4b	358	100
	ab	0	0		0	0
	bb	0	0			
HPA-15	aa	36	20.11	15a 15b	162	45.25
	ab	90	50.28		196	54.75
	bb	53	29.61			

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CORRELATION OF SERUM ERYTHROPOIETIN LEVELS WITH DEFERIPRONE AND DESFERIOXAMINE IN THALASSEMIC PATIENTS

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Background: In vitro studies have demonstrated that serum erythropoietin (EPO) levels may present a 3 to 10-fold increase with deferiprone or desferrioxamine. **Aims.** Our aim was to study whether EPO levels correlate with these iron chelants in thalassemic patients. **Methods.** We studied 36 patients (25 males-11 females) with median age 31.3 years (range 18-57 years). Twenty-seven patients had thalassemia major (TM), seven had thalassemia intermedia (TI), one had sickle-cell disease (S), and one double heterozygosity sickle-cell/thalassemia (S α). Splenectomy was performed in 15 patients. Thirteen patients had concomitant diseases, of which 8 patients were affected of hepatitis C virus (HCV), one patient had both HCV and HBV, one presented rheumatoid arthritis, and another one had systemic lupus erythematosus. Moreover, one patient had Hodgkin's disease, and one had low-grade non-Hodgkin's lymphoma. Patients presented median ferritin levels of 1616.05 ng/mL (range 210-9100 ng/mL), and median EPO levels of 107.24 mU/mL (range 28-200 mU/mL; normal values 2.6-34 mU/mL). Nineteen patients (52.8%) were using desferrioxamine (DSF, 50 mg/kg s.c., 5-6 days/week) as iron chelant, eleven patients (30.5%) were using deferiprone (DPO, 75 mg/kg/day p.os.) divided in three doses, and six patients (16.7%) were receiving combination therapy with both agents at doses described above. All patients were regularly transfused on schedule with 1-2 units of packed red cells of hematocrit 80-90% every 15-20 days in order to maintain hemoglobin levels of 9-10 gr/dL. No one presented impaired renal or liver function. **Results.** Erythropoietin levels were higher in the TI group than the TM group (120.3 mU/mL vs 102.5 mU/mL) independently from the iron chelation regimen. Median serum EPO levels in the DSF group were 121.48 mU/mL (range 28-200 mU/mL; 14% more than the DPO group), in the DPO receiving patients were 106.73 mU/mL (range 37-200 mU/mL), while patients receiving combination therapy presented EPO levels of 63.17 mU/mL (range 28-123 mU/mL; 48% less than the DSF group). Splenectomized patients presented EPO levels of 93.6 mU/mL, while non-splenectomized presented 117 mU/mL. **Conclusions.** EPO levels varied in the different therapeutic regimens. Iron chelants may influence EPO expression during EPO gene therapy (especially patients receiving the effective combined chelation treatment), and is probable that EPO levels and type of chelant taken together may predict, as in hematological malignancies, outcome of recombinant human erythropoietin treatment in thalassemic patients. However, further studies are needed to verify our observations.

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FLT3-ITD MUTATIONS IN ACUTE MYELOID LEUKEMIA: PREVALENCE AND PROGNOSTIC SIGNIFICANCE

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Treatment design in acute myeloid leukemia (AML) largely depends on patient prognostic profile, with special emphasis on karyotype. Detection of other disease parameters will help to better predict prognosis in these patients. Internal tandem duplication (ITD) of the FLT3 gene has been reported as an adverse prognostic factor, especially for disease-free survival (DFS). We evaluated the prevalence of FLT3-ITD mutations in 147 consecutive AML patients (80 men and 67 women), with a median age of 48 years (range, 8-73). FLT3-ITDs were detected in 43 patients (29%). More than one mutation was present in 12 patients, and loss of the wild-type allele in two. The presence

of FLT3-ITD was significantly associated with high white blood cell count, high serum dehydrogenase levels and normal karyotype. Most FLT3-ITD positive patients belonged to the M1, M2 and M5b subtypes. The prognostic significance of FLT3-ITD was assessed in 107 out of 147 patients treated for AML in our department; acute promyelocytic leukemia patients were excluded from this analysis. Remission rate was not influenced by patient mutation status and was 69% for both the FLT3-ITD-positive and -negative subgroups. The 33 FLT3-ITD positive patients had significantly worse 5-year DFS (8% vs 33%, $p=0,028$) and overall survival (OS: 12% vs 36%, $p=0,03$). These results were mainly attributable to the mutation effect in the intermediate risk karyotype group, in which the 5-year DFS for FLT3-ITD-positive patients was 10% versus 43% ($p=0,048$) with a median survival of 38 vs 14 months ($p=0,028$). On multivariate analysis, FLT3-ITD was an independent prognostic factor for DFS and OS. The mutation maintains its prognostic value even after relapse. Current DFS (comprises CR1, CR2 or CR after allogeneic stem cell transplantation) is significantly better for patients without ITD and half of them remain in CR at 5 years (DFS_{current}: 54% vs 18%, $p=0,026$). In conclusion, FLT3-ITD is a frequent genetic aberration in AML and represents an independent, adverse prognostic factor. Especially within the intermediate (cytogenetic) risk group, the presence of this mutation could possibly identify patients with need for more intensified treatment.

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THE EFFECT OF CMV INFECTION ON ENGRAFTMENT AND TRANSFUSION REQUIREMENTS IN PATIENTS AFTER STEM CELL TRANSPLANTATION IN DEPEND OF MYELOABLATIVE OR NONMYELOABLATIVE CONDITIONING REGIMENS

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Background. CMV infections are among the most important complications following stem cell transplantation. Few data have been reported about connection between CMV and delayed engraftment in depend of type conditioning regimen. Aim of the study: to evaluate the influence of cytomegalovirus (CMV) infection on autologous and allogeneic stem cell engraftment and transfusion requirements in patients (pts) with hemoblastosis in depend of preparing regimens: myeloablative (MA) or nonmyeloablative (NMA). **Methods.** From 1991 to 2003 year 107 pts, median age 26 (range 4-54) suffered from various hematological diseases (76 AL, 19 CML, 10 MM, 2 MDS) were underwent allogeneic ($n=38$, median age 24) or autologous ($n=69$, median age 27) haematopoietic stem cell transplantation (HSCT). Conditioning regimens in alloHSCT were MA in 28 pts and consist of Bu+Cy, Bu+Cy+VP-16, and NMA in 10 pts consist of Flu+Bu+ALG, Flu+Bu+Alk. Conditioning regimens in autoHSCT were MA in 28 pts and consist of Bu+Cy, Bu+Cy+VP-16, and NMA in 41 pts consist of BEAM, LACE, High dose Alkeran, TACC). **Results.** CMV was observed in 14 (20%) pts after autoHSCT and in 12 (32%) after alloSCT. The frequency of CMV was similar in autoHSCT pts with MA and NMA conditioning regimens: 6 (21%) and 8 (19,5%) accordingly. The frequency CMV in alloHSCT pts also was similar in groups with MA and NMA regimens: 8 (29%) and 4 (40%) accordingly ($p>0,05$). CMV (+) pts had delayed engraftment as in alloHSCT group so in autoHSCT group as compared with CMV (-) pts. Delayed engraftment was in CMV (+) pts as in MA group so in NMA group. CMV infection increased requirement of platelets and erythrocytes in autoHSCT group pts after MA conditioning regimens but did not influence for certain on requirement blood components in pts after NMA conditioning regimens. CMV infection after alloHSCT increased requirement of platelets and erythrocytes in NMA regimens group and increased requirement of erythrocytes in MA regimens group. **Summary.** These preliminary results suggest that CMV result in delayed engraftment independently from allogeneic or autolo-

gous stem cell and MA or NMA conditioning regimens. In group autoHSCT pts CMV increased requirement of donor blood in case using of MA regimens. In group alloSCT pts CMV more significantly influence on transfusion requirement in cases using of NMA regimens.

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CULTURAL CRITERIA TO THE PATHOGENESIS, DIAGNOSIS AND CLASSIFICATION OF MDS

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Classification, ethiopathogenesis and treatment of MDS are one of the important problems in modern hematology. The aim of our studies was to reveal some pathways of the development of MDS. Bone marrow and blood leukocyte culture methods worked out by us makes it possible to observe cyto kinetics of cells *in vitro*, to reveal proliferate activity of blast cells and help to predict the development of acute leukemia in patients with MDS. Cultural studies enable also to estimate the immune reactivity of organism according to macrophage-lymphocyte rosette (MLRos) formation and to reveal morphological inclusions specific for Chlamydia trachomatis in cells. Due to complexity of pathogenesis of MDS beside cytological and histological investigations blood and bone marrow leukocyte cultures were studied in 33 patients with MDS. Our data confirm the necessity of changing the classification of MDS from FAB to WHO: inclusion of the syndrome of refractory pancytopenia with multilineage dysplasia (RPMD), as 30% of our patients (10 cases) had this syndrome and exclusion of the syndrome of refractory anemia with excess blasts (RAEB) in transformation as in cultures of patients either with RAEB or RAEB-t according to FAB classification was revealed proliferation of blast cells. These patients (14 cases) were included in the group of RAEB and composed 43%. Patients with refractory anemia (9 cases) composed 27%, among them 1 patient was with ringed sideroblasts (RARS). In cultures of all patients with RAEB, 3 patients with RA and 5 patients with RPMD was detected active proliferation of blast cells that predicted the development of AML before its clinical onset. Cytochemical and immunological investigations of proliferated *in vitro* blast cells help to determine the type of malignant cells and therefore the variant of developing AML. High indices of MLRos *in vitro* was revealed in 70% of MDS patients that confirmed the opinion that immune conflict is one of the reasonable mechanism in the development of cytopenia. Notable that detection of the inclusions of Chlamydia trachomatis in macrophages, lymphocytes, erythrocytes and even in neutrophilic leukocytes in cultures of 5 patients (1 with RA, 2 with RCMD and 2 with RAEB) showed the existence of chlamydial infection. In the patient with RA and in 1 patient with RCMD antichlamydial therapy separately appeared most successful in increasing the Hb level and erythrocyte count in RA patient and causing the significant improvement of Hb level, erythrocyte and WBC count in RCMD patient. Both patients became transfusion independent for several months (7 and 2.5 months respectively) though antichlamydial treatment had to be repeated periodically to maintain remission and the patient with RCMD besides antichlamydial treatment also required immunosuppressive therapy with Cyclosporin A. Considering the complexity of the pathogenesis of MDS, we think that cultural studies including hemopoiesis *in vitro*, estimation of the functional activity of immunocompetent cells and revealing chlamydial inclusions as well is necessary to work out the right strategy of therapy in these patients. Chlamydia may have the triggering role in the development of MDS.

1011**IMMUNOREGULATORY CYTOKINE PRODUCTION AND POLYMORPHISM IN EARLY MYELODYSPLASTIC SYNDROME PATIENTS**

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Background. Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders characterised by trilineage dysplasia resulting in peripheral cytopenias. Autoimmune phenomena and alteration of apoptosis have been recently reported in MDS. It's known that immunoregulatory cytokines, both up-regulating (IL-2, IFN-gamma, IL-12, and TNF-alpha) and down-regulating (TGF-beta, IL-4, IL-6, IL-10 e IL-13), control the autoimmune cytotoxic response. Furthermore, in several autoimmune and inflammatory conditions, an association with functional polymorphisms, known to modulate the production of immunoregulatory cytokines, is described. **Aims.** We investigated IL-2, IL-4, IL-10, TNF-alpha, TGF-beta and IFN-gamma production in bone marrow and peripheral blood cultures from 20 patients with refractory anemia (RA) and RA with ringed sideroblasts (RARS), compared with 28 controls (miscellaneous haematological conditions). Moreover, we investigated regulatory variants of 4 cytokine genes (TNF-alpha, IFN-gamma, TGF-beta, and IL-10) in the same cohort of patients compared with normal controls. **Methods.** We cultured PBMC from whole blood from MDS patients and controls. Cells were separated by density gradient and plated with PHA (2 microg/mL) for 48 hrs. All cytokines were evaluated in supernatants from bone marrow and peripheral blood cultures by ELISA assay. Cytokine producer genotypes were detected by PCR-SSP. Genotypic frequencies of the patients were compared with a series of 363 healthy Italian blood donors and differences among frequencies were estimated using chi squared test. **Results.** We found that IL-2, IL-4, IL-10, TNF-alpha, and TGF-beta production was significantly higher, whereas IFN-gamma was significantly lower in early MDS patients than controls (Table). Concerning bone marrow cultures, IL-2, TNF-alpha, IL-10 and TGF-beta were reduced, although not significantly, in MDS compared with controls; IFN-gamma and IL-4 were comparable (data not shown). Statistical analysis performed with chi squared test showed no significant differences in the frequencies of cytokine producer genotypes between patients and controls (data not shown). **Conclusions.** We found that both Th-1 cytotoxic cytokines IL-2 and TNF-alpha and Th-2 inhibitory cytokines IL-4, IL-10, and TGF-beta were overexpressed in PB and not in BM in early MDS patients, suggesting that Th-1/Th-2 balance has no major role in BM failure and PB cytopenias. Consistently, neither cytokine producer genotypes demonstrated a shift towards a preferential Th profile.

	IL-2 pg/ml	IFN-gamma pg/ml	TNF-alpha pg/ml	IL-4 pg/ml	IL-10 pg/ml	TGF-beta pg/ml
MDS patients	1733 ± 168*	1200 ± 9*	1076 ± 151*	202 ± 8*	1359 ± 156 [‡]	7435 ± 1576*
Controls	504 ± 52	8969 ± 795	233 ± 36	46 ± 6	973 ± 133	2430 ± 184

Mean ± SE of 20 patients and 28 controls. * p < 0.01; [‡] p < 0.06 patients vs controls.

1012**ANTI-PHOSPHOLIPID SYNDROME IN PATIENTS WITH MULTIPLE MYELOMA**

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Introduction: in patients with multiple myeloma (MM) the prothrombotic tendency has been observed. In some cases the presence of monoclonal immunoglobulin and/or light chain paraprotein with a lupus-like anticoagulant activity and/or anti-cardiolipin activity were confirmed. Aim of the study was to evaluate the frequency of laboratory abnormalities suggesting the antiphospholipid syndrome in patients with advanced MM. **Material and Methods.** study group consisted of twenty newly diagnosed, consecutive MM patients (13 males and 7 females; median age 58.8 years, range 37-71 years) eligible for the study and recruited from October 2003 to December 2004. All patients had stage III of disease according to Durie-Salomon classification (including two cases with renal insufficiency). In 15 cases the protein M was IgG (mean concentration 56.4 g/L) and in 5 cases IgA type (mean concentration 30.9 g/L). All patients studied had normal platelets count. In two women (mean age 54 years) deep vein thrombosis was confirmed by colour Doppler ultrasound. Bleeding complications in the study group were not noted. APTT was determined with sensitive to anti-phospholipid antibodies reagent(Actin; Dade Behring). Diluted Russel viper venom time (dRVVT) was performed using standard method. Mixing plasma studies were performed by measurement of APTT (Actin) in mixture of patient plasma with normal plasma in proportion 1:1, 1:4, 1:9 before and after 1 hour incubation at 37°C. Independently, the LA test and LA1/LA2 ratio (Dade Behring) were determined. Anti-cardiolipin (ACA) antibodies titre was evaluated using ELISA test(American Diagnostica). Activated protein C resistance test(APC-resistance) was performed using a kit(Chromogenix). The FV Leiden mutation was determined using genomic DNA and polymerase chain reaction as described by Ripoll et al. The diagnosis of APS was confirmed when clinical symptoms in individual person were correlated with abnormal results of laboratory tests (prolongation of APTT (Actin), abnormal result of LA1 test and LA1/LA2 ratio >1.3, persistence of the prolongation of clotting time in mixing studies). **Results.** in two cases abnormal prolongation of APTT was confirmed (IgA kappa, IgG kappa class). A significant prolongation of dRVVT was found in 6 other cases (2 IgA lambda, 1 IgA kappa, 3 IgG kappa). LA1/LA2 ratio was normal in all cases. ACA were found in 3 cases. Mixing plasma study revealed prolongation of APTT value upon addition of normal plasma in two patients. In all patients studied APC-resistance test results were normal and FV Leiden mutation was not present. In one patient with DVT the diagnosis of APS was confirmed (prolongation of APTT, dRVVT, and increase ACA concentration in the plasma). In the next, the prolongation of dRVVT was only found. **Conclusions.** laboratory clotting abnormalities which mimic APS may be present in MM patients. However, its relation to monoclonal gammopathy should be further investigated.

1013**ANTI-ERYTHROBLAST ANTIBODIES AND DEFECTIVE APOPTOSIS IN BONE MARROW OF EARLY MYELODYSPLASTIC SYNDROME PATIENTS**

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Background. Autoimmune phenomena, particularly directed against RBC, and alteration of apoptosis have been reported in MDS. We recently described a new method for the detection of anti-RBC antibodies in mitogen-stimulated whole blood cultures, named mitogen-stimulated-DAT (MS-DAT), which is able to disclose a latent anti-RBC autoimmunity in different diseases (autoimmune hemolytic anemia in clinical remission and in B-CLL). **Aims.** We investigated MS-DAT positivity and apoptosis in bone marrow and peripheral blood cultures from 23

patients with refractory anemia (RA) and RA with ringed sideroblasts (RARS), compared with 21 controls (miscellaneous haematological conditions). *Methods.* MS-DAT was performed by stimulating whole blood and marrow cultures with PMA, and anti-RBC or anti-erythroblast antibodies were detected by competitive solid phase ELISA. As apoptotic markers we evaluated NF- κ B and Caspase-3 activity by ELISA and enzymatic assay, respectively. *Results.* As shown in table, the amount of anti-erythroblast antibodies was significantly greater in BM cultures of MDS versus controls, and the test was strongly positive in 9/23 patients (not shown). The anti-apoptotic marker NF- κ B was significantly increased in MDS versus controls, and consistently, caspase-3 activity decreased, although not significantly. On the contrary, PB of MDS displayed no MS-DAT positivity and no significant alterations of apoptotic and anti-apoptotic markers investigated (not shown). *Conclusions.* These findings suggest the existence of an anti-erythroblast autoimmunity in bone marrow of early MDS patients. This could be related to the observed defective apoptosis, which in turn determines survival of auto-reactive marrow effectors.

	MDS		Controls	
	medium	PMA	medium	PMA
Anti-erythroblast (pg/ml)	330 \pm 76*	342 \pm 72*	79 \pm 13	126 \pm 40
NF- κ B (OD units)	246 \pm 33*	310 \pm 52	92 \pm 25	121 \pm 48
Caspase-3 activity (OD units)	176 \pm 16	167 \pm 20	186 \pm 28	178 \pm 21

* denotes statistical significance versus controls. Values are the mean \pm SE

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A WIDE SPECTRUM OF CLOTTING ABNORMALITIES IN UNTREATED PATIENTS WITH ADVANCED MULTIPLE MYELOMA

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Introduction: in patients with multiple myeloma (MM) the presence of different coagulation abnormalities not related to the treatment applied was described. In the pathogenesis of hemorrhagic diathesis in patients with lymphoproliferative diseases autoimmune thrombocytopenia and the protein M related platelet storage pool deficiency, abnormal platelet adhesion and stimulus response coupling should be considered as well. The monoclonal gammopathy in patients with MM may also be responsible for acquired prothrombin deficiency, antithrombin activity, abnormal fibrin structure, impaired tissue plasminogen activator mediated fibrinolysis and acquired von Willebrand's disease. In patients with MM bleeding diathesis may also result from related to amyloidosis acquired deficiencies of factor IX, factor X, and vasculopathy. Aim of the study was to evaluate the relation between monoclonal gammopathy and coagulation abnormalities which predispose to bleeding in MM patients. *Patients and Methods.* Between October 2003 and June 2005, 20 newly diagnosed consecutive MM patients with advanced stage (III) were eligible for the study: 13 males and 7 females; mean age 58.8 years (range 37-71 years); stage B - 2 patients. Type of myeloma: 15 patients had IgG (mean concentration 56.4 g/L) and 5 patients IgA (mean concentration 30.9 g/L). In 1 case we observed hypercalcemia. The blood samples were drawn on admission day, before any treatment. The routine coagulation tests were performed using standard methods. Bleeding time was performed according Ivy method. Fibrinogen level was evaluated using biuretic method. Factor IX (FIX) activity was

determined using coagulometric method and factor IX deficient plasma. Von Willebrand factor antigen (vWF:Ag) level was evaluated with a help of Asserachrom vWF (Diagnostica Stago Roche). *Results.* in the study group evident bleeding complications were not observed. The routine coagulation tests showed prolonged activated partial thromboplastin time (APTT) in 2 cases, shortened APTT in 1 case; and prolonged prothrombin time in 15 cases. The activity of FIX and vWF:Ag level were decreased in 55% and 5% of patients, respectively. There was a significant correlation between monoclonal immunoglobulin level and PT value ($p=0,002$, $r=0.73$). There was a negative correlation between FIX activity in the plasma and immunoglobulin concentration but not significance (probably due to small group of patients). *Conclusions.* Our results may confirm the hypothesis that high concentration of monoclonal immunoglobulin may interfere with coagulation process. The documented high frequency of factor IX is probably related to MM-associated amyloidosis.

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PROGNOSTIC FACTORS INFLUENCING RELAPSE IN CHILDREN WITH ALL

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It is well known that children with acute lymphoblastic leukemia (ALL) have a 25% risk for relapse. In order to investigate the prognostic factors influencing the development of relapse, 195 children with ALL diagnosed from 11/1992 to 12/2002 treated according to BFM 90 and 95 and followed up to 2/2005, were retrospectively studied. Of the 194 children, 50 relapsed, 33 in the bone marrow (BM), 6 in the central nervous system (CNS), 5 in the testes and in 6 combined relapse was documented 3-82 months from diagnosis (med 24). Factors studied were age, sex, WBC, leukemic burden, Hb, platelet count, cytogenetics, immunophenotyping, risk group on diagnosis (dx). Also absolute blast cell count(ABC) on day 7, bone marrow status on day 15 and chemotherapy delay on induction and reinduction were evaluated. From these factors WBC on dx ($p=0.01$), leukemic burden ($p=0.000$), risk group ($p=0,003$), and ABC on day 7 and BM day 15 were statistically significant(SS) for relapse ($p < 0.05$). In particular ABC < 100 was SS compared to 100-1000/mm³ ($p=0.029$) and complete remission (M1) in bone marrow on day 15 of chemotherapy in comparison with M2 or M3 ($p=0.000$). It is concluded that intensification of ALL protocols may have modify the prognostic significance of other factors such us immunophenotype and sex.

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INCIDENCE OF EVI1 OVEREXPRESSION IN HEMATOLOGICAL MALIGNANCIES

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Background. EVI1 proto-oncogene is located in chromosome 3q26 and are involved in the pathogenesis of acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) with dysmegakaryopoiesis, as well as in the progression of the chronic myeloid leukaemia (CML). When a patient is diagnosed with a hematological neoplasm, a combination of methods is essential to determine the prognosis and, in most cases, this information is difficult to interpret. In the past few years, the role of EVI1 gene overexpression (associated or not to so called 3q26 syndrome) has been discussed. Recent studies, applying microarray technology, indicate that high levels of EVI1 expres-

sion are detected in 10% of patients with AML and, in these cases, it is associated with poor survival. *Aims.* To analyze the overexpression of the EVI1 in hematological malignancies, with the purpose of studying its value at diagnostic and evolution. *Methods.* The study was performed retrospectively on a total of 135 patients (49 AML, 19 MDS, 20 CML (16 CP, 2 AP and 2 BC), 5 chronic myeloproliferative syndromes (CMPS), 24 ALL, 4 NHL, 3 multiple myeloma, 5 CLL, 6 HD). Also 7 healthy volunteers were studied. Expression of EVI1 gene was examined in bone marrow samples and/or peripheral blood at diagnosis and during follow-up by RT-PCR. Survival curves in the cases of AML patients were plotted following the Kaplan Meier method and differences between the curves were analysed with the Log Rank test and Breslow test. *Results.* Of the 135 patients 28 overexpressed EVI1, all of them belonging to the myeloid group, with the exception of three cases of ALL-Ph+. In the group of 49 AML, 12 (24.4%) overexpressed EVI1 (1 M0, 1 M2, 3 M4, 6 M5, and 1 M6), 6 in the CML group (2 in CP with adverse features and 4 in AP or BC), 2 CMPS (also in progression) and 5 MDS. Among the AML patients that overexpressed EVI1 with available karyotype, two del 7, two inv16 and one t(8;21) were observed. There were no significant differences in overall survival and disease free survival. *Conclusions.* 1) The higher expression of EVI1 gene was clearly associated with myeloid malignancies. 2) In AML samples a greater than expected incidence of overexpression of EVI1 was observed (mainly in relation with subtypes M4 and M5), without prognostic significance, which it disappears with the complete remission. 3) EVI1 overexpression was clearly associated to progression of CML and other CMPS. 4) The high EVI1 expression observed in a subgroup of ALL-Phi+ could correspond with CML in lymphoblastic blast crisis and not with ALL properly.

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CHARACTERISTICS AND OUTCOME OF RELAPSE IN CHILDREN WITH ALL TREATED WITH BFM PROTOCOLS

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Characteristics and outcome of relapse in children with Acute Lymphoblastic Leukemia (ALL) diagnosed from 11/1992 to 12/2002 treated according to BFM 90 and 95 and followed up to 2/ 2005, were evaluated. Fifty relapses among 194 (25.8%) children, 108 boys and 86 girls, were retrospectively studied. Of these, 31 occurred in boys (28.7%) and 19 in girls (22.1%) 3-82 months (med 24) from diagnosis. Of these relapses 7 occurred very early, 31 early and 12 late. Of 50 relapses 33 occurred in the bone marrow (BM), 6 in the central nervous system (CNS), 5 in the testes and 6 had combined relapses (BM+CNS 3, BM+testis 3). Relapsed children were treated as per the relapse protocols BFM 90 (22/50), BFM 95 (22/50) and other (6/50). 2nd complete remission (CR) was achieved in 35/50 (70%) and of them 15 underwent bone marrow transplantation (BMT), 10 MSD and 5 MUD, and 20 continued with conventional chemotherapy. Of these 35 children 16 (45.7%) continued in CR2 5-108 months from relapse (med 41 mo), 1 died of toxicity after BMT and 18 experienced 2nd relapse (BM 14, CNS 1, combined BM+CNS 3), 3-42 mo after the first relapse (med 8.5 mo). CR3 was achieved in 4/18 children and 1 is alive free of disease for 41 mo after 2nd relapse. In total 17/50 relapsed children are alive (CR2 16, CR3 1) 28-141 mo from diagnosis (med 78) and 5-108 from the last relapse (med 41). From this group of patients it was shown that elevated white blood count on diagnosis ($p=0.019$) and very early relapse ($p=0,035$) have statistical significant prognostic value for the outcome after relapse.

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CYTOTOXIC T-LYMPHOCYTE ANTIGEN-4 (CTLA-4) GENE POLYMORPHISM AND SUSCEPTIBILITY TO B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA

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Background. The gradual accumulation of malignant cells in B-CLL is probably due to disturbances in the balance between programmed cell death and cell proliferation. It has been shown that inhibitory CTLA-4 (cytotoxic T-lymphocyte antigen 4) molecule may prolong the progression through the G1 phase of the cell cycle by upregulation of cyclin D2 and inhibition of cyclin D3, cdk4 and cdk6 production. Recently CTLA-4 overexpression in leukemic CD19+/CD5+ cells has been reported. It has been reported that polymorphic alleles may be related to the expression levels of CTLA-4 molecules. *Aims.* The study was undertaken to examine the genotypic distribution of CTLA-4 gene polymorphisms at position -319 in the promoter region and the dinucleotide (AT)_n repeat polymorphism in the 3' untranslated region, which may influence the level of expression of CTLA-4 molecule and susceptibility for B-CLL development. *Methods.* One hundred ten B-CLL patients and 105 healthy subjects were studied. Genomic DNA was isolated from whole frozen blood using the NucleoSpinR Blood kit (MARCHEREY-NAGEL, Germany). Allele identification was achieved by PCR amplification. The amplified product for SNP loci was purified and minisequenced using the commercial kit SnapShot(PE Applied Biosystems). The dinucleotide repeat polymorphism was studied by PCR and fluorescence based technique. The products were analyzed on the ABI PRISM 310 Genetic Analyzer (ABI PRISM 310 capillary electrophoresis system). *Results.* Genotype analysis showed that the T allele at position -319 in the promoter region was over-represented in patients with B-CLL ($p=0.005$, OR=2.105, 1.235<95% CI<3.590) than in controls. The analysis of (AT)_n repeat polymorphism showed that the (AT)8/ (AT)other genotype was the most represented in both examined groups. The genotype distribution did not differ significantly between patients and controls ($p=0,227$) even though a higher frequency of CTLA-4 (AT)8 homozygosity was observed in B-CLL patients. *Conclusions.* Our findings indicate that the T allele at position -319 in the promoter region may confer susceptibility to B-CLL.

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PHENOTYPIC ABNORMALITIES OF BONE MARROW B LYMPHOCYTES IN MYELODYSPLASTIC SYNDROMES

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Background. It has been hypothesized that myelodysplastic syndromes (MDS) are originated from hematopoietic stem cells. However, accumulating evidence supports an origin at a more immature cell with ability to differentiate into both myeloid and lymphoid cells. *Aims.* to search for the distribution and phenotypic characteristics of the different maturation-associated compartments of bone marrow (BM) B-cells in MDS. *Methods.* Bone marrow from newly-diagnosed patients with MDS was studied by multiparameter flow cytometry. Diagnosis was based on peripheral blood counts, bone marrow cytology and cytogenetics. Exclusion of deficiency anemias, HIV infection hepatic and renal failure and autoimmune disorders was made. After acquisition of 3x10⁵ events, B - lymphocytes were separated in the CD45/SSC low gate and CD19+. Characterization of CD34+ and CD34- B-cell precursors and mature B-lymphocytes was performed. Antigen expression was measured by mean fluorescence intensity. BM samples from 14 normal individuals (BMT donors) were used as controls.

Results. CD34+/CD45lo B-cells were absent in 5/22 RA/RARS and in 14/19 RAEB (p=0.0001). Mature B-lymphocytes were always present. Intermediate cells were found in all RA/RARS but only in 13/19 patients with RAEB. In RA/RARS, less intermediate cells were seen than in controls. However, the proportion of mature cells was higher. In RAEB, in the few cases where immature cells were seen, a 'shift to the left' was observed. There was a higher proportion of fully mature B-lymphocytes. CD19+/CD79a- cells were found in 7/11 cases of RA/RARS and 6/18 cases of RAEB. Abnormal expression for at least one of the antigens was found in 91% of RA/RARS and in 74% of RAEB cases. Underexpression of TdT and CD79a were most frequently seen in CD34+/CD19+ cells, and that of CD45 in more mature cells. Asynchronous antigen expression was restricted to the CD19+/cCD79a- phenotype on CD34+ B-cell precursors. **Conclusions.** Our results show evidences of an abnormal B-cell maturation in MDS patients. Supported by FAPESP

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DIRECT IMMUNOLABELLING OF ZAP-70 PROTEIN IN B CHRONIC LYMPHOBLASTIC LEUKAEMIA, A NEW ROUTINE FLOW CYTOMETRIC ANALYSIS

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Background. Status of the variable region of the immunoglobulin heavy-chain gene (IgVH) analysis is an important prognostic factor in B chronic lymphocytic leukaemia (B-CLL). However, IgVH sequencing is difficult to perform in a routine use. Recently, Crespo et al 1 described overexpression of ZAP-70 protein in the unmutated status of IgVH in B-CLL patients. This high expression correlates with progressive disease and poor survival. **Aims.** We attempted to simplify the method of flow-cytometric analysis for routine assessment of ZAP-70 expression in B-CLL patients. Patients. 59 B-CLL patients with F/M ratio 24:35; mean age 60 years (age range 40-79 years) were studied. Staging according to Binet was A and B in 91.5, 8.5% patients respectively. Matutes score of 5, 4, 3 was in 54.2, 30.5, 15.3% patients respectively. **Methods.** and materials. Flow-cytometric analysis was performed on mononuclear cells from B-CLL patients. After isolation of mononuclear cells from fresh peripheral blood samples or frozen samples, they were stained with conjugated CD5-specific, CD19-specific, CD3-specific and CD56-specific monoclonal antibodies. For assessment of ZAP-70 expression, after permeabilization, the cells were stained with a conjugated monoclonal antibody specific for ZAP-70 (clone 2F3.2) **Results.** Using flow cytometry, a continuum in the levels of ZAP-70 expression was observed in 59 B-CLL samples (CD19+CD5+) ranging from 0.8% to 92%.

stage B (p=10-2). According to National Cancer Institute Working Group criteria, 18 of 54 patients with Binet stage A required a therapy. Median time to treatment in patients with Binet stage A was significantly shorter when ZAP-70 protein was expressed (median 42.6 months versus median not reached months) (p=10-4). **Conclusions.** Assessment of ZAP-70 protein expression by using a direct immunolabelling method on flow-cytometric analysis is feasible and easy. The use of this analysis permits to select high risk patients in routine laboratory diagnosis of B-CLL patients which could benefit an adequate therapeutic strategy.

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THE COMPARISON OF IMPENEM WITH PIPERACILLIN/TAZOBACTAM AND AMIKACIN COMBINATION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES IN THE TREATMENT OF FEBRILE NEUTROPENIA

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Background. Infectious complications are the most important cause of death in neutropenic patients. **AIM:** To compare the efficacy of imipenem with the combination of piperacillin/tazobactam plus amikacin in patients with hematological malignancies in the treatment of febrile neutropenia. **Methods.** Forty patients were randomized in two therapy groups as imipenem and piperacillin/tazobactam plus amikacin combination groups. When the patients who became afebrile by the 3rd day of the therapy, it was considered as clinical response. Therapy was reassessed for the febrile patients after the 3rd day of the therapy. **Results.** Response rates to therapy were quite similar in both groups (65% for monotherapy and 60% for combination therapy, respectively (p>0.05). Vancomycin and amphotericin were added in 20% and 10% patients in both groups. These drugs was changed with cefepime, because there were resistance to imipenem and piperacillin/tazobactam and amikacin in four patients. Response to therapy was faster in imipenem group (p<0.01). Nausea and vomiting were seen in 55% and 20% of patients treated with imipenem and combination therapy, respectively (p<0.05) Neither imipenem nor combination therapy was stopped because of adverse effects. **Conclusions.** Imipenem was as effective as antipseudomonal β -lactam and aminoglycoside combination treatment in the patients with febrile neutropenia.

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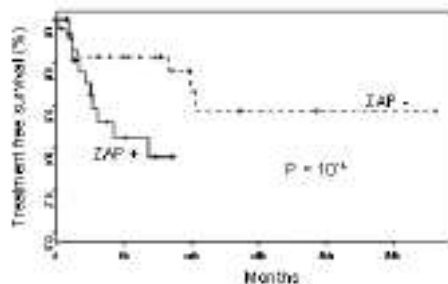
THE OUTCOME OF CHILDHOOD ALL IN A SINGLE CENTER FROM TURKEY: THE RESULTS OF 9 YEARS' EXPERIENCE WITH ALL BFM 90 AND 95 TRIALS

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Background. Dramatic progress in the treatment of childhood ALL has been achieved during the last two decades in Western countries where the 5 year event free survival rate (EFS) has risen from 10 to 70%. However, this high cure rate has not been always occurred in every center in developing countries due to limited sources. **The Aims.** In this study we evaluated the treatment results of ALL BFM 90 and 95 chemotherapy protocols used between 1993 and 2002 in our center. **Methods.** A retrospective analysis of 121 children diagnosed as ALL (M/F: 75/46; mean age: 6.2±3.7 years) was performed. The overall (OS) and event free survival rates (EFS) according to age, sex, initial leucocyte count, chemotherapy response and risk groups were analysed by Kaplan Meier survival analysis and Cox regression test. BFM -90 or 95 ALL chemotherapy protocols were given to children. Methotrexate (MTX) dose was reduced as 1g/m² in the protocol II of BFM 90 (n:37; 30,5%) whereas children in BFM 95 protocol (n:77; 63,6%) received the original dose of MTX (5g/m²). MTX could not be given to 7 children due to their loss during the induction phase. **Results.** EFS and OFS were not

Treatment free survival of 54 B-CLL patients with Binet stage A



According to prior studies, the ZAP-70 levels of 20% were an optimal threshold for classifying B-CLL patients as ZAP-70 positive. 28 (47.4%) B-CLL patients were ZAP-70 positive, including 23 patients with Binet stage A and all patients with Binet

significantly different according to age, sex and initial leucocyte count ($p > 0.05$). However, children who had good response to prednisolone in the first 7 days of treatment achieved significantly better OS and EFS ($p < 0.05$). Patients showing good response in BM aspirates to chemotherapy at 15 days of induction and also the ones achieving complete remission at day 33 had also significantly better OS and EFS ($p < 0.05$) than the others. Children in standard and median risk groups obtained better survival rates comparing to the patients in high risk group ($p < 0.05$). Five years EFS and OS in children given reduced dose of MTX were 65% and 76%, in respectively. Although the children who received original dose of MTX (5g/m²) had better 5 year EFS (84%) and OS (86%) than the others, the difference between the two was not significant ($p > 0.05$). Death occurred in 27 patients out of 121 (22.3%). None were lost due to drug toxicity. **Conclusions.** BFM 90 and 95 protocols in our center were successfully applied with no severe drug toxicity causing death. In our study, an improved outcome was obtained with 5g/m² MTX.

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SERUM LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN PATIENTS WITH NON-HODGKIN LYMPHOMA

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Recently, angiogenesis has been proposed as a useful prognostic factor in melanoma, breast, prostate, gastrointestinal and ovarian cancers. A pathogenetic role has been proposed for angiogenesis in a number of haematological malignancies. Increased serum and/or cellular levels of Vascular Endothelial Growth Factor (VEGF), as the most potent and specific angiogenic factor, were reported in acute and chronic leukemia and multiple myeloma and also predict poor prognosis in these haematological malignancies. Little is known about angiogenesis and VEGF levels in Non-Hodgkin lymphoma (NHL). The aim of this study was to investigate the serum level of VEGF in patients with NHL. **Materials-Methods.** Blood samples were obtained from fourteen patients, five women and nine men (median age 65,6 years) with NHL. Three patients with high grade B-NHL, seven with follicular lymphoma and four with CLL. Six of the 14 patients were at the onset of the disease, while eight patients were in partial or complete remission. In addition, a healthy control group including fifteen individuals was included in the study. VEGF was measured by ELISA immunoassay (R&D Systems, Minneapolis, USA) on patients' and healthy individuals' sera. **Results.** The level of VEGF was higher in patients at the onset of the disease compared to normal healthy individuals. A marked significant difference was elucidated between patients at the onset of the disease and controls, the mean values recorded were 683,76±372,05 pg/mL and 342,32±146,57 pg/mL ($p=0.019$), respectively. No significant difference was detected in the level of VEGF in patients with complete or partial remission and controls (356,17±210,6 pg/mL, 342,32±146,57 pg/mL, $p=0.87$). **Conclusions.** The present study revealed an increased VEGF serum levels in patients at the onset of the disease in comparison to healthy individuals. On the other hand, there was no difference in VEGF serum levels between patients with complete or partial remission and controls. These findings suggest a role for angiogenesis in the pathogenesis/progression of this malignant haematological disorder. Further studies with larger number of cases are warranted to further explore the role of VEGF and other angiogenic factors in the pathogenesis, progression and response to therapy in patients with NHL.

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HETEROGENEITY OF THE BETA - GLOBIN ALLELES IN BETA - THALASSEMIA IN ROMANIA

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Beta-thalassemia is a worldwide inherited disorder characterized by a reduction or complete absence of a beta globin expression. This study was designed to identify the beta-thalassemia mutations in Romanian population. Hematological data were collected with automated cell counters (Coulter). Quantitation of hemoglobin was done by cation exchange HPLC and by agarose gel electrophoresis. Analysis of the mutation in the beta - globin gene has been performed using the PCR based **Methods.** Amplification Refractory Mutation System (ARMS) and Denaturing Gradient Gel Electrophoresis (DGGE). 44 patients with beta - thalassemia were included, 40 cases with beta - thalassemia minor, 4 cases with beta - thalassemia major. DNA of 42 non related patients revealed 9 different mutations: IVS I-110 (21 alleles), CD 39 (9 alleles), IVS II-745 (5 alleles), IVS I-1 (4 alleles), IVS I-6 (3 alleles), CD 6 (2 alleles), -87 (2 alleles), CD51 (1 allele), polyA (1 allele). The most frequent mutations were: IVS I-110 (G/A)[41,3%] and CD 39 (C/T) [19,5%]. These results show that the origin of beta - thalassemia in Romania is Mediterranean as we expected. In our country preventive programs for thalassemia, based on the detection of heterozygote carriers and genetic advice, are not sufficient to reduce the incidence of newborns with major thalassemia. Prenatal diagnosis of thalassemias has given a new dimension to the prevention of these, but in order to implement this, a knowledge of the mutations and the incidence of these, is essential.

This work was supported by grant 217 / 2003 VIASAN.

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TUMOR LYSIS SYNDROME IN PATIENTS WITH ACUTE LEUKEMIAS

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Background. Tumor lysis syndrome (TLS) is a complication of hematological malignancies that is observed in patients who are receiving intense doses of chemotherapy followed by rapid lysis of cells. Often, TLS is asymptomatic, being detected only by metabolic lab abnormalities, including hyperkalemia, hyperuricemia, hyperphosphatemia and hypocalcemia. **Aims.** To evaluate the incidence of TLS and its impact on outcome and survival of patients with acute leukemia (AL). **Patients and Methods.** We studied, retrospectively, 174 patients (81 males, 93 females) with diagnosis of AL, in a single institution. There were 143 myeloid AL and 31 lymphoid AL. The median age was 45.5 years (ranges 17-81 years). Clinical aspects, hematological (white blood cells, percent of blasts in peripheral blood and bone marrow) and blood chemistry (potassium, calcium, phosphate, uric acid, creatinine) parameters were analyzed. **Results.** TLS was present in 57 (32.75%) of 174 patients, 33.56% in myeloid AL and 29.03% in lymphoid AL. 63.15% of patients had a grade I, 24.56% a grade II, and 10.52% a grade III TLS. TLS was found after induction therapy in 52.63% of cases, in 33.33% of cases in first relapse of AL, 10.52% in AL with multiple relapses, and in 3.50% of patients who had an initial refractory AL. TLS was present in 36.84% of cases treated with standard chemotherapy, whereas 63.15% of patients receiving intense doses of chemotherapy developed a TLS. Most often (35.08%) TLS was detected in cases with leucocytes count > 80,000/mm³, and in cases with > 80% blasts in bone marrow. Forty-four patients (77.19%) were asymptomatic, and only 13 patients (22.80%) presented with nausea, vomiting, weakness, and renal colic. In 3 cases a reversible renal failure was diagnosed. The complete remission rate was lower in patients with TLS (40.35%) than in patients without this syndrome (52.13%).

Early deaths occurred in 19.29% of cases with TLS, and in 11.96% of patients without TLS. One-year survival rate was 36.84% in patients with TLS in comparison with patients lacking TLS. Three-year survival rates were 5.26% and 11.96%, respectively. *Conclusions.* TLS has a high incidence in patients with AL and most often it is associated with intensive systemic chemotherapy. TLS is a true hematological emergency influencing the outcome and survival of AL. It is essential to recognize patients at increased risk and to initiate preventive therapy in a timely manner in order to prevent the morbidity and mortality associated with this syndrome.

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QUANTITATIVE T-CELL ZAP-70 EXPRESSION IN CHRONIC LYMPHOBLASTIC LEUKEMIA: CORRELATION WITH CLINICAL COURSE AND STAGE OF DISEASE

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Background. ZAP-70, a protein tyrosine kinase, which plays an important role in T-cell receptor signaling, was recently shown to serve as a surrogate marker for the mutational status in chronic lymphocytic leukemia (B-CLL). *Aims.* In the light of the T-cell aberrations and dysfunction known to exist in B-CLL, we evaluated T-cell ZAP-70 expression in B-CLL patients. *Methods.* ZAP-70 levels in circulating T and B-cells from 35 CLL patients and 8 normal controls were measured by quantitative flow-cytometry analysis. *Results.* In CLL, circulating T-cells overexpress ZAP-70 compared to healthy individuals ($p < 0.0005$). In addition, T-cell ZAP-70 levels are higher in the 'high ZAP-70 CLL' subgroup (using cut-off of $\geq 20\%$ ZAP-70 positive B-CLL cells) compared to the 'low ZAP-70 CLL' subgroup ($p = 0.015$). A positive linear correlation also exists between ZAP-70 expression in the leukemic B-cells and the T-cells derived from the same patients ($r = 0.902$). Furthermore, 'high T-cell ZAP-70' CLL patients have a shorter time to clinical progression compared to the 'low T-cell ZAP-70' CLL subgroup ($p = 0.0019$), and patients in Binet stage C express more T-cell ZAP-70 than those in Binet stages A+B ($p = 0.048$). *Conclusions.* In CLL, T-cell ZAP-70 overexpression correlates with clinical stage and disease progression.

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LEUKOCYTE ACTIVATION IN MYELOPROLIFERATIVE DISEASE

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Background. The pathogenesis of thrombotic events in myeloproliferative disorders (MPD) is still unclear. Despite different large studies, it was not possible to correlate the incidence of thrombotic events with either platelet number or their *in vivo* or *ex vivo* activation status. In any case it is accepted that both platelets and leukocytes from MPD patients circulate in an activated status. On the other hand, tissue factor (TF) plays a central role in the formation of thrombi. *Aims.* We analyzed the expression and blood cell content of TF and other markers of cellular activation in 12 MPD patients: 6 with essential thrombocytemia (ET) and 6 with polycythemia vera (PV). *Methods.* The expression and/or cellular content of TF, fibrinogen (Fg), myeloperoxidase (MPO), CD11b, CD40 were determined using three-colour flow cytometry. *Results.* The results obtained were expressed as means \pm SEM of positive cells, compared with those obtained from 12 comparable healthy volunteers. In all patients up regulation of the following functions was observed: CD11b on PMN ($78 \pm 6\%$ vs $28 \pm 4\%$), TF expression on PMN ($6 \pm 1\%$ vs $1 \pm 0.7\%$) and on monocytes (27 ± 12 vs 0.5 ± 0.1), Fg association to PMN surface ($14 \pm 5\%$ vs $2.2 \pm 0.4\%$) and to mono-

cyte surface (9 ± 3 vs 0.5%), CD40 expression on PMN surface (18 ± 9 vs 8 ± 1) and on monocytes surface (24 ± 5 vs 11 ± 1). Intracellular content of TF in PMN was higher only in 10 patients ($82 \pm 7\%$ vs $15 \pm 5\%$), while, in the other two (TF content $< 1\%$) a dramatic PMN degranulation was found and confirmed by MPO ($< 3\%$). *Summary/Conclusions.* Our data confirm PMN and monocyte activation in MPD patients. The up regulation, as well as the increase in TF content in PMN might suggest an important role of this factor in the thrombotic tendency of MPD patients.

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IDENTIFICATION OF GENE EXPRESSION PROFILING IN A CML CELL LINE, K562, AFTER TREATMENT WITH SKI606, A DUAL INHIBITOR OF SRC/ABL KINASE

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STI571 is a selective inhibitor of Bcr-Abl and is currently used to treat CML patients, although treatment STI achieves complete haematological remission in $> 95\%$ of patients, complete cytogenetic and molecular responses are observed in only a minority of patients. For this reason we tested a new drug, SKI606, a potent Src/Abl kinase inhibitor and with antiproliferative activity against CML cell lines in culture. Here we describe the gene expression profile of a CML line treated with this new drug. To analyze earlier biological effects of SKI606, we used four oligomers microarrays, using slides containing more than 20,000 genes (MWG). We adopted a microarray co-hybridization to compare gene expression profiles of K562 treated with SKI606 for 8 hours at the concentration of 1mM with K562 of control. We have labelled the amplified aRNA of the reference with green (Cy3) and that of the treated with red (Cy5) and we applied the dye swap to confirm our data. Our approach to design comparisons utilizes analysis of variance (ANOVA) models and a filter to obtain the genes most up and down-regulated. We found out 71 up-regulated genes and 2 down regulated. Ontological information on the cellular function of these transcripts suggests differential expression of genes associated with a wide range of cellular roles, including transcriptional regulation, signal transduction and cell death. However, our data provide a first evidence that SKI606 treatment is also effective in up-regulation of apoptosis genes (CD5L, LY86, MAEA). It is also involved in the cell cycle regulation (DBC1 and BRCA2) and transduction of signal (TLR5 and IL1RL1). Moreover we observed an up regulation of transcription regulation activity (MYST2 and ZNFN1A1); an increase of AVPR1A, a protein kinase C-binding. Currently we are analyzing the most interesting modified genes to validate our data. A combination of genome-wide expression-level analysis and cell-biology research in cultured leukemia cells is expected to be a useful functional-genomic approach to characterize the mechanism of action of this drug.

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2004, by the University of Bologna (grants 60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R), Fondazione del Monte di Bologna e Ravenna.

1030**THE ROLE OF HEREDITARY AND ACQUIRED THROMBOFILIA IN WOMEN WITH OBSTETRIC COMPLICATIONS**A. Skordilaki¹, A. Livadiotaki², S. Sfiridaki², S. Samiotaki¹, C. Kidona¹, N. Tsagarakis¹¹General Hospital Hania, HANIA, Greece; ²Transfusion Center - Venizelio, HERAKLION, Greece

The progress that has been achieved in molecular biology and in the study of antiphospholipid syndrome has helped a lot in the research and knowledge of thrombophilia's causes. AIM: The aim of our study was to evaluate the thrombophilia's test results in women with obstetric complications who have attended our unit MATERIAL-METHOD: 82 women were examined with mean age 33,3 years old (range of age 20-43) who have come from 1/4/04 until 31/12/04. The reasons were recurrent abortions (more than two) or fetal loss or intrauterine growth reduction or preeclampsia. The blood tests included: PT, APTT, FIB, DD, FDP, PrC, PrS, ATIII, APCR, FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, APA, ACA, antib2GPI, PTT-LA, dRVVt, tHCy, PAI-1, t PA and genetic control with PCR for mutations FVLeiden, FIIG□ A 20210, MTHFR. *Results.* We found 9 (10,97%) heterozygous FVLeiden, 7 (8,53%) heterozygous MTHFR, 6 (7,31%) homozygous MTHFR, 17 (20,7%) Antiphospholip Syndrome, 4 (4,87%) het.FVLeiden and het. MTHFR, 1 (1,22%) homozygous FIIG□ A 20210 and heterozygous MTHFR, 1 (1,22%) heterozygous FIIG□ A 20210 and 37 (45,12%) normal. *Conclusions.* It seems that a big percentage of these complications is due to hereditary thrombophilia. Nevertheless more important is the presence of antiphospholipid antibodies that causes acquired thrombophilia. It is therefore necessary in all cases with obstetric complications to fully investigate for hereditary or aquired thrombophilia, since their definition will determine the right therapeutic choice.

1031**INCREASED CYTOSOLIC FREE CALCIUM IN PLATELETS AT PREECLAMPSIA**

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Background. Platelets play an important role in the development of preeclampsia. Platelet activation occurs in pregnancy of women with risk of preeclampsia. Because of the intracellular Ca²⁺ is a major determinant of cellular function it may contribute in pathogenesis of disease. *Aims.* Our purpose was to investigate concentration of intracellular Ca²⁺ in platelets of healthy pregnant women, with risk of preeclampsia and with preeclampsia and thus elucidate the role of [Ca²⁺]_i in mechanisms of preeclampsia development. *Materials and Methods.* Peripheral blood was collected from 20 healthy pregnant women, 20 women with risk of preeclampsia and 20 preeclamptic women. Measurement of intracellular Ca²⁺ was carried out with the dye fura-2-AM by means of fluorescence method. *Results.* Resting intracellular free Ca²⁺ concentration was higher in platelets from women with risk of preeclampsia (90±17 nM) and preeclamptic women (107±18 nM) than in platelets from healthy pregnant women (77±19 nM). The ionomycin-induced increase in [Ca²⁺]_i without external Ca²⁺, an index of the size of internal Ca²⁺ stores, was significantly higher in platelets of women with risk of preeclampsia. *Conclusions.* These results suggest that increased platelet [Ca²⁺]_i may be associated with preeclampsia.

1032**CD38 EXPRESSION IN PATIENTS WITH CHRONIC LYMPHOBLASTIC LEUCAEMIA IN DIFFERENT HEMOTHERAPY REGIMENS, INCLUDING FLUDARABINE**

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Background. one of the most important prognostic factors in the treatment of chronic lymphocytic leucaemia is CD38 expression. The aim of this work was to examine the CD38 expression in the patients with CLL in different chemotherapy regimens, including fludarabine. Patients and methods. This treatment included 240 CLL patients aged from 32 to 76 years (the average age was 58 years). Among those patients, there were 139 males, 101 females. The diagnosis of CLL was confirmed by morphological and immunophenotyping of the peripheral blood, bone marrow and biopates of lymph nodes. The CD38 expression was estimated immunocytochemically and by cytofluorimetric assay. According to chemotherapy regimens, the patients were divided into two groups: the patients of the first group received FCM combination treatment: fludarabine 25 mg/m² intravenously (IV), d 1-3, cyclophosphamide 200 mg/m² IV, d 1-3, mitoxantrone 6 mg/m² IV, d 1. The patients of the second group received FC combination treatment: fludarabine 25 mg/m² intravenously (IV), d 1-3, cyclophosphamide 300 mg/m² IV, d 1-3. The average number of courses given was not more than 6, with the 28-days intervals. Besides that, the patients of the first and the second groups were divided into two subgroups, which depends on the expression of the CD38 more than 30% and CD38 less than 30%. *Results.* The expression of the CD38 on the more than 30% cells were marked in 64% of the patients, and CD38 on the less than 30% cells in 36% patients. The median overall sur-vival in the patients with the CD38 >30% was 69 months, but with CD38 < 30% - 98 months. By treating patients with CD38>30% on the FCM program, the complete responses were achieved in 58% of patients, partial responses in 32% of patients. On the FC treatment, complete responses were achieved in 27%, partial responses-in 49%. The median Disease-Free Survival (DFS) on the group of CD38 positive patients with the FCM program, was 35 months, and in patients on the FC treatment-21 month. The median OS and DFS of the CD38-negative group was not reached, which was not depended on the regimen of the chemotherapy. *Conclusions.* consequently, The high CD38 expression makes an impact on the Over-all and Disease-Free Survival of the CLL patients. If such adverse prognostic factor, as CD38 expression more than >30%, was presented on the CLL cells, the best results of the therapy were obtained with the introducing of the FCM program.

1033**A STUDY FOR ASSOCIATION BETWEEN MUTATIONS AND IRON METABOLISM IN PATIENTS WITH TYPE 2 DIABETES MELLITUS**

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Background. Diabetes mellitus is a recognized consequence of hereditary hemochromatosis (HH). Hereditary hemochromatosis is an autosomal recessive disorder in which affected individuals absorb elevated amounts of iron from the diet, resulting in accumulation of excess iron in internal organs. This overload of iron in various organs especially in the liver, the heart and pancreas, ultimately leading to liver cirrhosis, diabetes, arthritis, cardiomyopathies and premature death. AIM: The aim of this study was to investigate a possible association between i genes mutations HFE, TFR2 and FPN1 and iron metabolism in patients with type 2 diabetes mellitus. *Methods.* We studied 74 patients (35 men and 39 women) aged 40 to 75 years old, with type 2 diabetes mellitus (T2DM). Our method was based on the reverse-hybridisation principle. Genomic DNA was isolated from anticoagulated blood. Gene fragments

containing mutations were amplified from genomic DNA by polymerase chain reaction technique (PCR). The PCR products were selectively hybridised for the detection of 12 mutations in the HFE gene (V53M, V59M, H63D, H63H, S65C, Q127H, P160delC, E168Q, E168X, W169X, C282Y, Q283P), 4 mutations in the TFR2 gene (E60X, M172K, Y250X, AVAQ594-597del), and 2 mutations in the FPN1 gene (N144H, V162del). **Results.** From the 74 patients 70,25% were found to be wild type, 2,70% were Homozygotes for H63D mutation, 22,95% were heterozygotes for H63D mutation, 2,75% were Heterozygotes for S65C mutation, and 1,35% were Heterozygotes for W169 X mutation of the HFE gene. **CONCLUSIONS** All these mutations might have important role in the pathogenesis of type 2 Diabetes mellitus and they could be used as prognostic markers in the clinical course of the disease.

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ALLOGENEIC HLA-IDENTICAL SIBLING TRANSPLANTATION FOLLOWING NON MYELOABLATIVE PREPARATIVE REGIMEN (FLUDA/TBI) IN PATIENTS WITH CHRONIC HEMATOLOGICAL MALIGNANCY

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Background. Non myeloablative conditioning regimens (NM) are increasingly used for allogeneic stem cell transplantation (allo-SCT). NM has been shown to allow engraftment with minimal early transplant-related mortality (TRM). However, most of reported studies were heterogeneous in terms of donor type, HLA-matching, source of stem cell, underlying diseases and/or GVHD prophylaxis. **OBJECTIVE:** In this report, we analyzed the outcome of 25 consecutive patients with chronic hematological malignancy, who were referred to our center for an HLA-identical sibling allo-SCT after NM including fludarabine (30mg/m²/d x 3) and TBI (2Gy). **Methods.** From October 2001 and June 2004, 18 males and 7 females received an allo-SCT after NM conditioning for NHL (n=10), CLL (n=7), HD (n=4), MM and WM (N=2) and MDS (n=2). At the time of allo-SCT, 14 patients had a responsive disease (3 CR and 11 PR) and 11 had a progressive disease (4 stable, 4 refractory, 2 relapse and 1 untreated). Median age was 49 years (range, 34-62). Donors were HLA-matched siblings and source of SC was peripheral blood stem cell in all of them. The median CD34+ cell infused was 5.4 x 10⁶/Kg (3.1-12.4). Sex mismatch (male patient undergoing transplantation with HSC of female donor) was recorded in 7 patients. GVHD prophylaxis consisted of CSA and MMF. Three patients with minor ABO incompatibility received MTX instead of MMF. The same supportive care measurements including anti-infection prophylaxis were used in all patients. **Results.** Four and 9 patients had never experienced neutrophil count <500/mm³ and/or Platelet count < 50000/mm³, respectively. For the other patients, sustained neutrophil and platelet recovery occurred at a median of 18 days and 16 days, respectively. No patient had autologous reconstitution in the 100 days post-transplantation. Full donor chimerism (FDC) was obtained progressively. Thus, while there were 10 patients with FDC at day28 they were 16 patients at day100. Statistical analyses were performed at the reference date of February 28, 2005. With the median follow-up of 769 days (range, 244-1231), the estimated 2-year overall survival (OS), event free survival (EFS), relapse and transplant-related mortality (TRM) rates were 53%, 45%, 39% and 27%, respectively. The cumulative incidence of grade II-IV and grade III-IV acute graft-versus-host disease (aGVHD) were 56% and 28%, respectively. Sixteen patients developed chronic GVHD (cGVHD) including 6 with extensive stage. In GVHD setting, cGVHD showed a protective effect on survival and disease progression with better OS and EFS for patients with cGVHD as compared to those without cGVHD (p=.03 and p=.0004, respectively). On the other hand, OS and EFS were adversely influenced by grade III-IV aGVHD (p=0.0001 and .033, respectively). Finally, the absence of FDC at day 28 was associated with a higher risk of relapse (p=0.039).

Conclusions. Collectively, these results confirm the efficacy of allo-SCT with NM in patients with chronic hematological malignancy and highlight the benefit of the GVL effect. Better control of aGVHD is needed to reduce TRM and afterward to improve results.

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NON-HODGKINYS LYMPHOMA PRESENTING WITH EPIDURAL SPINAL INVOLVEMENT

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Background. Spinal cord compression and radicular involvement are infrequent events in the natural history of Non-Hodgkin's lymphoma. They are usually secondary to invasion of spinal extradural space and rarely are the presenting manifestations of this condition (varying from 0.1% to 6.5%). The spinal tumours are frequently high-grade B lymphomas. Patients and **Methods.** Between 1984 and 2004, 7 patients (6 F, 1M) with a median age of 48.8 years (range 28-68) were diagnosed. Symptoms of presentation consisted in dorsal, cervical or lumbar pain (during the last 3 months); progressive neurological impairment (paresthesias, esphinteral compromise and paraplegia). B symptoms were present in 3 patients in whom CR was not obtained. The dorsal segment was the most affected (5/7); cervical (1/7); lumbar (1/7). All the patients were HIV negative. Anaemia was present (7/7) with a medium haemoglobin value of 99 g/L (89-115). Bone marrow was not affected (0/7). Histological subtype: diffuse large B-cell lymphoma (DLBCL) (7/7). IPI 2/3. The treatment consisted in decompressive laminectomy, local radiotherapy and chemotherapy (CHOP-like and R-CHOP in one case) **Results.** 7 out of 7 patients responded (4 CR and 3 PR). Within complete responders, 2 are alive with a median duration of response of 54 months (6-96); 2 were lost in follow up. Neurological improvement was observed after treatment, depending on the duration of symptoms. 2/7 patients with paraplegia at diagnosis did not improve. Partial responders are dead (because of complications during treatment or progression) with a median follow up of 11 months (3-18) **Conclusions.** Spinal cord compression as initial presentation is rare in high-grade lymphomas, but it may be taken in account in a patient with chronic pain and progressive neurological impairment. Early diagnosis and treatment are critically for preventing irreversible neurological impairment.



Figure. MNR: Epidural involvement and cord compression.

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PATIENTS AT RISK OF VITAMIN B12 DEFICIENCY IN THE AREA OF CRETE

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Background. Vitamin B12 deficiency is frequently observed in older people and is asymptomatic or may present with hematologic and neuropsychiatric manifestations. The aim of our study was to identify patients at risk of Cobalamin (B12) deficiency among the general population of Crete using a screening

test based on serum vitamin B12 levels. *Methods.* We investigated 268 patients who had been referred from general practitioners, with anemia or clinical findings suspected of cobalamin deficiency, in relation to their age, gender, the presence of anemia and other clinical manifestations. Patients were classified into three groups in correlation with the vitamin B12 levels. Group I, patients with low serum B12 level <120pg/mL, group II patients with borderline serum B12 level 121-200pg/mL and group III patients with normal >201pg/mL B12 levels. Serum vitamin B12 levels were determined by the AIA-600 analyzer (Biochem), by using a competitive immunoassay method and automated complete blood cell counts were measured by the Abbott 3700 analyzer. *Results.* Of the 268 patients included in our study 81 (30.22%) were males and 187 (69.77%) were females. The median age was 59,84 years with an age range of 10-90 years. Vitamin B12 deficiency was found in 15 patients (5.59%) with B12 values between 60-119 pg/mL, mild vitamin B12 deficiency was found in 13 patients (4.85%) with values between 125-200pg/mL and normal B12 values 201-950pg/mL were found in 240 (89.5%) patients. 110 patients of the total population were anemic, 11 (73.33%) for group I, 7 (53.84%) for group II and 92 (38.33%) for group III. The correlation of gender, age and the presence of anemia are shown in table 1. *Conclusions.* The prevalence of cobalamin deficiency in our group of patients was 5.59% and this agrees with the prevalence of 5-15% in the elderly population that has been reported in other studies. The determination of serum cobalamin is a first line diagnostic test to discriminate patients at risk of B12 deficiency. To sum up, patients with low and borderline B12 levels require further studies for a definite diagnosis.

Group	Male	Female	Patients	Age	Anemia
I	5	10	15 (5.59%)	61.86 (±16.06)	11 (73.33%)
II	4	9	13 (4.85%)	55.80 (±15.94)	7 (53.84%)
III	72	168	240 (89.56%)	59.83 (±16.68)	92 (38.33%)
Total	81	187	268	59.84 (±16.80)	110 (41.04%)

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RITUXIMAB IMMUNOTHERAPY: A PROMISING ALTERNATIVE TREATMENT IN PATIENTS WITH ANEMIA AND THROMBOCYTOPENIA OF IMMUNE ORIGIN

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Background. Immune thrombocytopenic purpura (ITP) and autoimmune hemolytic anemia (AIHA) are two distinct immune disorders in which platelets and erythrocytes are destroyed by self-reacting immune mediated autoantibodies and or/ circulating immune complexes. Standard treatment modalities include corticosteroids, intravenous immunoglobulin (IVIg), splenectomy and immunosuppressive agents. Rituximab (anti-CD20) is a novel investigating therapeutic agent which causes B lymphocytes depletion and contributes to successful treatment of the underlying immune disorders especially those of relapsed or refractory type. *Aims.* We assessed the clinical outcome and tolerance of Rituximab administration in five male patients with refractory autoimmune hematologic disorders. Patients and *Methods.* Two ITP, 2 AIHA patients and 1 patient with Evan's syndrome, all refractory to first line therapies, were documented between 1/2003 and 6/2004. Their median age was 43 years (range: 35-52) and all had been treated with

steroids and IVIG as first line treatment. Vinca alkaloids and cyclophosphamide were used as second line therapies in 2/5 and 3/5 respectively without response. Rituximab 375mg/m² iv weekly was administered in four to six consecutive courses as an alternative treatment modality. *Results.* A complete response was achieved in all of them and is sustained 9- 20 months post-Rituximab administration (median time: 18 months). No serious adverse events were reported. *Conclusions.* Our early findings suggest that Rituximab could be a promising alternative therapeutic agent in refractory/relapsed cases of immune cytopenias. Further studies including more patients could elucidate the clinical benefit of Rituximab and the algorithm of therapeutic strategy in autoimmune cytopenias.

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PERICYTE COVERAGE OF ABNORMAL VESSELS IN THE MYELOFIBROSIS BONE MARROW

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Background. Increased angiogenesis, with large tortuous vessels, is a recognized feature of the myelofibrosis (MF) bone marrow, but the pathogenic mechanisms are not well understood. It has been claimed that relative pericyte deficiency, suggested to be a sign of vessel immaturity, may characterize vessels of solid tumors, which also often are tortuous. *Aims.* To see if vessels in healthy and myelofibrotic bone marrows are deficient in pericytes and, thus, immature. *Methods.* We studied microvessel density (MVD), vessel morphology and pericyte coverage in bone marrows from nine myelofibrosis patients and nine controls with confocal microscopy techniques, including 3-D imaging reconstructions. *Results.* As reported, mean MVD was 6.2-fold higher in MF marrows compared to controls (p<0.001). Likewise, the vessels of myelofibrosis displayed much larger mean perimeters (89±61 µm vs. 19±9 µm; p<0.001). The number of VEGF-A165 positive cells in MF bone marrows were significantly higher than in control marrows. Pericytes (defined as multibranched alpha-smooth actin-positive pericapillary cells) covered the vessels. In myelofibrosis, 99±3% of all vessels was pericyte-coated (compared with only 51±20% in controls; p<0.001). Especially the large tortuous vessels of MF were covered by PC. Moreover, a small proportion of MF (but not control) pericytes expressed PDGF receptor $\alpha 1$, but none expressed desmin. *Conclusions.* Thus, human bone marrow vessels are covered with pericytes and, in contrast to solid tumors, intense pericyte coverage of the aberrant vasculature in myelofibrosis suggests active recruitment of these cells, related to the disease process. This might be a starting point for targeting the recruitment of pericytes as part of treatment for MF.

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EFFECT OF CYCLINE-DEPENDENT KINASE INHIBITOR (ROSCOVITINE) ON LEUKEMIC CELL LINES

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Background. Roscovitine is a purine analogue and cyclin-dependent kinase inhibitor. The drug has been shown to possess a good effect on both brain tumors as well as breast cancer in animal models. *Aims.* The present study aims to investigate the cytotoxicity of roscovitine in leukemic cell lines P39, Jurkat and K562 in terms of viability, proliferation, apoptosis, and cell cycle analysis. *Methods.* The P39 (myeloid), K562 (CML) and Jurkat (lymphoblastic) cell lines were cultured in RPMI1640 supplemented with 10% FBS. Cells were treated with Roscovitine in concentrations of 5µM, 25µM and 50µM up to 24 hours. Cells were harvested at several time points for investigation. Following method were used: trypan blue exclusion for cell viability, 3H-thymidine incorporation for cell proliferation, morphologi-

cal staining according to May-Grunwald-Giemsa on cytospined slides for apoptosis. Cell cycle was studied using propidium iodide staining and FACS analysis. **Results.** Cell viability in all untreated cells was > 95% at 24 hrs. Cells treated with roscovitine 5 µM did not show decrease in cell viability compared to control in all cell lines. Roscovitine at 25 and 50 µM showed a significant effect on cell viability in both P39 and Jurkat cells while little effect was observed in K562. Decrease in cell proliferation was observed in all cell lines. The decrease in cell proliferation was both time and concentration dependent. However, the effect was most pronounced in P39. Roscovitine in concentration of 5 µM did not induced apoptosis in any of the cell lines. Roscovitine in concentrations of 25 and 50 µM induced apoptosis in time and concentration dependent manner in all three cell lines, however, the sensitivity differed among the cell lines (roscovitine at 50 µM at 24 hours induced apoptosis in about 95% of P39 and Jurkat cells, but only in about 25% of K562 cells). In parallel, the sub-G1 peak was detected when cell cycle was studied using FACS. Changes in cell cycle distribution were detected already 3 hours after the incubation start. The cell cycle arrest at G2 phase was observed in K562 cell line. **Summary.** Roscovitine has affected the leukemic cell lines in concentration- and time-dependent manner. The cytotoxicity of roscovitine has resulted in apoptosis in all studied cell lines. The sensitivity of the cells has differed according the cell type, the myeloid cell line P39 being most sensitive.

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PERCUTANEOUS VERTEBROPLASTY FOR PATIENTS WITH MULTIPLE MYELOMA

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STUDY DESIGN: Biomechanical properties of spine in MM determinate the quality of life and social activity of the patients. Progressive destruction of spine is a major cause of morbidity in patients with MM. We evaluated the safety and clinical results of percutaneous transpedicular vertebroplasty (PV) for multiple myeloma (MM). **Methods.** For stabilization involved spine and restore its mechanical stiffness combination biphosphonates and PV were used. The investigation is based on the 19 patients at the age of 47-61 years with MM in the stage²²⁻⁶ and ²²²⁻¹³ patients. All the patients underwent complex investigation (include clinical examination, CT, MRI). SPECT bone scan with technetium-99m were performed in 5 cases. Quality of life of the patients was estimated according three criteria: back pain (VAS), social activity, depending from medication. The patients suffered from local pain in thoracic and lumbar spine due to vertebral compressive fractures (VCF) and lytic of vertebral body without lysis of posterior wall of vertebral body (G2,G3) at the level Th11-L3 of areas in vertebral body (1-3 lytic foci with diameter more than 2cm). Tactic of treatment depended of type of MM. All the patients were divided on two groups: 1. Patients with small lytic foci (less than 2 cm). 2. Patients with big lytic foci (more than 2 cm) With the first group, the unilateral transpedicular access was chosen, and in cases of second group of patients, we used a bilateral transpedicular puncture of the vertebral body. Uninvolved bone tissue was augmented in all cases. PV was performed only in cases with acute VCF that led to pain syndrome. PV was performed as 'come and go' procedure. Operations were performed under fluoroscopic control and local anesthesia. Duration of PV-17-40 min. Control CT investigations were performed immediately after an operation and after 3 and 12 months. **Results.** Complete pain relief immediately after operation was revealed in 18 patients and partial pain relief in 1 of cases (less than 1 score on Pain Score Scale). In all patients decreasing of analgetic taking was noticed and increasing social activity. Complications were not obtained. Using VP did not influence on standard treatment. CT and MRI characteristics and degree of cement filling were assessed. **Conclusions.** 1. PV is a safe and effective procedure for relief of pain, increases quality of life and disability in patients

with VCF for MM. 2. Indications for PV in MM are acute VCF in cases of local or local-diffuse MM. 2. PV can enlarge therapeutic 'window' for chemotherapy. 3. Combination of chemotherapy, biphosphonates and VP provide the best results for treatment patients with VCF due to MM.

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VARIATION OF TELOMERE-TELOMERASE COMPLEX COMPONENTS IN MYELODYSPLASTIC SYNDROMES: TELOMERE LENGTH AND EXPRESSION OF THE hTERT GENE SEEM TO BE THE MOST VALUABLE FOR DISEASE PROGNOSIS

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Background. Chromosomal ends telomeres and telomerase, ribonucleoprotein synthesizing repetitive sequences onto telomeres, became intensively studied topics in tumors. Presence of eroded telomeres and enhanced activity of telomerase in malignant cells as well as knowledge of their regulation offer possibilities for molecular target therapy (MTT). Expression studies of gene encoding catalytic sub-unit of enzyme telomerase - human telomerase reverse transcriptase (hTERT), telomerase regulation gene tankyrase (TNKS) and gene encoding telomere associated protein (TEP1), together with estimated levels of telomerase activity and telomere length determination can bring important sign into molecular characteristic of the disease. **Aims.** Changes of telomere-telomerase complex components were studied in patients with myelodysplastic syndromes (MDS) with the aim to evaluate its association with clinical outcome and disease progression. **Methods.** The study was performed on 26 samples of 22 patients with MDS dividing according to FAB criteria as follows: 12 x refractory anemia (RA), 2 x RA with ringed sideroblasts (RARS), 5 x RA with excess of blasts in transformation (RAEB-t), 1 x CMML. Protein extracts, DNA and RNA were earned from bone marrow or peripheral blood mononuclear cells. Protein extracts were tested for activity of enzyme telomerase using modified TRAP (Telomeric Repeat Amplification Protocol) Assay - TeloTAGGG Telomerase PCR ELISAPLUS kit (Roche Molecular Biochemicals). Expressions of hTERT, TEP1 and TNKS genes were assayed by quantitative real-time PCR with specific Taq-Man probes. Average telomere length from all chromosome ends TRF (Terminal Repeat Fragment) was determined performing TeloTAGGG Telomere Length Assay (Roche Molecular Biochemicals). Mononuclear bone marrow cell of age matched donors provided control samples. Telomere-telomerase characteristics were discussed together with karyotype findings (G-binding, FISH, mFISH) and risk score established according to the International Prognostic Scoring System (IPSS). **Results.** Positive levels of telomerase activity/expression of hTERT gene were obtained in 40/50% patients with early forms of MDS (RA, RARS) and in 50/75% patients with advanced forms of MDS. Significantly reduced telomere lengths were detected in 66% of MDS patients: in all patients with positive levels of telomerase activity and also in some patients with negative telomerase activity. In sequential samples taken at the time of diagnosis and during the course of the disease different expressions of the hTERT gene were observed. In patients with RAEB and RAEB-t notable increase of the hTERT expression was found in contrast to no significant changes of telomerase activity. Disease progression does not seem to be associated neither with changes of TNKS gene expression, nor with telomerase activity showing only low variability in the course of MDS. Also, the TEP1 gene shows only small expression differences depending on clinical outcome. **Summary/conclusions:** Although enhanced telomerase activity appears already in early stages of MDS (RA, RARS), it represents later event than telomere erosion and increased expression of hTERT gene. The TEP1 gene seems to play no important role in regulation of telomere length. For further evaluation of role of all studied telomere-telomerase components, which might have importance for disease prognosis, more extended study is required. Grants IGA MHCR NC7606-3 and NK7713-3 supported this work.

1042**MARKERS OF ANGIOGENESIS IN POLYCYTHAEMIA VERA**S.T. Theodoridou¹, T. Vyzantiadis¹, I. Venizelos², I. Klonizakis¹, A. Papadopoulos³, S. Vakalopoulou¹, E. Mandala¹, V. Perifanis¹, V. Garipidou¹¹Hippokraton Hosp., Aristotelion Univ., THESSALONIKI, Greece; ²Hippokraton Hosp., THESSALONIKI, Greece; ³Ahepa Hospital, Aristotelion University, THESSALONIKI, Greece

The role of angiogenesis is critical for tumor and haematopoietic malignancies progression and is under investigation for myeloproliferative diseases. Few are reported on angiogenesis in Polycythaemia Vera (PV), the significance of angiogenic stimulators as Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF) and the microvessel density (MVD) in trephine biopsies of polycythaemic patients. Therefore, we report our observations in patients with PV concerning the serum levels of VEGF and bFGF, as well as the microvessel density in bone marrow samples immunostained with anti-CD34 monoclonal antibody. We evaluated VEGF and bFGF levels in sera of 23 patients with polycythaemia vera (PV duration 1-17 years) with a mean age of 58,2±3,6 years (mean±SEM). Twelve patients received hydroxyurea, ten were managed with phlebotomy and one was treated with interferon. No one had clinically detected spleen enlargement. In twenty two of them we estimated the bone marrow MVD by counting the number of vessels per 200x high power field (HPF) using light microscopy. The control group consisted of 20 healthy subjects (mean age 54,4±1,6 years). The age difference between the two groups was statistically not significant (p=0,9). Serum VEGF levels were found very significantly increased in the group of patients compared with the control group (569,7±101,2 and 164,7±32,8 pg/mL respectively; p=0,001). No differences were found in serum bFGF concentrations between patients and the control group (2,6±0,19 vs 2,8±0,16 ng/mL respectively; p=0,65). In the patient group we found no correlation between serum VEGF and bFGF levels and platelet count, Hb, WBC count, age or therapeutic regimen. On the contrary, a very interesting positive correlation was found between bFGF and VEGF levels only in the group of polycythaemic patients (r=0,54, p=0,02). Although we found no difference between the serum bFGF levels of the two groups, the above correlation gives a possible evidence of the interaction of the two factors in the angiogenic procedure in PV. The MVD was found 4,2 vessels/HPF (reported reference values ≤ 3). The results provide a profile of increased angiogenic tendency in PV and suggest the need for further research.

1043**EXTRAMEDULLAR HEMATOPOIESIS AND IFEBRILE PLEURITIS IN THALASSEMIA INTERMEDIA**

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Background. While TM (thalassemia Major) is characterized by severe anemia, life-long transfusion, iron-induced ventricular dysfunction and heart failure, TI (thalassemia Intermedia) has a later clinical onset with milder anemia, high cardiac output state, left ventricle remodeling and age-related pulmonary hypertension. Bone deformities, extramedullar hematopoiesis (EMH), spleen and liver enlargement are the hypoxia and enhanced erythropoiesis consequences in both. Pleural effusion in thalassemia is rarely referred in the literature. **Aims.** We present our experience on this complication of thalassemia intermedia. **Methods.** We present 7 thalassaemic patients, hospitalized in our Department with massive pleural effusion, within the last 8 years. Data is collected by medical files. **Results.** Our group included 7 TI patients (4 men, mean age 37 yrs). The com-

mon clinical findings were dyspnoea and apyrexia. In the majority, effusion was unilateral. It was exudative in 6, and aspiration revealed massive hemothorax in one case. Cytologic fluid analysis was negative for malignancy, cultures and serology were negative for viral, bacterial infection and tuberculosis. Echo and clinical study excluded congestive heart failure and pulmonary embolism. Although thoracentesis could not control the effusions, pleurodesis with bleomycin or tetracycline was successful. Radiology revealed intrathoracic EMH in all cases. **Conclusions.** Accumulation of ifebriple pleuritis cases in TI, introduces a possible pathogenetic relation between EMH and effusion, allowing doctors to reconsider transfusions indications, including there the suppression of hematopoietic masses.

1044**EVALUATION OF DENSITY AND DEFORMABILITY OF ERYTHROCYTES IN COMPLETE REMISSION OF AIHA**O.F. Nikulina, N.V. Tsvetaeva, E.S. Shurhina, N.D. Khoroshko
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Autoimmune hemolytic anemia (AIHA) results from the production of autoantibodies towards membrane antigens of red blood cells which leads to their premature destruction. Deformability of erythrocyte is the property to change their forms, while moving through the vessels. **Aims.** To analyze density deformability of erythrocyte during different periods of disease, to monitor degree of hemolyses that can influence on applied treatment. **Methods.** The density distribution of RCBs was determined by Danon-Marikowsky phthalate method. We determined middle density of erythrocyte, I max of erythrocytes, which was found to be of 0,004 g/mL, light fraction of erythrocyte - % erythrocyte with density 1,086 g/mL, heavy part of erythrocyte-with 1,112 g/mL. Deformability of erythrocyte was studied by applying special filter with different osmolarity. **Results.** 29 patients with AIHA were observed including monitoring in different periods of disease. 6 patients were observed with the certain hemolytic clinical picture. We observed increase of the light part of erythrocyte equal to 1,1±1,2%; Ucr 185±10 mOsm (critical osmolarity). The heavy fraction of erythrocytes was increased by 50%. During remission of AIHA Ucr changed up to 194±2 mOsm, the light part of erythrocytes-3,5±0,8%, middle density of erythrocyte was 0,97±0,01% (N = 1,098±0,001%) and I max-40,8±6,1% (N = 42,9±4,5%). At the beginning of disease we noted the increasing of light part of erythrocyte which turned out to be 20-40% bigger than normal that can be explained with reticulocytes. It reaches 20,5±5,9% (N = 0,6±0,3%). The heavy fraction of erythrocyte had value of 22,6±1,56% (N = 0,3±0,3%) with the increase of 20-30% in comparison with normal value. Deformability of erythrocyte with middle density was equal to 1,102±0,002% (N = 1,099±0,001%); Ucr 237±31 mOsm. Patient was resistant for many types of treatment. After receiving the course of Rituximab 375 mg/m² the deformability of erythrocyte was normalized. Ucr 187±2,3 mOsm. The light part of erythrocyte became near the normal 2,7±0,2% (N = 0,6±0,3%). The density of heavy part of red cells abnormally changed to 4,2±1,7%, however Hb and Rt took normal value. After 7 months the heavy part of erythrocyte had increased by 7,8% without clinical symptoms of hemolyses. After this change the Rituximab was repeated in the same regime. **Conclusions.** Monitoring of density and deformability of erythrocytes could show changes in different periods of AIHA and can be regarded as a prognostic factor of hemolyses to correct treatment.

1045**EFFICACY, SAFETY AND TOLERABILITY OF ANAGRELIDE IN THE TREATMENT FOR THROMBOCYTHEMIA IN CHRONIC MYELOPROLIFERATIVE DISORDERS**

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Background. Thrombocythaemia in chronic myeloproliferative disorders (CMPDs) has an associated risk of thrombotic and haemorrhagic complications, which can be diminished by efficient control of the platelet count. Anagrelide is a cytoreductive drug, which selectively reduces the platelet count. However, the informations about tolerability and efficacy during long-term use of anagrelide are still limited. **AIM:** To evaluate the efficacy, tolerability and safety of anagrelide in patients with chronic myeloproliferative disorders. **Methods.** Twenty nine patients with CMPDs (26 [89,6%] patient with essential thrombocythaemia [ET] and 3 [10,4%] patients with polycythaemia vera [PV]; 25 females, 4 males) with a median age of 59 (range 25-80) were included into the study. Most of them were previously treated with one or two other agents (Hydroxyurea [16 patients; 55%] or Hydroxyurea and Interferon alpha [5 patients; 17,2%]). Eight patient had no other treatment. The median follow-up time was 24 months (range 1-48) **Results.** Anagrelide in an average dose of 2 mg/day reduced platelet count from a mean $815 \times 10^9/L$ to $379 \times 10^9/L$ ($p < 0,001$) in a median time of approximately 4 weeks and maintained this level. Levels of white blood cells and hemoglobin are not significantly decreased. Most often side effects included headaches (34,48%), palpitations (20,7%), ostealgia (13,8%), weakness (24,1%), paresthesias (13,8%), diarrhoea (10,3%), skin itching (10,3%) and others (vertigo, nasal bleedings, hypertension, lumbalgia, exacerbation of circulatory failure, somnolence, gingivorrhoea, oedema, flatulences, abdominal pain, nausea and venous thrombosis). All symptomatic patients (8 patients; 27,6%) had improvement in symptoms attributable to thrombocythaemia. Thrombocythaemia complication during the anagrelide treatment were observed in 5 patients and included: superficial thrombophlebitis (2 patients), myocardial infarction (1 patient), carcinoma basocellulare (1 patient), adenocarcinoma endometrium, atrial fibrillation, oesophageal varices hemorrhagia (1 patient). During the follow-up time 6 patients finished the treatment one because of the transformation to myelofibrosis and death, one because of failure of treatment, three because of no access to the treatment, one of unknown reason. **Conclusions.** Anagrelide is valuable alternative for treatment of thrombocythemia in CMPDs. Minor side effects are common, however, they tend to occur early and resolve spontaneously in most cases.

1046**INFLIXIMAB FOR THE TREATMENT OF REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE IN TWO PAEDIATRIC PATIENTS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION**R. Parasole¹, G. Menna², S. Buffardi², G. Zanotta², V. Poggi², M. Ripaldi²¹Santobono-Pausilipon' Hospital, NAPLES, Italy; ²Santobono-Pausilipon' Hospital, NAPLES, Italy

Background. Acute graft-versus-host disease (aGVHD) is a severe complication of bone marrow transplantation (BMT) and in steroid-refractory grade III-IV GVH is cause of morbidity and high mortality. Recently, new molecules are under investigation to treat severe aGVHD. Infliximab (Remicade) is a chimeric human and murine antibody that inhibits TNF-alpha, a key cytokine in the inflammatory cascade of acute GVHD. **Aims.** We evaluated efficacy and toxicity of infliximab in two paediatric patients with intractable aGVHD grade IV. **Methods.** Two children of median age 10 years, males, who underwent an allogeneic BMT from familial HLA-identical donors (father and sister, respectively), showed a precocious attachment (median day +8) and a rapid appearing of aGVHD grade IV, nevertheless

GVHD prophylaxis. The first patient with chronic myeloid leukaemia (CML) in chronic phase was conditioned by Bu-Cy, while the second with acute promyelocytic leukaemia (APL) in second complete remission with Bu-Cy-L-PAM. Table 1 resume the characteristics of patients. The patients had previously received treatment with 5 mg/kg/day steroid in addition to tacrolimus, mofetil mycophenolate (MMF) and ATG (only pts 1), without any response. The aGVHD started as skin extension that suddenly evolved in severe gut involvement (grade IV) complicated by gastrointestinal bleeding with haemorrhagic shock. The situation required repeated red blood cell and platelets transfusions for maintaining platelet counts $> 50.000/mm^3$ plus repeated infusions of rFVIIa for stopping intestinal haemorrhage. In this phase patients received infliximab at the dosage of 5 mg/kg weekly for three doses. **Results.** All children showed improvement of intestinal aGVHD (from IV to I grade) with disappearing of gut bleeding and decreased transfusion request. Unfortunately, both patients progressed to liver aGVHD, that needed other treatments (extracorporeal photopheresis). The infliximab therapy was well tolerated and no adverse events were reported. No fatal infections occurred during the treatment. One patient (pts 1) died at +3 months from BMT for thrombotic thrombocytopenic purpura (TTP), nevertheless the disappearing of aGVHD. The second patient was alive, disease-free, at + 12 months from BMT with chronic GVHD (skin and liver). **Conclusions.** These results suggest that infliximab is effective to improve severe gut aGVHD but is not sufficient to stop the progression of GVHD. Probably TNF-alpha is only a step of the complex inflammatory cascade in the pathogenesis of GVHD. We suggest that infliximab may be used in association with other treatment in refractory, advanced GVHD after bone marrow transplantation. Besides for its well tolerability, the drug should be used more extensively in paediatric field. Probably a more precocious application of this treatment, in early GVHD grade II or III, should be investigated.

Table 1. Characteristics of Patients

Characteristic/Pts	Pts 1	Pts 2
Age (years)	14	8
Male/Female	M	F
Underlying disease	CML	AML
Conditioning regimen	Bu-Cy	Bu-Cy-L-PAM
GVHD prophylaxis	CSA + MTX	CSA
aGVHD	Int-GI-Liver	Int-GI-Liver
Time of onset GVHD	+1	+8
Total grade aGVHD	IV	IV
Time of start infliximab (days)	+28	+10
DFS (months)	+3*	+12

1048**THE EFFECT OF ACUTE MENTAL STRESS ON IMMUNE SYSTEM**

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Background. A wide variety physical and psychological stressors have profound effects on immune system through to activation of hipotalamo-pituitary axis and sympathetic nervous system. The current understanding of these effects at the cellular and molecular levels, however, is not sufficient. **Aims.** To analysis of the effects of acute mental stress on the immune system **Methods.** Forty healthy subjects who are students in Trakya University Medical Faculty (20 females, 20 males, mean age 22) were included. Blood samples were taken 10 days before the

exam (no exam stress) and immediate before the exam (exam stress). Cell counts were measured by Coulter counter analysis. Lymphocyte subsets were determined; fluorochrome-labeled monoclonal antibodies to CD3, CD4, CD7, CD8, CD16, CD19, CD20, CD22, CD56 (Immunotech, USA) were utilized in two or three-color immuno fluorescence analyses by using a Coulter Epics XL flow cytometer (Beckhman Coulter, USA). The cytokines; interleukin (IL) 4, IL5, interferon (INF)- γ (Diacclone Research, France); and prostaglandin (PG)E2 (R&D Systems, UK) plasma levels were detected by ELISA assay. Paired samples t-test was used for comparisons of the data. **Results.** Mean leukocyte count increased but mean absolute lymphocyte count decreased with the exam stress. Total T cell count slightly decreased with the exam stress, and both of CD4 and CD8 T cells decreased with the exam stress, but decreasing of CD4 T cells were more profound, and thus, CD4/CD8 ratio increased. Natural killer (NK) cell, were characterized with CD3 (-)/CD16 (+)/CD56 (+) or CD16 (+)/CD56 (+) cells, significantly increased with the exam stress. The ratio of B cell markers in the total lymphocyte population did not change with the exam stress. Plasma levels IL4 and IL6, which is showed a preference toward the Th2-type responses, increased and INF- γ , which is showed a preference toward the Th1-type responses, decreased with the exam stress. PGE2 which is a immunosuppressive metabolite of arachidonic acid, is also decreased. **SUMMARY/Conclusions.** Acute mental stress has suppressive effects on adaptive cellular immunity with changing levels of cytokines but it increases innate immunity with augmentation NK cell count

1049**THE CONCENTRATION OF ANTI-ASPARAGINASE ANTIBODIES CORRELATES WITH L-ASPARAGINASE ACTIVITY DURING THE SECOND EXPOSURE ON THE DRUG**

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The enzyme L-asparaginase is an important agent in the treatment of acute lymphoblastic leukemia (ALL). It is a foreign protein for humans; the common adverse effects of its use are allergic reactions, which occur due to production of anti-asparaginase antibodies. However, there is still not clear, if the antibodies may influence the activity of childhood ALL. We examined 48 children, aged 2-18 years, with newly diagnosed ALL. All children were treated according the Polish pediatric Leukemia-Lymphoma Group protocol, based on ALL-BFM 95. There are two series of L-asparaginase in this protocol. In the induction phase of treatment the patients received 8 doses of L-asparaginase (5000 IU/m²); and in the reinduction phase 4 doses (10000 IU/m²), every third day in each phase. Before the administration of the drug, blood samples for measurement of L-asparaginase activity were collected. The enzyme activity was estimated using the spectrophotometric method. On the last day of L-asparaginase therapy in both phases of the treatment, the concentration of anti-asparaginase antibodies in the sera of patients was assessed, using ELISA assay. In the induction phase antibodies in class IgM in 28% and in class IgG in 18% of patients were found. There was no correlation between the presence of anti-asparaginase antibodies and enzyme activity in this phase of treatment. We also did not found the correlation between the presence of anti-asparaginase antibodies in the induction phase and the occurrence of allergic reactions during the second exposure on the drug. In the reinduction phase of treatment we found anti-asparaginase antibodies in 45% of patients (IgM class) and 50% (IgG class). During the second exposure on the enzyme anti-asparaginase antibodies correlated with the level L-asparaginase activity (R -0,07 for IgG antibodies and R -0,52 for IgM antibodies, p=0,003 and p=0,008, respectively). We concluded that anti-asparaginase antibodies influence L-asparaginase activity during the second course of treatment with the drug. There is a significant correlation between anti-asparaginase concentration and L-asparaginase activity during the reinduction phase in children with ALL.

1050**FLAG-MITOXANTRONE AS SALVAGE THERAPY FOR RELAPSED AND REFRACTORY ACUTE MYELOID LEUKEMIA**

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Background. In patients with AML who are refractory to induction of remission or have relapsed in a short period after induction of remission, there is indication for salvage chemotherapy. One of the best regimens introduced recently is FLAG-idarubicin or (mitoxantrone) protocol. **Aims.** We evaluated the efficacy and toxicity profiles of the combination of fludarabine, high-dose cytosine arabinoside, mitoxantrone and granulocyte stimulating factor (G-CSF) as a salvage regimen in those patients with primary refractory or relapsed AML. **Materials and Methods.** During 1 year, 16 patients with AML with a mean age of 31 years (range: 19-52) were treated with FLAG-Mito (fludarabine 30 mg/m², Ara-C 1-2g/m² over 2-3 hr for 5 days, Mitoxantrone 12 mg/m² for 3 days and G-CSF 5 μ g/kg from day +6 until neutrophil recovery). 12 patients were in relapse and 4 patients had refractory disease after conventional chemotherapy including (cytarabine 100 mg/m² and daunorubicin 45 mg/m²). **Results.** Complete remission (CR) was obtained in 8 patients (50%) and partial remission (PR) in 4 (25%). 2 patients died of mucormycosis early after treatment, 1 died of CNS hemorrhage and another of bowel perforation. Median duration for overall survival was 9 months. Recovery of neutrophil and platelet required a median duration of 17, and 20 days, respectively, from the beginning of chemotherapy. Fever >38.5 was observed in 15 patients (93%), mucormycosis in 3 (19%), positive culture for pseudomonas in 2 (13%), pneumonia in 1 (6%) and fever of unknown origin in 9 (56%). After complete remission was achieved, 2 patients received allogeneic stem cell transplantation that are in complete remission after a median follow-up of 15 months. **Conclusions.** Our data are similar to other studies and suggest that the FLAG-mitoxantrone protocol is feasible and can be safely performed in our patients. This regimen has shown high efficacy and acceptable toxicity in patients with relapsed or refractory AML.

1051**CORONARY ARTERY DISEASE AND THE BETA-THALASSEMIA GENE**

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Background. Beta-thalassemia trait is accompanied by hypcholesterolemia, the effects of which, if any, on the development of coronary artery disease (CAD) have not been clearly evaluated. **Aims.** Our purpose was to establish whether beta-thalassemia trait confers any protection against CAD. **Methods.** We studied 638 patients with clinical symptoms and/or electrocardiographic findings suggestive of stable angina pectoris. All patients underwent coronary arteriography and hemoglobin electrophoresis in order to determine the presence of beta-thalassemia trait. Multiple logistic regression was used to assess the effect of the latter and of smoking, hypertension, cholesterol and diabetes mellitus on the development of CAD. **Results.** A significantly higher frequency of beta-thalassemia heterozygosity was detected between patients with normal coronary arteriography, in comparison to those with arteriographically detected coronary stenosis (p<0.007). The presence of the beta-thalassemia trait was found to exert a protective effect against CAD (odds ratio 0.39). At the same time we reconfirmed the importance of the currently established risk factors. **Conclusions.** The lower incidence of CAD in Mediterranean and Asian areas has been widely attributed to local dietary factor. In view of our results, the increased prevalence of the beta-thalassemia gene in the same areas might also play an important role.

1052**GENE FREQUENCIES OF HUMAN PLATELET ANTIGENS IN THE MACEDONIAN POPULATION**

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Background. Human platelet antigen (HPA) systems consist of more than twelve bi-allelic antigen polymorphisms in which a base pair substitution leads to change in an amino acid of a membrane glycoprotein expressed on the platelet surface. Due to these polymorphisms, human platelet-membrane glycoproteins can be recognized as alloantigens or autoantigens and can cause different clinical conditions such as: post-transfusion refractoriness to platelets (PTR); post-transfusion thrombocytopenic purpura (PTTP) and fetomaternal alloimmune thrombocytopenia. PLA or HPA-1 alloantigen is the one most frequently implicated in the syndromes of immune-mediated platelet destruction. HPA typing is important for the diagnosis and treatment of a variety of disease and also for obtaining population genetic data. HPA typing can be performed by serology or PCR based molecular methods. The molecular method of genotyping the platelet antigens are preferred to serology. **Aims.** The purpose of this study was to investigate the distribution of platelet human antigens in Macedonian population. **Material and method:** Gene frequencies for the human platelet antigens HPA-1, -2, -3 and one silent polymorphism in GP Ia (Bgl II) were determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using a DNA isolated from peripheral blood from healthy volunteers. We genotype 216 healthy volunteers for HPA -1 or PLA platelet antigen polymorphism; 84 for HPA -2; 114 healthy volunteers for HPA -3 and 217 for Bgl II. **Results.** Distribution of HPA genotypes in Macedonian population were: a/a=166/216 (76,8%), b/b=46/216 (21,3%) and b/b=4/216 (1,9%) for HPA-1 or PLA; a/a=60/84 (71,4%), a/b=24/84 (28,6) and b/b=0/84 (0%) for HPA-2; a/a=41/114 (36%), a/b=50/114 (44%) and b/b=23/114 (20%) for HPA-3; and for Bgl II (+/+) =16/217 (7,4%), Bgl II (+/-) =107/217 (49,3%) and Bgl II (-/-) =94/217 (43,3%). The allele frequencies were 0.87 for HPA-1a, 0.13 for HPA-1b, 0.857 for HPA-2a, 0.143 for HPA-2b, 0.579 for HPA-3a, 0.421 for HPA-3b, 0.32 for Bgl II (+) and 0.68 for Bgl II (-). **Summary: Results.** of our study were similar to the results from other populations in Europe and were not significantly different from the frequencies reported in the most of the other studies. Our population displayed slightly, but in the most cases, not significantly higher frequencies for the HPA-2b and HPA-3b alleles from those reported in other Caucasian population.

1053**QUANTITATIVE ASSESSMENT OF PRV-1 GENE EXPRESSION IN PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA AND IDIOPATHIC MYELOFIBROSIS-A TOOL FOR DIFFERENTIAL DIAGNOSIS?**

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Background. Essential thrombocythaemia (ET) and idiopathic chronic myelofibrosis (IMF) are Ph⁻, and BCR/ABL negative myeloproliferative disorders (MPD) characterized by an increased production of mature blood cells and induction of the bone marrow fibrosis. Sometimes it is very difficult to establish a proper diagnosis, and in proportion of cases it is impossible to distinguish between ET/IMF, especially when diagnosed in early phases. The differential diagnosis of MPD should include also clinical conditions with secondary thrombocytosis, erythrocytosis, bone marrow fibrosis and/or splenomegaly. The hematopoietic cell surface receptor polycythemia rubra vera 1 (PRV-1) is overexpressed in granulocytes from patients with polycythemia vera (PV), ET and IMF if compared with healthy

controls but not in patients with secondary erythrocytosis, or thrombocytosis. **Aims.** The aim of the study was to analyze the expression of PRV-1 mRNA in patients with ET, IMF compared with healthy controls. The second aim was to check if the level of PRV-1 mRNA expression varies significantly between studied groups of patients and could be the molecular marker useful to distinguish between them. **Methods.** We investigated the level of PRV-1 mRNA expression in 66 unfractionated PB samples from 22 patients (12 with ET and 10 with IMF). The mean age was 48,2 and 55,2 in the ET and IMF group respectively. The male to female ratio was 1,1:1 for ET and 1:1,75 for IMF. ET and IMF diagnosis was established according to the WHO criteria. We used a RealTime-Quantitative-PCR assay based on a specific set of primers and probe (Assays-on-Demand, Gene Expression Products). The values obtained, were normalized using Abelson (ABL) as a control gene and the results were expressed using the $\Delta\Delta Ct$ method as the target and endogenous reference were determined and found to be equal. **Results.** The expression of PRV-1 transcript in normal samples was quite constant (mean value of $2^{-\Delta\Delta Ct} = 1,26$) and PRV-1 mRNA expression in PB from ET and IMF patients was statistically significantly elevated. The mean value of $2^{-\Delta\Delta Ct}$ was 503 (range 0,31-4629) ($p=0,036$) in ET and 250 (range 0,15-2568) ($p=0,055$) in IMF. The differences between PRV-1 mRNA expression in ET and IMF were not significant, however the tendency to higher expression of PRV-1 transcript in ET could be noticed. **Summary/conclusions:** Our results are in accord with other reports and confirm that PRV-1 is a sensitive molecular marker for diagnosis of ET and IMF, useful to distinguish between myeloproliferative disorders and secondary polyclonal disorders characterized by thrombocytosis and/or increased bone marrow fibrosis. The differences in PRV-1 mRNA expression among ET and IMF patients were not statistically significant, but observed tendency of higher expression level of PRV-1 transcript in patients with ET should be further explored and analysed in larger group of patients. Our results show that the quantitative assessment of PRV-1 transcript can be performed using unfractionated PB samples, making the procedure easier and faster when compared to analysis of granulocyte fraction.

1054**SUCCESSFUL TREATMENT OF REFRACTORY TO STEROIDS, AUTOIMMUNE HEMOLYTIC ANEMIA DUE TO WARM IGG AUTOANTIBODY, WITH ANTI CD-20 MONOCLONAL ANTIBODY, IN A YOUNG PATIENT WITH CVID AND HISTORY OF SPLENECTOMY**

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Autoimmune hemolytic anemia due to warm IgG autoantibody may sometimes present with dramatic fall of hematocrit and hemoglobin levels, which may not respond to corticosteroid and/or immunoglobulin therapy. We present the case of a 20 year-old boy, with a three-year history of Common Variable Immunodeficiency. The last two years, he receives monthly infusions of immunoglobulin because of recurrent sinusopulmonary infections. He had been subjected to splenectomy ten years ago because of relapsing episodes of autoimmune thrombocytopenic purpura. The patient was admitted to another hospital because of fatigue, anemia and jaundice. He was diagnosed as suffering of autoimmune hemolytic anemia and he received a four-day course with immunoglobulin (400 mg/kg/d) and prednisone 1 mg/kg/d without signs of improvement. He was then sent to our unit for further investigation and treatment. He was admitted with Hgb 3.9g/dL, Hct 9.7% tachycardia and tachypnoea with very strong positive Direct Coombs (+++) (PV+++), 1:128, IgG+++), 1:32, C3d+++), 1:64, eluate +++), LDH 1830 IU/L, TBil 2.2mg/dL, IndBil 1.7 mg/dL and reticulocyte count 17% (151000 u/L). We increased the dose of prednisone up to 2 mg/kg/d and transfused him, totally, with six units of packed red cells, still without indirect signs of improving hemolytic procedure. Ten days after corticosteroid initiation

and taking into consideration the young age of the patient, we decided to treat him with the monoclonal antibody anti-CD20 at the dose of 375 mg/m²/w. Four days after infusion of monoclonal antibody we noticed that LDH levels, reticulocyte count and hemoglobin levels were gradually improving. We then continued with five more weekly infusions of rituximab. After the last infusion the hemoglobin was 14g/dL with Hct 42.5%, reticulocyte count 1.5% and slightly elevated titles of IgG (+) to the direct antiglobulin test (DAT). Six months after the last infusion the hemoglobin levels were stable with negative DAT but still with very low count of B-lymphocytes owed to the rituximab infusion. To the last follow up, eight months after the last rituximab cycle, the autoimmune process is absent and the B lymphocyte count became normal. What is more, the patient, during this period, continued receiving his monthly infusions of immunoglobulins and he did not experience any serious infection. Despite the lack of a huge number of cases, we may take into consideration the eventual outcome of this case, for selected young patients with autoimmune hemolytic anemia and concomitant CVID as a second line treatment option. Reviewing the literature someone notes the need of further experience of rituximab in similar cases to exact more safe conclusions for its efficacy and also for the degree of therapy toxicity.

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CYTOKINES, LYMPHOCYTE ANTIGENS, AUTOIMMUNE ANTIBODIES AND LUPUS ANTICOAGULANT PROFILES IN ACUTE AND CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA

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Background. Cytokine levels, lymphocyte antigens, autoimmune antibodies and lupus anticoagulant positivity are seen in some immunological based thrombocytopenia. **Aims.** The aim of this study was to evaluate cytokine levels, lymphocyte antigens, autoimmune antibodies and lupus anticoagulant in 70 patients with acute and chronic idiopathic immune thrombocytopenic purpura (ITP). **Methods.** Cytokine levels were measured before and after treatment. Thirty three (47%) of the patients were acute ITP and 37 (53%) of the patients were chronic ITP. Male/Female ratio was 1/3.5 in acute ITP and 1/2.3 in chronic ITP. Median years of the patients was 25 (15-83 years), 30 (18-63 years) in acute and chronic ITP, respectively. **Results.** In initial laboratory, lymphocyte antigens (CD4, CD8 and CD19), autoimmune antibodies (antinuclear antibody and anti-double strand DNA), and lupus anticoagulant were measured. Mean values of cytokine levels in acute ITP pre- and post-treatment consecutively are IL-1: 5.0±0.0 and 5.0±0.0, IL-2: 874±565 and 782±370 (p=0.180), IL-6: 6.2±11.5 and 6.6±7.5 (p=0.276), TNF-alpha: 5.9±3.3 and 5.7±2.5 (p=0.593). These results in patients with chronic ITP are IL-1: 5.0±0.0 and 5.0±0.0, IL-2: 768±370 and 714±236 (p=0.068), IL-6: 4.2±3.9 and 4.3±3.7, TNF-alfa: 7.4±7.7 and 4.7±1.7 (p=0.180). Lymphocyte antigens was found that CD3:46%, CD4:36%, CD8:22%, CD19:20% in acute ITP and CD3:56%, CD4:33%, CD8:27%, CD19:14% in chronic ITP. ANA positivity was found in 30% of acute ITP, Anti-ds DNA was not found in these patients. In patients with chronic ITP, ANA positivity was found in 40% of patients, Anti-ds DNA was found in 1% of patients. Lupus anticoagulant was found in 24% of patients with chronic ITP. **Summary/Conclusions.** We conclude that some immunologic parameters may be changed during period of disease in acute and chronic ITP.

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CONCOMITANT SICKLE CELL ANEMIA WITH GAUCHER DISEASE: A CASE REPORT

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Background. Sickle cell disease is one of the most common blood disorder caused by a unique mutation in the beta-globin gene, resulting in the production of a structurally abnormal hemoglobin called HbS. Gaucher disease is the most common lysosomal storage disease, which is characterized by accumulation of beta-glucocerebrosides in the reticuloendothelial system due to a deficiency activity of the lysosomal enzyme beta-glucocerebrosidase. The most common clinical subtype is the type I (adult chronic non-neuronopathic form). These two disorders are rare and transmitted by an autosomal recessive inheritance pattern. To our knowledge, we here describe the first adult case of Gaucher disease (type I) occurring in a sickle cell patient. **Methods/Results.** The patient is a female known to have a mild anemia because of heterozygosity for sickle cell disease (A/S) and a tendency for iron deficiency. The diagnosis of Gaucher disease was established, at the age of thirty-two-year-old, by bone marrow examination during the investigation of thrombocytopenia without any clinical manifestation. Detection of the characteristic Gaucher cells in the marrow was made and this diagnosis was further confirmed by biochemical demonstration of reduced leukocyte beta-glucocerebrosidase activity (1.9 µkat/kg). Molecular analysis of the patient's genomic DNA revealed identification of the N370S / N370S (1226G / 1226G) genotype. Enzyme replacement therapy with alglucerase (Cerezyme[®]) was started with 60 units/kg body weight every two weeks because of severe bone symptoms. The evidence of inherited Gaucher disease was made: her parents and her sister were subsequently found to be heterozygous for this mutation N370S. **Conclusions.** A wide range of clinical appearance is described in Gaucher disease type I from nearly asymptomatic patients to patients who have severe complications with cytopenia, hepatosplenomegaly and pathologic fractures. Furthermore, the mutation displayed of this patient (N370S) is considered as a mild mutation because it produces a relatively conservative aminoacid substitution, and, indeed the residual enzyme is fairly active. In our case, the patient suffered from thrombocytopenia and bone pain but these manifestations can be caused by sickle cell disease as well as Gaucher disease. These characteristics should explain that the patient has a mild clinical course, and was not diagnosed until she was thirty-two-year-old. These two inherited disorders have never been described in association in the same patient. This case demonstrates that Gaucher disease type I could be easily overlooked and should be considered in any patient presenting with lytic bone disease and thrombocytopenia.

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FLUDARABINE, CYCLOFOSFAMIDE AND MITOXANTRONE (FMC) IN RELAPSED OR REFRACTORY INDOLENT LYMPHOMA

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Background. The indolent non-Hodgkin's lymphoma are usually chemosensitive at diagnosis but become progressively refractory to further therapies. **Aims.** The aim of the study was to evaluate the activity and toxicity of a combination of Fludarabine, Cyclophosphamide and Mitoxantrone (FMC) in patients (pts) with relapsed or refractory indolent lymphoma. **Methods.** Twenty eighth 28 pts received FMC (Fludarabine 25 mg/m², days 1 to 3, Cyclofosfamide 300 mg/m², days 1 to 3 and mitoxantrone 10 mg/m², day 1, delivered every four weeks. 11 (39,28%) females and 17 (60,72%) males (median age 58, rang-

ing from 41 to 71 years) was treated with the FMC salvage regimen. There are 15 pts with LLC/B-lymphocytic lymphoma, 9 with follicular lymphoma and 2 with mantle cell lymphoma. All pts has previously treated with two to five prior regimes (median three); fifteen were pretreated with anthracyclines, and five received purine analogous. *Results.* At diagnosis four pts were in stage I-II and twenty four in stage III-IV, whereas by the time of FMC treatment all pts are in stage III-IV. Only six patients had B symptoms at this time. When starting FMC, no pts were considered low risk according to IPI, eighteen pts were intermediate and ten were high risk. Minimum four cycles were planned in each pts and prophylaxis with Co-trimoxazol was given as recommended. In a total of 88 cycles delivered to the pts (since five pts progressed during therapy). The major toxicity observed was mielosuppression. Seventy-one per cent of pts experienced granulocytopenia and seven pts (25%) needed G-CSF during treatment. Despite growth-factor support, neutropenia (<500 neutrophils/ μ L) was severe in three. Although twenty eighth febrile neutropenic episodes were reported only nine was infectious documented episodes. Anemia (<10g/dL) and thrombocytopenia (<100 000/ μ L) were observed in seven and nine pts, respectively but only four needed erythrocyte transfusions. No pt died during therapy. Of twenty seven pts evaluable for response, 15 pts (53,57%) entered a complete and seven pts (25,9%) a partial remission with a response rate of 79,47%. Another five pts progressed and lately died after failing one or two more chemotherapy regimens. With a median follow-up of 16 months, 11 pts have progressed, with a 24-month failure-free survival of 74,07%. *Conclusions.* The FMC salvage regimen is effective and may allow a high rate of remissions with an acceptable toxicity in heavily pretreated, intermediate and high-risk pts with indolent lymphoma. This regimen should be compared to other, more conventional regimens. Also, whether anti-B cell monoclonal antibodies (e.g., rituximab) add efficacy to FCM should be evaluated.

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FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA AND PLASMA CELL LEUKEMIA

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Fms-like tyrosine kinase 3 (FLT3) is a member of the class III receptor tyrosine kinase family. It is preferentially expressed on hematopoietic progenitor cells and mediates stem cell differentiation and proliferation. FLT3 activating mutations have been detected in acute myeloid leukemia (AML). FLT3 internal tandem duplication (ITD) and Asp835 (D835) point mutation, occur in 20%-30% of AML cases. We analyzed 123 patients with AML for mutations in FLT3 gene and 21/123 (17,1%) were positive for one of two FLT3 gene alterations. We used polymerase chain reaction-restriction length polymorphism (PCR-RFLP) assay for detection of D835 point mutation in exon 20, and PCR method for detection of FLT3 ITDs located from exon 14 to 15. FLT3/ITD mutation was detected in 15/21 patients (71,4%), D835 mutations in 6/21 patients (8,6%) and both type of mutations were detected in 1 patient(4,8%). According to FAB subtypes, FLT3/ITD mutation positive cases comprised M0 4/21 pts, M2 4/21 pts, and M3 4/21 pts. Of 21 patients with FLT3 mutations a normal karyotype was found in 14 patients, 4 patients had t(15; 17), 2 patients had inv (16) and one patient tetraploid karyotype. All patients received standard induction chemotherapy but out of 21 pts with FLT3 mutations, only 4 patients achieved complete remission (one patient with M0 and normal karyotype, one with M5a and also normal karyotype, and 2 patients with M3 and t(15; 17). We also analyzed 12 patients with primary plasma cell leukemia: 7 male and 5 female, median age 67 year (range 40-73 years), with white blood cell count ranging from $6,3 \times 10^9/L$ to $98 \times 10^9/L$ (median $11 \times 10^9/L$), with bone marrow plasmoblastic/plasmocytic infil-

tration in range of 50% to 90% (lymphoplasmocytic morphology in 5 patients, asynchronous in one patient, mature plasmocytic in 3 and plasmoblastic in 3 patients). There were 5 patients with IgG κ , one with IgG λ , 2 with Bence Jones kappa light chains and one with lambda light chains. All patients had a poor outcome with median survival of 6 months. None of the 12 patients were either FLT3/ITD or FLT3/D835 positive. *Conclusions.* In contrast to other reports, incidence of FLT3/ITD and D835 mutations are lower in our AML patients, though the study group is small. FLT3 mutations were equally distributed among M0, M2, M3, M4 and M5 subtypes of AML. The frequency of complete remission in this cohort of patients is low confirming a poor prognosis. FLT3 mutations were not detected in patients with plasma cell leukemia.

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CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA PRESENTING AS MARROW HYPOPLASIA: PROGRESSION OF THE SAME LEUKAEMIC CLONE

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Background. In rare pediatric cases, transient marrow hypoplasia can precede acute lymphoblastic leukaemia. This episode is defined as a 'preleukaemic state' which ineluctably leads a frankly leukaemic phase. We report a case of a child who had repeated episodes of marrow suppression which evolved five months later into an acute lymphoblastic leukaemia. DNA index and molecular techniques showed that the leukaemic clone was present at the initial presentation with aplasia. *Methods/Results.* A twenty month-old boy developed an acute lymphoblastic leukaemia whose initial presentation was characterized by two episodes of pancytopenia following infection. The first episode was spontaneously resolved after transfusion. Bone marrow examination showed fibrosis and no blast was detected. The second episode appeared two months later, bone marrow examination and peripheral blood revealed the presence of atypical cells (respectively 10% and 15% of total cellularity). DNA index of marrow cells found a hypoploid clone (0.94) and a hyperploid one (1.14). Rearrangements of the immunoglobulin heavy chain genes were detected. Cytogenetic study revealed no abnormalities and all other laboratory tests were normal. Because of concomitant severe abdominal infection, G-CSF treatment was given and blood count returned to normal over the next seven days. Finally, three months later, the diagnosis of acute common B lymphoblastic leukaemia was established. The same immunoglobulin heavy chain gene rearrangements and DNA index (0.96 / 1.17) were present and cytogenetic examination found a hyperdiploid clone of 54 chromosomes. *Conclusions.* Diagnosis of leukaemia is based on morphological examination of bone marrow, but when only a few cells is present in a hypocellular marrow, the sensitivity is strongly decreased. Then, detection of monoclonal gene rearrangement and/or abnormal DNA index is suggestive of neoplasia. In our case, we initially considered that the first transient marrow aplasia was induced by infection. The second aplastic episode was characterized by detection of a population of immunoglobulin heavy chain genes and of a hyperploid clone; consequently we conclude that a low level of leukaemic population was probably present. Furthermore, the same results were found at the time of diagnosis and indicate that this leukaemic clone was already present during transient marrow suppression. In literature, molecular detection of a leukaemic clone at the time of hypoplasia has been realised subsequently while our patient had been studied in the phase of apparently idiopathic marrow suppression. In conclusion, patients with marrow hypoplasia in whom a monoclonal rearrangement gene is detected, carried an increased risk for the subsequent development of acute leukaemia.

1060**ONCE WEEKLY EPOETIN BETA EFFECTIVELY MAINTAINS HB LEVELS IN ELDERLY HODGKIN'S LYMPHOMA PATIENTS TREATED WITH A NEW BACOPP SCHEDULE - INTERIM RESULTS OF A MULTICENTER PHASE-II TRIAL OF THE GHSG**

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Background. Therapy related anaemia severely affects quality of life of oncology patients. Moreover, low Hb levels before and during cancer therapy correlates often with a lower response to treatment. In this analysis we present the first results on the efficacy of Epoetin beta in elderly Hodgkin's lymphoma (HL) patients treated with the new BACOPP schedule, a BEACOPP schedule with an increased dose of doxorubicin while omitting etoposide. **Methods.** From December 2003 to February 2005, 41 HL pts with a median age of 66 (range 61-75 years) and newly diagnosed HL in unfavourable clinical stage (CS) I, II and CS III, IV were prospectively recruited to receive 6 to 8 cycles of bleomycin (10 mg/m²), doxorubicin (50 mg/m²), cyclophosphamide (650 mg/m²), vincristine (1.4 mg/m²), procarbazine (100 mg/m²), and prednisone (40 mg/m²) (BACOPP) followed by local radiotherapy to residual lymphoma. 30.000 IE Epoetin beta (NeoRecormon®) once weekly was administered by Hb < 14 g/dL to maintain Hb-levels between 12 and 14 g/dL throughout the therapy. **Results.** Up to day, 23 pts (11 males and 12 females) were evaluable for treatment response. All pts presented with Hb < 14g/dL and hence received Epoetin beta (NeoRecormon®) therapy. Average Hb-levels were 11.2 g/dL (staging), 12.2 g/dL (1st restaging) and 11.7 g/dL (2nd restaging). **Summary/conclusion:** This first interim analysis on the efficacy of 30.000 IE Epoetin beta in elderly patients with HL treated with the new BACOPP schedule showed that once weekly Epoetin beta effectively corrects and maintains Hb levels throughout the therapy. Average Hb levels were at all points maintained above baseline levels and the pts remained transfusion free.

1061**PROGNOSTIC SIGNIFICANCE OF CELLULAR VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN CHRONIC MYELOID LEUKEMIA**

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Background. The chronic myeloid leukemia (CML) progenitor cells have been recently suggested to arise from a hemangioblastic progenitor cell, the malignant progeny of which constitute leukemic blood cells and genotypically clonal endothelial cells. Provided this assumption were correct, the malignant endothelial cells might play role in the increased marrow vascularity in CML with the vascular endothelial growth factor (VEGF) being potentially linked to CML development. **Aims.** In this study we examined cellular VEGF expression in the bone marrow of patients in different phases of CML and assessed its prognostic significance. **Patients and Methods.** Eighty five CML patients (M/F ratio 41/44) were studied (median age 54,5 yr, range: 16-75 yr). Twenty nine patients were in chronic phase CML, 25 in accelerated, and 31 in the blast crisis. We evaluated the expression of VEGF with an immunohistochemical technique utilizing monoclonal antibody to VEGF (c-1, sc-7269, Santa Cruz) and streptavidin-biotin method for visualisation. The expression of VEGF was given as percentage of positive cells per 1000 randomly analysed cells. The values of Hb, white blood cells count, the platelet count, the percentage of blasts and basophils in blood, as well as the clonal evolution of the karyotype were followed up. Duration of each phase of CML was examined. **Results.** The mean expression of VEGF in all patients was 18,65±12,235%; in the chronic phase patients 17,41±9,318%, in the accelerated phase 23,84±11,757%, and

in patients with the blast crisis 15,61±13,930%. There was a significant difference in VEGF expression among patients in different phases of CML (ANOVA test, p=0,033). The VEGF expression correlated with smaller spleen size (p=0,011) and lower percentage of blasts (p=0,018) (Pearson). The patients with a clonal karyotype evolution showed a significantly lower level of VEGF expression (p=0,037, Spearman). VEGF expression is positively correlated also with the duration of chronic phase CML (log-rank test, p=0,0304). **Conclusions.** These data suggest that VEGF is involved in the biology of CML and that VEGF inhibition should be investigated in CML. To obtain more valid results further investigation in a larger cohort of patients are warranted.

1062**ERYTHROID PROGENITOR CELLS FROM PATIENTS WITH DIABETES MELLITUS TYPE II AND NORMOCHROMIC-NORMOCYTIC ANEMIA (PRELIMINARY RESULTS)**

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Aim of this study was the evaluation of the erythroid progenitor cells of patients with Diabetes Mellitus (DM) type II and normochromic-normocytic anemia. **Methods.** Mononuclear cells were isolated from the bone marrow of 8 patients with DM type II (median age 66 years) and normochromic-normocytic anemia (median Hb 9 gr/dL, MCV 92 fl). All probable causes of anemia like iron, vitamin B12, folate deficiency, blood loss, hemolysis or bone marrow infiltration were excluded. Patients were receiving oral antidiabetic treatment with poor glycemic control (median HbA1C 9,1%). All patients' renal function was normal. Mononuclear cells 105/mL were plated in semisolid cultures (Methocult HC 4435) for the growth of progenitor cells. In some cultures erythropoietin (Epo) was also added in an excess dose of 5 U/mL. These cultures were microscopically scored for erythroid colony formation (BFU-E and CFU-E) after 14 days of incubation at 37°C in a fully humidified atmosphere with 5% CO₂. **Results.** Diabetic patients' median BFU-E were 80 BFU-E/105 bone marrow mononuclear cells while CFU-E were 20 CFU-E/105 cells. In the cultures with Epo in excess the median BFU-E cells remained constant (85 BFU-E/105 cells) while a triplicate increase of CFU-E cells was observed (60 CFU-E/105 cells). CFU-E cells are the most mature erythroid progenitors and their proliferation and differentiation is erythropoietin dependent. **Conclusions.** The preliminary results of our study show that erythroid progenitor cells of diabetic patients with anemia increase with Epo addition. As the *in vitro* cultures system reflects the *in vivo* events, we suggest that Epo administration may be useful in diabetic patients with normochromic-normocytic anemia without any obvious cause for this anemia.

1063**HEALTH CARE REFORM IN SERBIA**

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It has been three years since the beginning of the Health Care Reform in Serbia: drug production and distribution, human blood derivatives (new laws 2004-2005). **Methods.** Drugs, blood derivatives and components; Voluntary blood donors; blood and plasma. New drug production and distribution laws (2004) and new blood security laws (2005). Law instruments: haemovigilance and pharmacovigilance systems; traceability. Recommendations and Directives 2000/83CE and 2002 (98)EC implementation. Committees of Transfusion, SOPs, GMP, revision of all clinical indications (blood, derivatives and components)-Gazzetta 2002,2004,2005. **Results.** In 2004, Ministry of Health has formed a Drug Agency in Belgrade responsible for certification after laboratory controls of all drugs. In 2005 Ministry of Health,

European Agency for Reconstruction (EAR) and NBTI are forming Transfusion Security Center for blood derivatives and components with maximum functions of surveillance and alert. Blood Agency will control 47 Transfusion Centers by means of their operations, quality control, surveillance, blood transfer and transparency of reform system on national, regional, and local level. National Center for Plasma Fractionation in Serbia remains under control of both agencies, and will provide plasma fractionation from 8 West Balkan Countries in albumin. Virus inactivation in FFP with SD and MB will be available (new technologies). 50 Standard Operating Procedures (SOPs) for blood collection, processing, testing, clinical transfusion practices and quality are applied (2003-2004). Revision of all indications of good clinical practices has been done. Transfusion Committees are forming in hospitals. Staff education is complete. European Agency for Reconstruction (EAR) has provided equipment (2004-2005). In period 2000 - 2004 there were 1 149 503 voluntary blood donors in Serbia (306 941 in NBTI). Strategy of control (interior and exterior) is applied according to law (TQM). Quality control is independent. Creation of large information system is on the way. *Conclusions.* Technology transfer, application of new Health Care laws and Staff Education will provide higher safety for patients and better life quality.

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RETROSPECTIVE ANALYSIS OF TREATMENT OF PRIMARY PROGRESSIVE AND/OR RELAPSED HODGKIN'S DISEASE IN 1996 - 2004

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Background. Depending on stage and risk factor profile, up to 95% of patients with Hodgkin's disease at first presentation reach complete remission after initial standard treatment. However, 5% to 10% of patients fail to obtain a complete remission and up to 30% of responders usually relapse and require additional therapy. These patients represent a therapeutic challenge considering the poor outcome of most cases. *Aims.* To analyze the treatment of primary progressive and/or relapsed Hodgkin's disease at the Department of Clinical Hematology in Hradec Kralove between January 1996 and September 2004. *Methods.* 128 adult patients with Hodgkin's lymphoma were treated at the Department of Clinical Hematology. The group included 62 males and 66 females with median age 30 years (range, 17-74). Primotherapy of choice was chemotherapy alone (29%), chemotherapy and radiotherapy (69.5%) and radiotherapy alone (1.5%). All patients (n=128) and group of patients with primary progressive disease and/or relapse (n=29) were evaluated retrospectively. The median of the follow up was 41 months (range, 4-107). For survival analysis, Kaplan-Meier method was used, significance was tested using the logrank test. *Results.* 111 patients (87%) achieved complete remission after primotherapy, 12 patients (11%) subsequently relapsed. The failure of primotherapy (primary progressive disease) was observed in 17 patients (13%). 62% patients with primary progressive disease and relapse were treated with high-dose chemotherapy followed by autologous stem cell transplantation. The probabilities of 4-year overall survival and disease free survival are 92% and 86%. The group of patients who achieved complete remission after chemotherapy had significantly longer overall survival (p<0.001). Longer overall survival was observed in patients with relapse vs. primary progressive disease (p=0.002). *Conclusions.* The success of lymphoma therapy depends on accurate staging to determine the patient's individual prognosis and select direct the most effective and least toxic treatment.

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PROGENITOR HAEMOPOIETIC CELLS IN THE PERIPHERAL BLOOD OF THALASSEMIC PATIENTS WITH DESFERIOXAMINE OR DEFERIPRONE CHELATION THERAPY

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Aim of this study was to evaluate the effect of iron chelation therapy with desferioxamine or deferiprone on the peripheral haemopoietic progenitor cells (CFU-G, CFU-GM, CFU-GEMM, BFU-E) of the patients with thalassemia major. We also investigated the possible growth of autonomous erythroid colonies from the peripheral blood of the same patients. *Material-Method:* Sixteen patients with thalassemia major (median age 26 years) were studied. Nine patients (Group A) received chelation therapy with desferioxamine subcutaneously (45 mg/kg/24hr, 5 days/week) while seven patients (Group B) were on deferiprone treatment (75 mg/kg/day per os). All patients were seronegative for hepatitis C. Three patients in group A and four in group B had been splenectomised. Mononuclear cells were isolated from 10 ml peripheral blood of thalassemic patients and of healthy individuals (Group C-control group). 2x10⁵/mL mononuclear cells were plated in semisolid cultures with and without erythropoietin (Epo). In the control cultures we added 100 µl of serum of patients of both groups. These cultures were microscopically scored for colony formation after 14 days of incubation at 37°C in a fully humidified atmosphere with 5% CO₂. *Results.* A statistically significant increase of erythroid progenitor cells (BFU-E) in group A (p=0,01) and group B (p=0,03) in comparison to the control group was observed. A statistically significant increase of CFU-GEMM in group A (p=0,01) in comparison to the control group was observed as well as a significant increase of granulocyte progenitor cells (CFU-G) in group A in comparison to group B and control group. Moreover the growth of CFU-G in cultures of the control group was inhibited under the influence of a patient's serum, who underwent deferiprone treatment. Unexpectedly, BFU-E was grown in cultures without Epo (median BFU-E 30/2x10⁵ cells). *Conclusions.* Thalassaemic patients with desferioxamine or deferiprone chelation therapy have increased erythropoietic activity due to chronic hemolysis. Patients who received deferiprone have less granulocyte progenitor cells, probably due to inhibition of maturation of these cells by deferiprone. Progenitor haemopoietic cells in the peripheral blood of thalassemic patients with desferioxamine or deferiprone chelation therapy.

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SIMULTANEOUS COEXPRESSION MEASURING FOR PRETHERAPUTIC IDENTIFICATION OF DOUBLE- POSITIVE CD33/CD64 AML, IMMUNOPHENOTYPING AND CLINICAL CORRELATIONS

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Background and Objectives: Leukemia cells from patients with acute myeloid leukemia (AML) commonly express certain myeloid-specific antigens such as CD33 and CD64. Considerable efforts and attention have focused on targeting these antigens with monoclonal antibodies (mAb) for therapeutic effects, and an understanding of the activity of the anti-CD33 X anti-CD64 bispecific antibody (BsAb) on myeloid cells has potential clinical significance. *Design and Methods.* Hundred patients with primary AML (70 *de novo* AML, and 30 relapsed AML), diagnosed according to FAB criteria and immunological marker studies, were examined for the dual expression on their blast cells of the CD33/CD64 immunophenotype coexpression by direct immunofluorescence assay using dual staining combination flow cytometry. The immunophenotype of AML was determined by flow cytometry. CD2, CD3, CD5, CD7, CD10, CD11b, CD11c, CD13, CD14, CD15, CD19, CD20, CD33, CD34, HLA-DR, TdT expression were analyzed. *Results.* In

89/100 AML patients analyzed (89%), blast cells coexpressed the CD33 and CD64 antigens. Moreover, in 52 of them, i.e., 52/89 (58% of the 89 positive CD33/CD64 cases) (52% of AML cases), leukemic blasts coexpressed the CD33 and CD64 antigens by a positivity of $\geq 60\%$. There were no significant correlations between the FAB subtype, clinical data (*de novo* or relapsed), or age group and the expression of CD33 and CD64 antigens in AML ($p > 0.05$), however, most (21/32, i.e., 66%) of AML cases with monocytic element (M4 and M5) expressed higher levels of CD33/CD64 ($\geq 60\%$). *Conclusions.* Considerable efforts have been made to identify molecular parameters, such as cytogenetic abnormalities, mutations, or cell surface markers as prognostic factors, in an effort to better stratify AML patients for adequate therapy. Immunophenotyping is not only helpful for diagnosis but is of independent significance for prognosis, and may be useful for risk stratification in AML patients. An understanding of steps involved in the anti-CD33 X anti-CD64 BsAb-induced signaling cascade in myeloid cells is of potential clinical significance. Inhibition of leukemia cell growth initiated by BsAb may have therapeutic value for the treatment of AML, and a direct inhibitory effect of the BsAb on AML cell proliferation and colony formation has been proved. Successful immunotherapy for cancer and, in particular, mAb-based therapy are most likely to succeed first for the hematopoietic neoplasms because of their biology and because of the pharmacology of mAb, and the availability of antibodies reactive with antigens expressed only by hematopoietic cells has provided clinical investigators with new tools for use in developing therapies for AML.

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THE INCIDENCE OF THE THROMBOTIC EVENTS IN THE PRESENCE OF ANTICARDIOLIPIN AND ANTIPHOSPHOLIPID ANTIBODIES

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Anticardiolipin (ACL) and antiphospholipid (APL) antibodies presence, epiphenomena or pathogenic phenomena, requiring prophylactic anticoagulant therapy, is another dilemma of the actual medicine. Studies proving the presence of these antibodies to: 25% of patients with unexplained recurrent deep thromboses of the peripheral veins; 60% of patients with cerebrovascular thrombosis; 37% of patients with transient ischemic attack (TIA); 18% of patients with premature coronary thrombosis; 60% of patients with recurrent fetal loss - patients with Budd-Chiari syndrome, occlusion of mesenteric artery, retinal vascular occlusion. In opposition with the studies proving the absence of the association with thrombophilia in subjects with a transitory presence of ACL and APL antibodies or subjects with ACL, APL antibodies associated to VHC infection, make very difficult to answer to this question. For this reason, we investigated the incidence of the thrombotic events to 100 randomized subjects with ACL antibodies. The diagnostic of these was: Degenerative pathology in 28%; Systemic lupus erythematosus (SLE) in 22%; Malignant diseases in 9%; Thrombocytopenia in 7%; VHC infections in 7%; Autoimmune thyroiditis in 6%; Vasculitis in 5%; Overlap syndrome in 5%; Psoriasis in 3%; Rheumatoid arthritis in 3%; Myelodysplastic syndrome (MDS) in 3%; Sjögren syndrome in 2%. At these 100 subjects with ACL antibodies the presence of thrombotic events was demonstrated in 27%, as follows: deep venous thrombosis in 9%; cerebrovascular thrombosis in 6%; aseptic necrosis of hip in 5%; coronary thrombosis in 3%; fetal loss in 2%; thrombotic thrombocytopenic purpura (TTP) in 2%. We point out that 33% from the subjects with thrombotic events had SLE; in other words, at 41% from the subjects associating SLE-ACL antibodies, the evolution was complicated with thrombotic events. *Conclusions.* there is a high risk for thrombotic accidents in the subjects associating SLE-ACL antibodies. To appreciate the opportunity of prophylactic anticoagulant therapy for the others groups, is required the testing of mark-

ers more specific for the thrombotic risk. Anti beta2-GP-I antibodies, IgG, IgM, IgA nature of these antibodies, complement fixing, ACL antibodies. We point out the aseptic necrosis of hip in association with ACL antibodies.

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ALANYL-GLUTAMINE-DIPEPTIDE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA UNDERGOING INTENSIVE CHEMOTHERAPY, A RANDOMIZED, DOUBLE-BLIND, CONTROLLED STUDY

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Alanyl-glutamine-dipeptide in patients with acute myeloid leukemia undergoing intensive chemotherapy, a randomized, double-blind, controlled study. Rodríguez P, Martínez, T., Cerda G., Cao C. Hematology-Oncology Department, Dipreca Hospital - Santiago de Chile. **OBJECTIVE:** Mucositis is a very common complication undergoing chemotherapy; this favours bacterial translocations from the altered gut leading to sepsis; glutamine is known for its proteoanabolic effects and as an energy source for the enterocytes and the immune system. Glutamine has also stimulating effects on lymphocytes and, as demonstrated, improves the clinical course of patients after bone marrow transplantation and in the critical illness. This study investigated the clinical and immunologic effects of parenteral alanyl-glutamine supplementation in patients with acute leukemia receiving intensive conventional chemotherapy without bone marrow transplantation. *Methods.* A randomized double-blind, controlled study compared the addition of alanyl-glutamine by a peripheral or central vein, (DipeptivenR, Fresenius Kabi, Bad Homburg, Germany) containing 20 g of glutamine in adult patients with acute myeloid leukemia undergoing conventional chemotherapy. Clinical endpoints included the incidence of mucositis and the duration of neutropenia. *Results.* Forty adult patients entered the study and were randomized to receive or not parenteral glutamine: 33 were evaluated; 15 received glutamine and 18 did not. The median duration of neutropenia was 16,4 d. (range 11-21 d) in the glutamine group vs. 19d. (range 10-26) in the control group, ($p = 0.045$); mucositis was graded 0,46 (range 0-2) in the glutamine group vs. 0,83 (range 0-3) in the control group. Gut integrity was suggested with the observation that patients receiving glutamine had septic episodes with Gram (+) bacteria ($n=5$) and Gram (-) bacteria ($n=5$), and patients in the control group had septic episodes with Gram (+) bacteria ($n=1$) and Gram (-) bacteria ($n=7$), Hospital stay was 25 days vs. 23 days, respectively, and the mortality rate was higher (22%) in the control group vs. 13% in the glutamine group. *Conclusions.* In patients with acute myeloid leukemia supplemented with alanyl-glutamine the neutrophils recovery after intensive myelosuppressive chemotherapy was faster; gut integrity was preserved with few episodes of Gram (-) infections, meaning less microbial translocation from gut, this, perhaps was reflected in a fewer mortality rate in the glutamine group (13% vs. 22%).

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THE INCIDENCE AND ETIOPATHOGENESIS OF PANCYTOPENIA IN HIV INFECTION

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The pancytopenia frequency in a cohort of 147 HIV seropositives subjects (39 adults and 108 children) during 10 years was evaluated to 15%, meaning an incidence of 1,5/100 pers/year (18% in adults and 14% in children). Significant differences ($p < 0,001$) was observed, between the frequency of pancytopenia to the subjects having a number of Ly T CD4+ $< 200/\text{mm}^3$ (37.5%), and the frequency to the subjects with a number of Ly T CD4+ $200-400/\text{mm}^3$ (11%). The main identified mechanisms were: 1. HIV infections of the bone marrow CD34+ population. 2. Viral persistence infection of stem cells, and stromal cells

(EBV, PV-B19, CMV, HHV8, HTLV I, II). 3. Disturbance of cytokines and interleukins synthesis and activity. 4. Bone marrow involvement by lymphoma, Kaposi's angiosarcoma and granulomatous disease. 5. Mielotoxic effects of ARV-therapy. The main diagnosed entities were: Pancytopenia by bone marrow aplasia-hypoplasia in 13.63%. Pancytopenia with myelodysplastic changes in 31.82%. Pancytopenia with hemophagocytic syndrome in 13.63%. Pancytopenia by bone marrow involvement in lymphoma and Kaposi's angiosarcoma secondary to infection with opportunistic oncogenic virus (EBV-LMNH, HHV8-Kaposi's angiosarcoma) in 13.63%. Pancytopenia secondary to infections involving the bone marrow e.g. *Mycobacterium avium* - intracellulare, *Mycobacterium tuberculosis*, *Toxoplasma Gondii*, *Histoplasma Capsulatum*, *Cryptococcus*, *Leishmania Donovanii*, bone marrow necrosis (27%). Conclusively, we can sustain the etiopathogenesis' complexity in HIV pancytopenia; this require an appropriate consideration.

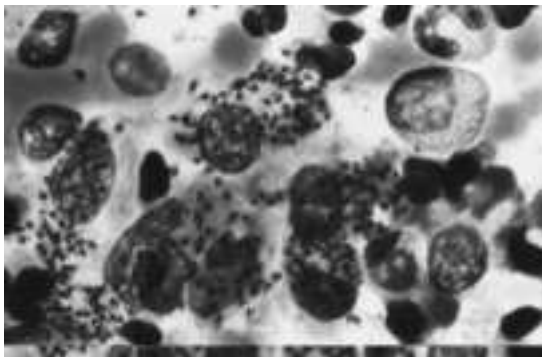


Figure. *Leishmania Donovanii*

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HIGH-DOSE METHOTREXATE KINETICS IN THE MANAGEMENT OF HIGH-GRADE LYMPHOMAS

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Background. In our institute we introduced a special folin-acide rescue, suitable for adult patients. We applied 78 treatments during 10 years. **Aims.** We investigated the kinetics of two different types of high-dose Methotrexate infusion, a three-hour's and a 24 hour's type. We compared three different doses of Methotrexate as well. **Design and Methods.** We applied 24-, 36-, 42-, 48 hours and even more daily sampling as long as the level of Methotrexate lowered below the toxic range. Statistical calculations: We used Anova single factor and correlation analysis. **Results and Conclusions.** 1. We have observed very few non-hematological side-effects in negligible number. 2. In the aspect of hematological side effects and Methotrexate kinetics, the three-hour infusions showed an unambiguous favourable character. 3. All these effects were dose-independent. 4. We find our rescue method suitable for adult patients.

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GROWTH PATTERN AND ENDOCRINE COMPLICATIONS IN BETA-THALASSEMIA MAJOR

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Introduction: Despite regular transfusions and iron chelation, endocrine disturbances are common in β -thalassemia major (THM). We evaluated the growth pattern and endocrine complications among patients with THM. **Patients/Methods.** Twen-

ty-three THM patients (13 females and 10 males, age 7.5-20 years) were evaluated. The patients underwent growth assessment, pubertal status and biochemical evaluation. In six patients GH provocative tests were performed. **Results.** In 70% of the patients, growth retardation and/or delayed puberty were found. Growth velocity decreased from a mean of 5.5 cm/year before puberty to 3 cm/year at puberty. The decline in growth velocity and delay in bone age were more significant in older patients ($P < 0.001$). No pubertal spurt was observed, except in three girls with spontaneous puberty. In most patients the decrease in BMI (SD) and in weight gain began at age 9-10 years ($p < 0.001$). All patients had low IGF-I (SD) levels with 40% values below -2.5 SD. IGF-I levels were lower in the older patients ($P < 0.001$). Low peak GH response was found in all five patients that underwent stimulation test. Height SD values were inverse correlated with pre pubertal (6 to 10 ys) ferritin levels: $r: -0.642 - p < 0.01$ and less significant to pubertal (11 to 20 ys) levels: $r: -0.482 - p < 0.05$. Weight SD values were inverse correlated with pre pubertal (6 to 10 ys) ferritin levels: $r: -0.681 - p < 0.01$ and no correlated to pubertal (11 to 20 ys) levels. All adult patients had hypogonadism except for three females. Hypoparathyroidism and Diabetes Mellitus were diagnosed in one patient. None have hypothyroidism. Five patients (22%) had elevated PTH levels with normal calcium, phosphate and vitamin D. No correlations were found between ferritin and growth parameters. **Conclusions.** A significant percentage of the patients show reduction in longitudinal growth and decreased IGF-I levels as they progress towards puberty. Growth retardation in THM is attributed to several factors: 1) Lack of pubertal growth spurt, 2) Hypogonadotropic hypogonadism, 3) Disturbance in GH-IGF-I axis, 4) Low weight gain during puberty, probably due to hypermetabolic state and undernutrition. Our data confirms the high prevalence of impaired growth and pubertal failure in children with THM. Pre pubertal chelation treatment is crucial to provide normal growth in β TM. The treatment of β TM patients with new oral iron chelators with better compliance may reduce these late complications. Better dietary recommendations may also play a role in growth impairment among thalassemia patients.

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FLUORESCENCE IN SITU HYBRIDIZATION FOR THE DETECTION OF 20Q DELETION AND TRISOMIES 8 AND 9 IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

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Essential thrombocytosis (ET) and polycythemia vera (PV) are clonal myeloproliferative disorders in which chromosomal aberrations are not very common at the time of diagnosis but are increasing with disease progression. With fluorescence in situ hybridization (FISH), interphase cells can be evaluated and clones can be detected even though not revealed by conventional cytogenetic methods. The aim of the present study was to determine by FISH the incidence of trisomy 8, trisomy 9 and 20q deletion in bone marrow samples from ET and PV patients during the course of the disease and to assess their clinical utility. Eighteen samples from 17 ET patients with median age 69 (range 41-86) years and 22 samples from 19 PV patients with median age 67 (range 44-81) years were studied using FISH, utilizing probes for centromere enumeration of chromosomes 8 and 9 and for 20q12 locus. Bone marrow samples from twelve healthy volunteers were used as assay validation controls. The median duration of the disease at the time of analysis was 62 (range 2-137) months. Thirty-five patients were treated with hydroxyurea for a median period of 48 (range 2-125) months. Four patients during the course of their disease received interferon whereas one with ET treated only with aspirin and another with PV with phlebotomy. Three out of 18 ET samples

(16,6%) presented the 20q deletion detected by FISH but none the trisomy 8 or 9. Furthermore, three of the 22 PV samples presented the 20q deletion (13,6%) but none the trisomy 8 or 9. Concurrent conventional marrow cytogenetic karyotype did not reveal the above aberrations. One patient had initially (63 months since disease diagnosis) normal sample but FISH detected a 20q deletion with disease progression 21 months later. There was no correlation between the presence of a marker and age, interval between presentation and FISH analysis, disease progression and therapy. Our experience in concordance with other studies showed that interphase FISH analysis can detect some karyotypic abnormalities that are not detected with conventional cytogenetics. Although there was not any prognostic value of FISH in our cohort of patients, the fact that one patient developed with disease progression a detectable aberration by FISH could be indicative of the need to follow up ET and PV patients with this method.

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EVALUATION OF MEAN CORPUSCULAR VOLUME IN IRON-DEFICIENCY AND COMBINED COBALAMIN-IRON DEFICIENCY ANEMIA

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Background. Simultaneous deficiencies of cobalamin and iron, may be a common cause of combined-deficiency anemia. Megaloblastic anemia is a classical manifestation of vitamin B12 (cobalamin) deficiency. When iron deficiency coexists with cobalamin deficiency, the megaloblastic changes can become compromised. **Aims.** To investigate if patients with iron-deficiency anemia (IDA) are prone to develop cobalamin deficiency and if there is a difference for this manifestation. **Methods.** Ninety-six patients with peripheral blood count, ferritin, serum iron levels, serum vitamin B12 levels determination performed in our hospital were reviewed. Anemia was diagnosed according to the WHO criteria and IDA was considered when iron and serum ferritin levels were inferior to 37mcgr/dL and 59ng/mL in women or to 45 mcgr/mL and 65ngr/mL in men, respectively. We excluded patients with malignant diseases. **Results.** 38 patients had IDA: 30F/8M. A total 58 cases had simultaneously deficiency of cobalamin and iron: 35F/23M. Mean corpuscular volume (MCV) in IDA was 77,2+-9,2fl in women and 79,9+-7,5fl in men. The sensitivity and specificity of MCV <83fl was 100% and 50% respectively. In patients with combined deficiency anemia MCV was 80,2+-12,9fl in women and 81,9+-12,9fl in men. Most of this patients had low MCV, little had macrocytosis and 10% no morphologic evidence to suggested the coexistence of cobalamin deficiency. **Summary.** Simultaneous deficiencies of cobalamin and iron, may be a common cause - 60% in our study, of combined-deficiency anemia. Mean corpuscular volume is a parameter with high sensitivity for diagnosis of IDA, but in patients with coexists cobalamin deficiency, anemia was dimorphic. Therefore, screening for vitamin B12 deficiency should be done routinely in anemic patients.

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INCIDENCE AND PROGNOSTIC VALUE OF FLT3 CONSTITUTIVE ACTIVATION IN MDS PATIENTS

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The FMS-related tyrosine kinase-3 (FLT3) is a receptor tyrosine kinase expressed in early hematopoietic progenitors that plays an important role in hematopoietic development. Multiple studies have shown that activating mutations of FLT3 are common in blasts from patients diagnosed with AML and are strongly associated with poor prognosis. Internal tandem dupli-

cation (ITD) of the JM domain-coding sequence of the FLT3 gene (FLT3/ITD) is found in 20%-30% of patients with AML and in about 3% of those with myelodysplastic syndrome (MDS). FLT3-Asp835 mutations have been reported in 7% of AML and 3% of MDS cases. The most common substitution is Asp835Tyr, but other substitutions, including Asp835Val, Asp835His, Asp835Glu, and Asp835Asn, have also been reported. The aim of this study is to explore the incidence of flt3 mutations in MDS patients and their role in disease progression. Patients and methods. DNA was isolated from the bone marrow of 97 MDS patients at diagnosis and during their disease progression. The WHO classification was: RA: 13, RARS: 2, RAEB: 46, MDS/MPD: 4, CMML: 6, Unclassified: 26 (hypocellular 7). The IPSS was as follows: LOW 18 INT-I 25 INT-II 30 HIGH 24 Two patients have been found to be positive for FLT3 ITD at diagnosis (2,1%). Both patients progressed to AML in 2 and 6 months respectively. Four patients who were ITD negative at diagnosis (4,1%) became ITD positive 4-36 months later at disease progression to AML (total 6 patients-6,2%). Asp835 point mutations were not present in any of the 97 MDS at diagnosis; 2 patients (2,1%) became Asp835 mutation positive at disease progression to AML 24 months after diagnosis. The other MDS patient became Asp 835 positive 8 months after diagnosis and died from refractory cytopenia with no evidence of AML. One MDS patient(1,1%) was double positive at diagnosis for both ITD and Asp835 mutation and he progressed to AML in 3 months. Forty two patients evolved to AML (43,3%) including the 2 patients who originally were ITD positive (both IPSS score High) and the patient who carried both mutations. The IPSS of the 42 patients who evolved to AML was as follows: LOW 6 INT-I 15 INT-II 13 HIGH 8 ITD was found in the bone marrow specimen of 4 patients (9,5%) during transition to AML (3 with IPSS INT-I and 1 with INT-II), Asp835 was found in 1 (2,4%-IPSS INT-I) and 1 patient(2,4%) carried both mutations (IPSS High). In total 6 (14,3%) of the 42 MDS patients who evolved to AML carried ITD, Asp835 or both mutations. **Conclusions.** FLT3 mutations have low prevalence in MDS patients at diagnosis, or at follow up (9,4% of Patients) but they seem to dictate disease evolution to AML. be one of the crucial genetic alterations during evolution to AML especially to those patients who present with intermediate prognostic factors.

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DARBEPOETIN ALFA ADMINISTERED ONCE WEEKLY ALLEVIATES ANAEMIA IN CHILDREN WITH SOLID TUMORS AND LYMPHOPROLIFERATIVE MALIGNANCIES

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Anaemia is a common occurrence in patients with cancer and currently can be treated in several ways. Darbepoetin alfa is an erythropoietin that has been shown, to be safe and clinically active when administered to patients with cancer every 1, 2 or 3 weeks. This study was designed to assess the safety and efficacy of darbepoetin alfa in paediatric patients with solid tumours or lymphoproliferative malignancies. We retrospectively analyzed 32 paediatric patients (median age 7,5 years), 20 with solid tumours and 12 with non-Hodgkin's lymphoma (NHL) and anaemia (Hemoglobin \geq 11,0 g/dL) who were receiving cytotoxic chemotherapy. Children were treated with darbepoetin alfa as a subcutaneous injection once per week at dose 4,5mcg/Kgr for 12 weeks. A haematopoietic response, defined as an increase in hemoglobin level of 2,0g/dL, was achieved in 14 (70%) patients with solid tumours and 8 (66%) with NHL without requiring blood transfusion. Hemoglobin level increased from 8,8 \pm 0,4 to 11,3 \pm 0,6g/dL at the end of treatment. No adverse reaction related to Darbepoetin alfa therapy was observed. Darbepoetin alfa appears to be well tolerated. The results indicate that darbepoetin alfa is effective and safe administered once weekly in children with solid tumours, and lymphoproliferative malignancies.

1076**STEM CELL TRANSPLANTATION IN 281 PATIENTS OVER 60 YEARS WITH ONCO HEMATOLOGIC MALIGNANCIES: A SINGLE INSTITUTION EXPERIENCE**

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The median age of diagnosis of malignancies is usually beyond 60 years but the impact of high dose chemotherapy and stem cell support has only been validated in younger patients assuming that elderly patients could not tolerate intensive regimens. We report here the experience of a single center from 06/1982 to 12/2003. Two hundred and eighty one patients (163 males) received autologous (AuSCT) and /or allogeneic (AlloSCT) transplantations as part of first line treatment (61%) second line treatment (21%) or salvage therapy (17%). There were 140 (50%) multiple myelomas (MM)-including 3 amyloidosis-62 (22%) lymphomas (HL/NHL), 51 (18%) acute myeloid leukemias (AML), 24 (8%) solid tumors (ST)-mostly advanced breast carcinomas- and 4 chronic myeloid leukemias. Two hundred and sixty nine patients -median age: 64 y (60-77) - received one Au SCT (28/269 received a second Au SCT in med of 3 (2-7) months), 1 patient (71 y) received a double syngeneic transplant, 11 patients -median age: 62 (60-68) received AlloSCT. Conditioning (condreg) for the first AuSCT included: Melphalan 140mg/m² (Mel140) n=168; BCNU/Etoposide/AraC/Melphalan (BEAM) n=41; Melphalan 200mg/m² n=29; Mel 140/Busulfan (8 to 16 mg/kg) or Mel 140/TBI 8 grays n=18; Cytosar 200 mg/kg/Melphalan 140 n=9; TBI 8 Grays n=7; BCNU 600mg/m² n=3. Cond reg for the second AuSCT included Mel 200 n=20; Mel 140 n=4; BCNU 600 n=4. Cond reg for the double syngeneic transplant were Mel 140 then Bu 8 Mel 140. Cond reg for the AlloSCT were Fludarabine/Busulfan/ATG (FBS) based reduced intensity regimen in 11 cases. Ninety percent of the patients received peripheral blood stem cells-med 5.3.10⁶ CD34/kg (2-40)- 10% of the patients transplanted before 1994 received bone marrow cells. Tolerance was good with only 10/281 transplant related deaths: 7 early deaths in aplasia (D2-D21), 2 respiratory failures (D30-D80), 1 AGVH (D 60). These deaths occurred in 6/141 patients aged 60-64 years and 4/140 patients older. These patients presented 2 amyloidosis, 4 refractory MM, 3 CR NHL, 1 OR1 MM, These deaths occurred after FBS in 1 pts, BEAM in 3 pts, Mel 140 in 5 pts, Mel 200 (second transplant) in 1 pt. Engraftment (>1.10⁶ neutrophils, 50.10⁶ platelets) was obtained in all cases except one patient with refractory myeloma. With a median follow up of 20 months from transplant, 10/281 second cancers had occurred: 3 AML, 1 ALL, 2 NSC pulmonary carcinoma, 1 colon cancer, 1 uterin carcinoma, 1 melanoma, 1 prostatic cancer within 13 to 144 months. One hundred and eighty patients had relapsed in a median of 12 months (1-92) from transplant. Median overall survival (OS) from transplant for the 281 patients is 37 months. Median OS for MM, NHL/HL, AML or ST are 40 months, 45 months, 18 months and 23 months respectively. When analysis is restricted to the 98 first line MM median OS is 55 months without difference between pts aged 60-64 years or older. However relapse free survival (RFS) was 29 months and 18 months (p=0,01) for patients aged 60-64 years and older patients respectively. These data support the idea that high dose alkylating agents and PBSC support can be incorporated safely in the treatment strategy of elderly patients up to 75 years. The higher relapse rate observed in first line myeloma patients older than 64 years needs prospective studies.

1077**IDENTIFICATION OF NOVEL MARKER GENES FOR B-CELL CHRONIC LYMPHOBLASTIC LEUKAEMIA**

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Background. Chronic lymphocytic leukaemia (CLL) is the most common form of leukaemia in the Western world. It is a malignancy of mature B-cells that have escaped programmed cell death. The clinical course of the disease is heterogeneous where many patients survive for decades without treatment, while others have a rapidly progressive disease with poor response to treatment. Despite several prognostic markers, prognosis in early stage patients is difficult to predict. The heterogeneity of the disease suggests that there are distinct subgroups with different biological features that might need different treatment. **Aims.** In the present study the aim is to identify susceptibility genes for CLL using DNA microarray technology, and to identify different marker genes that distinguish indolent CLL from aggressive CLL. **Methods.** Blood samples were collected from 6 patients with aggressive CLL (Rai III-IV), 6 patients with indolent CLL (Rai 0) and 6 age-matched healthy controls for conventional cytogenetic studies, immunofenotyping by flow cytometry, and B-cell isolation by density gradient centrifugation and immunomagnetic cell separation. RNA was isolated from the B-cells, then biotin-labelled cRNA was synthesized and hybridized to DNA microarrays (Human Genome U133A; Affymetrix). This gene chip covers more than 20 000 human genes. Genes were clustered and selected using Genespring 5.0. RNA transcribed to cDNA was also used for amplification of IGVH-genes with an IGH gene clonality assay (InVivoScribe Technologies). **Results.** The diagnosis of the patients was confirmed by immunophenotyping. Follow up time since diagnosis was at least 18 months (mean 80, range 18-154 months). Chromosome analysis revealed trisomy 12 in one patient with indolent disease and one patient with aggressive disease. In addition, one patient in the aggressive group had a translocation involving 11q. Sequencing of the IGVH-genes of the tumors showed that all tumours except one in the aggressive group had undergone somatic hypermutation. 2656 of the analysed clones differed in expression between CLL cells and peripheral B-cells. Among these genes Bcl-2, PKCbeta1 and LYN were upregulated, and immunoglobulin genes, CD22, CD49d and CD38 were decreased in CLL cells compared to peripheral blood B-cells. 753 clones were differently expressed between indolent and aggressive CLL. Several genes involved in cell migration and metastasis, and two putative tumor suppressor genes were upregulated in indolent disease. The expression of several of these gene products is currently studied in a larger group of CLL patients and compared to clinical features and IGVH mutational status.

1078**ZOLEDRONIC ACID PROLONGS SURVIVAL IN THE INA-6 SCID MOUSE MODEL AND BLOCKS PROTEIN PRENYLATION IN PLASMACYTOMAS**

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Background. Nitrogen-containing bisphosphonates such as zoledronic acid (ZOL) are effective at preventing osteolytic bone disease in patients with multiple myeloma (MM). ZOL inhibits bone resorption by inhibiting FPP synthase and preventing the prenylation of small GTPases. In vitro studies have demonstrated previously that ZOL can also directly affect the growth and viability of myeloma cells and several other malignancies by inhibiting protein prenylation and therefore could exert direct anti-tumour effects in addition to osteoclast inhibition. **Methods and Results.** To explore this in more detail, the effect of

ZOL was investigated on 11 human malignant B-cell lines (six MM, four body-cave based lymphomas [BCBL], one Burkitt's lymphoma). ZOL caused concentration-dependent growth inhibition in all MM and BCBL cell lines, including the IL-6 dependent plasma cell line INA-6. Sensitivity ranged from 20 to 285 μM (IC50 values). The potential anti-tumour effect of ZOL on INA-6 cells *in vivo* was studied in a SCID mouse xenograft model, in which mice injected intraperitoneally with INA-6 cells develop plasmacytomas. In several experiments involving more than 50 mice, ZOL was administered subcutaneously (sc) or intravenously (iv). ZOL, at a dose of 8 μg or 2 μg three times per week for two weeks after inoculation of INA-6 cells, significantly reduced tumour burden and increased survival of the mice ($p = 0.002$). The effect of ZOL on protein prenylation in plasmacytomas dissected from the mice was measured by western blotting to specifically detect the unprenylated form of the small GTPase Rap1A. Unprenylated Rap1A was virtually absent from tumour samples of untreated animals, while a single iv injection of 8 μg ZOL induced a marked accumulation of unprenylated Rap1A in the tumours after 24-72 hours. *Conclusions.* These studies are the first to demonstrate that a single injection of ZOL can inhibit protein prenylation in plasmacytomas *in vivo*. Given the decreased tumour burden of mice and the better survival, these data suggest that ZOL may inhibit plasma cell tumour growth by inhibiting protein prenylation and hence interfering with intracellular signalling. Thus, ZOL may have therapeutic potential at least in MM beyond the treatment of osteolytic lesions.

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EVALUATING BIOSIMILARS: A COMPARISON OF BIOSIMILAR EPOETINS

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Background. Patents of innovator biopharmaceutical products, such as epoetin, are starting to expire potentially allowing biosimilar versions of these products to enter European and American markets. Copies of these products, termed biosimilars or follow-on biologics, are not truly equivalent and cannot gain market approval through the procedure typically applied to low-molecular weight generic drugs. *Aims.* This study compared 8 biosimilar epoetin products currently marketed outside the USA and Europe to epoetin alfa and evaluated the quality and rigor of clinical studies performed with these products in light of recently implemented European Agency for the Evaluation of Medicinal Products (EMA) guidelines. *Methods.* Epoetin preparations marketed outside western markets were analyzed by high performance liquid chromatography, western blot, radioimmunoassay, *in vitro* and *in vivo* bioassays, and isoelectric focusing. Clinical studies conducted by these manufacturers were identified by literature searches in the USA, Asia, Europe, and Africa. The studies were assessed according to target population, study scope and design, and quality and rigor. *Results.* Products differed widely in composition, did not always meet self-declared specifications, and exhibited batch-to-batch variation. Total protein content was not in compliance for 5 samples. Potency, as measured by *in vitro* bioassays, exceeded specifications for 3 samples (136-192%). *In vivo* bioactivity exceeded specifications for 3 samples (137-226%) and was below specifications for 2 preparations (71-75%). Isoform deviations identified included additional basic isoforms and some products exhibited batch-to-batch variation. Products were within the specifications except where noted with some testing comparable to epoetin alfa. Most of the studies were small (median 41 patients, range 18 to 1079) and of short duration (median 12 weeks, range 6 weeks to 1 year). The clinical studies were conducted mainly with chronic kidney disease patients, 2 were with premature infants, 1 was with pregnant HIV+ women, 1 was with HIV+ children, and 2 were with cancer patients. Although several clinical studies demonstrated correction of anaemia with biosimilar epoetins using open-label or placebo-controlled studies, only 4 of 22 studies were competitor con-

trolled. Clinical experience with epoetin shows that the dose required to achieve similar haemoglobin levels varies between patients, making it impossible to demonstrate bioequivalence without a comparator. *Summary/conclusions:* The epoetins analyzed were variable and often mislabeled, which could cause under or overdosing of patients and undesired clinical effects. Although some products may be interchanged if haemoglobin values are closely monitored, they are not interchangeable on a dose-by-dose basis and safety profiles remain a major concern. The clinical studies evaluated were not rigorous enough to demonstrate safety and efficacy of a biopharmaceutical product.

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RESISTANCE TO IMATINIB: WT-1 AS A POSSIBLE MARKER FOR THE EVALUATION OF UPFRONT RESISTANCE, ROLE OF KINASES OTHER THAN BCR-ABL IN SECONDARY RESISTANCE

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Background. Wilms tumor suppressor gene (WT1) is overexpressed in a variety of hematologic malignancies and solid tumors as well. It is a possible prognostic marker of chronic myeloid leukemia (CML). WT1 expression at the mRNA level can serve as a disease progression monitoring tool as well as act as an indicator of hematologic relapse versus response to treatment. Preliminary results show that the expression of WT1 is influenced by therapeutics such as imatinib, and the treatment leads to the decrease of WT1 levels. However, there is evidence of patients with an upfront resistance to imatinib, who show no response to the treatment. During treatment a secondary resistance caused by mutations in the ATP-binding site, BCR-ABL amplification, or involving the Src family kinases who take over the role of BCR-ABL in the cell pathogenesis can also occur. *Methods.* In our study an *in vitro* short term cultivation (24 - 48 hours, 0,1 - 20 μM imatinib) was used to evaluate the possible upfront resistance. Cell conditions were further characterized by monitoring cell vitality, proliferation (expression of the proliferative marker Ki-67), apoptosis (activity of the apoptotic marker caspase-3) and expression of BCR-ABL and WT1 genes. Gene expression was measured by quantitative real-time RT-PCR. Peripheral blood cells of the BCR-ABL positive CML patients before imatinib therapy ($n > 30$), BCR-ABL positive (imatinib sensitive) human leukemia cell line K-562, BCR-ABL negative (imatinib resistant) human leukemia cell line ML-2 and BCR-ABL negative peripheral blood cells of healthy donors ($n = 5$) and patients with acute myeloid leukemia ($n = 5$) were used. *Results.* *Results.* of our study show that imatinib induction led to cell vitality deterioration, increased activity of caspase-3 and Ki-67, BCR-ABL, and WT1 down-regulation in BCR-ABL positive cells (K562 cell line and most of the CML patients) in contrast to the BCR-ABL negative cells. This phenomenon was dose dependent, 1 μM imatinib was chosen for comparison. In responding patients the expression of WT1 did not exceed 40% of the expression in non-affected control cells. Peripheral blood cells of the CML patients, whose cells did not respond to the *in vitro* imatinib induction or who became resistant during the treatment, were further incubated *in vitro* with Src and Jak family kinases inhibitors. This incubation resulted in cell vitality and proliferation deterioration as well as WT1 down-regulation. *Conclusions.* Our results show a possible use of WT1 expression monitoring to evaluate the upfront imatinib resistance. The results also suggest that in case of imatinib resistant patients some other kinases could at least partly take over the role of BCR-ABL in the cell pathogenesis. Further monitoring of the involved patients is necessary to determine the usefulness of this approach for clinical practice. This study was supported by grant IGA NC/7560-3 MZCR and IGA NC/3721 MZCR

1081**CLONAL INSTABILITY IN A CASE OF ACUTE BIPHENOTYPIC LEUKEMIA WITH TRISOMY TEN**

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Trisomy 10 in acute leukemia is a rare numeric chromosomal abnormality. The majority of patients reported had atypical AML with CD 7 antigen expression. A 56 year old male patient was admitted with tiredness, jaundice and flu-like symptoms. The only relevant disease in his past medical history was a virus hepatitis type A after a holiday 7 years ago. An acute biphenotypic leukemia without a sign of MDS prephase with trisomy 10 as a sole cytogenetic abnormality was diagnosed. Full blood count at admission showed a white blood cell count of $6,2 \times 10^9$ /dL with 69% blasts, hemoglobin 10,5 g/dL, reticulocytes 60×10^9 /dL and platelets 141×10^9 /dL. Bone marrow showed an infiltration with immature blasts without any granulation, AML-M0/1 (70%). By immunophenotyping CD 34 positive blasts co-expressed CD 2, CD 7, TdT and myeloid surface antigens (CD 33, CD 11b). MPO and CD 56 were negative. TCR gamma PCR for clonal rearrangement was negative. The investigation of the cerebrospinal fluid revealed a meningeosis leucaemica without clinical symptoms. Following an ALL induction regimen (GM-ALL 6/99) including cerebral irradiation the patient achieved a partial remission. Only a small part of the blasts now co-expressed myeloid and lymphatic markers together, the major part showed myeloid differentiation only. Subsequently, AML induction treatment led to a complete morphological and cytogenetic remission. Despite continuous AML maintenance therapy, relapse with the original biphenotypic blasts occurred four months later. By cytogenetic analysis a deletion of the long arm of chromosome 7 [del (7) (q22)] and of the short arm of chromosome 1 [del (1) (p32)] in addition to the trisomy 10 were detected. An AML reinduction-therapy induced an aplastic bone marrow followed by a partial remission with another shift of the immunophenotype (CD 13, CD 33, CD 34 and CD 117 pos., CD 7 neg). The persisting blasts lacked trisomy 10 and del (1) (p32) in chromosomal analysis but the deletion of the long arm of chromosome 7 [del (7) (q22)] persisted. As shown by the course of this unusual case, a clonal instability may result in clonal changes together with a gain of abnormal surface markers at relapse.

1082**AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR DIFFUSE LARGE B CELL LYMPHOMA - A SINGLE CENTRE EXPERIENCE**

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Background. ASCT is an established treatment for chemotherapy sensitive relapsed or refractory diffuse large B cell lymphoma (DLBCL). We have evaluated outcome of 41 patients (pts) with DLBCL including 17 pts with primary mediastinal B cell lymphoma (PMBL) who underwent high dose therapy (HDT) and ASCT in first remission or relapsing disease between 11/1997 and 11/2004. **Methods.** Pts characteristics: median age was 47 (18-64). 23 pts (57,5%) presented with clinical stage III/IV, 23 pts (57,5%) had IPI score ≥ 2 and 28 pts (71,8%) had bulky disease. 36 pts (87,8%) received CHOP as a first-line treatment, whereas 5 pts (12%) with PMBL and unfavorable prognostic factors were treated with more intense regimens. 35 pts (85,4%) responded to the first-line chemotherapy with 17 pts (41,5%) achieving CR and 18 pts (43,9%) PR. Among responding pts 17 (42,5%) relapsed and had second and third-line chemotherapy leading to complete or partial remission. The median number of chemotherapy regimens administered prior to transplantation was 2 (range: 1-4). 35 pts (85,4%) were transplanted in

chemosensitive disease status (17 pts in CR, 18 pts in PR), among them 16 pts (39%) in first remission. 6 pts were transplanted in refractory disease. The following prognostic and treatment-related factors influencing overall survival (OS) and progression free survival (PFS) were analysed by Cox proportional hazard regression model: CS, IPI, bulky disease, response to the first-line treatment, disease status at transplantation, monoclonal antibody therapy. **Results.** The median follow-up of pts who are still alive at the time of analysis is 51,3 months (range 3-90). Probability of survival at 5 years (yrs) for the entire group is 44,1, 95% C.I. [26,5; 61,7]. Probability of time to progression (TTP) at 5 yrs is 42,2, 95% C.I. [24,9; 59,5]. Death risk for pts with primary refractory disease was 18 times higher than for pts responding to the first-line chemotherapy. Significant difference ($p < 0,001$) in OS between these two groups of pts was found. The median survival for pts with chemosensitive disease had not been reached at the time of this analysis, whereas pts with primary refractory disease had a median survival of 5,45 months. Pts transplanted without achieving remission had 29 times higher risk of post-transplantation disease progression ($p < 0,001$). The median PFS for refractory pts was 2,69 months, for other pts-18,76 months. The median PFS for pts transplanted in first remission had not been reached at the time of this analysis, for pts with one or more relapses before ASCT the median PFS was 9,72 months ($p = 0,074$). There was no significant difference for the median OS between these two groups of pts. **Conclusions.** The outcome of ASCT in pts with chemosensitive disease is good-probability of survival at 5 yrs for these pts is 52,7%. Primary refractory aggressive lymphoma remains an important clinical problem-salvage chemotherapy followed by HDT and ASCT failed to induce lasting remissions. ASCT carried out in first remission leads to longer PFS but has no impact on OS.

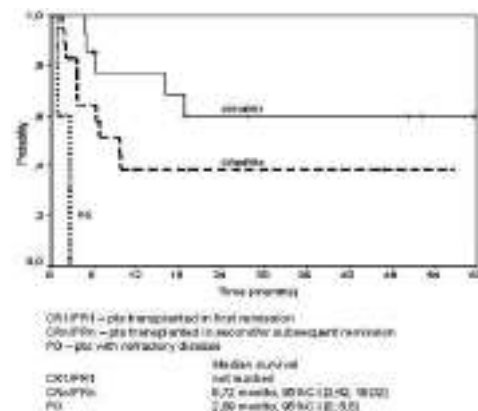


Figure. Progression free survival depending on disease status at transplantation.

1083**TREATMENT WITH RFXVIA IN A TODDLER WITH NEUROBLASTOMA, DIC AND LIFE-THREATENING BLEEDING**

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Haemorrhagic disseminated intravascular coagulation (DIC) associated with the presence of underlying advanced or

metastatic tumors is often difficult to control. We describe a sixteen-month old boy with metastatic bilateral neuroblastoma and evidence of DIC at the time of diagnosis. The boy presented with pallor and abdominal distension due to a large palpable mass. Imaging studies revealed an unresectable extending bilaterally tumor with lymph node involvement. MIBG scan was negative, but ⁹⁹Tc bone scan positive. Bone marrow was infiltrated by metastatic cells on diagnosis. Fine-needle biopsy confirmed the diagnosis of neuroblastoma and the boy started chemotherapy with the Rapid-COJEC protocol (vincristine, carboplatin, etoposide, dexamethasone). DIC was documented clinically (petechiae) and laboratorywise (thrombocytopenia, increased INR and D-dimers levels and decreased fibrinogen levels) and was initially treated with RBC, PLT and FFP transfusions. Worsening of the patient's condition (echymoses, bleeding tendency) lead to the initiation of daily administration of antithrombin III and cryoprecipitants, as well as blood product transfusions. The patient did not respond to the above DIC treatment, despite concurrent treatment of the underlying malignancy, over a ten-day period. Life-threatening bleeding with severe thrombocytopenia ($<20 \times 10^9/\text{ul}$), extremely elevated D-dimers levels ($>10000 \text{ ng/mL}$) and very low fibrinogen levels ($<50 \text{ mg/dL}$) continued, so introduction of rFVIIa at a dose of $60 \mu\text{g/kg/d}$ as well as administration of fibrinogen (haemocomplettan) at a dose of 100 mg/kg/day was decided. The patient responded with cessation of bleeding and improvement of all coagulation parameters over a 48 hour-period. Treatment with fibrinogen continued two days and rFVIIa for seven days. Prothrombin time, activated thromboplastin time and fibrinogen levels normalized within 48h of therapy. D-dimer levels normalized only ten days later. Our patient completed the chemotherapy protocol successfully and no further complications were observed. Recent data from the literature provides evidence that rFVIIa can be used safely to control haemorrhagic episodes associated with DIC, with close monitoring of patients. This has been our impression, too, with our patient.

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PROGNOSTIC VALUE OF SERUM CONCENTRATIONS OF INTERLEUKIN-10 AND SOLUBLE INTERLEUKIN-2 RECEPTOR IN PATIENTS WITH HODGKIN DISEASE

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Background. Although Hodgkin Disease (MH) represents subject of investigation for almost two centuries, till nowadays some aspects are still unknown. Latest researches shows that immunosuppression based on the domination of Th-2 (IL-10, IL-4) cytokine activity over the Th-1 (IL-2, IFN γ) cytokines might have important role in the pathogenesis of MH. Some biologically active substances, such as interleukin-10 (IL-10) and soluble interleukin-2 receptor (sIL-2R) decreases cells antitumor activity, leading to disease progression. **Aims.** To measure serum concentrations of IL-10 and sIL-2R in patients (pts) with MH and than their values correlate to clinical, biohumoral and histological prognostic parameters, i.e. course and outcome of disease. **Methods.** We have measured IL-10 and sIL-2R concentrations with commercial ELISA tests in 35 untreated MH pts and 20 healthy persons. **Results.** Average concentrations of IL-10 and sIL-2R were significantly higher in the pts group ($7,39 \pm 5,44 \text{ pg/mL}$; $1,14 \pm 1,05 \text{ ng/mL}$, respectively) comparing to control group ($2,52 \pm 0,38 \text{ pg/mL}$; $0,18 \pm 0,14 \text{ ng/mL}$). Also, IL-10 and sIL-2R values were significantly higher in pts with an advanced clinical stages, presence of 'bulky' disease and B symptoms ($p < 0,001$). Comparing with appropriate group of pts with favorable prognosis IL-10 and sIL-2R values positively correlates with the sedimentation ($r=0,55$; $r=0,88$, $p,0,001$), CRP ($r=0,58$; $r=0,75$, $p,0,001$) and negative linear correlation with the hemoglobin value ($r=-0,66$; $r=-0,68$, $p,0,001$), albumins ($r=-0,45$; $r=-0,75$, $p,0,001$) and lymphocyte count ($r=-0,45$; $r=-0,43$, $p,0,05$). We haven't found correlation of IL-10 and sIL-2R concentrations with the LDH level and histology type of MH. Pts with high concentrations of IL-10 and sIL-2R had significantly lower per-

centage of remissions and higher frequency of relapses than those with lower values of tested cytokines. **Conclusions.** Besides well known clinical and biohumoral parameters, serum concentrations of IL-10 and sIL-2R could predict MH activity. These cytokines might be used for the selection of high risk pts that needs aggressive initial therapy and also in the precise monitoring of the remission.

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THE COMBINATION USE OF ⁹⁹MTC-MIBI SCINTIGRAPHY, SERUM BETA-2-MICROGLOBULIN AND SERUM THYMIDINE KINASE IN THE EVALUATION OF PATIENTS WITH HODGKIN'S DISEASE

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Background. In patients with Hodgkin's disease exact diagnosis and staging is important for the treatment and the relapse identification of these patients. ⁹⁹mTc-MIBI is currently used to study myocardial perfusion, but also been reported to be localized in various types of malignant tumours, including lymphomas. With previously used visualized techniques it has been extremely difficult to differentiate active tumour tissue from posttherapy fibrosis. Serum beta-2-microglobulin and serum thymidine kinase levels have recently been used to detect tumour regression and progression in malignant lymphomas. The aim of the study was to assess the clinical value of ⁹⁹mTc-MIBI scintigraphy, serum beta-2-microglobulin and serum thymidine kinase and the correlation between them for diagnosis, staging and therapy control of patients with Hodgkin's disease. **Material and Methods.** Eighty two patients (44 male, 38 female, median age 43 years) with Hodgkin's disease underwent ⁹⁹mTc-MIBI scintigraphy. The scintigraphic results were compared with the data of conventional methods (clinical examination, x-ray, CT, sonography). Beta-2-microglobulin levels were measured by radioimmunoassay using the Immunotech international microtest. Thymidine kinase in serum was determined using Prolifigen radioenzyme assay (Sangtec, Sweden). **Results.** ⁹⁹mTc-MIBI scintigraphy was positive in 64 patients. From 93 detected lesions, 55 were with lymph node involvement in mediastinal, neck, supraclavicular, axillar and inguinal area. Extranodal localization was found in 38 lesions. Lesions, identified in the lung as a primary tumour were 18. The number of residual masses after treatment that were correctly identified with ⁹⁹mTc MIBI was 11. ⁹⁹mTc MIBI scintigraphy showed also an extent of tumour in primary thyroid lymphoma in 3 patients. In 4 patients in whom a medullary biopsy showed lymphomatous infiltration, ⁹⁹mTc MIBI scintigraphy showed abnormally increased uptake. Bone lesions were identified on ⁹⁹mTc-MIBI scintigraphy only in 2 patients. The mean tumour-to-background ratio calculated 20 minutes after tracer injection was 1,5. Patients with advanced clinical stages are those who display the highest serum levels of the tumour markers. Significant correlation was found in patients with true positive ⁹⁹mTc-MIBI scintigraphy and higher serum beta-2-microglobulin and thymidine kinase levels in patients with advanced disease. Fifteen patients had negative ⁹⁹mTc-MIBI scan after chemotherapy. In these patients, the levels of the serum beta-2-microglobulin and serum thymidine kinase were near to the normal levels. In 3 patients with false negative scans, the results were compared with the data of other visualization methods. Positive clinical findings and increased value of serum beta-2-microglobulin and serum thymidine kinase were found in these patients. Negative ⁹⁹mTc MIBI scintigraphy could be used as a good prognostic factor in these patients to determine primary drug resistance. **Conclusions.** ⁹⁹mTc-MIBI is a promising tracer in determining disease activity, site of active lesions and the follow up of the patients with Hodgkin's disease. The uptake score correlated significantly with clinical status and stage of disease and with disease activity as determined by beta-2-microglobulin and serum thymidine kinase.

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GROWTH AND PUBERTY DISORDERS IN CHILDREN AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION - A SINGLE CENTRE LONGITUDINAL STUDY

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Background. High dose chemotherapy and cranial irradiation are important risk factors for late endocrine complications after HSCT. **Aims.** The aim of this study is to evaluate late endocrine deficiencies (focusing growth and puberty disorders) in children treated with high dose chemotherapy (HDCT) or/and cranial irradiation observed after haematopoietic stem cell transplantation (HSCT). **Methods.** 27 pts (17 girls and 10 boys) age 2-20 years (average 11,8 years) underwent HSCT between 1995-2003. Autologous transplantation was performed in N=7, allogeneic grafting with match sibling donor N=10, match unrelated donor (MUD) N=6 and HLA-mismatch related donor N=4. Indications for HSCT were AML (N=12), ALL (N=5), CML (N=4), MDS (N=2), NHL (N=2), JMML (N=1) and SAA (N=1). Cranial irradiation (CI) prior to HSCT with 18 Gy [N=10] or 24 Gy [N=4] was performed in 14 children. The preparative regimens consisted of HDCT usually BU/MEL (N=6), CY/BU/VP-16 (N=6) and total body irradiation (TBI) 12 Gy in 2 patients. Prolonged high steroid doses (at least 28 days) received 14 children. All patients were divided according to their stature in subgroups: Short (<10 percentile) and abnormal growth velocity (decrease at least 1 SD). Evaluation of the endocrine functions following HSC was performed in average 31,59 +/-20,54 months after transplantation. **Results.** Three independent variables (below) were evaluated using Multiple Regression Analysis and of the dependent variables determining abnormal growth velocity (decrease at least 1 SD in centile position), which proved statistically significant $p=0,046$ negative dependence ($R^2=0,55$): 1. Cranial irradiation (CI) 2. Busulphan (BU) 3. Cyclophosphamide (CY) The influence of CI on growth was more significant than high-dose-chemotherapy (piecemeal correlations of CI=0,54; of CY=0,30; of BU significantly lower). No effect of given CI dose was observed. There was a positive, but statistically not significant, correlation between of above variables and growth deficiency after therapy. Analysing the influence of evaluated variables (CI, CY, BU) on abnormal gonadal function (late onset puberty) or BMI there was any significant dependence shown in the investigated group. **Conclusions.** This short time observation on endocrine disorders in children after HSCT was not sufficient to reveal the deficiency of the stature. There was, however, statistically significant decrease of growth velocity. Our study does not confirm the influence of busulphan consisting preparative regimen on growth's disorder, as reported by others. Increased number of patients in investigated group is necessary to achieve more accurate assessment of dose effect and to enumerate a threshold value of CI. Decreased growth velocity in children after HSCT is potentially useful for prognosis of terminal height with special focus on the catch-up effect.

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KARYOTYPIC CHANGE ADDITIONAL TO THE PH' CHROMOSOME AND THEIR PROGNOSTIC RELEVANCE IN CHRONIC MYELOID LEUKEMIAA.M. Liberati¹, A. Bassetti¹, M. Gubbiotti¹, P. Ferrara¹, M. Merluzzi¹, V. Capparella², E. Gentileschi², C. Ardisia¹, G. Venti¹, E. Donti¹¹Internal Medicine and Oncology Science, PERUGIA, Italy; ²Oncologia Ospedale di Rieti, RIETI, Italy

Background. Karyotypic abnormalities additional to the Ph' translocation have been described at the time of blastic phase (BP) in the 75-80% of patients with chronic myeloid leukemia (CML). To evaluate whether different chromosome changes are associated with specific clinical evolutions and even whether the time of their appearance could influence the prognosis, a

cytogenetic study was performed on 108 CML pts in chronic phase at diagnosis and during the course of the disease. Patients and method: twenty-five metaphases were analysed in each examination; a clone was identified according to ISCN recommendation. **Results.** at diagnosis, 12 pts showed one or more additional karyotypic abnormalities. Ph'-variant or additional translocations were detected in 3 and 2 pts respectively while in one case both types of translocations were observed. The other cytogenetic change were: -Y (4 pts), +8 (2 pts), i (17 q) (1 pt), i (Ph') (2 pts) and 19p- (1 pt). A complete karyotypic remission (CKR) in response to STI (1 pt), to IFN α (3 pts) or to combined IFN α -STI (2 pts) therapy was documented. In one pt relapsing, after IFN α discontinuation, a second CKR was achieved with STI therapy. Transient minor or no KR were observed in other six pts, two of which showed a further CE; one died 4 mths after the appearance of +8, +Ph', 12p- and the other developed a duplication of Ph' 9 years after diagnosis, followed by a 6q deletion 2 years later. He died in BP 7 mths later. The median overall survival of these 12 pts was 52,5 mths (10-147), while the median survival of the subgroup of cytogenetic responsive patients was: 58,5 + mths (11-147) with 4 pts still alive. In other 23 pts of the remaining 96, a total of 34 mutational events [i.e., i Ph' (2 pts), 2 Ph' (2 pts) 9q+ (1 pts), 22q- (1 pts) variant or additional translocation (3 pts) i 17 (1 pts)+ 8 (6 pts) monosomies (6 pts), -Y (3 pts) other structural rearrangements (9 pts)] were seen after diagnosis. In two cases a second CE occurred 1 after the first and was followed by a rapid BP and death. A third patient survived 10y,8y after the first and 2y after the second CE. Simultaneous occurrence of CE and clinical progression was seen in 6 pts. The median overall survival of these 23 pts was 74 mths (10-235) while the median survival after CE was 21,5 mths (3-100). **Conclusions.** the presence at diagnosis of karyotypic abnormalities does not prevent the achievement of cytogenetic response and prolonged survival in CML pts. Trisomy 8, duplication of the Ph', i (Ph'), and variant-translocations, whenever occurring, seems to be associated with a good prognosis. Instead monosomy of part or whole chromosomes, complex karyotype aberrations and multiple or subsequent CE appear to have a poor prognostic significance. Finally the lack of the Y seems to be associated with a good prognosis if present at diagnosis, but has a worse significance when occurring during the course of the disease.

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POSSIBLE NEUROPROTECTIVE EFFECT OF ERYTHROPOIETIN IN ACUTE INFLAMMATION INDUCED BY EXHAUSTIVE EXERCISEM. Tsironi¹, E. Premetis², K. Kariyannis³, K. Skenderi³, G. Assimakopoulos⁴, A. Stamoulakatou², G. Chrousos², I. Pappasotiriou²¹Medical School, University of Athens, ATHENS, Greece; ²'Aghia Sophia' Children's Hospital, ATHENS, Greece; ³'Harokopio' University, ATHENS, Greece; ⁴Sparta General Hospital, SPARTA, Greece

Background. Erythropoietin is a glycoprotein hormone produced mainly by interstitial fibroblasts in the kidneys of the adult and by hepatocytes of the foetus. Released into the circulation, erythropoietin makes its way to the bone marrow, where it regulates red cell production by preventing apoptosis of erythroid progenitor cells. Recently, erythropoietin has emerged as a multifunctional growth factor that plays a significant role in the central nervous system (CNS). Both erythropoietin and its receptor are expressed throughout the brain in glial cells, neurons and endothelial cells. Hypoxia and ischaemia have been recognised as important stimuli of erythropoietin expression in the brain. Erythropoietin has potent neuroprotective properties *in vivo* and *in vitro* and appears to act in a dual way by directly protecting neurons from ischaemic damage and by stimulating endothelial cells. S-100b, a glial-derived protein is a well-established biomarker for severity of neurological injury and prognosis for recovery. Cell-based and clinical studies have implicated S-100b protein in the initiation and maintenance of a pathological, glial-mediated pro-inflammatory state in the CNS.

Aims. We studied the possible association between erythropoietin and S-100b in 18 athletes participated in the ultra-distance foot race of the 246Km 'Sparthatlon'. This race consists of continuous, prolonged, brisk exercise. We have reported that IL-6, CRP and SAA levels markedly increase (8000-, 152- and 108-fold, respectively) over the baseline at the end of the race and that IL-6 levels return to normal within 48h, while CRP and SAA remain elevated 48h post race. **Methods.** Erythropoietin and S-100b protein levels were determined by means of chemiluminescence (Nichols Institute Diagnostics, CA, USA and Sangtec Medical AB, Bromma, Sweden respectively). The measurements were performed before (phase I), at the end (phase II) and 48h post-race (phase III). **Results.** Erythropoietin levels at phase I (13.5 ± 6.3 IU/L) were increased significantly at phase II (52.0 ± 52.0 IU/L) and subsequently decreased at phase III (21.0 ± 14.6 IU/L). At the same time period S-100b protein followed the same pattern (phase I: 0.13 ± 0.01 mg/L, phase II: 0.29 ± 0.01 and phase III: 0.13 ± 0.01 mg/L). A significantly positive correlation between erythropoietin and S-100b protein was found at phase II ($r=0.521$, $p<0.03$), while this correlation was absent in the other two phases ($p>0.40$ and $p>0.08$ respectively). Simultaneous measurements of haemoglobin levels showed only a significant drop of haemoglobin at phase III (143.4 ± 12.4 , 140.4 ± 15.0 and 124.3 ± 9.5 g/L respectively). **Conclusions.** Exhaustive exercise induces inflammation and CNS injury, promoting an erythropoietin anti-inflammatory response. We suggest that in this particular inflammatory process, erythropoietin secretion is associated with brain lesions, as they are expressed by the S-100b protein release in the circulation. This is strongly supported by the lack of any association between erythropoietin values and haemoglobin levels.

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THE POTENTIAL ROLE OF KIR GENOTYPE FOR GVHD IN ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Actions of NK cells are mediated by activating and inhibitory signals induced by cell surface receptors (KIR: killer immunoglobulin-like receptors) binding to HLA class I ligands, thus regulating NK cell response in clinical conditions such as infections, cancer and allogeneic tissue. NK cells will be alloreactive towards cells lacking their KIR ligand, and will be tolerant to cells expressing the same or additional KIR ligands. In allogeneic stem cell transplantation, matching of donors and recipients is focused on HLA polymorphisms. In the future, additional consideration of KIR polymorphisms and thus the potential NK cell alloreactivity might also play an important role in order to improve transplant outcome (e.g. enhancement of antileukemic effect, reduction of GvH-effect) as shown in recent studies. **Methods.** In a monocenter pilot study starting in 2004, we collect data of KIR genotype of donor-recipient pairs in allogeneic stem cell transplants. These data are mainly correlated to HLA genotype and transplant outcome, esp. incidence and severity of GvHD and survival. HLA alleles were determined by sequence-based typing. KIR typing was performed by PCR-SSP. Especially the presence/absence of inhibitory KIR 2DL1, 2DL2, 2DL3 and 3DL1 is considered for outcome analysis. Incidence and severity of acute GvHD was analysed by Chi2- test. **Results.** To date, results of 24 allogeneic transplants (6 related, 18 unrelated; 20 with 10/10 HLA allele match, 4 with 9/10 HLA allele match) comprising different hematological malignancies and hemoglobinopathies are available. Considering relevant HLA epitopes only, i.e. with potential for donor-versus-recipient NK cell allorecognition, only 1 out of 24 transplants showed donor KIR ligand (HLA-C *03) missing in recipient (HLA-C *07), i.e. the potential of the donor's NK cells to lyse the recipient's cells and to cause GvHD. In another approach using KIR genotype data of the donors, we analysed

the presence or absence of recipient HLA ligand (HLA-Bw4, HLA-C group 1 and 2) for donor inhibitory KIR. In 18 of 24 transplants, there were one or more recipient HLA epitopes missing for the identified donor inhibitory KIR. Considering 20/24 cases evaluable for GvHD, we did not yet find any correlation between incidence and severity of GvHD and KIR genotype. **Conclusions.** Due to our preliminary data with a restricted number of patients and data from different categories (e.g. donor: related/unrelated, disease: myeloid/lymphoid/other), additional data are needed in order to define a KIR matching strategy for improved transplant outcome.

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PROGNOSTIC SIGNIFICANCE OF C-KIT RECEPTOR (CD 117) EXPRESSION IN AML

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Background. Acute myeloid leukemia (AML) is a heterogeneous group of disorder with different clinical, morphologic, immunologic and molecular characteristics. The prognostic significance of c-kit receptor (CD117) that is thought to play an important role in regulation of proliferation and differentiation of hematopoietic cells with other lineage specific growth factors has been controversial in AML. **Aims.** to investigate prognostic significance of CD117 and to determine importance of CD117 in diagnosis of AML. **Material and Methods.** 28 newly diagnosed denovo AML and 14 ALL patients were included in this study. Bone marrow aspirate or peripheral blood sample of patients were analysed by flow cytometry using a panel of monoclonal antibody (MoAbs). Cytogenetic examination and molecular analysis of t(8;21), t(15;17) and inv (16) by RT-PCR were performed in 21 of 28 AML patients. Several clinical characteristics such as age, sex, white blood cell count (WBC), hemoglobin level, platelet count, LDH level and FAB subtypes were identified. All patients were given remission induction therapy. After completion of remission induction therapy, patients were evaluated for response of therapy. **Results.** CD 117 expression was positive in 19 of 28 (% 68) AML patients but all of ALL patients were negative. In AML patients CD117 expression was detected in all FAB subtypes. We did not find a clear correlation between CD117 expression and complete remission (CR) rate. We found no association between CD117 expression and other prognostic factors such as age and WBC. CD34 expression was correlated with CD 117 expression in AML patients. **Conclusions.** CD 117 is an important marker for differentiating of AML from ALL. Our results suggest that CD 117 expression has no prognostic significance in patients with AML. But large studies with more patients that include all prognostic factors are necessary to conclude whether CD 117 has prognostic significance.

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ALLOGENEIC-SCT AFTER TOXICITY-REDUCED CONDITIONING: MYELOBLASTIC CHARACTER AND ANTI-LEUKEMIC EFFICACY IN ELDERLY PATIENTS

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Despite the introduction of reduced-intensity conditioning there is still much dispute if patients aged 60 years or more should be considered as candidates for allogeneic stem cell transplantation (allo-SCT) and the experience with this patient group remains limited. This report summarizes our cumulative experience in a cohort of 28 selected elderly patients (age 60-66, median 61 years) with hematological malignancies treated with, depending on the underlying disease, a variety of toxicity-

reduced conditioning regimens followed by allo-SCT. All but two patients received mobilized peripheral blood stem cells as graft and 12 patients had a sibling and 16 an unrelated donor (MUD), respectively. In the majority of patients the underlying disease was acute leukemia with only 25% being in complete remission at the time of transplant. The remaining 8 patients suffered from either chronic leukemia (CML, n=4; CLL, n=1), osteomyelofibrosis (n=3) and multiple myeloma (n=1). For this patient cohort the conditioning regimens applied were all myeloablative as evidenced by full donor chimerism at day +30 in all patients. The time to recovery of absolute neutrophil count $\geq 0.5 \times 10^9/L$ was 10-34 days (median 19 days). Despite the high relapse risk in the majority of patients we observed only 3 deaths because of recurrent leukemia. Severe acute GVHD (grade 3 and 4) occurred in 29% and the non relapse mortality was 36% with 5 patients each dying from GVHD or infection. With a median follow-up of 11 months (range 1-61m) for surviving patients the Kaplan-Meier procedure estimates a 40% probability of survival. Interestingly, there is no difference what so ever in survival if patients had an identical sibling donor or a MUD. Our data support the notion that toxicity-reduced conditioning followed by allo-SCT may be still myeloablative in the elderly, nevertheless it can safely be applied and has excellent anti-leukemic efficacy. However, further efforts are needed for control of GVHD and infectious complications, especially in this high-risk population.

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SERUM LEVELS OF IL-6, IL-8 AND IL-10 IN PATIENTS WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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Background. Cytokine deregulation has been found in various hematological malignancies, including non-Hodgkin's lymphoma (NHL). This abnormal or unbalanced production of cytokines is likely to participate in the development or evolution of the malignant process. The aim of this study was to investigate and to correlate the serum levels of interleukin-6 (IL-6), IL-8 and IL-10 in the pretreatment period and outcome in patients with aggressive NHL. **Methods.** Serum IL-6, IL-8 and IL-10 levels were measured by enzyme-linked immunosorbent assays from 46 patients and 30 healthy controls. Serum levels of cytokines were also assayed after the treatment. In 43 cases initial treatment consisted of CHOP and in three of ACVBP regimens. The median follow-up duration was 45 months (32-61 months). Cytokine levels were correlated with clinical features and overall survival. **Results.** Significantly elevated pretreatment serum levels of cytokines (IL-6, IL-8 and IL-10) were found in patients with aggressive NHL ($p < 0.001$; $p < 0.01$; $p = 0.01$ respectively) as compared to healthy controls. In 32.6% of patients all three cytokines were elevated in parallel. Patients who were effectively treated had a significant reduction in cytokine levels ($p < 0.05$). Serum levels of IL-6, IL-8 and IL-10 were higher in patients with higher (≥ 2) International Prognostic Index (IPI). Using univariate analysis, overall survival (OS) in all patients was affected by presence of systemic symptoms, Ann Arbor stage (≥ 3), performance status score (≥ 2) and IPI (≥ 2), elevated levels of beta-2 microglobulin, pretreatment serum levels of cytokines (IL-6, IL-8 and IL-10) and the number of cytokines increased (0-1 vs. 2-3). When univariate analysis was performed only elevated serum level of IL-6 after the treatment showed negative prognostic significance for OS. **Conclusions.** In patients with aggressive NHL, serum IL-6, IL-8 and IL-10 levels are elevated and correlate with adverse disease features and shorter survival. These markers could be used in identification of high risk patients who would need more aggressive therapy and early minucious disease monitoring.

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PERSISTENT POLYCLONAL B-CELL LYMPHOCYTOSIS: REVIEW OF 9 CASES

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Background. Persistent polyclonal B-cell lymphocytosis (PPBL) is a rare entity of unknown aetiology affecting mainly women, usually cigarette smokers. It is characterized by a chronic B lymphocyte increase, typical binucleated lymphocytes on peripheral blood smears and elevated serum IgM. **Aims.** We studied clinic characteristics and evolution in nine patients (8 females, 1 male) with PPBL diagnosed between 1994 and 2004. **Patients.** Median age at diagnosis was 42 years (range 36-48). All patients were cigarette smokers. Four of them showed mild splenomegaly, and two hepatomegaly, but adenopathy was not detected in any of them. The lymphocytosis at diagnosis varied between 3.3 and $11.6 \times 10^9/L$ (median $7 \times 10^9/L$), with normal Hb in all patients and mild thrombocytopenia in three. Peripheral blood examination showed atypical lymphocytes and all presented typical binucleated lymphocytes. The ESR was elevated in 5 patients. A polyclonal increase of IgM levels was observed in all patients (median 749 mg/dL; range 438-1420 mg/dL); in three patients a mild decrease of IgG was detected. Immunophenotyping findings were consistent with a polyclonal expansion of the B-lymphocyte pool, as evidenced by a normal k/L ratio, reaching 27-71% of all lymphocytes. Five patients underwent peripheral blood cytogenetic analysis showing a normal karyotype, and seven patients tested showed absence of clonal rearrangement of IgH gene. PCR Bcl-2/Ig gene rearrangement was present in 7 out of 8 patients, but none of the 8 patients tested showed this rearrangement by FISH. The median follow-up in all patients was 38 months (range 6-16 months). Two of the patients were initially diagnosed with NHL with leukaemic expression, but neither of them received therapy before the establishment of LBPP diagnosis. For this period, the clinical course and the hematological parameters were stable. **Conclusions.** PPBL is a rare hematological disorder of unknown aetiology and indolent evolution, which must be considered in the differential diagnosis of B-cell lymphocytosis. It is probably underdiagnosed and underreported, as most patients are asymptomatic and show mild lymphocytosis. Recognition of this disorder is important in order to prevent unnecessary therapy.

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COMPARATIVE QUALITY OF LIFE STUDY BETWEEN PATIENTS WITH LOW GRADE NON-HODGKIN'S LYMPHOMA USING RITUXIMAB AND CONVENTIONAL CHEMOTHERAPY WITH ALKYLATING AGENTS

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Background. Low grade non-Hodgkin's lymphoma (NHL) is an indolent form of disease with a generally slow course of progression. Although still usually incurable, low grade disease has shown responsiveness to some of the newer chemotherapeutic and non chemotherapeutic treatment options. In the absence of a cure, the variety of therapeutic options available for patients with indolent NHL range from 'watchful waiting' to high-dose therapy (HDT). There is no current consensus on standard treatment. However, since cure remains elusive, and since many patients with low grade NHL may have few or even no symptoms initially, the decision about whether or not to initiate treatment logically must include quality of life (QoL) issues. QoL has become an essential outcome measure in the evaluation of treatment for low grade NHL patients. Knowledge about QoL may be of help in decision-making in clinical practice, may give important information about long term effects in patients with low grade NHL and help to identify potential adjustment problems. **Purpose:** The aim of this study was to compare the QoL between patients using Rituximab (Mabthera) maintenance to treat low grade NHL (A Group) and patients that received con-

ventional treatment (alkylating agents; B Group). All patients were in advanced stage of disease (III or IV). *Methods.* The European Organization of Research and Treatment of Cancer Quality of Life Questionnaire C-30 (EORTC QLQ-C30) was selected as the main QoL measure for the present study. It is a multidimensional, cancer specific, reliable, valid, and sensitive to changes in QoL over time. Two groups of 21 patients responded to the QLQ-C30. *Results.* In response to the disease and treatment related questions, 16% of the patients in the group A reported they had at least some problems with chills and nausea during the infusion and 68% patients in the group B reported some problems with chills and nausea during the treatment with alkylating agents and 72% reported fatigue. In the group A 90% reported an improvement in their family life and/or social life after start the treatment and in the group B only 25% reported an improvement in their family life and/or social life after start the treatment. *Conclusions.* This analysis was realized in patients using Rituximab (support for the healthy security) and patients without healthy security (B group) This issue raises an important question: outside the setting of a clinical trial, should patients be treated aggressively with therapies that do not yet have proven curative ability? This study considers the evidence and relative merits for the use of Rituximab maintenance treatment to indolent NHL, in order to maintain a normal quality of life in these patients and also illustrate the effects of this agent on QoL.

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TREATMENT OPTIONS IN HEMANGIOMAS OF INFANCY

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Background. Hemangiomas are the most common benign tumors of infancy; however, the management is controversial due to the wide spectrum of clinical disease, rapid change and potential toxicity of treatment in early infancy. Various treatment modalities are used as steroids and interferon although most hemangiomas will resolve without treatment. Age of the patient is critical for determining therapy. Objective: Our purpose was to review our experience with this challenging problem by evaluating the clinical features, management, and therapeutic responses of infantile hemangiomas. *Methods.* A retrospective analysis of infantile hemangiomas at the University of Istanbul, School of Medicine, Department of Pediatrics, Division of Hematology, and Oncology from 1996 to 2004 was performed. *Results.* The medical records of 29 patients were evaluated. Twenty-one female and 8 male patients were seen with a female/male ratio of 2.6:1. Seventeen patients were below 6 months and 12 patients were above 6 months of age. The time of occurrence of hemangioma was mean 3.8 weeks (range: 0-60 weeks), admittance to hospital was 10 month (range: 1-12 m). Head and neck was the most frequently involved site determined in 20 cases (69%). Other lesions were in extremities, and trunk and extended in the body in 3 (10%), 2 (7%), 4 (14%) patients respectively. Because of their auto-involutive nature, sixteen patients were followed-up without treatment. In 17 patients were below 6 months while 6 of these patients were followed-up without any treatment, remaining 11 patients having proliferation and risk of loss of function were started steroid therapy. Five patients had regression (45%, 4 marked, 1 partial regression). Lesions were stable in 2 patients. Interferon alfa-2a was started in 4 patients (14%) due to inadequate response or side effects of steroid therapy after 6 months. Two patients over 6 months of age at admission were also applied interferon therapy. Five of the 6 patients (83%) receiving interferon therapy was recovered (1 complete, 2 marked, 2 partial regressions). Treatment complications like ulcerations and infections were seen in 4 patients (2 patients with steroid and 2 patients with interferon therapy) but they were successfully treated. Six patients below 6 months and ten patients over 6 months of age were followed-up without treatments. They had no regression

during 1-36 months follow-up period. Long term results in natural prognosis of the diseases could not be obtained in patients without treatment due to lost to follow-up cases. In conclusion, steroid therapy is preferred in management of patients with hemangiomas required treatment below 6 months of age in our clinic. In these patients interferon therapy may be used effectively and safely in infants over 6 months of age. Only interferon can be applied as first line therapy in severe hemangiomas over 6 months of age.

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EFFICACY AND SAFETY OF INTRAVENOUS IRON SUCROSE IN BARIATRIC SURGERY SECONDARY ANEMIA

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Background. Iron deficiency secondary to malabsorption is one of the problems associated with bariatric surgery for morbid obesity. Intravenous iron preparations such as iron sucrose are suitable alternatives because of their quick effect on erythropoiesis. *Aims.* In this retrospective study we report our experience of treating 47 consecutive patients with documented iron deficiency anemia after bariatric surgery with parenteral iron sucrose from 2002 to 2005. *Methods.* Iron sucrose was given in an outpatient setting in doses of 100-200 mg at variable intervals. Nine patients were also treated with erythropoietin beta (3000-6.000 unit/wk) at some point during the period of observation. Efficacy in correction of anemia and secondary effects were evaluated. Complete response to treatment was defined as an increase in hemoglobin (Hb) to normal levels (>12 g/dL for females and >13 g/dL for males). *Results.* Total number of patients was 47 (42 females, 5 males). Mean age was 43.5 + 8.6 years. Iron deficiency anemia before surgery was detected in 16 patients (34%). Surgical procedure consisted in biliopancreatic diversion in 42 patients (89.4%). Median interval between bariatric surgery and parenteral iron therapy was 33 months (range 3-145 months). Forty-three patients (91.5%) had received oral iron previously without response, 7 of whom did not tolerate it. The median cumulative administered iron dose was 1.300 mg, with a range of 400-4.200 mg. All patients but one showed an increase in Hb level. The mean Hb pretreatment was 9.1 + 1.6 g/dL and posttreatment was 12.1 + 1.5 g/dL. Complete responders (24 patients) received a median cumulative dose of iron sucrose of 1600 mg (600-4200), while it was 1100 mg (400-3200) in 23 patients not reaching a normal Hb (p<0.05). There were no differences between patients receiving only iron or iron plus erythropoietin. There were 5 mild adverse events attributed to iron sucrose (1 constipation, 1 abdominal pain and 3 discomfort with chills), and two side effects non drug-related (1 catheter associated thrombosis and 1 vasovagal syncope). *Conclusions.* Intravenous iron sucrose therapy is a safe and effective procedure in the treatment of iron deficiency anemia secondary to bariatric surgery. Poor response is associated to inadequate iron administration. Association of erythropoietin does not seem to improve the results. Further studies are required to evaluate the routine use of intravenous iron in these patients, both for replacement previous to surgery and for treatment of iron deficiency after surgery and subsequent maintenance of iron stores.

1097**INTRATHECAL TREATMENT OF NEOPLASTIC MENINGITIS (NM) DUE TO LYMPHOMA WITH LIPOSOMAL CYTARABINE (DEPOCYTE®)**

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Background. NM is a devastating complication that appears in almost 10% of patients with leukemia or lymphoma. The prognosis is extremely poor and effective treatment of the neurological symptoms may improve quality of life. DepoCyte® is a sustained-release formulation of Ara-C; a single intrathecal (IT) injection of 50 mg maintains cytotoxic concentrations in CSF for 14 days. **Aims.** To confirm the efficacy and safety of DepoCyte® in patients with Lymphomatous Meningitis (LM) **Methods.** Eight patients were included (5 males and 3 females), aged 21-48 years (median 39 years) and diagnosed of systemic lymphoma (6) and Primary CNS lymphoma (2). Treatment was given for the management of LM in all cases. Five patients had previously received IT chemotherapy as prophylaxis or treatment for CNS infiltration. All had received one or more lines of systemic chemotherapy. Treatment consisted of the IT administration of 50 mg of DepoCyte® every 14 days. In 7 patients this treatment was provided concomitantly to systemic chemotherapy or radiotherapy. **Results.** A total of 19 doses were administered (1 patient 1 dose, 3 patients 2 doses and 4 patients 3 doses). Some grade of clinical improvement and/or cytological response were elicited in the 8 patients, although DepoCyte® didn't induce effect on its survival. Headache was recorded in 33% of the patients. Other side effects (vertigo, nausea, vomiting, neurological defects) were reported in fewer than 15% of cases. **Conclusions.** DepoCyte® administered once every 14 days seems to induce a good response rate. The post puncture side effects were few and of limited magnitude. The use of this treatment as front line therapy, and its role in the prevention of neoplastic meningitis, should be further explored by larger studies.

1098**INDIVIDUAL FOLLOW-UP OF P-GLYCOPROTEIN ACTIVITY: A VALUABLE TOOL IN THE DEFINITION OF THE OVERALL MANAGEMENT STRATEGY FOR MM PATIENTS**

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Background. Multiple myeloma (MM) is a disease where we would like to know the future: some patients present early with a heavy form of the disease needing an aggressive therapy, or transplantation, while others live for years after the diagnosis without any of the symptoms. During this time, we wait for the first symptoms to start the treatment, knowing that the most favorable outcome have those patients who achieve a complete or near complete remission following the first therapy, before the development of chemoresistance associated with the relapse of MM following an initial responsiveness to chemotherapy, and with the incurability of MM and overall poor prognosis for the patient. All the research on MM so far couldn't elucidate what makes MM behave in a specific manner: a key event in the development of the disease, or an intrinsic characteristic of the patient. While the quest for an answer continues, also in the realm of the genetic polymorphism, we would like to remind that individual follow-up of the P-glycoprotein (P-gp), the first multidrug resistance (MDR) protein discovered, can help in the overall follow-up of the MM patient, and give us a hand in the therapeutic management of the individual MM patient. **Aims.** Previous studies have suggested an association between the increase of expression/activity of P-gp and the development of

MDR/chemoresistance and relapse of MM. Still, due to a relative small number of patients, sample size, of the single studies for the individual variability of the constitutive expression of P-gp, and as seen lately, the polymorphism of the P-gp encoding MDR1 gene, no clear cut conclusions and recommendations regarding MDR proteins were made. Here we would like to present a long-term follow-up of a patient with MM from the diagnosis through the relapse into the remission, highlighting the influence of therapy protocols on the change of activity of P-gp. **Methods.** The standard flow-cytometric Rhodamine 123 (Rh123) dye functional efflux assay with Cyclosporine A (CsA) as the P-gp modulator has been used, and the P-gp activity expressed as the ratio of mean fluorescence (RMF) with and without the use of CsA. **Results.** i) P-gp activity acts in response to treatment i.e. change in therapy, irrelevant of the initial/constitutive level of P-gp activity. ii) P-gp activity increases with the use of the MDR therapy protocols. iii) Steady P-gp activity increase is concurrent with poor response observed on the clinical level. **Conclusions.** P-gp activity is an intrinsic characteristic of an individual, and needs to be individually monitored to allow for any inference. If individually monitored, P-gp activity can have a prognostic value regarding response to therapy of MM. As almost all patients with MM will relapse, the individual follow-up of P-gp activity can be a valuable tool in the definition of the overall management strategy and planing of the treatment of relapse for MM patients.

1099**ABCG2 V12M AND Q141K POLYMORPHISMS IN PATIENTS WITH DIFFERENT HEMATOLOGICAL MALIGNANCIES**

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ABCG2, which confers multidrug resistance to cancer cells, appears to be expressed in the hematopoietic pluripotent stem cells at an early stage. Its expression is sharply down regulated at the point of commitment to lineage-specific development. ABCG2 is presumed to play a protective role against a broad spectrum of toxic substrates. On the other hand, BCRP may be responsible for the efflux of small lipophilic substrates, such as steroids, and its expression may play a regulatory role in inducing growth, differentiation or apoptosis. On the other hand, BCRP may be responsible for the efflux of small lipophilic substrates, such as steroids, and its expression may play a regulatory role in inducing growth, differentiation or apoptosis. Alteration of the protein function may result in hematopoietic stem cell disorders. The V12M and Q141K mutations are the most common single nucleotide polymorphisms (SNP) in the coding region of ABCG2. The results of investigations, regarding expression levels, localization and functionality of the polymorphic variants are strongly contradictory, some indicate decreased expression of Q141K or abnormal intracellular processing of V12M. The aim of this study was to identify the allele frequency of V12M and Q141K SNP-s in 158 patients with chronic myelogenous leukemia (CML), in 119 patients with acute myelogenous leukemia (AML), and in 113 patients with myelodysplastic syndrome (MDS) comparing to healthy controls (n=149). V12M and Q141K SNP-s were investigated by PCR-RFLP and LightCycler allelic discrimination methods. The V12M and Q141K allele frequencies were 4.0±2.3% and 9.4±3.4% in the Hungarian control population. The allele frequencies of the two SNP-s in the patient groups were the following: CML: 4.7±2.4% and 8.9±3.2%; AML: 4.2±2.6% and 9.7±3.8%; MDS: 5.7±3.1% and 10.6±4.1%. No allele frequency changes could be observed in younger patients (<50 years), or in AML patients who relapsed during the course of treatment. Since no significant difference was observed between the control and the patient populations, our data indicate that BCRP V12M and Q141K polymorphisms are unlikely to play a role in the pathogenesis of the hematological malignancies examined.

1100**HIGH-DOSE IMMUNOGLOBULIN FOR ADULTS OUTPATIENTS WITH SEVERE AUTOIMMUNE THROMBOCYTOPENIC PURPURA AS PREPARE FOR SPLENECTOMY**

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Backgrounds: Intravenous immunoglobulins (IVIg) can be used in down-regulation of the immune response in autoimmune diseases. The mechanisms of action of IVIg in the modulation immune system are far from being clearly defined. Blockade of the Fc receptors of IgG, which are present on cells of the reticuloendothelial system, could be major mechanism by which IVIg demonstrates a therapeutic efficacy in this AITP. IVIg are effective but expensive treatment for patients with autoimmune thrombocytopenic purpura (AITP). There is general agreement that high-dose immunoglobulin should be administered in emergency situations and that it is safe preparation for surgery. **Methods.** We report results of administration of IVIg in 9 outpatients with poor response to corticotherapy. All patients (1 male, 8 females) with severe AITP were diagnosed according to standard criteria. The average platelets number was less than 20 in all patients without life threatened bleeding manifestations. In the aspect of emergency situation - high risk of bleeding-patients were prepared to splenectomy by administration high dose IVIg 1g/kg/bw and day. The initial response was observed in all women. The higher dose 2g/kg/bw was given in male during subsequent 3 days for inadequate effect of treatment. The initial response was observed in all patients. The increase of platelets in the range 60-110 was in all patient during subsequent 3 days after the IVIg administration. Splenectomy was performed in all patients after the platelets increase. **Results.** The complete therapeutic response was observed in 6 patients-platelets more than 150 after 3 months after splenectomy. The partial responded was observed in 3 patients-platelets more 50. **Conclusions.** Application of high-dose IVIg is successfully effective, less expensive in outpatient conditions and it is evaluated by outpatients as positive-quality of life.

1101**INCREASED CA-15.3 LEVELS IN THE SERUM OF PATIENTS WITH HOMOZYGOUS μ -THALASSEMIA AND SICKLE CELL/ μ -THALASSEMIA**

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Background. Glycoprotein CA-15.3 is produced by epithelial cells and is found increased in the serum and serosal effusions of patients particularly with breast cancer, but also in other malignancies of epithelial origin (ovarian, lung, liver, prostate, colon cancer etc). Less frequently CA-15.3 is found elevated in hematological malignancies, as well as in some not malignant diseases and conditions, including pregnancy, elderly and various diseases associated with a serosal effusion. **Methods.** We measured serum CA-15.3 in 105 adult patients with homozygous μ -thalassemia (μ -thal) (56 female, 49 male, median age 28, range 17-67 years) and in additional 33 patients (16 female, 17 male, median age 36, range 20-63 years) with sickle cell/ μ -thalassemia (S/ μ -thal). Eighteen thalassemic patients (17%) had the intermediate phenotype of the disease, and among them 12 had never been transfused. **Results.** Ninety-nine patients with μ -thal (88.6%) and 27 with S/ μ -thal (82%) exhibited increased levels of CA-15.3. In 38 patients with μ -thal and 9 with S/ μ -thal (36.2% and 27.3% respectively), the increase of serum CA-15.3 was more than 3-fold the upper normal limit (i.e. $>85 \mu\text{U/mL}$, normal range 0-28 IU/mL). There was no difference between male and female patients, and no correlation with age, past splenectomy, serum CRP and ferritin levels, presence of HCV

infection, diabetes, heart, liver, or thyroid disease was observed. Patients with intermediate phenotype had significantly higher CA-15.3 levels, as compared to transfusion-dependent patients ($\bar{x} \pm \text{SD}$: 105.1 ± 60.8 vs 74.2 ± 42.2 IU/mL, $p=0.028$). Among μ -thal patients, CA-15.3 was negatively correlated with MCV ($r = -0.389$, $p < 0.01$), and positively, with serum total bilirubin ($r=0.583$), unconjugated bilirubin ($r=0.572$) and LDH ($r=0.547$, for all $r: p < 0.001$). Reevaluation of CA-15.3 in 48 of the patients revealed a significant fluctuation in 15 of them. In all cases serum CA-15.3 was higher among patients inadequately transfused and therefore preserving their own hematopoiesis, as manifested by increased reticulocyte count, lower MCV and higher serum unconjugated bilirubin and LDH. All female patients were monitored closely, and 17 of them underwent mammographic imaging, which revealed fibrocystic lesions in 5 cases. After a median follow-up of 62 months no patient developed any kind of neoplastic disease. **Conclusions.** It is concluded that increased CA-15.3 levels are commonly found in the serum of patients with μ -thal and S/ μ -thal, and this finding is not associated with the presence of any malignant disease, but is correlated with the degree of marrow ineffective erythropoiesis. The increased serum amounts of the mucin might be the result of CA-15.3 release by the apoptosing erythroblasts in the bone marrow of the patients.

1102**T CELL-ASSOCIATED SURFACE MARKERS CAN BE USED TO DERIVE A ZAP70-CORRELATED PROGNOSTIC FACTOR FOR CLL**

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1. **Background.** Prognostic factors in chronic lymphocytic leukemia (CLL) include clinical staging and various parameters, such as lymphocyte doubling time, beta2 macroglobulin, and chromosomal aberrations. ZAP70 has emerged in the last few years as a powerful prognostic factor, although the exact mechanism by which it leads to progression of disease is not clear. Previous studies in a chimeric NOD-SCID mouse model of CLL have shown the importance of T cells in the survival of the CLL clone. Depletion of T cells leads to better engraftment and survival of the CLL clone, whereas enrichment with T cells leads to early apoptosis of the engrafted cells. We previously found (in a study conducted on 625 CLL patients) that the ratio of the B-CLL clone to the total T cell population was significantly associated with disease severity ($p < 0.01$). 2. **Aims.** To assess the correlation between the expression of ZAP 70 and the ratio of the B-CLL clone to the total T cell population. 3. **Methods.** ZAP70 expression was assessed by RT-PCR in 46 patients; the ratio of B-CLL to T cell population was calculated using (CD5-CD3)/CD3. 4. **Results.** A statistically significant correlation ($p < 0.05$) was found between ZAP70 positivity and a high ratio. Among the 13 patients with negative ZAP70 the mean ratio was 2.3, whereas among 33 patients with a positive ZAP70 the ratio was 10.7. 5. **Conclusions.** Our results thus show that there is a significant correlation between the expression of ZAP70 and the ratio of the B-CLL clone to the T cell population. Therefore the above ratio, which is easily obtained in all patients with CLL, may be used as a powerful prognostic tool. Furthermore, the relative decrease in the T cell clone in ZAP70-positive patients suggests a mechanism by which ZAP70 expression confers poor prognosis: the normal T cell population is possibly depleted through interaction with the CLL clone.

1103**COMPLEMENTARY AND ALTERNATIVE MEDICINES (CAM) IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES**

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Background. The complementary and alternative medicines

(CAM) are increasing among patients with different diseases, and they might become an important issue in hematological disorders. *Aims.* To assess the use and diffusion of complementary and alternative medicine among hematological patients, along with their practice, perception and patterns of usage. *Methods.* A questionnaire was applied to 136 patients (56 male and 80 female) with different hematological malignancies (lymphoma: 73, myeloma: 14, leukemia: 24 and others: 25) in order to compile their self-evaluation on different aspects of the use of CAM. *Results.* Of the patients surveyed, 108 (79.4%), admitted the use of some practice or product related with CAM. The products used, mostly from vegetal origin, included: *Matricaria chamomilla* (83.3%), *Tilia vulgaris* (66.6%), *Valeriana officinalis* (25%), Royal Jelly (17.5%), soya (16.6%), green tea (12.9%), Brewer's Yeast (9.2%), wheat germ (8.3%), ginseng (7.4%) and aloe vera (5.5%); Cat's Claw, *Hypericum Perforatum* (St. John's Wort), *echinacea*, grape seed, milk thistle (*Carduus marianum*), *graviola* and *marihuana* (less of 5%). Among the practices of patients, the commonest were prayer (18.5%), relaxation (10.1%), Aromatherapy Massage Oil (7.4%), acupuncture (5.5%), imposition of hands (4.4%) and music therapy (3.7%). Other mean, including meditation, yoga, homeopathy, healer consultation, chiropractic, were used in less than 3% of the cases. Visualization, hypnosis, biofeedback, chromotherapy, reflexology or therapies from humor, movement or dance were not used, as well as internet navigation. Among the benefits sought by patients were complement for traditional medicine (24.2%), the content of relatives (3.7%), decreasing untoward effects of treatment (14.8%) or a fast recovery of the disease (24%). Only 32.4% of them knew the meaning of CAM. The qualification of the personnel providing the alternative measures was unknown to 80.5% of the patients. A total of 65 patients (60.1%) consider that the National Health Systems should provide these treatment measures. *Conclusions.* Information about the use of CAM in our environment is reported in this paper. Physicians should try to achieve some knowledge on CAM as patient's formation so that those aspect of such therapies that can help the follow-up of patients can be integrated in our therapeutical armamentarium.

1104

IDENTIFICATION OF HEREDITARY HYPERFERRITINEMIA-CATARACT SYNDROME IN GREECE

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Hereditary hyperferritinemia-cataract syndrome (HHCS) is an autosomal dominant disorder characterized by bilateral cataracts and increased serum L-ferritin, in the absence of iron overload. Under physiological conditions, ferritin synthesis is finely regulated at the translational level by iron availability. This regulation is achieved by the high-affinity interaction between cytoplasmic mRNA-binding proteins (iron regulatory proteins, IRPs), and mRNA stem-loop structures, known as iron responsive elements (IREs), located in the untranslated regions (UTRs) of the mRNAs. A single IRE is located on the 5' UTR of L-ferritin, and the IRE-IRPs binding repress L-ferritin translation. The deregulation of ferritin production responsible of HHCS is caused by heterogeneous mutations in the iron regulatory element (IRE) of L-ferritin that interfere with the binding of iron regulatory proteins, disrupting the negative control of L-ferritin synthesis and causing the constitutive up-regulation of ferritin L-chains. We report here the identification of the HHCS in Greece. The proband an apparently healthy 21-year-old woman showed persistently high ferritin levels (900ug/L) in routine blood tests. The patient had no personal history of disease and no liver or inflammatory disease suggesting hyperfer-

ritinemia. Serum iron (12.3umol/L), TIBC (60umol/L), transferrin saturation (22%) levels, ferritin-iron content (16ug/L), non-transferrin-bound iron (non-detectable) levels were normal, while sTfR levels were elevated (2.8ug/L). Examination of the eyes revealed an early bilateral cataract in the lens periphery. The patient had a family history of bilateral cataract. Iron metabolism parameters were also assessed in proband's mother and her brother. Both had high ferritin levels 667 and 1500ug/L respectively. Interestingly, the mother showed significant reduction of iron metabolism parameters (iron deficiency anemia), while these parameters were within normal range in her son. The entire iron-responsive element sequence of the L-ferritin subunit gene was amplified by polymerase chain reaction using appropriate primers and both sense and antisense were directly sequenced. An heterozygous C39G substitution was detected in the apical loop of the iron-responsive element from the proband, her mother and brother. The C39G mutation is located in the conserved hexanucleotide apical loop of the IRE and is computationally predicted to significantly alter the base pairing within the loop, which is critical to IRP binding. This mutation has been recently described in a French family. A clinical variability among subjects sharing the same mutation has been observed in our probands including the ferritin levels, iron metabolism parameters and age of onset. These findings suggest that, besides the L-ferritin IRE genotype, additional factors are likely to modulate the lens involvement and the rate of progression to severe cataract in HHCS patients. The identification of HHCS in Greeks further supports the worldwide distribution of the syndrome. HHCS should be included in the differential diagnosis of persistent hyperferritinemia in patients of Greek origin.

1105

IMATINIB IN MYELOPROLIFERATIVE DISORDER WITH FIP1L1-PDGFR A REARRANGEMENT AND IDIOPATHIC HYPEREOSINOPHILIC SYNDROME

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Idiopathic hypereosinophilic syndrome (HES) is a rare hematological disorder characterized by persistent eosinophilia greater than 1,500 cells/L for longer than 6 months, absence of other apparent etiologies for eosinophilia and symptoms of organ involvement. We studied 13 patients with HES (m/f=1/2) to identify the clonality of the disease. All patients were studied by conventional cytogenetic analysis. Normal karyotype was found in all cases. All patients were negative for the BCR-ABL and ETV6-PDGFRB rearrangements. Three patients (all men) were positive for FIP1L1-PDGFR A rearrangement therefore they should be classified as having chronic myeloproliferative disorder rather than HES. We treated eight patients (7 men, 1 woman), including the three with FIP1L1-PDGFR A positive ones, with imatinib mesylate (100 mg/daily). Median follow up of treatment was 10 months (range 4,5-14). Complete clinical and hematological responses to imatinib therapy were recorded in 7 patients (87,5%): all the three FIP1L1-PDGFR A positive patients and four with HES. In all cases with FIP1L1-PDGFR A rearrangement molecular response was obtained after 4 months of therapy. Taking into consideration durability of response more than 4 months we try to reduce the dose to 200 mg per week in one patient with FIP1L1-PDGFR A rearrangement and two pts with HES. Complete remission was preserved 5 months, but in patient with FIP1L1-PDGFR A rearrangement FIP1L1-PDGFR A transcript appeared again after 3 month since the beginning low dose treatment, therefore he returned to initial regimen of the therapy. Of the other two pts in maintenance one refused of the therapy, and in the second case we stopped the therapy after overall one year complete remission. This patient (woman) is in remission during two months without treatment. To the best of our knowledge, this is the only woman with HES, that has achieved a stable remission in imatinib therapy.

1106**THE PROGRAM THERAPY OF PH⁺-POSITIVE CHRONIC MYELOID LEUKEMIA AT EARLY CHRONIC PHASE**

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In order to develop the program of chronic myeloid leukemia (CML) treatment, including the induction, consolidation and maintenance treatment, 14 Ph⁺-positive CML chronic phase patients received the combination therapy. The induction consisted of oral imatinib mesylate, 400 mg daily and cytosine arabinoside, 10 mg/m² bid s/c for 10 days of every month until the major cytogenetic response (MCR) was achieved. After that cytosine arabinoside was substituted by pegylated alpha-interferon 2b, 50 mcg s/c weekly (consolidation therapy). The cytogenetic response (CR) was monitored every 3 months until the MCR achievement, then — every 6 months. The patients (5 male:9 female) were 21—52 years old; 8 were classified to low-risk, 1 — to intermediate and 5 — to high-risk groups. The induction phase lasted 3—12 months. After 3 months of therapy the complete hematological remission was achieved in all patients, MCR — in 64.2% (complete cytogenetic response, CCR in 35.7%); 21.4% achieved minor CR and 2 patients (14.3%) had no response. After 6 months of therapy 85.7% patients had MCR (64.3% — CCR), 7.1% achieved minor CR and 1 patient had no response. After 9 months of therapy all patients except one from high-risk group (92.3%) had MCR and after 1 year 82% achieved CCR. 11 patients has received consolidation therapy (imatinib + alpha-interferon). All of them has kept the achieved hematological and cytogenetic response. In 71.4% patients the combination of imatinib and cytosine arabinoside was associated with hematological toxicity grade I-II; no grade IV toxicity was observed. 28.6% had toxicity grade III; among them 14% had granulocytopenia, 14% had thrombocytopenia and 7% — bleeding. In 3 patients the therapy was thus interrupted. 21% patients had non-hematological toxicity grade III (no cases of grade IV toxicity was observed). The combination of imatinib and alpha-interferon was associated with hematological toxicity grade I-II in 8%, grade III — in 16% patients. There were no cases of grade IV toxicity. The described combination therapy needs further investigation. It can be concluded now that it's side effects are comparable with imatinib alone, while it gains an advantage in time to CR achievement and remission stability.

1107**CIRCULATING ENDOTHELIAL CELLS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA**

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The circulating endothelial cells (CEC) are proposed to be a noninvasive marker of angiogenesis. The number of CEC in peripheral blood of patients (pts) with acute myeloid leukemia (AML) have not been investigated so far. We evaluated the count of resting (rCEC) and activated (aCEC) CEC and circulating endothelial progenitor cells (CEPC) by 4-color flow cytometry in 33 AML pts at the time of diagnosis and 29 healthy controls. Additionally, in 12 pts measurements were performed after the first course of induction treatment. The levels of CEC were correlated with the disease status and known prognostic factors. In untreated AML pts we observed significantly higher CEC level (Me 36,7/μL) than in the control group (Me 3,2/μL) $p < 0,0000001$. The numbers of both aCEC (Me 15,9/μL), rCEC (Me 13,5/μL) and CEPC (Me 0,7/μL) were significantly higher in AML pts when compared to healthy controls (aCEC Me 0,9/μL, rCEC Me 1,6/μL $p < 0,0000001$ and Me 0,1/μL; $p < 0,001$ respectively). CEC count was significantly higher in AML pts

with WBC > 15G/L, elevated LDH level and higher (over median) absolute blast count (ABC) in PB than in the group with WBC < 15G/L ($p < 0,03$), a normal LDH level ($p < 0,03$) and lower (below median) ABC ($p < 0,05$). The positive correlation between CEC and WBC ($p = 0,356$; $p < 0,05$) and a trend toward a positive correlation between CEC and ABC ($p = 0,051$) were observed. The CEC counts did not correlate with hemoglobin and platelet count as well as percentage of bone marrow blasts and LDH level. The CEC numbers evaluated after the first course of chemotherapy were significantly lower than at the time of diagnosis in pts who achieved complete remission ($p < 0,001$) and do not differ significantly in pts refractory to treatment. In conclusion, the CEC levels are significantly higher in AML patients than in healthy subjects and correlate with disease status and response to treatment. Further investigation should be undertaken to better determine their prognostic and therapeutic value.

1108**PERSISTENCE OF EXTRAMEDULLARY ERYTHROPOIESIS (EXE) IN MODERATELY AND HYPERTRANSFUSED THALASSAEMIC PATIENTS RECEIVING IRON CHELATION THERAPY**

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Background. Extramedullary Erythropoiesis (ExE) has been described in about 15% of cases of thalassaemia major (TM) or intermedia (TI). ExE is characterized by emergence of haematopoietic tissue-like masses in various organs giving symptoms and signs of pressure on adjacent healthy tissue, usually on the lower thoracic spinal cord. Factors contributing to ExE are anaemia, blood transfusion regimen and Hb-F inducers. **Aims.** Evaluation of the blood transfusion regimen in conjunction with ExE in moderately and hypertransfused thalassaemic patients undergoing iron chelation therapy with Desferrioxamine (DFO) or Deferiprone (DFP) or both. **Methods.** We studied 33 adults with TM and 5 with TI. All TI and 25 TM were splenectomized. 18 patients received DFO, 10 DFP and 10 combined DFO + DFP. We examined body weight, pre and post transfusion hemoglobin level, daily hemoglobin fall, annual blood consumption (RCCs), serum ferritin, and liver and cardiac iron measured by Magnetic Resonance Imaging (MRI). Allo and auto immunization and antibodies against HLA were also examined. X-ray tests and MRI were performed to detect ExE. **Results.** All TM patients received prestorage leucodepleted RCCs over the last three years. Their mean Hb was 10.2 gr/dL before transfusion and 13.8 gr/dL after. Two TI patients were transfused sporadically. No patients had alloimmunization or autoimmunization. X-ray negative but MRI positive ExE masses were persistently detected in 11 clinical free TM patients over the study period. Clinically active ExE was observed in 2 TI patients and 2 TM, while ExE was inactive in the other 9 TM cases. ExE was not correlated with splenectomy, blood consumption, Hb indices, iron load or chelation therapy. **Case report.** A 45 year old male TI patient was splenectomized at 33 years and maintained mean Hb 12.5 gr/dL receiving Erythropoietin 300 iu/kg/10 days x 3 years. Periodical X-rays for ExE were negative. He complained suddenly of lumbago and inability to walk. Clinical examination revealed signs of paraparesis while spinal MRI showed ExE like masses near and parallel to lumbar vertebrae, compressing the spinal cord. Erythropoietin was stopped and the patient was treated with supertransfusion regimen and intensive iron chelation. Hydroxyurea was administered 20 mg/kg/d x 3 months and then decreased to 10 mg/kg/d. The patient recovered after 6 months. Therapy continued for two more years, with reduced but not completely inactive ExE masses. **Conclusions.** Persistent ExE was diagnosed in many adequately transfused TM patients as well as in sporadically transfused TI patients. Careful analysis of clinical data, long-term monitoring of each patient, individualized blood transfusion regimens and concurrent treatment with hydroxyurea are required. Investigation and follow-up of ExE should be based on MRI rather than simple X-rays.

1109**INTERIM ANALYSIS OF RESULTS WITH HYPER-CVAD IN THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN A TERTIARY HAEMATOLOGY CENTER**

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Background. Hyper-CVAD represents an intensified program for the treatment of acute and chronic lymphoid malignancies. Hyper-CVAD has been proposed as a highly efficient treatment for adult ALL with an acceptable toxicity profile in younger patients. **Aims.** Hyper-CVAD was initiated in September 1999 in our institution as the primary treatment of adult ALL. Twenty two consecutive ALL patients received Hyper-CVAD and were analyzed focusing on the efficacy and the toxicity of the program. **Methods.** Our patient population consisted of 22 *de novo* ALLs (7 T-cell, 15 B-cell) with median age of 41.5 yrs (range 16-68 yrs). The M/F ratio was 10/12 and seven (7/22 31.8%) patients were older than 50yrs. Hyperleucocytosis $\geq 100 \times 10^9/L$ was present in 6/23 (26%) cases (3 T-cell, 3 B-cell) while splenomegaly, hepatomegaly and bulky disease were documented in 17/22, 15/22 and 1/22 cases respectively. Cytogenetic analysis was performed in 21/22 patients: in 9/21 it was normal, in 1/21 showed del (12), in 1/21 revealed just polyploidy and failed in 10/21 cases. Bcr-abl transcripts were detected in three cases. None of our patients presented with CNS disease (morphology and immunophenotyping). Median follow up to date is 17.8 months (range 4-58). Treatment consisted of four cycles of Hyper-CVAD, alternating with four cycles of methotrexate/cytarabine. All patients received intrathecal CNS prophylaxis and G-CSF support. Maintenance therapy consisted of two years of treatment with mercaptopurine, methotrexate, vincristine and prednisone (POMP). Imatinib was added in bcr-abl (+) cases. **Results.** Haematological CR was achieved in 19/22 (86.3%) *de novo* ALL cases: (11pts $\leq 28d$, 8pts $> 28d$). Primary resistance was documented in 2/22 cases, which subsequently received other therapeutic protocols and eventually deceased. One patient died in early induction. From the group of remitters 8/19 are alive in CR after a median DFS of 16mo (range 3-57). Another 6/19 remitters - including one post-autologous transplantation- relapsed after median of 8mo and four of them deceased. 3/19 remitters underwent allogeneic transplantation (2 alive in CR, 1 deceased from complications). Regimen-related toxic deaths occurred in 3/19 (15.7%) cases whilst in remission status. 6/7 \emptyset ALLs entered CR but half of them latter relapsed (two in consolidation and one in maintenance). CNS involvement during therapy was not detected in the subgroup of resistant/progressive patients. **Conclusions.** Within the limitations of the small patient number and the short follow-up we confirm the effectiveness of hyper-CVAD in *de novo* ALL. In contrast, results in relapsed ALL are poor. Infectious complications were significant despite the administration of growth factors and prophylactic antibiotics. Hyper-CVAD can prevent leukemia extension to CNS in both responders and non-responders.

1110**THE EFFECT OF COMBINING OF A HISTONE DEACETYLASE INHIBITOR WITH ARSENIC TRIOXIDE OR IMATINIB ON MYELOGENOUS LEUKEMIA CELLS**

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Introduction. Histone deacetylase inhibitors (HDI) are emerging as a class of anticancer therapeutic agents and have been demonstrated to induce differentiation in some myeloid leukemia cell lines. Butyric acid (BA) is a known differentiating

agent and HDI. Pivaloyloxymethyl butyrate (PIVANEX) a BA derivative was developed in our laboratory and recently it is used in combination with docetaxel in a multi-center randomized phase IIb study on NSCLC. PIVANEX was shown to induce histone hyperacetylation, apoptosis, and differentiation at several cell models. However, the most important effect of PIVANEX was the reduction in BCR-ABL protein levels in the K562 cell line, a model of Chronic Myelogenous Leukemia (CML). This hybrid protein is the translation product of t9;22 translocation existing in most CML patient cells, which has a high tyrosine kinase (TK) activity. Imatinib is a TK inhibitor which is effective for most CML patients. However, some patients in the chronic phase will develop drug resistance to Imatinib and most patients in the accelerated and blast crisis phase will not respond to it. To overcome these disadvantages several approaches were suggested among them combination of imatinib with chemotherapeutic drugs, HDI and combination of other drugs such as arsenic compounds. Arsenic trioxide (AS₂O₃) has been recently confirmed to be efficacious and safe in patients with first and subsequently relapsed or refractory acute promyelocytic leukemia (APL). Studies had shown that AS₂O₃ activity is required prior to inhibition of HD in order to induce differentiation in tRA differentiation resistance APL cells. It was shown that HDI as well as arsenic compounds act synergistically or additively with Imatinib in cytotoxic induction of K562 cells. **Aims.** To investigate the effect of PIVANEX combined with Imatinib or AS₂O₃ on K562 cells. **Methods.** K562 cells were cultured in the absence or the presence of PIVANEX, AS₂O₃, Imatinib and combinations of AS₂O₃ or Imatinib with PIVANEX at different concentrations and periods of exposure time. **Results.** Herein we show that in K562 cells PIVANEX induces viability reduction, differentiation enhancement, activation of caspase-3 and apoptosis. Moreover, it reduced the levels of the hybrid BCR-ABL protein. The combination of PIVANEX and Imatinib was additive but a synergistic effect on the reduction of BCR-ABL protein levels was seen. The combination of AS₂O₃ with PIVANEX was additive at all parameters. **Conclusions/Summary.** These results may suggest that PIVANEX in combination with Imatinib or AS₂O₃ compounds may offer an alternative treatment for CML patients which are resistant to Imatinib.

1111**IMMUNOHISTOCHEMICAL FINDINGS AND DIGITAL IMAGE ANALYSIS OF BONE MARROW ANGIOGENESIS IN MULTIPLE MYELOMA PATIENTS BEFORE AND AFTER FIRST LINE CHEMOTHERAPY**

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Tumor- induced neoangiogenesis plays an important role in tumor growth, progression, and metastases. Recently much attention has been devoted to the role of angiogenesis in the development of multiple myeloma. Digital image analysis (DIA) is a method of quantitative evaluation of a computer provided image. The main problem in applying DIA for morphometry is how to define the area for measurements. Immunohistochemistry allows to select specific antibodies and in an effect is suitable for precise determination of the object for evaluation. This study is an attempt of applying the DIA for quantitative evaluation of bone marrow angiogenesis. To study angiogenesis in multiple myeloma (MM), we evaluated bone marrow biopsy from 22 patients (12 females and 10 males), in the age range 41-75 years. To evaluate the effect of first line chemotherapy on angiogenesis, we analyzed bone marrow biopsy specimens before and after standard chemotherapy. Thin sections of bone marrow biopsies were stained by indirect immunohistochemical technique with monoclonal antibody anti-CD34 to highlight endothelial cells. The intensity of staining of each sample was examined by 3 independent investigators, who identified area of neovascularization. Digital image analysis of bone marrow angiogenesis was estimated in three ways. Initially, entire specimens were scanned using light microscope. Each slide was assigned an angiogenesis grade (+1 to +3) based on visual assess-

ment. Each slide was scanned at x100 magnification to determine an area of maximum numbers of microvessels (hot spot). Microvessels were measured at x400 magnification. Finally each slide was measured at x400 magnification in three areas without previous preselection of spot. Quantity and area of CD34 stained object were measured and statistically analysed. In the bone marrow of an untreated MM, we showed in 8 patients (pts) mild density (+1) microvessels, in 11 pts moderate (+2), and in 3 pts severe density (+3) microvessels. In the post-treatment analysis 15 patients (68%) did not reveal any changes in the density of microvessels. In 4 patients (18%) we observed an increased density, and in 3 patients (14%) decreased density of microvessels. We showed that microvessel density of the bone marrow correlated with the clinical course and with survival of myeloma patients. First results show quite good usefulness of digital image analysis for objective evaluation and statistic analysis of bone marrow angiogenesis grade as well as observation of its dynamics. In the present study, we have shown that the primary, conventional chemotherapy may be not effective for impairing tumor-angiogenesis in the bone marrow of MM patients.

1112

REVERSAL OF ACUTE MULTIPLE MYELOMA (MM) - INDUCED RENAL FAILURE BY BORTEZOMIB-COMBINATION THERAPY. A REPORT OF THREE CASES

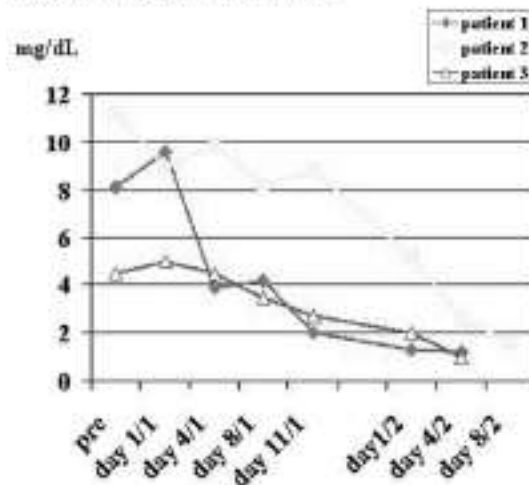
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Background. Acute renal failure (ARF) is a severe complication of MM and may occur de-novo in newly diagnosed pts or in pts with known MM and progressive disease. Only rapidly effective myeloma therapy can prevent progression into chronic renal failure and dependence on chronic haemodialysis. **Aims.** To evaluate the efficacy of Bortezomib based regimens (B-DD = Bortezomib 1.3mg/m², Doxorubicin 9 mg/m², Dexamethasone 40 mg on d 1, 4, 8, and 11 of each 21 cycle, or B-D = Bortezomib 1.0mg/m², Dexamethasone 40 mg on d 1, 4, 8, and 11 of each 21 cycle) in reversing acute myeloma-induced renal failure. **Patients, Methods, and Treatment:** Three pts with myeloma-induced ARF were studied. Two were admitted with ARF (creatinine 11.2 mg/dL and 8.1 mg/dL, respectively) and newly diagnosed MM (pt 1 and 2). Another pt (pt 3) developed myeloma-induced ARF during progressive disease (creatinine 4.5 mg/dL) after failing 3 treatment lines (Thalidomide, VAD, Dexamethasone). The 2 newly diagnosed pts were immediately treated with B-DD and initially put on haemodialysis. In the patient with progressive disease Bortezomib was combined with Dexamethasone alone (B-D). **Results.** All three patients responded to the Bortezomib based combination therapy. A reduction of proteinuria by >50% was seen within 2 weeks in all patients. Two patients finally entered CR and one very good PR (pt 3). Creatinine concentration normalized in all three patients (creatinine 1.1 mg/dL, 1.2 mg/dL and 1.3 mg/dL) (Figure 1) and hemodialysis could be discontinued within 2 weeks in pts 1 and 2. After a median follow up of 3 months (range, 2-5 months) all pts are in remission with normal or near normal renal function. **Summary/conclusions:** Myeloma-induced renal failure is the consequence of nephrotoxic light chains that lead to apoptosis of tubular lining cells and tubular obstruction by cell debris and protein casts. If this process can not be stopped rapidly, renal damage becomes irreversible. In the pre-Bortezomib era, myeloma therapy was neither very effective nor fast acting. Hence, only a small number of patients with myeloma-induced ARF were salvaged from haemodialysis. Bortezomib combination therapy has previously been shown to be highly active in newly diagnosed pts with an overall RR of 93% (Cavenagh JD et al. ASH 2004). We used a variation of this combination with 2 or 3 drugs given sequentially (Dex∂Dox∂B or Dex∂B, respectively) on each treatment day in order to fully exploit Bortezomib's potential to inhibit DNA repair. The rapid decline in paraprotein excretion observed in our patients during B-DD or B-D therapy

was accompanied by a concurrent improvement in renal function. These encouraging results render hope that many more pts with myeloma-induced ARF may in the future be salvaged by Bortezomib combination therapy, although confirmation of these promising findings is needed.

Figure 1 Creatinine Serum-Levels in Patients Treated with Bortezomib + Adriamycin + Dexamethasone (Pts. 1 and 2) or Bortezomib + Dexamethasone (Pt. 3)



1113

HIGH PREVALENCE OF HEPATITIS C VIRUS (HCV) INFECTION AND FAVORABLE PROGNOSIS IN PRIMARY HEPATIC LYMPHOMA (PHL)

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Background. Primary Hepatic non-Hodgkin lymphoma (PHL) is a rare disease, representing 0,4% of extranodal non-Hodgkin's lymphomas. To date, less than 150 cases have been published. We report eight patients with PHL diagnosed between 1995 and 2004 at our center, with a study of the viral status and the result of cytotoxic treatment. **Results.** Eight patients with PHL were identified. The disease occurred in middle-aged men (median age: 58 years). The main presenting complaint was right upper quadrant abdominal pain (4/8 patients). Tumour markers (α-fetoprotein and CEA) were normal in six patients tested. Liver scans demonstrated either a solitary nodule or multiple lesions. Pathologic examination revealed diffuse large cell lymphoma in 6 patients, a case of centrocytic lymphoma and a case of T cell lymphoma. Five patients were HCV-positive. Seven patients received chemotherapy (CEOP), while a patient with a single focal lesion received surgical treatment. The complete remission rate was 100% (8/8); one patient, who had HCV-related cirrhosis, died of hepato-renal syndrome in complete remission from lymphoma, and another patient died of myeloid leukemia. Median survival is 70 months (from 6 to 108 months). **Conclusions.** The outcome of patients with PHL who are treated with combination chemotherapy seems excellent. The frequent association of PHL with HCV infection suggests a possible role of this virus in lymphomagenesis. HCV-infection does not appear to influence the outcome of therapy.

1114**ACUTE LYMPHOBLASTIC LEUKEMIA; A SINGLE CENTER EXPERIENCE**

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Background. Acute lymphoblastic leukaemia (ALL) has a standard of treatment in conventional protocols for adults and children which generally do not include bone marrow transplantation as up-front therapy, except for those patients who have t(9;22) or t(4;11) or are at high risk for other factors. The cure is almost obtainable in about 25% of adults and 50% of children. **Aim.** To demonstrate if the early therapeutic approach with bone marrow transplantation (BMT) is curative in a higher number of patients. **Patients and Methods.** Since 1993 we treated a total of 43 patients with ALL with an age of 60 years or less, 11 patients were children. The GIMEMA for adults or AIEOP protocols for children were applied to all patients but those with high risk disease for t(9;22) or t(4;11) were addressed to BMT following consolidation therapy as up-front therapy and those without bad karyotypic characters were addressed to BMT at relapse following salvage therapy. Those patients with high risk characters without available donor underwent ABMT in remission. Twenty-one patients received only conventional therapy, 9 patients at high risk for karyotypic characters or relapsed without donor received conventional therapy and ABMT, 13 patients at high risk or relapsed received BMT from family or unrelated donor. **Results.** Among the 21 patients who received only conventional therapy 8 are in CR, 12 to 120 months from the therapy; 5 of them are children. One patient, relapsed from conventional therapy, is waiting for MUD transplantation and 11 patients died for disease progression and one for concurrent Gram- sepsis during induction. Among the 9 patients at high risk who received high dose therapy following remission and ABMT only one is alive and in CR 16 months from the procedure and 8 patients died for disease progression. Among the 13 patients who received BMT 8 patients are in CR 6 to 64 months from transplantation; 3 of them are children. The type of BMT was from matched family member in 5 patients with 4 of them in CR, matched unrelated in 6 patients and 4 of them in CR and two Aploidentical from family member, both died for infection. **Comment and conclusions.** Our experience, although limited to a small number of patients clearly indicated that fully matched BMT is curative in a higher proportion of patients as compared to conventional and high dose therapy followed by ABMT. Due to low incidence of complications BMT should be recommended in young adult patients when a matched donor is available as up-front therapy. The BMT in children should be addressed following relapse to conventional therapy.

1115**NOVEL CHROMOSOMAL TRANSLOCATIONS, T (14;17) (Q32;Q21) AND T (2;16) (P23;P13), IN LYMPHOID MALIGNANCIES**

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Background. Chromosomal aberrations play an important role in diagnosis and prognosis of hematological malignancies. **Aims.** We report two cases of lymphoid malignancies (acute B-lymphoblastic leukemia and anaplastic large-cell lymphoma) showing two undescribed reciprocal translocations, t(14;17) (q32;q21) and t(2;16) (p23;p13), respectively. **Methods.** Conventional cytogenetic analysis (CCA) was performed on 24-hour unstimulated bone marrow cells (case 1) and lymph node cells (case 2). Chromosomes were G-banded with Wright stain. Karyotypes were described according to the International System for

Human Cytogenetic Nomenclature (1995). Fluorescent in situ hybridization (FISH) technique was carried out on destained G-banding metaphases using a dual-color IgH break-apart probe (case 1) and a dual-color ALK break-apart probe (case 2), following conditions provided by the manufacturer (Vysis, Downers Grove, Illinois, USA). **Results.** The first patient was diagnosed with an acute B-lymphoblastic leukemia. CCA revealed a 47,+10,t(14;17) (q32;q21) karyotype in all 20 metaphases. Metaphase-FISH with dual color probe for IgH locus showed split hybridization signals on the der (14) and der (17), confirming rearrangement of IgH due to t(14;17). The second patient presented an anaplastic large-cell lymphoma. CCA showed a complex karyotype including a variant t(2;16) (p23;p13). FISH analysis using a dual-color ALK break-apart probe displayed a fusion hybridization signal on the normal chromosome 2 whereas the 3' portion of the ALK gene was translocated to chromosome der (16) and the 5' portion of the ALK gene was deleted. Cytoplasmic ALK expression was demonstrated on paraffin-embedded lymph node section by ALK-1 immunohistochemical staining. **Conclusions.** To our knowledge none of the above chromosomal translocations have been described previously in association with acute lymphoblastic leukemia and anaplastic large-cell lymphoma. Molecular characterization is currently in progress.

1116**COMPARISON OF FOUR LABORATORY TECHNIQUES FOR DETECTION OF PNH CLONE**

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal hemopoietic stem cell disorder with chronic, intermittent intravascular hemolysis. A defect in GPI anchor synthesis results in loss of GPI anchored proteins from cell surface. DAF and MIFL detected by CD55 & CD59 respectively are reduced on PNH cells and their loss results in increased sensitivity to complement mediated lysis. Ham's test and sucrose lysis tests are based on increased sensitivity of PNH cells to complement mediated lysis. Gel card test and flow cytometric methods are based on loss of GPI anchored proteins on cell surface. **Aims.** In this study we compared four **Methods.** Ham's test, sucrose lysis test (SLT), gel card test (GCT) containing CD55 & CD59 antibodies and flow cytometric (FCM) immunophenotyping for detection of PNH clone. **Methods.** Forty-five patients clinically suspected to have PNH defect were evaluated by all the four methods i.e. Ham's test, Sucrose lysis test, GCT and FCM using CD55 & CD59 monoclonal antibodies. **Results.** A PNH clone was detectable in 9 patients by FCM and in 7 by GCT. Two cases, which were negative by GCT, had Type III cells and type II cells ranging from 0.6-8% and 5.5-35.5% respectively. Ham's and SLT were positive in 4 cases that were also positive by GCT. In five cases, negative for Ham's and SLT, type III cells deficient in CD59 ranged from 0.1-13.8% (Figure-1). **Conclusions.** Ham's test and SLT showed concordance and could detect PNH when type III cells with CD59 deficiency were more than 18%. Gel card test is a sensitive method as compared to Ham's and SLT, more sensitive to type III cells and results correlate with total number of type III and II cells rather than type III cells alone. Flow cytometry is, however, superior for detection of small clones especially in cases with low number of type III cells or cases with type II cells alone.

1117**TRANSITION OF POLYCYTEMIA VERA TO CHRONIC MYELOID LEUKEMIA**

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Background. Clinical course of Polycythemia Vera (PV) is char-

acterized by a variable risk of disease transformation into myelofibrosis with myeloid metaplasia (MMM), myelodysplastic syndrome (MDS) and less often to acute leukemia (AL). The development of chronic myeloid leukemia (CML) in the course of PV is extremely rare. **Aim.** To cytogenetically and molecularly characterize the transition of a case of PV to CML. **Case Report.** A 77-years-old Caucasian female was diagnosed as having a PV in September 1996. She showed only a just palpable spleen. Hb 13.3 g/dL, Htc 50, WBC $10.7 \times 10^9/L$ with normal differential. Platelets $712 \times 10^9/L$. LAP score 98 (n.v.40-60). Red blood cell mass 55mL/Kg. Arterial oxygen saturation 96.6%. Bone marrow biopsy and aspirate showed marked erythroid and prominent megakaryocytic hyperplasia. Cytogenetic study on bone marrow cells showed a normal female karyotype. RT-PCR analysis of RNA extracted from bone marrow cells showed no rearrangement of bcr gene. Phlebotomies and hydroxyurea (HU) (0.5-1 g/day) were started. One year later, a moderately enlarged liver (3 cm below costal margin) and spleen (0.5 cm) were found. Polycythemia was controlled by phlebotomies and intermittent course of HU for 8 years. On July 2004, the patient suddenly showed the typical features of CML. The physical and ultrasound examination showed an enlarged spleen (3 cm) and liver (3 cm). Hb 10.8 g/dL, Htc 36.8, WBC $160 \times 10^9/L$ with neutrophils 51%, eosinophils 1%, basophils 1%, lymphocytes 4%, monocytes 14%, promyelocytes 1%, myelocytes 7%, metamyelocytes 15% and blasts 6%: Platelets $39 \times 10^9/L$. LAP score was 8. Bone marrow aspirate showed an huge granulocytic hyperplasia. Cytogenetics showed the 46,XX,t(9;22) karyotype in the majority of examined bone marrow cells (Table 1). Rt-PCR revealed the presence of the BCR-ABL chimeric transcript, b2a2 subtype (p210). The patient developed a refractory bilateral pneumonia and died nine days later. **Discussion.** In the setting of chronic myeloproliferative disorders, a transition from one subgroup to another is possible, either as natural evolution or owing myelosuppressive treatments. Among the seven reported cases of transition of PV to CML, five have not been investigated for the presence of chromosome or bcr rearrangement during the PV phase. Five of such cases underwent radioactive phosphorus or chlorambucil, but one developed CML in absence of any therapy. Radiations and alkylating agents are known to predispose to leukemogenesis. For the seven reported cases and included our own, and in absence of a marker for the Ph-negative diseases, whether the transition of PV to CML might be a natural or clonal evolution, a treatment dependent progression or a fortuitous occurrence of two distinct disorders, remain to be elucidated.

1118**IMMUNOPHENOTYPE FEATURES AS PROGNOSTIC FACTORS IN ACUTE MYELOID LEUKEMIA**

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Background. the recent WHO classification for acute myeloid leukemias (AML) separates entities by recurrent cytogenetic abnormalities and immunophenotypic features, with prognostic importance. **Aims.** to examine the distribution and impact on survival of patients with AML of the expression of several lineage and maturation linked antigens used in routine immunophenotyping. **Methods.** only patients with *de novo* AML were studied. Cases were diagnosed by peripheral blood counts, bone marrow cytology, cytochemistry, cytogenetics and immunophenotyping (CD45, CD117, CD33, CD13, myeloperoxidase, HLA-DR, CD34, CD7, CD19, CD14, and CD56). Antigen expression was measured by mean fluorescence intensity (MFI) by flow cytometry (Paint-a-gate software). Overall survival was recorded. **Results.** 35 patients entered the study. Median age: 51 years (11-79). By morphology/cytochemistry: AML without maturation: 8 cases; AML with maturation: 10 cases; promyelocytic leukemia: 4 cases; myelomonocytic leukemia: 12; acute erythroid leukemia: 1 case. Median peripheral leuko-

cyte count: $16.8 \times 10^9/L$ (0.8-150) MFI of CD45 was 16.1 (0.5-79.6) CD13 was positive in all cases, and CD33 in all but one. CD34+ was positive in 15 cases, CD7+ in 5 cases, CD19+ in 2 cases, CD14+ in 5 cases, CD56+ in 12 cases. Myeloperoxidase was negative in 12 cases. In 21 patients, only one population could be individualized by immunophenotyping, but in 13 cases there were 2 sub-clones. The case of erythroid leukemia presented 3 sub-clones. According to karyotype (26 cases) 7 were low risk: 4 t(15;17), 1 t(8;21) and 1 inv (16); 14 were intermediate risk and 5 were high risk. Survival analysis by the Kaplan-Meier method for categorical variables was significant only for karyotype ($p=0.05$) and presence of expression of CD14 (independent of the positivity for alpha-naphthyl-esterase). In the Cox model, age, peripheral leukocytes at diagnosis, MFI of CD45 and CD14 were independent parameters of a worse survival. **Conclusions.** besides karyotype, clinical and phenotype features (expression of CD45 and CD14) were important for the outcome of AML. Measuring the fluorescence intensity of the antigens, we could detect prognostic parameters (MFI CD45) as well as the presence of sub-clones. These did not influence survival, but may complicate studies of minimal residual disease by flow cytometry, as chemotherapy may select one of the sub-clones. Supported by FAPESP and CNPq

1119**INCREASED INCIDENCE OF HERPES ZOSTER IN RELAPSED MULTIPLE MYELOMA PATIENTS RECEIVING BORTEZOMIB: SINGLE INSTITUTION EXPERIENCE**

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Background. Recently, BORTEZOMIB, a potent inhibitor of proteasome, has been introduced into clinical use: it inhibits the activation of NF-kappaB, which controls the genes encoding IL-6, TNF- α and other cytokines and growth factors. **Methods.** In our experience 5 patients (2 F, 3 M; median age 61 yr) with relapsed Multiple Myeloma after standard therapy and ABMT were treated with BORTEZOMIB at a dose of 1,3 mg/m² on days 1, 4, 8 and 11 of a 21-day cycle, for a maximum of eight cycles. **Results.** Four of five patients completed the full treatment and were evaluated for response; one patient did not complete the therapy because of PD. Three patients achieved a PR and 1 patient a minimal response according to the EBMT/IBMT criteria. Two of four patients presented severe neutropenia which required the use of G-CSF; only 1 patient was hospitalized for septicemia. In 3/4 cases platelet infusions were performed for severe thrombocytopenia. But, in 3/4 patients the most clinically relevant adverse event was 3° WHO disseminated Herpes zoster; 1 patient developed viral infection 16 days after the end of treatment while in 2 patients Herpes zoster showed during treatment; in these two cases BORTEZOMIB was momentarily discontinued. The clinical picture resolved completely after valaciclovir therapy. In our experience no CMV reactivation was found. All our patients, evaluated before treatment with BORTEZOMIB, showed a deep depletion of CD4+ T-cells (median 150 cells/mL [normal values 500-1300/mL]) for immunosuppressive properties of previous regimens; but, despite an increased risk of developing opportunistic infections, during the long-term follow-up, no evidence of viral infections was detected. **Discussion.** In our single clinical experience Herpes zoster was the most clinically relevant adverse event. These observations are probably of particular clinical relevance for viral complications might not be a simple coincidence. In our patients high-dose myeloablative chemotherapy for ABMT and previous conventional regimens were certainly responsible of T-helper depletion; nevertheless, the decreased levels of CD4+ were not never associated with severe viral infections. The subsequent treatment with BORTEZOMIB did not contribute a further reduction/depletion of the total number of CD4+ cells. Authors demonstrated that BORTEZOMIB suppresses *in vitro* T-cell responses, inhibits cytokine production. Thus, CD4+ T-cells of our patients might be dysfunctional; if compared to memo-

ry T-helper of normal individuals they might show reduced capacity to secrete cytokines; their anergy might be the consequence of the inhibition of the ubiquity proteasome system; Further studies of BORTEZOMIB and additional clinical observations are now needed to establish and explore its full spectrum of activity. **CONCLUSIONS.** The high incidence of Herpes zoster in our small cohort of patients, suggests that viral infections might be probably related to an intrinsic defect of memory CD4+ T-cells specific for VZV, induced by BORTEZOMIB. Because of an increased risk of developing opportunistic infections, we recommend anti-infective prophylaxis with antiviral drugs and trimethoprim-sulfamethoxazole all therapy long and until at least three months after drug discontinuation as well as monitoring CD4+ cell levels.

1120

BRUCellosis OCCURRED DURING THE COURSE OF FEBRILE NEUTROPENIA DUE TO AGRANULOCYTOSIS: CASE REPORT

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Background. Brucellosis is one of the leading diseases in the differential diagnosis of fever of unknown origin in Turkey. It can cause to treatment failure because of slow growth in blood cultures and late appearance of signs and symptoms in patients with febrile neutropenia (FN) who were unresponsive to empirical antibiotic treatment. Agranulocytosis is generally a clinical condition of typically profound neutropenia (<500/ μ L) with concomitant severe infection and mucositis. Infectious agents as well as non chemotherapeutic drugs are reported in the etiology of agranulocytosis. **Aims.** In this study we aim to present the clinical findings and molecular characterization of an adult patient with brucellosis occurred during the course of FN due to agranulocytosis originated from the use of metimazol. **Case:** H.T. 21-year-old female patient admitted to our hospital with 15 days of fever (39.2°C) and mucositis complaints in September 2004 and hospitalized for further investigation. On her first physical examination; her general appearance was poor; with multiple white aphthous mouth lesions and hepatosplenomegaly of 1 cm was detected. White blood count was 7/ μ L, hemoglobin: 9,6 gr/dL, Htc: 32%, platelet: 201000/ μ L on her hemogram. Her bone marrow aspiration revealed significant decrease of granulocytes site but normal ranges of erythroid and megacaryocyte sites. The patient had been administered metimazol sodium for analgesic and/antipyretic purposes during her septic operation 2 weeks ago. Agranulocytosis was diagnosed depending on all these findings and treatment with filgrastim 300 μ g/day as set on. Empirically, Imipenem/amikacin therapies were also begun for profound neutropenia and fever. Prior to antifungal treatment, the patients were re-evaluated because of the history of unpasteurized milk ingestion without overt signs and symptoms. Nevertheless, fever couldn't be controlled within 5 days, so amphotericin B was added to therapy. On the 14th day of the medical therapy, while neutropenia and fever were going on, Serum agglutination tests of brucellosis were performed and were negative. *Brucella* spp was identified in multiple blood cultures. Empiric antibiotic treatment was stopped and rifampisin 600 mg/day (42 days) /doxycycline (200 mg /day (42 days) were given. Follow-up examination neutropenia and fever subsided in 2 days. **Conclusions.** Pancytopenia, are mainly reported in adults with brucellosis. However, agranulocytosis is a rare feature of the infection. In our case, we studied Codon 54 and codon 57 polymorphisms in the exon 1 of the MBL gene which are important for the susceptibility to infections with PCR-RFLP on the patient's DNA samples and heterozygote MBL gene polymorphism (AB allele) was shown. To our knowledge, no searches about this mutation in febrile neutropenia associated with brucellosis are studied. Thus, longitivity of neutropenia and increase of bloodstream infections were reported in FN linked

with this mutation. In our country, 10 cases with FN derived from *Brucella* spp. were reported. The difficulties of mainstay of diagnosis and its association with MBL gene was discussed on this paper. Due to high incidence of brucellosis in some geographic areas, especially in Turkey, brucellosis should be kept in mind in the differential diagnosis of FN.

1121

THERAPEUTIC IMPLICATIONS OF PET CT STUDIES IN HODGKIN LYMPHOMA PATIENTS TREATED WITH ESCALATED BEACOPP REGIMEN- CENTER FIRST EXPERIENCE

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Introduction: A typical problem of post treatment assessment of HD is a differential diagnosis of residual mass: fibrosis versus partial regression. CT scans can answer this question only indirectly, showing the (lack of) dynamic change in subsequent examinations. 18 FDG PET is the method of choice, demonstrating metabolically active lesions. **Methods.** 18 - FDG PET was performed in 18 high risk HD patients (IIBX-IV) with a residual mass after the end of first line therapy with escalated BEACOPP regimen. **Results.** 18-FDG PET confirmed complete response in the all 8 patients with a residual mass stable in a repeated CT scans, 12-36 months after the end of therapy. One out of further 2 patients, examined at the time of suspected relapse, suggested in CT studies, occurred positive in PET scan. 2 out of 8 patients examined after the end of the first line therapy, were PET positive. Metabolic activity did not correlate with dimensions of the residual mass: PET scans were positive in 1 out of 4 patients with lesions < 3 cm, and 1 out of 4 patients with lesions greater than 7 cm. **Conclusions.** In our study 18-FDG PET correlated with all CT results indicating stable residual mass. When performed at suspected relapse or after the first line therapy, PET results, changed therapeutic decisions in 5 out of 10 cases. Further therapy (high dose therapy supported by autologous SCT) was not initiated in 4 out of 6 patients (1 of 2 with suspected relapse, 3 of 4 with suspected inadequate partial regression). The additional therapy was initiated in 1 out of 4 patients with relatively small residual mass.

1122

ANALYSIS OF THE FVIII GENE IN PATIENTS WITH HIGH PLASMA FACTOR FVIII:C LEVELS IN SERBIAN POPULATION

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Background. Venous thrombosis is a multicausal disease, in which more than one inherited and acquired factor may be involved. High FVIII:C levels have previously been shown to be an independent risk factor for venous thrombosis, but the mechanisms that cause high factor VIII:C levels are unclear. **Aims.** We hypothesized that increased activity of FVIII protein may be caused by possible presence of polymorphisms in the FVIII gene, especially in the regions important for protein inactivation and clearance. Therefore, we analyzed parts of gene coding for residues responsible for interaction with activated protein C (APC), thrombin, FIXa, and FXa. We also analyzed regions coding for residues involved in binding with low-density lipoprotein receptor-related protein (LRP), and heparan sulphate proteoglycans (HSPGs). **Methods.** A study was carried in the group of 45 unrelated consecutive patients with clinical features of the inherited thrombophilia and FVIII:C > 1.5 u/mL. Patients with malignancy, antiphospholipid antibody syndrome, and defects of antithrombin, protein C and protein S were excluded from the evaluation. We screened exons 8, 10 and 11 of the FVIII gene using conformation sensitive gel electrophoresis (CSGE).

Results. and Conclusions. No polymorphisms were identified, however further studies with larger samples of venous thrombosis patients are recommended. It would also be useful to analyze other FVIII gene regions that may influence on FVIII:C level, that is on FVIII expression.

1123

MICROVASCULAR DENSITY IN MULTIPLE MYELOMA, INFLUENCE ON PROGNOSIS

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Background. Multiple myeloma (MM) is disease with median of survival from 3 to 5 years. It is disease with wide interval of overall survival (OS). One of the newest prognostic factors is microvascular density of bone marrow (MVD), which evaluates degree of angiogenesis. **Aims.** Influence of MDV on OS in patients with MM. **Methods.** MVD was evaluated from bone marrow by antibody anti-CD 34. We used samples of spongiuous bone from iliac crest at the time of diagnosis assessment. MVD was graded as low, middle or high in 32 patients with MM. All the patients were treated by autologous stem cell transplant. The watching period was minimally 5 years in all patients. **Results.** 10 patients had high grade of MVD and their median of OS was 38 months, 14 patients had middle grade of MVD and median of OS was 62 months in this group and 8 patients had low grade of MVD and median of OS was 78 months. **Conclusions.** There were statistical significant different in OS in patients with different grade of MDV. Patients with high grade of MVD had significantly shorter OS than patients with low and middle grade of MVD.

Supported by grant No. 8076-3 from Ministry of Health of Czech Republic.

1124

CHINESE HERBS DECOCTION BEVERAGE INDUCING SIGNIFICANT RESPONSE IN PROGRESSIVE B-CLL AND IN VITRO APOPTOSIS ON B CLL CELLS

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An article published recently in Leukemia Research (2003, 27) reported a complete remission in a CLL patient treated only by Chinese herbs which origin was not defined. Biological studies performed at the DFCI in Boston found that these herbs induced a significant apoptosis of B-CLL cells *in vitro*. A 80-year-old biologist was followed in our Institute of Hematology for several years because of B-CLL that progressed slowly to stage Binet B, including high lymphocyte count ($80 \times 10^9/L$) and splenomegaly. This patient who by himself read this article, was very interested to try these Chinese herbs and he asked the article authors to refer him to this Chinese medicine, unsuccessfully. Finally, he decided to ask a well-known Israeli herbalist to prescribe him Chinese herbs. These herbs mixture had to be decocted in water and this dark bitter liquid extract was recommended to be taken every day at least 0.6 liter per day. We should emphasize that our Institute was not involved in the decision of the patient to take this medication. But during the routinely examination we observed a slowly decrease of the lymphocyte count dropping down to $6 \times 10^9/L$ after 6 months of this treatment. The physical status of our patient currently is excellent, the spleen is palpable only on inspiration. In view of the exceptional effect of the herbal extract in this patient, we attempted to study the effect of the extract on apoptosis *in vitro* on B CLL cells in culture for 20 hours to 5 days with 1:10, 1:20 and 1:100 dilutions of the extract using a phosphatidyl serin detection kit for flow cytometry. We studied samples from 9 B CLL patients, all in advanced stages. In five cases a significant apoptosis (30-100%) was noted after 20 hours, and in 4 other

cases up to 90% of apoptosis was obtained after 3 to 5 days by adding the herbal extract every day. The optimal effect was equally with 1:10 and 1:20 dilutions. In comparison, the control cultures of B CLL cells remained viable after 5 days. In conclusion, a Chinese herbal decoction beverage (which formula is not yet known) induced a significant response in a progressive B CLL patient and apoptosis *in vitro* on B CLL cells from patients with advanced disease. We believe that this outstanding response looks out of interest, and in the next steps we will focus on segregation of the potential effect of these herbs.

1125

HUMAN CD154 PROTEIN TRANSFERRED ON TO CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL) B LYMPHOCYTES ELICITS AUTOLOGOUS ANTITUMOR ACTIVITY IN VITRO AND IN VIVO IN NOD-SCID MICE

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Background. CD154-based gene therapy, a promising tool in cellular immunotherapy of many hematological malignancies may, by activating the CD40 pathway, increase the malignant cell capacity to present tumor antigens. CLL is a good candidate for CD154-based gene therapy because leukemic B lymphocytes, by inhibiting T-cell production of CD154, become ineffective antigen presenting cells (APCs) due to the resulting lack of costimulatory molecules, such as CD80 and CD86, which are necessary for efficient T-cell activation. Several studies show tumor-reactive T cells exist in CLL, particularly in the early stages. Were their numbers and activation states to be enhanced, they could potentially mediate therapeutic responses. **Aims.** To prove that CLL B lymphocytes, expressing human CD154, are efficient APCs *in vitro* and *in vivo* in a NOD/SCID mice model. **Methods.** We transferred the hCD154 molecule by co-culturing CLL B lymphocytes with the HFL-1/hCD154+ cell line. Multi-parametric analysis with anti-CD45, CD3, CD5, CD19, CD40, CD154, CD80, CD86, CD54, CD95 was performed on B cells before and afterwards. After stimulation with hCD154+ B lymphocytes, autologous T cell proliferation was assessed by [³H]thymidine incorporation and cytolysis of CLL B lymphocytes by the annexin V assay. Control experiments were based on autologous CLL B lymphocyte stimulation of T cells. NOD/SCID mice were injected intraperitoneally with 40×10^6 hCD154+ B lymphocytes (group 1) or 40×10^6 T + 40×10^6 hCD154+ B lymphocytes (group 2). After 1 month flow cytometry checked intraperitoneal lavage fluid for engraftment and Minimal Residual Disease (MRD). **Results.** Values are expressed as mean percentages of positive cells. CLL B lymphocyte co-culture determined the transfer of hCD154 protein (from 0.1 beforehand to 73 afterwards. Immune accessory molecules were upregulated (CD80 from 3.4 to 70; CD86 from 8 to 71, the adhesion molecule CD54 from 8 to 72 and the apoptotic molecule CD95 from 5.7 to 81. Mean stimulation index of autologous T lymphocytes cultured with CD154+ B cells was 56.9 vs 10.6 in controls. Mean T cell cytotoxicity against autologous B-CLL, expressed as percentage of annexin V positive cells, was 42.6% vs 7.3% in controls. In NOD/SCID mice the mean percentage of engrafted human CD45+ cells was 13.11% in group 1 and 7.39% in group 2. Mean percentage of MRD (as evaluated by CD19+/CD5+ cells) was 44.9% in group 1 and 9.5% in group 2 ($p < 0.007$). Residual CD3+ cells in group 2 were 5.14%. **Summary/conclusions:** Successful intercellular transfer of hCD154 protein induces CLL B lymphocytes to become efficient APCs as we showed the *in vivo* leukemic burden is significantly reduced in the NOD/SCID mice model. Confirming this observations is a significant upregulation of co-stimulatory, adhesion and pro-apoptotic molecules which is associated *in vitro* with enhanced autologous T cell proliferation and cytotoxic activity against autologous B lymphocytes. This *in vivo* finding suggests that intercellular CD154 transfer on to B CLL lymphocytes, might provide a suitable cellular vaccine for use in clinical trials.

1126

THE EFFICIENCY OF NEW HEMOCORRECTOR IN COMPLEX TREATMENT OF IRON DEFICIENCY ANEMIAK.K. Bakirkhanova¹, N.M. Holmatova², M.K. Bakirhanov²¹Institute of Hematology and Transfusion, TASHKENT, Uzbekistan;²Institute of Hematology, TASHKENT, Uzbekistan

Iron deficiency anemia (IDA) takes the leading place in the Central Asia among all other pathologies. The frequency of IDA in women and children in some regions of Uzbekistan is about 80-90%. Purposes: 1. To investigate the indexes of ferrokinetic in fertile women suffering from IDA (first degree) 2. Treatment of fertile women suffering from IDA (first degree) Materials and Methods. 25 women with the first degree of IDA have been examined. Diagnosis of IDA verified according to the generally accepted clinic-lab criterions. The calculation of peripheral blood cells has been done on the hematological cell counter. The index of hemoglobin was conducted by colorimetric ferrocyanide method. Serum ferritin and transferrin were determined by immunofluorimetric method. Results. The patients were subdivided into 2 groups. 1st group comprised 10 women which received only ferrotherapy. The second group-15 women, got complex treatment(ferrotherapy plus Succinasol). Patients complained about general weakness, quick fatigability, giddiness, palpitation, paleness and dryness of skin, weak nails, hyperkeratosis, intensive hair falling and growing them dim, stomatitis, pica chloratica etc. Average indexes of hemograms: hemoglobin-100,8±4,3; erythrocytes: 3,6±0,5*10¹²; color index: 0,7±0,1; average maintenance of hemoglobin in erythrocyte was 21.5mkg, of reticulocytes: 4,1±1,8%. Considerable changes of thrombopoiesis and leucopoiesis have not been detected. The maintenance of serum iron is 7,1±1,5 mmoles/ liter. Content of serum transferrin was increased and coefficient of transferrin saturation decreased to 5,1±1,05%. Low level of ferritin testifies about emaciation of depot storage of iron (16,1±2,3 mkg per liter). Thus, in fertile women with IDA the serious dysfunctions of ferrokinetic have been established that expressed in decreasing of iron storage of an organism. In complex treatment of IDA in the 2nd group of patients we used ferriferrous medicines as well as a new blood substitute hemocorrector Succinasol that was invented in the Institute of Hematology and Blood Transfusion of Uzbekistan. The treatment course comprised 15 days, number of transfusions of Succinasol per course-3 times (1 injection per 5 days). A single dose of Succinasol - 200 ml Intravenously. The patients noticed improvement of their general physic condition; we detected an instant and stabile normalization of peripheral blood and ferrokinetic indexes. Treatment the 2nd group of patients was not effective for that period (15 days), normalization of the indexes hasn't been determined. Succinasol has a component of Krebs cycle-the amber acid, which has an unique ability to join in process of oxidation in conditions of hypoxia. As a result in a unit of time amber acid assists to synthesize more adenosine triphosphoric acid than during oxidation of other substrates. This priority makes Succinasol the most effective remedy in complex treatment of IDA. Learning the long term results (3 month) showed stability of achieved results in the 2nd group of patients. Summary. 1. In fertile women with IDA revealed profound dysfunctions of metabolism 2. Using the new hemocorrector Succinasol in complex treatment of IDA showed better results than traditional treatment.

1127

PERCUTANEOUS VERTEBROPLASTY (PVP) AN ALTERNATIVE THERAPY FOR PAIN RELIEF CAUSED BY VERTEBRAL COMPRESSION FRACTURES (VCF) IN PATIENTS WITH MULTIPLE MYELOMA. A PRELIMINARY REPORT

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Background. Percutaneous injection of bone cement in the ver-

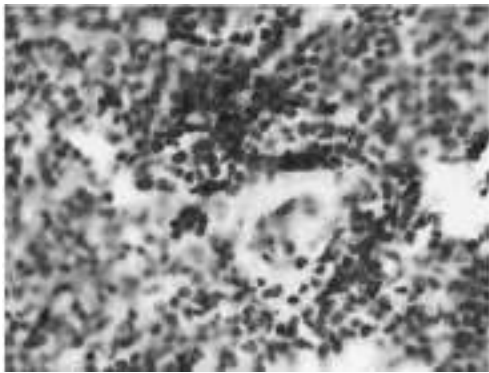
tebral body (PVP) is known to give pain relief and reinforce the vertebra in 80% of patients with VCF due to malignant destruction. There have been relatively few reports of the use of PVP in patients with multiple myeloma (MM). We describe the preliminary experience of treatment with PVP in four patients with MM. Aims. To evaluate the safety and effectiveness of PVP in the treatment of vertebral pain due to osteolytic destruction in patients with MM. Material: Four patients (two males and two females) between 47 - 78 years old and in different phases of MM received PVP treatment. Two patients were in first partial response, and two in relapse treatment. The vertebral destructions were localized to C2 and dens in one case and in Th10; Th7 and 8; Th 9 and L2 respectively in the remaining three patients. Methods. Patients with vertebral pain and corresponding VCF visualized by skeletal X-ray and CT/MRI, which did not respond well to conventional analgesics, were offered PVP. The study has been approved by the Regional ethical review board. The percutaneous injection of bone cement was performed according to local routines. General anaesthesia was required in one case (patient with C2 destruction); the remaining procedures were performed with conscious sedation. The patients were discharged from the hospital 24 hours after the procedure. The follow-up included a telephone call one week after PVP and the clinical controls at the outpatient clinic according to requirements for MM treatment. Results. Three out of four patients experienced pain relief after PVP. The follow-up showed a stable improvement, which lasted for 4 months, +7 months, +2 years respectively. In one patient no improvement was observed. There were no clinical complications. Conclusions. PVP resulted in rapid and stable pain relief without clinical complications in three out of four patients. The procedure may be considered as a valuable and safe option for symptomatic treatment of vertebral pain due to osteolytic destructions in selected myeloma patients. However, the criteria for treatment should be lead by a strict correlation between clinical and radiological features.

1128

CHEMOKINE RECEPTOR CXCR4 (CD 184) EXPRESSION IN LYMPH NODES OF LYMPHOMAS AND LYMPHOID REACTIVE TISSUEG. Mazur¹, T. Wrobel², A. Halon³, M. Jelen³, K. Kuliczowski²¹Wroclaw Medical University, WROCLAW, Poland; ²Dept. of Haematology, WROCLAW, Poland; ³Dept. of Pathology, WROCLAW, Poland

Background. Chemokines are proteins responsible for leukocyte migration and activation. Many of them have been identified within tumour tissues as a secretory product of tumour cells and/or inflammatory cells. Some chemokines induce tumour progression by interacting with the specific receptor expressed on the tumour tissue. Chemokine receptor CXCR4 have been shown to be expressed on normal and malignant B lymphocytes recovered from patients with different B-cell malignancies. CXCR4 and its ligand CXCL12 are widely expressed in normal tissues and play a fundamental role in foetal development, mobilisation of haematopoietic stem cells, and trafficking of lymphocytes. There are some reports that CXCR4 is expressed on a subset of B-cell lymphomas but little is known about T-cell lymphomas and reactive lymph nodes. Aims. The aim of the study was evaluation of expression of CXCR4 in lymph nodes of different types of lymphomas and lymphoid reactive tissue. Methods. Samples of the studied lymphomas (37) and reactive lymph node tissues (9) were fixed in 10% buffered formalin and then embedded in paraffin. The preparations were stained with hematoxylin and eosin and evaluated histopathologically. Deparaffinised sections were boiled in the citrate buffer to unblock antigenic determinants. In all studied cases sections were incubated with antibodies against CXCR4 (R & D Systems Inc., USA), CD3, CD20 (DAKO, Denmark). The DAKO Fast Red Substrate System was used as a substrate and chromogen in immunocytochemical staining procedures utilizing alkaline phosphatase. In each case the control was included, in which the specific antibody was omitted. For the CXCR4

evaluation slides were scanned in the light microscope at 400x magnification and 15 areas of lymph node were identified and cells with positive staining of CXCR4 expression were counted. For each slide mean number of CXCR4 was calculated per 1.0 mm². Statistical analysis was made with the ANOVA-rang Kruskal-Wallis test. Differences were considered statistically significant at p < 0.05. **Results.** Mean expression of CXCR4 in reactive lymph nodes was 143,43±113 per mm². In the group of B-cell aggressive lymphomas CXCR4 mean expression was 176,13±191,22 per mm² and in the group of B-cell indolent lymphomas was 158,74±218,81 per mm² and these group did not differ statistically. In T-cell lymphomas CXCR4 mean expression was 56,27±22,73 per mm² and was statistically lower than the others group. **Conclusions.** CXCR4 expression in all lymphoid tissues is observed. In T-cell lymphomas this expression is significantly lower than in B-cell lymphomas and reactive tissue. Expression of CXCR4 was slightly higher in aggressive lymphomas than in indolent lymphomas.



1129

NON- HODGKIN LYMPHOMA (NHL) AS A RARE MANIFESTATION OF IMMUNE RECONSTITUTION SYNDROME IN HIV-1 POSITIVE PATIENTS

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Background. The administration of highly active antiretroviral therapy (HAART) is associated with significant decrease in plasma HIV RNA and rise of CD4 T cells. This immune restoration has resulted in the decline of AIDS morbidity and mortality observed to a lesser extend in severely immunosuppressed individuals. However, improvement of the immunocompetence can lead sometimes to unexpected, paradoxical, atypical inflammatory reactions called IRS (immune reconstitution syndrome, especially in individuals with advanced immunodeficiency. The association of HIV-1 infection, HAART and non-Hodgkin lymphoma (NHL) has rarely been reported. IRS-NHL can be an example of paradoxical deterioration of the immune response. **Aims.** We report 4 cases of HIV-1 positive patients (pts) receiving HAART who developed NHL related to immune restoration syndrome. **Methods.** Clinical data and therapy are presented in tables 1. **Results.** There are patients who commence HAART and occasionally develop NHL despite effective antiretroviral therapy. This can be due to IRS. We observed 4 (25%) cases of IRS-NHL among 12 individuals with AIDS related lymphoma. All the patients were severely immunocompromised with very low CD4 (+) T lymphocyte count and high VL at the beginning of HAART, with no signs and symptoms of lymphomas. They reached viral suppression; in 1 case to undetectable levels by PCR, and in 3 cases partial, but with significant inhibition of viral replication. Mean time of achieving suppression of viral replication was 15 weeks. This was followed by CD4 (+) T cells rise within 16,5 weeks. Mean time to the occurrence of NHL after HAART was introduced was 31,5 weeks, after immune

restoration was established. This fact can indicate that immune reconstitution may have been a predictive factor for the development of NHL in our patients. The onset of the disease in our patients was abrupt and from the very beginning the disease was advanced and prognosis was poor, indicating that NHL had developed some time earlier and was not recognized. We did not perform any additional immunological examinations for better understanding of the events. The pathogenesis of AIDS related NHL is multifactorial, yet not well understood. In addition, pathogenesis of IRS-NHL seems to be more complicated. The mechanisms involved in IRD are not similar for all the diseases. There are only scanty reports concerning IRS-NHL, it's pathogenesis, manifestation and frequency. Our presentation is one more description of such an occurrence of NHL. **Conclusions.** One can expect improvement after effective HAART even in a patient with advanced immunodeficiency. The unexpected deterioration of the clinical state can be misdiagnosed. In any case of lymphadenopathy generalized or limited to the abdomen or periphery, IRS-NHL should be suspected within a few months after potent HAART had been initiated. These patients should be examined more often to avoid any delay in proper diagnosis if the symptoms occur and to start adequate chemotherapy as soon as possible.

Table. Data concerning HIV infection/AIDS and NHL.

Patient, age	Diagnosis type	Date	CD4 cell count (x10 ⁶ /L)	Viral load (log ₁₀ ml)	Chemotherapy	Date of death
			at the diagnosis of lymphoma			
F 28yo	Immunoblastic diffuse B cell lymphoma (CD20+) (CD43-)	11.2007	25	3.88	1 x CHOP (CHAD, 2MC, 1P) 03.07.2008	26.07.2008
F 58yo	Blastic T-cell lymphoma III (CD45+) (CD20+) (CD43+) (CD30+) (CD55) (CD56) (-)	01.2008	60	2.28	None	09.02.2008
M 58yo	Diffuse T-cell lymphoma (CD45+) (CD20+) (CD43-)	01.2008	81	4.58	1 x CHOP (CHAD, 2MC, 1P) 03.08.2008	24.02.2009
M 42yo	Follicle lymphoma (CD20+) (CD43+)	01.2008	87	2.88	1 x CHOP (CHAD, 2MC, 1P) 03.08.2008	28.03.2009

Patients, n°	Time (in weeks) from beginning of HAART to:			Survival time from diagnosis:		
	viral suppression	immune restoration	onset of lymphoma	HIV free (years)	AIDS months	Lymphoma months
F 28-yr	36	36	36	0	7	7
F 58-yr	6	11	44	12	Antipy	Antipy
M 58-yr	3	18	14	13	5	5
M 42-yr	NA	4	32	1	12	5

1130

SINGLE ORAL DOSE OF ICL670 HAS NO EFFECT ON SAFETY, TOLERABILITY AND STEADY STATE PHARMACOKINETICS OF DIGOXIN IN HEALTHY VOLUNTEERS

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Background. ICL670 (deferasirox) is a once-daily oral iron chelator in Phase III clinical development that has demonstrated good efficacy and tolerability in patients with transfusional iron overload. Digoxin is commonly used for treatment of heart failure, a common complication of α -thalassemia. It was important to establish whether a drug-drug interaction could impact the co-administration of digoxin and ICL670. **Aims.** To determine the effect of a single 20 mg/kg oral dose of ICL670 on the multiple-dose pharmacokinetics (PK), safety and tolerability of digoxin. **Methods.** Sixteen healthy male volunteers between

18–45 years were enrolled in this single-center, open-label, two-treatment, two-period crossover study. All received daily oral doses of digoxin-0.5 mg on days 1 and 22 and 0.25 mg on days 2–8 (period 1) and days 23–29 (period 2). Subjects also received a single oral dose of 20 mg/kg ICL670 immediately following the digoxin administration, either on day 8 or 29 depending on the treatment sequence. PK samples were collected pre- and up to 72 hours post-digoxin administration on days 8 and 29. Safety assessments included physical examinations, ECGs, vital signs and laboratory evaluations. *Results.* Fourteen subjects completed the entire study period and evaluable PK samples were available from 15 subjects. The steady-state digoxin PK parameters AUC and C_{max} were within previously reported ranges (Table 1) and were bioequivalent when administered alone or in combination with ICL670, since the 90% confidence intervals (CI) around their geometric mean ratios were within the limits of 0.8 and 1.25: AUC ratio 0.908, 90% CI [0.827, 0.997]; C_{max} ratio 0.933, 90% CI [0.822, 1.059]. ICL670 plasma concentrations, measured 2 and 3 hours post-dose (expected t_{max}), were within the range of previous clinical findings in patients with iron overload. Five subjects reported adverse events (AE) which were mild and not drug-related. No subjects discontinued due to an AE. There were no serious AEs, no clinically relevant changes in vital signs, laboratory parameters or creatinine levels. Nine ECG abnormalities were noted in six subjects; eight occurred on digoxin alone. None were clinically significant and all returned to normal by study end. *Conclusions.* A single oral dose of ICL670 following daily administration of digoxin has no notable effect on digoxin PK, safety or tolerability in healthy volunteers.

Table 1. Summary of digoxin pharmacokinetic parameters, with and without ICL670 (n=15)*

Parameter (mean ± SD)	Digoxin and ICL670	Digoxin alone
C _{15min} (ng/mL)	1.55 ± 0.31	1.75 ± 0.60
C _{30min} (ng/mL)	0.83 ± 0.18	0.69 ± 0.19
C _{60min} (ng/mL)	0.46 ± 0.17	0.46 ± 0.13
t _{1/2α} (h) †	1.50 (0.50, 2.50)	1.00 (1.50, 2.00)
AUC ₀₋₂₄ (h·ng/mL)	15.21 ± 4.45	16.62 ± 4.57
Fluctuation index (%)	189.76 ± 84.94	182.2 ± 36.43
Urinary excretion (%)	30.54 ± 12.00	44.37 ± 9.96
Clearance/fluctuation (L/h)	18.74 ± 9.57	16.09 ± 5.04
Renal clearance/fluctuation (L/h)	6.73 ± 2.35	7.12 ± 2.14
Non-renal clearance/fluctuation (L/h)	12.01 ± 7.98	8.47 ± 4.96
*Median (interquartile range)		
t _{1/2α} (h) †	1.50 (0.50, 2.50)	1.00 (1.50, 2.00)
AUC ₀₋₂₄ (h·ng/mL)	15.21 ± 4.45	16.62 ± 4.57
Fluctuation index (%)	189.76 ± 84.94	182.2 ± 36.43

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TREATMENT OF REFRACTORY ACUTE MYELOGENOUS LEUKEMIA WITH ARSENIC TRIOXIDE AS MONOTHERAPY AND IN COMBINATION WITH CHEMOTHERAPY

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Introduction. The prognosis in patients (pts) with resistant and relapsed acute myelogenous leukemia (AML) is very poor. Among new drugs, tested in therapy of refractory AML is arsenic trioxide (ATO). ATO induces complete remission (CR) in 85% of relapsed pts with acute promyelocytic leukemia (APL). There are *in vitro* evidences that ATO induces apoptosis, exerts anti-proliferative effect and overcomes multidrug resistance to chemotherapy in blast cells from pts with non-APL AML. *Methods.* Based on these promising reports we applied ATO in pts with resistant and relapsed AML. In our group there were 8 pts (3M/5F with median age: 43.0 years; range: 18-68). In 4 pts (1M/3F with median age: 53.7 years; range: 36-68; the first group) we applied ATO as single agent (0.25 mg/kg, admin-

istered intravenously over 2 hour for four-five weeks - 20-25 doses). Three of these pts had primary resistant and one had relapsed AML. In 4 younger pts (2M/2F with median age: 32.2 years; range: 18-55; the second group) we applied ATO (0.25 mg/kg/day - 10 doses) with ICE regimen (idarubicin, cytarabine, etoposide). *Results.* The most often adverse event in the first group was elongation of QT in ECG. In all pts disease progressed during ATO therapy. The median survival following the first dose of ATO in this group was 2.6 months (2.0-4.0). In the second group the oldest pt died because of sepsis in aplastic phase (F; age: 55 years) and in one there was leukemia regrowth (M; age: 18 years). CR was obtained in 2 pts (50% pts treated with ICE+ATO). In one of these pts (F; age: 36 years) AML relapsed after allogeneic bone marrow transplantation (alloBMT), and one (M; age: 20 years) had primary resistant leukemia. First pt underwent second alloBMT and second pt is now prepared for alloBMT. Toxicity in ICE+ATO group included infection (WHO III/IV - 75%), mucositis (WHO II - 50%) and atrial flutter after ATO infusion (1 pt). The median survival following the first dose of ATO in ICE+ATO group was 8 months (range: 2.0-16.0), the median disease-free survival (DFS) in 2 pts, who obtained CR was 9 months (range: 5-13). *Conclusions.* Our preliminary results in very small series of haevily pretreated pts with refractory AML show that single ATO therapy is not effective. The ATO+ICE regimen has a significant activity and may be very valuable, especially for pts expecting a suitable donor for alloBMT. Further studies on a larger group of pts are warranted to confirm our observations.

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MOLECULAR DETECTION OF BONE MARROW (BM) INVOLVEMENT IN PRIMARY CUTANEOUS B CELL NON-HODGKIN LYMPHOMA (PCBCL) AT DIAGNOSIS

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Background. Although the skin is the second most common site for extranodal lymphomas, only 20% of cutaneous NHL are of B cell origin. A wide range of B cell lymphomas can occur as primary cutaneous tumours but their clinical course can not always be predicted by histology alone. PCR-based analysis of immunoglobulin genes is commonly used to assess clonality in B cell lymphoproliferations. However, the incidence and significance of the presence of clonal B cell populations in the BM of affected patients at the time of diagnosis are presently unknown. We sought to determine the incidence of occult BM involvement in a series of 12 patients (3 women and 9 men, median age 54.5, 28 to 77 years old) diagnosed with PCBCL between August 2000 and May 2004, for whom skin biopsies, BM aspirates and clinical data were available. **Methodology:** Patients' diagnosis, according to the WHO classification were marginal zone B cell lymphoma (5 cases), diffuse large B cell lymphoma (5 cases), follicular lymphoma (1 case) and NHL NOS (1 case). In all cases clinical evaluation, CT scans from the thorax, abdomen and pelvis as well as BM trephine biopsies failed to demonstrate extracutaneous involvement by NHL. Moreover, a maximum of 6% of polyclonal CD19+ B cells were present in the BM aspirates, as demonstrated by flow cytometry. B cell clonality was assessed in paired BM and skin biopsies (9 paraffin-embedded and 3 fresh samples) through PCR amplification of the IGH and IGK genes followed by heteroduplex and genescan analysis, using the Biomed2 primers and protocols. *Results.* B cell clonality was demonstrated in 10 out of the 12 skin biopsies: 2 cases were IGH positive, 5 were IGK positive and 3 were IGH and IGK positive. In two additional cases (paraffin embedded biopsies), no clonal rearrangements were detected by PCR. In four (33%) out of the 12 BM samples

analysed by PCR, six clonal rearrangements were detected: 3 complete and 3 incomplete IGH gene rearrangements. However, all rearrangements found in the BM were different from those detected in the skin biopsies. The differences concerned the type (complete vs incomplete IGH rearrangements) and size of the rearrangement. The BM clonal rearrangements occurred in three DLBCL and one MZL. Two of these patients are still under treatment and the other two (with DLBCL) remain in complete remission 9 and 12 months after first line therapy. **Conclusions.** In this small series of patients with PCBCL we detected four (33%) cases with clonal B cell rearrangements in the BM. The rearrangements were different from those identified in the skin, strongly suggesting that they were unrelated to the lymphoma. Moreover, the clinical course of the positive cases did not differ from the whole series. Our data shows that bone marrow B cell clones in PCBCL should be interpreted carefully and should not change the patients' staging based on clinical, phenotypic and radiological criteria.

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ACUTE PROMYELOCYTIC LEUKEMIA DEVELOPING IN A PATIENT WITH CHRONIC LYMPHOBLASTIC LEUKEMIA PREVIOUSLY TREATED BY CHLORAMBUCIL

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Background. The development of acute promyelocytic leukemia (APL) in patients with chronic lymphocytic leukaemia (CLL) is extremely rare. Most cases of therapy-related APL regard patients affected by solid tumours treated by chemotherapy, frequently including topoisomerase inhibitors. **Aims.** and **Methods.** To describe clinical and laboratory findings in a very rare case of APL diagnosed at our Institution in a patient with CLL. **Results.** We describe a case of APL developing in a 68-year-old woman, with a diagnosis of B-CLL in stage IIB (Rai/Binet) lasting from 1996 and treated with monthly chlorambucil and prednisone until March 2004. She was admitted to the hospital in June 2004 because of anemia (hemoglobin level 9.3 g/dL) with macrocytosis (mean corpuscular volume 103 fl), neutropenia with absolute lymphocytosis (white cell count $5.1 \times 10^9/L$, neutrophil count $0.6 \times 10^9/L$, lymphocyte count $4.4 \times 10^9/L$) and mild thrombocytopenia (platelet count $84 \times 10^9/L$). The coagulation profile was normal except for elevation of D-dimer. Bone marrow trephine biopsy showed a maturative arrest of myelopoiesis with about 20% of atypical hypergranular promyelocytes and a B-lymphoid infiltrate consisting of small mature cells accounting for 10% of cellularity. Immunophenotypic characterization by flow cytometry confirmed the suspicion of APL showing a 30% of promyelocytes with a negative staining for HLA-DR and CD34 and positivity for CD33 and MPO. Lymphocytes coexpressed CD19, CD20, CD23 and CD5. Cytogenetic study on bone marrow aspirate demonstrated a t(15;17) in 13% of the metaphases. Additional chromosomal abnormalities were absent. PML-RAR- α (bcr 3) transcript was revealed by RT-PCR. The patient was treated by All-Trans Retinoic Acid (ATRA) alone from July to October 2004 achieving a complete morphologic remission of APL (with persistence of a CD5+/CD19+/CD23+ monoclonal lymphoid population) but no disappearance of PML-RAR- α rearrangement. A consolidation with idarubicin and ATRA was then started in January 2005. **Conclusions.** This report shows a unique case of APL diagnosed in a patient affected by CLL and previously treated only by an alkylating agent. With respect to response to therapy and clinical outcome, the prognosis seems to be similar to that of *de novo* APL, despite the persistence of CLL.

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EPOETIN BETA (NEORECORMON®) THERAPY IN ANAEMIC CANCER PATIENTS RECEIVING PLATINUM AND NON-PLATINUM CHEMOTHERAPY

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Background. Patients with cancer often experience anaemia as a consequence of the malignancy or resulting from the myelo-suppressive effects of their chemotherapy (CT). Anaemia is particularly common with platinum (Pt)-based CT, which has led to much research into the use of recombinant human erythropoietin in patients receiving this type of CT. However, anaemia is a common complication of all forms of CT, including non-Pt-based CT. **Aims.** The analysis was set up to investigate whether there is a difference in efficacy outcomes as a result of CT type from the pooled results of three controlled clinical trials (Boogaerts et al 2003; ten Bokkel Huinink et al 1998; Oberhoff et al 1998). **Methods.** Patients with solid tumours receiving either Pt-based CT or non-Pt-based CT who had been randomised to epoetin beta treatment or standard care were included in this analysis. Patients were assigned to treatment groups according to the treatment received. The primary endpoint for this meta-analysis was haemoglobin (Hb) change. Secondary endpoints included transfusion requirements, time to survival and tumour progression. Thromboembolic events (TEEs) and other adverse events (AEs) were recorded and analysed. **Results.** A total of 255 patients who received epoetin beta and 199 control patients were included in the analysis. Median increases in Hb levels from baseline to week 16 were observed in all groups receiving epoetin beta versus standard care (all patients 1.5 g/dL versus 0.0 g/dL; Pt-based CT 1.4 g/dL versus -0.2 g/dL; non-Pt-based CT 1.9 g/dL versus 0.6 g/dL). A median Hb of 12.2, 12.5 and 11.8 g/dL was achieved in all patients, patients receiving Pt-based CT and those receiving non-Pt-based CT after 16 weeks of epoetin beta treatment, respectively. All patients responded rapidly to epoetin beta treatment, showing a median Hb increase of ≥ 1 g/dL from baseline at week 4 (all patients = 1.1 g/dL; Pt-based CT = 1.2 g/dL; non-Pt-based CT = 1.0 g/dL). A total of 157 transfusions were observed (epoetin beta n = 67; control n = 90), with risk reductions associated with epoetin beta treatment of 53% (p < 0.0001), 61% (p < 0.0001) and 26% (non significant) being observed for all patients, Pt-based CT and non-Pt-based CT patients, respectively. Overall, for all three populations there were no risks identified for tumour progression or overall survival. There was a slightly higher, though statistically non-significant, incidence of TEEs (5.9% vs 4.5%) in the epoetin beta group compared with control. No marked differences were observed between the treatment groups in terms of the frequency or types of AEs reported. **Conclusions.** Epoetin beta is safe in anaemic cancer patients receiving Pt or non-Pt-based CT. Furthermore, CT type has no impact on the ability of epoetin beta to rapidly increase Hb in patients with solid tumours and CT-induced anaemia.

Boogaerts M, et al. *Br J Cancer.* 2003;88:988-995. Oberhoff C, et al. *Ann Oncol.* 2002;9:255-260. ten Bokkel Huinink W, et al. *Med Oncol.* 1998;15:174-182.

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LACK OF ASSOCIATION BETWEEN THE PDCD1 GENE AND NON-HODGKIN'S LYMPHOMA (NHL) SUSCEPTIBILITY

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A dysregulation of the endogenous immune system is important in the pathogenesis of B cell malignancies. The 2q33 chro-

mosomal region harbours several genes encoding for molecules that play a pivotal role in the T-lymphocyte activation. Among these genes, CTLA-4 encodes for a receptor involved in the downregulation of the immune response. We have recently reported that a functional polymorphism in the CTLA-4 gene is associated with NHL and may have a role in genetic susceptibility to the disease. Since linkage disequilibrium of CTLA-4 with other adjacent genes cannot be excluded, the analysis was extended to the closely located PDCD1 gene, which is involved in the same pathway for the maintenance of immune homeostasis with inhibitory function similar to that of CTLA-4. Here we report the analysis of the PDCD1 gene in NHL patients. PDCD1 is a type 1 trans-membrane protein and a member of the immunoglobulin supergene family and it is known to regulate peripheral self tolerance in T and B cells. We focused the analysis on the PD1.3 SNP located in an enhancer-like structure in intron 4 of the PDCD1 gene that alters the binding site for transcription factors involved in hematopoietic differentiation and inflammation. DNAs from 44 patients with NHL and 76 healthy controls from central Sardinia (Italy) were studied for PD1.3 A/G SNP using a PCR-RFLP assay. We found that allele PD1.3 G is the most common allele in the examined population. Neither the allelic nor the genotype distribution differed between patients and controls ($P>0.05$). The PD1.3 AA genotype was very rare. The lack of association of PDCD1 gene and NHL, further indicates a major role of CTLA-4 gene variants in the susceptibility to NHL. In order to further clarify the role of CTLA-4 gene in NHL a comprehensive analysis of the polymorphisms in the 2q33 chromosomal region is underway.

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CTLA-4 GENE POLYMORPHISMS AND MULTIPLE MYELOMA

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Multiple Myeloma (MM) is a B-lineage malignancy with unknown aetiology characterized by an accumulation of clonal neoplastic plasma cells in bone marrow. It has been considered that predisposing genetic factors might be implicated in the disease and susceptibility genes involved in the immune regulation of MM may be involved. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene encodes for a receptor that provides a negative signal to the T-cell once an immune response is initiated and completed. It has been associated with susceptibility to MM and monoclonal gammopathies of undetermined significance (MGUS) in Swedish patients. In this study, the CTLA-4 SNP +49A*G and the 3'UTR (AT)_n polymorphisms were analysed in 51 MM, 15 MGUS together with 128 healthy controls, all originated from central Sardinia (Italy). Genomic DNA was genotyped by PCR-RFLP assay to detect +49A*G polymorphism, while the dinucleotide (AT)_n polymorphism was studied by a fluorescence based technique on genetic analyzer ABI PRISM 310. Genotype and allele frequencies were compared in patients and control groups by Chi-squared test with Yates correction. The distribution of the allele and genotype frequencies did not show any statistically significant difference in MM and MGUS with respect to the control group (p value >0.05). All genotypes were in Hardy-Weinberg equilibrium both in patients and controls. The allele +49A is the most represented in the studied population and the homozygosity for +49A allele was increased in MM and MGUS patients with respect to the controls, however this difference did not reach statistical significance. The 3'UTR (AT)_n polymorphism genotyping resulted in 21 different alleles and 18 genotypes. The (AT)⁸² allele was the most represented in the studied population and more frequent among MM and MGUS than controls, however both allele and genotype distributions were not significantly different between patients and controls (p value >0.05). The frequency of the genotype (AT)⁸²/ (AT)⁸² was higher in MM and MGUS than controls, even though it did not

reach statistical significance ($p=0.058$). Thirty one different haplotypes were reconstructed from genotypic data. The haplotype distribution was not statistically different among MM patients and controls ($p=0.23$), with the most frequent haplotype being the +49A- (AT)⁸². Our results showed that CTLA-4 polymorphisms are not associated with MM and MGUS at least in Sardinian patients, in contrast with a previous report that showed the CTLA-4 microsatellite polymorphism as a susceptibility locus for MM and MGUS. This indicates that ethnic differences may be playing a role. We have recently reported that a functional polymorphism in the CTLA-4 gene is associated with Non-Hodgkin's lymphoma (NHL). The association of CTLA-4 polymorphism with NHL but not with MM and MGUS in our population further underlines that different genetic factors are acting in the susceptibility to lymphoproliferative disorders. labematologia.nuoro@tiscali.it

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WARM TYPE AUTOIMMUNE HEMOLYTIC ANEMIA WITH C3 SPECIFICITY POSITIVE ANTIGLOBULIN TEST

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Background. approximately 13% of patients with warm autoimmune hemolytic anemia have positive direct antiglobulin tests due to sensitization with complement, without IgG, IgM or IgA being detected on the red cells. Since it is known that macrophages do not clear efficiently RBCs coated with C3 alone the hemolytic process in these cases is mild. However, not much is known about C3-DAT positive hemolysis. *Aims.* Determine the frequency of C3-DAT positive cases and the clinical significance. *Methods.* A retrospective study. The study population consisted of all the patients in the blood bank registry of DAT results. *Results.* During a 2-year period (2003-2004), 277 positive DATs were recorded, 19 (6,8%) were positive for C3 alone. The clinical and laboratory data of these 19 patients were the following: 8 patients had a normal hemoglobin level. 2 patients had anemia due to others etiologies without evidence of hemolysis and 3 patients had a high titer cold agglutinins producing active intravascular hemolysis. The remaining 6 patients (of which 2 had CLL, 1 infectious mononucleosis, 1 SLE) experienced warm type hemolysis of diverse severity. In 2 patients a moderate degree of hemolysis was noted (Hb range between 8-10 g/dL), with a good response to prednisone therapy. In the other 4 patients severe, life-threatening hemolysis was encountered (Hb range between 4-7 g/dL and reticulocytopenia), requiring multiple courses of corticosteroids, IVIG splenectomy and even a vincristine loaded platelet infusion in one case. *Conclusions.* The clinical manifestations spectrum of DATs positive patients with C3 specificity varied from no clinical evidence of hemolysis (52%) to severe life threatening hemolysis, (30%) a similar picture to the one seen in DAT positive patients with a combination of IgG and C3. Interestingly reticulocytopenia demonstrated at diagnosis is an ominous prognostic sign and might disclosed a subset of patients with severe hemolysis refractory to conventional treatment modalities.

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HAIRY CELL LEUKEMIA: PROGNOSTIC FACTOR ANALYSIS AND TREATMENT RESULTS IN A MONOCENTRIC EXPERIENCE OF 151 PATIENTS

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Background. Few data are available on factors predicting treatment response and survival in Hairy Cell Leukemia (HCL). The aim of this study was to analyze clinical and laboratory features

at presentation in correlation to treatment response and overall survival, and the role of different treatment approaches. *Methods*. The data of 151 consecutive HCL patients observed between 1982 and 2004 were retrospectively analyzed. *Results*. Median age was 53 (range 30 to 80) years, 126 males, 25 females. The following data at presentation were analyzed and compared with response, DFS, PFS and OS: Hb < 10 g/dL (observed in 27% of patients); Plt < 100.000/mL (72%); WBC > 10.000/mL (15%); splenomegaly (75%); bone marrow involvement > 70% (27%). At univariate analysis, only WBC > 10.000/mL resulted significantly correlated to a reduced PFS. 88 patients received as first line treatment α 2-interferon (IFN) alone, 49 purine analogues (PA) alone or in combination with IFN, 5 were treated with splenectomy. Among IFN treated patients, CR, PR and MS were obtained in 21.6%, 73.8% and 4.5% of the patients respectively; while among PA-treated patients in: 26.5%, 71.4% and 2.0%, respectively. Nevertheless, DFS was significantly prolonged in patients treated with PA with respect to IFN. No significant difference in OS was observed. The median PFS is 27.6 months, the median OS is projected at 238 months after a median follow-up of 131 months. *Conclusions*. Among the routine clinical and hematological baseline features, only the presence of a WBC count > 10000/mL was correlated at the lower PFS. First line treatment with purine analogues is associated with prolonged PFS and DFS with respect to IFN; nevertheless, no difference is observed in OS.

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T CELL - RICH B CELL LYMPHOMA: A FOUR YEAR EXPERIENCE OF A SINGLE INSTITUTION

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Background. The non-Hodgkin lymphomas (NHL) are a heterogeneous biological and clinical group of lymphoproliferative diseases that reflect the diverse cell types belonging to the immune system, and are characterized by the clonal expansion of malignant cells (B, T, NK, histiocytic cell origin). T cell rich B cell lymphoma (TCRBCL) is a histological variant of diffuse large B cell lymphoma (DLBCL). *Aims*. The aim of this study was to characterize the patients followed in our hospital between 2000 and 2004, in whom the diagnosis of TCRBCL was made and to analyse the therapeutic options and to define the outcome. Patients and *Methods*. We studied a group of 11 patients (5 males and 6 females, with a median age of 61 years; 2 were lost for follow up), with confirmed diagnosis of TCRBCL. *Results*. Most of the patients (7) were in an advanced clinical stage (IV/III Ann Arbor staging system) and 6 had a high / high intermediate IPI score. The first line of treatment was CHOP regimen in 7 patients and 2 underwent other chemotherapy regimens (1 had radiotherapy previously to chemotherapy). Complete or partial remission was achieved in 5 of the patients; 2 are still undergoing induction chemotherapy and 2 died during treatment. Among the 5 patients eligible for analysis, 4 relapsed or had disease progression and 1 refused to continue treatment. As second line of treatment, the ESHAP regimen was applied to 2 patients; 1 underwent a CHOP-like regimen and 1 COP. All died due to progressive disease. The overall survival at 2 years was 50%. *Conclusions*. TCRBCL is an aggressive form of NHL; the advanced age of this patients poses a special challenge. The standard treatment of Diffuse Large B Cell Lymphoma (CHOP) does not change the bad prognosis of this entity. Perhaps a best biological characterisation of this subtype of NHL may help to identify a tailored drug therapy with the potential to alter the outcome of this disease.

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THE ROLE OF ADDITIONAL RISK FACTORS FOR THE CLINICAL OUTCOME IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background. Acute lymphoblastic leukemia (ALL) is the most common neoplastic disorder in children and adolescent. During the past decades the significant progress in the treatment of childhood ALL happened, nowadays nearly 80% pediatric patients can be successfully cured. Several risk factors for the unfavorable course of the disease are known, such as white blood cells count, age of the diagnosis or the presence of translocations t(9;22) and t(4;11). The aim of the study was to assess the meaning of additional clinical factors, i.e. prolonged intervals in the anti-cancer treatment, severe infections during the treatment or the time of achieving of hematological remission for the clinical outcome in pediatric patients with ALL. *Methods*. In the retrospective study the data of 100 children (50 boys, 50 girls) aged 1-17 years, with newly diagnosed ALL were evaluated. The statistical analysis was performed using Fisher exact test, Mann-Whitney test and Kaplan-Meier method. *Results*. We observed, that the unplanned interval in the anticancer therapy (caused by infections or metabolic disturbances) was an important factor for overall survivor (0,92 vs 0,74, p=0,017). The occurrence of serious infections influence the overall survival in these children (p=0,02). When the hematological remission was achieved in the planned time, the overall survival was 0,91 vs 0,8, when it was not (p=0,03). *Conclusions*. Our observation indicate, that the additional clinical risk factors, such the unplanned intervals in the anti-cancer therapy, time of the achieving of hematological remission and the occurrence of severe infections influence the overall survival and further outcome in patients with ALL.

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SKIN ADVERSE EVENTS IN CML PATIENTS TREATED WITH IMATINIB: OCCURRENCE OF PECULIAR MANIFESTATIONS AFTER 1 YEAR OF THERAPY

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Skin reactions are well recognized adverse events occurring in CML patients treated with imatinib, and are usually related to drug dosage and disease state. We report on our experience relating to skin toxicities during imatinib therapy, which showed a wide range of early and late dermatologic manifestations. In a series of 164 chronic phase (CP) and 36 in accelerated/blastic phase (AP/BC) patients, we observed skin reactions in 24 patients (12%). Early events developed in 18 patients, and were represented by grade I dermatitis (dry desquamation) on the face in 2 CP patients, grade II dermatitis (moderate to brisk erythema) in 13 patients (3 AP/BC and 10 CP patients), grade III dermatitis (confluent moist desquamation) in 2 CP and a grade IV dermatitis that requires definitive suspension of the drug in 1 CP patient. Management of these events consisted of low dose oral prednisolone (10-20 mg/d) with temporary suspension and gradually re-start at full reaction resolution in 13 patients; no treatment was necessary in 5 patients and the lesions spontaneously resolved within 8 days from drug discontinuation. Late events occurred in 6 patients and were represented by: psoriasis in 2 CP patients, who required therapy with systemic retinoids and bethametasone dipropionate; hyaline cell syringoma, a rare benign skin neoplasm of unclear origin, in 1 CP patient, who was treated with surgical excision; malpighian epithelioma in 1 CP patient and basal cell carcinoma in 2 patients (1 CP and 1 AP), who were treated with only surgical therapy. These late events occurred after 54, 68, 76, 80, 98 and 101 weeks respectively of therapy. The observed incidence of 12% is in line with that reported in literature (7-21%); we also

noticed that early events were generally more common in patients with Ph+ cytogenetic status, with a relative preponderance of cases treated with higher imatinib dosages. In conclusion, we show that skin adverse events during imatinib are quite common and are usually easily manageable: however we suggest a careful monitoring of patients with prolonged therapy for possible occurrence of tardive skin manifestations.

1142

HEMOVIGILANCE DATA IN HAEMATOONCOLOGY PATIENTS IN THE HRADEC KRALOVE UNIVERSITY HOSPITAL

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Background. The aim of this study was to analyse the hemovigilance data collected from haematooncology (H-O) patients (blood components consumption, rate and types of post-transfusion reactions, near miss events) which were reported to the University Hospital Transfusion Department over three years (2002 - 2004) **Methods.** The total number of components transfused to H-O patients was 19696 TU: RCC 10589 TU (29% leucodepleted), plasma 6518 TU, apheresis trombocytes 905 TU (100% leucodepleted), buffy-coat trombocytes 1684 TU (2% leucodepleted). 22% of components were irradiated. **Results.** 28 adverse transfusion events were reported over three years (2002 - 2004): 7 transfusion reactions, 11 wrong identification of patients and samples, 10 near miss events. The transfusion order forms were incomplete in more than 5% (missing product type and amount, date of delivery, physician identification). 4 posttransfusion reactions were febrile (57%) and 3 allergic (43%) only. No severe reactions, no TRALI and no ABO incompatible reactions were reported. **Conclusions.** The rate of posttransfusion reactions reported from H-O patients is very low, underreporting is probable. The Hospital Transfusion Commission will develop an improvement strategy (staff education, new adverse event reporting form, instructions).

1143

HIGH RATE OF COMPLETE MOLECULAR RESPONSE ACHIEVED BY TREATING WITH IMATINIB MESYLATE VERY LATE CML PATIENTS IN STABLE CCR AFTER IFN- α

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Imatinib mesylate was given to 16 Philadelphia positive (Ph+) chronic myeloid leukemia (CML) patients who were in very late chronic phase (CP) and in stable complete cytogenetic response (CCR) after interferon-alfa (IFN- α), but still showed persistent molecular positivity under this treatment. Eight patients were males and 8 females; according to Sokal's score, 11 patients were low risk and 5 were intermediate risk. Median time from diagnosis was 124.5 mo.s (52-201), median CCR duration was 79 mo.s (9-148). Imatinib was administered at the standard dose of 400 mg/die, after stopping IFN for 1 week. Residual disease was measured on bone marrow (BM) cells at baseline, before starting Imatinib, at 3, 6, 12 mo.s by assaying BCR-ABL transcript using qualitative reverse transcriptase nested polymerase chain reaction (RT-nested-PCR) and quantitative PCR (RT-Q-PCR) approaches. **Results.** of the RT-Q-PCR were expressed as the ratio of BCR-ABL copy number/104 copies of ABL normal transcript. A progressive and consistent transcript decrease with respect to baseline was observed in almost all patients, from a median of 97.9 at baseline to 0.8 at 12 mo.s. In particular, BCR-ABL transcript undetectability was observed in 6/15 patients with evaluable analyses at 3 mo.s, in 9/16 patients analysed at 6 mo.s, and in 5/8 with available results also at 12 mo.s; of these latter patients, 4 were already negative in previous analyses and one become negative at 12 mo.s. Thus, altogether, 10/16 (62.5%) patients showed tran-

script undetectability within 12 months. Achievement of molecular response was significantly correlated with baseline transcript level (median 43.47 for patients achieving molecular negativity vs 582.6 for those who did not; $p = 0.002$), but not with other clinical/biological characteristics. Albeit obtained from a series of very selected patients, present results support the efficacy of combining Imatinib and IFN- α . Sequential therapy, which may overcome the additive toxicity of concurrent use, while possibly reducing the emergence of drug resistance, seems to be worth investigating in patients reaching stable CCR on Imatinib but still having detectable residual disease.

1144

COMBINED FLUDARABINE, MITOXANTRONE, DEXAMETHASONE AND RITUXIMAB (R-FMD) IS ACTIVE AND SAFE FOR NEWLY DIAGNOSED OR RELAPSED PATIENTS WITH FOLLICULAR LYMPHOMA

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Background. High response rates in follicular lymphoma (FL) with the fludarabine combination 'FMD' have been previously reported. The monoclonal anti-CD20 antibody rituximab has been shown to induce a high response rate in FL patients, and to improve outcome when associated with classical regimes (CVP or CHOP). **Aim and Methods.** In order to evaluate the benefit of the addition of rituximab to FMD from March 1994 to July 2004, 26 patients with FL were enrolled in the study. 21 patients received treatment as first line therapy and 6 patients received it as other lines of treatment. Treatment comprised 6 courses of R-FMD administered as follows: fludarabine 40 mg/m² oral days 1-3, mitoxantrone 10 mg/m² day 1, dexamethasone 20 mg days 1-5, and rituximab 375 mg/m² day 1. **Results.** Median age was 54 (32-72), 15 males and 12 females. Ann Arbor stages were: 11,1% stage I, 11,1% stage II, 22,2% stage III, 55,6% stage IV. Risk assessment according to FLIPI score was as follows: 51% low risk, 18,5% medium risk and 29,6% high risk. PCR molecular analysis was performed in 20 patients at diagnosis: 70,4% were bcl2 rearranged and 3,7% were not. Up to date 26 patients are evaluable. All 21 patients receiving treatment as first line therapy achieved remission, 20 complete remission and 1 undetermined complete remission. All 3 patients receiving R-FMD as second line achieved complete response as also do the 3 patients receiving treatment as third line. No differences were observed in terms of response between patients groups with different prognostic features at diagnosis. For all the patients in the study, after a median follow up of 24 months the median progression free survival was 15,1 months and the overall survival was 15,4 months. 17 (85%) of those patients receiving R-FMD as first line are actually in complete remission, 2 died in progression and one died in complete remission. Two patients receiving R-FMD as not first line died under progression, 2 progressed and received other treatment, one is in complete remission and the last one was lost from the study. The toxicity was mild with grade 3-4 neutropenia reported in 3 patients 11,3% who developed grade 3 infections. Rituximab toxicity was as expected. **Conclusions.** R-FMD is an active and safe regimen both for newly and relapsed patients with follicular lymphoma.

1145

PEGYLATED FILGRASTIM AS SUPPORT FOR OUT-PATIENT CHOP-TYPE CHEMOTHERAPY

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Single dose pegylated filgrastim (PF) has been reported to reduce the incidence of febrile neutropenia (FN) post cytotoxic chemotherapy, providing similar prophylaxis to 11 daily doses of conventional filgrastim. Here we have retrospectively stud-

ied our first 10 months of PF prophylaxis for outpatient CHOP/R-CHOP chemotherapy, compared to conventional filgrastim in the immediately preceding period. A comparative group receiving 36 courses of CHOP/R-CHOP without prophylaxis had 7 episodes (19.4%) of FN and subsequent chemotherapy was delayed in 7 (19.4%) instances. Median neutrophil count at day 14 was $< 1.0 \times 10^9/L$ in all patients. 25 patients received PF prophylaxis following 85 courses of CHOP/R-CHOP. Median age was 65 years (range 43-81). 18 patients received conventional filgrastim for a median of 5 days following 72 courses of chemotherapy. Median age was 68 years (33-79). One patient in the filgrastim group reported severe bone pain but no adverse effects were seen following PF. In the PF group, 9 courses (10.5%) were given at reduced dose due to previous non-vinca alkaloid toxicity compared to 7 courses (9.7%) in the filgrastim group. FN occurred after 6/85 courses (8.2%) in the PF group compared to 8/72 courses (11.1%) of the filgrastim group (chi-squared test, $p=0.38$). Subsequent dose reduction was not required in any of the PF group compared to 3 instances (4.2%) in the filgrastim group ($p=0.07$). Subsequent chemotherapy was delayed in 3 episodes (3.5%) in the PF group compared to 11 episodes (15.3%) in the filgrastim group ($p=0.02$). Median neutrophil count on day 14 was $6.3 \times 10^9/L$ (0.29 - 5.0) in the PF group, with all patients having a neutrophil count $> 1.0 \times 10^9/L$. Median neutrophil count on day 14 was $2.6 \times 10^9/L$ (0.29 - 5.0) in the filgrastim group, with 2 patients (6.25%) having a neutrophil count $< 1.0 \times 10^9/L$. In conclusion, PF is well tolerated, with a low rate of febrile neutropenia, chemotherapy delay and dose reduction following CHOP type chemotherapy and is likely to support CHOP-14. Limited conclusions can be drawn from this small retrospective analysis but it is evident that PF supported treatment intensity more effectively than 5 days of filgrastim. Study of a larger group of patients is indicated.

1146**RITUXIMAB-ESHAP AS SALVAGE THERAPY IN RELAPSED AGGRESSIVE B CELL LYMPHOMA**

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The addition of rituximab to CHOP chemotherapy has been shown to significantly improve CR rate and overall survival in patients with untreated Diffuse Large B cell non-Hodgkins lymphoma (DLBCL). Addition of rituximab to ICE chemotherapy as second line therapy prior to autologous stem cell transplantation (ASCT) has similarly been shown to improve CR rate in relapsed or refractory DLBCL. Here, we retrospectively describe our experience of using rituximab plus ESHAP (R-ESHAP) in relapsed aggressive B cell lymphoma. 18 patients were treated with a median of 3 cycles of R-ESHAP, compared to a historical control group of 18 patients treated with a median of 3 cycles of ESHAP. The R-ESHAP group comprised 12 cases of diffuse large B cell lymphoma (DLBCL), 4 transformed follicle centre lymphoma (tFCL), 1 mantle cell lymphoma (MCL) and 1 follicle centre cell lymphoma (FCL), median age 51 years. 7 patients had previously received rituximab. The ESHAP group comprised 13 cases of DLBCL, 1 tFCL, 1 MCL, 3 FCL, median age 53 years. 3 patients had previously received rituximab. Complete remission (CR) rate was 28% in the R-ESHAP group compared to 11% in the ESHAP group, ($p=0.2$, Fishers exact test) and overall response (OR) rate was 56% v 45% respectively ($p=0.5$). Febrile neutropenia was seen in 12% cycles for both regimes. Significant rituximab-related adverse effects occurred in one cycle of R-ESHAP, though no patient had R-ESHAP related toxicity precluding ASCT. PBSCH was attempted in 7 of the R-ESHAP patients (1 previously harvested in 1st CR) and was successful in 5; of the unsuccessfully harvested patients, one had a subsequent successful bone marrow harvest. There was no apparent difference in the ESHAP group, of whom 6 were harvested successfully although 2 required a repeat harvest. 5 R-

ESHAP patients progressed to ASCT, of whom 2 are in CR at 274 and 475 days, 1 has asymptomatic PET scan positive lymphadenopathy and 2 have relapsed. In summary, addition of rituximab to ESHAP appears to improve CR rates in relapsed / refractory aggressive B cell lymphoma though numbers in our study are too small to attain significance. This may improve the efficacy of high dose therapy and translate into prolonged disease free survival post-autograft, supporting the investigation of this approach in large randomised trials.

1147**THE USE OF SLOW RELEASE INTRATHECAL CYTARABINE TO IMPROVE THE QUALITY OF LIFE OF A PATIENT WITH LEPTOMENINGEAL RELAPSE, RECEIVING PALLIATIVE CARE**

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Background. As lymphomatous meningitis is generally incurable, palliative treatment to relieve neurological symptoms and increase symptom-free survival time is appropriate and the issue of quality of life during the palliative period particularly important to the patient. The clinical benefits of slow release intrathecal cytarabine (S-R IT ara-C) for the treatment of lymphomatous meningitis have been demonstrated and maintenance of cytotoxic levels for at least 14 days in most patients allows injections to be reduced to once every two weeks. This results in effective treatment with the added benefit of fewer lumbar punctures for the patient and reduced hospitalisation giving improved quality of life and decreased use of healthcare resources. We report a case where this approach was successfully employed. *Case:* A 63 year old female diagnosed with NHL-T-cell, Stage III B with lumbar vertebral involvement but no CNS symptoms was treated with CHOP every three weeks and clinically was progressing well. Prophylactic treatment for neoplastic meningitis was not given. After 4 courses of CHOP, Lymphomatous meningitis was diagnosed and confirmed by cytology & scan. Lumbar puncture revealed the presence of large blast-like lymphoma cells. Neurological symptoms at diagnosis included a 3 day history of visual deterioration and frontal headache for 5 weeks. The patient was treated with dexamethasone and IT methotrexate followed by high dose IV methotrexate 5 days later. 12 days after diagnosis, it was decided to follow the EORTC protocol for primary CNS lymphoma. The patient had a second lumbar puncture of IT methotrexate along with ara-C and a second IV dose of methotrexate 27 days post diagnosis. 3 months later the patient received craniospinal irradiation. A further 3 months on, the patient presented with symptoms of CNS relapse: ataxia, backpain and leg weakness and lymphoma cells were detected in the CSF. Treated with dexamethasone and high dose IV ara-C infusion plus triple therapy was started followed by IT triple therapy, methotrexate, hydrocortisone and ara-C resulting in the CSF being clear. However, the patient remained unwell. 3 week on the CSF was still clear but the patient developed diplopia. S-R IT ara-C was prescribed with concurrent dexamethasone. 2 weeks later a second injection of S-R IT ara-C was administered and 4 weeks later a third. During that 2 months period, the CSF remained clear and side effects were minimal (headaches and neurological deficits, which could have been attributed to the lymphoma). The patient remained clinically stable with no change of neurological symptoms at first but later the CNS signs deteriorated with confusion, diplopia, backpain and leg weakness. The patient died at home. *Summary.* Based on the palliative intent for this type of patient, the absence of concern over long-term toxicities, the added logistical problems bringing elderly patients to hospital twice a week for intrathecal injections and the improved quality of life during the palliative period through reduced time in hospital and fewer injections, the use of S-R IT ara-C is recommended.

1148

IRON DEFICIENCY AND ANEMIA IN YOUNG ATHLETES - THE USE OF THE SERUM TRANSFERRIN RECEPTORG. Landahl, M. Börjesson, S. Jacobsson, S. Rödger
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Background. Iron deficiency (ID) and iron deficiency anemia (IDA) are common findings among elite athletes as well as in a non athletes population, and especially affects women. Today several methods to diagnose ID are available. Usually transferrin saturation (TS) and serum ferritin are used and iron stained bone marrow smear is the golden standard. During the last years it has been possible to analyze soluble transferrin receptor (s-TfR) in clinical praxis. **Aims.** The purpose of this study was to explore the reference values of s-TfR for female and male adolescents and to compare s-TfR with TS and s-ferritin as a method for detection of ID in a screening situation. **Methods.** 231 students in a senior high school for athletes (59 women and 172 men) aged 16-19 years took part in the study. Venous blood tests were drawn in the morning in semi supine position. The subjects were fasting from midnight before the tests. Hb was analyzed the same day. Serum was kept frozen and analyzes of s-Fe, TIBC, and ferritin and s-TfR were done for all subjects at the same time. ID was defined as ferritin <16mikrog/L or TS <16%. For s-TfR ID was defined as a value above the upper reference value according to the method used at our laboratory, for males 5.0 mg/L and for females 4.4 mg/L. **Results.** The range for s-TfR for females were 2.4 -6.9, for males 2.0 -13.1. The number of subjects with ID diagnosed with the different methods using the limits mentioned above were as follows: Transferrin saturation; females n=23, males n=15. Ferritin; females n=30; males n=15, S-TfR; females n=17; males n=3. The number of females with ID diagnosed with ferritin were 30, the range of sTfR in this group was 3.0 - 6.9 Summary In this group of young athletes s-ferritin seems to be the most sensitive method to diagnose ID. The individuals who present with a high level of s-TfR, have an iron deficient erythropoiesis, the hemoglobin levels are lowered and the iron stores are empty. Only 20 of 45 subjects with ID according to their s-ferritin lowered as a sign of depleted iron stores, were found when using the s-TfR. Elevated s-TfR signals an ID of a more advanced stage, typically when the hemoglobin level has begun to fall. We recommend that s-ferritin is used as a screening test for ID in healthy individuals. S-TfR may be valuable in other situations, i. e. in patients with infection or inflammation.

1149

WHOLE BODY MRI VERSUS SKELETAL SURVEY IN MULTIPLE MYELOMAJ. McHugh¹, C. Johnston², J. Sargent¹, M. Ooi¹, P. Thornton¹, S. Eustace¹, P. O'Gorman¹¹Mater Misericordiae Hospital, DUBLIN, Ireland; ²Cappagh Orthopaedic Hospital, DUBLIN, Ireland

Background. Bone disease in myeloma is conventionally assessed by radiographic skeletal survey (plain x-rays of spine, skull, chest, pelvis and long bones). However this may not pick up bony disease in all patients who may then present with serious complications of myeloma bone disease such as spinal cord compression. Whole body MRI may be better than skeletal survey at evaluating myeloma bone disease. **Aims.** To compare the evaluation of myeloma bone disease by conventional radiographic skeletal survey (RSS) with whole body magnetic resonance imaging (MRI). **Methods.** Sixteen patients with multiple myeloma (median age 69 yrs, range 46-81) underwent conventional RSS and whole body MRI. Ten of the patients had newly diagnosed multiple myeloma and six had relapsed multiple myeloma. The extent of myeloma bone disease was evaluated in both RSS and MRI as follows: the body was divided up into ten areas: skull, cervical spine, ribs, thoracic spine, lumbar spine, pelvis, right arm, left arm, right leg, left leg. In each area the extent of myeloma bone disease was scored in both RSS and

MRI as follows: 0 = normal; 1 = one focus of abnormality; 2 = more than one focus of abnormality; 3 = diffuse disease. The scores for each of the ten areas were combined to give an overall score out of thirty for both RSS and MRI. **Results.** All except one of the sixteen patients had more extensive bone disease on MRI than RSS. The only patient in which there was no difference had no bone disease detectable on MRI or RSS. The mean score for the extent of myeloma bone disease on MRI was significantly higher than that for RSS (MRI mean score: 18.5 out of 30 (median 19, range 0-30); RSS mean score 6.2 out of 30 (median 2, range 0 to 24); p<0.001). MRI was superior to RSS in all ten areas evaluated both in terms of lesion detection and extent of disease. The greatest difference between MRI and RSS was seen in the cervical, thoracic and lumbar spine, while the smallest difference was seen in the ribs and skull. Of the 160 areas evaluated in total in the sixteen patients there was a higher score on MRI in 92 areas and a higher score on RSS in only 7 areas. Four of the patients had no bone disease detectable on RSS but did have bone disease on MRI and this resulted in upstaging on Durie-Salmon staging in two patients. **Summary/Conclusions.** MRI is superior to RSS in the evaluation of myeloma bone disease. MRI may lead to earlier detection and treatment of myeloma bone disease, in particular, spinal cord compression and pathological fracture.

1150

'BASOPHILIA' IN NEISSERIA MENINGITIDIS SEPSIS: A LABORATORY PITFALL

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Background. Automated haematology analyzers (e.g. ADVIA® by Bayer Health Care) are widely used in many laboratories and practices. Such instruments do not only provide highly accurate and precise numeric results but also qualitative information on subsets of cells. Leukocyte subsets can be distinguished based on the size of the cell and their peroxidase activity. On printouts eosinophils appear with the strongest peroxidase activity, whereas neutrophils show an intermediate activity, and monocytes can be separated by a weak staining. For the identification of basophils an aliquot of the blood sample is exposed to a lytic agent at low pH. In this milieu, basophils are resistant to lysis and no cytoplasmic stripping occurs. The size of the basophils is therefore conserved and allows to separate them from the rest of nuclear residues. Findings: We report on an analysis performed on the ADVIA. The blood sample was from a patient with a haemoglobin of 14.1 g per deciliter, a white cell count of 2,000 per cubic millimeter and a platelet count of 23,000 per cubic millimeter. The printout showed many neutrophils in the peroxidase image while a much lower percentage of neutrophils was detected in the basophil image. Compensatorily, a marked basophilia was observed. In contrast, the peripheral-blood smear only had a low percentage of basophils, but many neutrophils with abundant diplococci in the cytoplasm. These microorganisms seem to affect the properties of the cells to lytic agents. As a result neutrophils could not be stripped and appeared in the basophil gate. Additional information obtained from the clinicians revealed that the patient was a 17-year-old woman who was admitted to the hospital with a brief history of fever and petechial lesions. She had to be transferred rapidly to the intensive care unit due to septic shock. Despite immediate treatment with ceftriaxone and intensive therapeutic interventions, disseminated intravascular coagulation and multi-organ failure could not be controlled and the patient died the same day. Meningococemia was later confirmed by positive cultures of blood and cerebrospinal fluid (serogroup C Neisseria meningitidis). **Conclusions.** Newer haematology analyzers do not protect us from misinterpretation of laboratory data. Careful evaluation of results is mandatory. In situations of abnormal findings and discrepancies a visual blood smear should be performed and reviewed thoroughly.

1151**ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE PLUS RITUXIMAB (FCR) IS A SAFE AND EFFECTIVE SALVAGE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA AND FOLLICULAR LYMPHOMA**L. Smolej, P. Zak, D. Belada, V. Maisnar, J. Maly, J. Voglova
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Background. Combination of rituximab and fludarabine has been recently shown to confer survival benefit over fludarabine alone in the treatment of B-cell chronic lymphocytic leukemia (B-CLL). Combination of fludarabine, cyclophosphamide and rituximab (FCR) has an excellent activity in patients with low-grade lymphoid malignancies. Standard treatment of relapsed/refractory B-CLL and follicular lymphoma (FL) has not been established yet. **Aims.** To determine efficacy and safety of oral FCR regimen as salvage treatment in patients with relapsed/refractory B-CLL and FL. **Patients and Methods.** Between October 2003 and December 2004, 11 patients were treated at our institution with oral FCR regimen. The group included 5 patients with relapsed/refractory B-CLL and 6 patients with relapsed/refractory FL (median age 57, range 51-68). The regimen consisted of oral fludarabine 40 mg/m² day 1-3, oral cyclophosphamide 250 mg/m² day 1-3, and rituximab 375mg/m² intravenously on day 1. The dose of rituximab was escalated to 500mg/m² from cycle 2 in patients with B-CLL. Treatment was repeated after 28 days. Oral setrons were used in the prevention of nausea/vomiting, antimicrobial prophylaxis included cotrimoxazole 960mg tiw during therapy and 6 months thereafter. **Results.** Out of 10 patients eligible for evaluation as of March 2005, 8 achieved complete remission after 3 cycles of oral FCR. One patient with FL achieved partial remission after 3 cycles and will receive additional 3. Treatment was very well tolerated. Toxicity was mainly hematological (grade III-IV neutropenia and grade III thrombocytopenia). However, there was no episode of severe infection requiring hospitalization. All patients remain in CR, median follow-up is 8 months (range, 3-13). **Summary.** Our pilot study shows that chemoinmunotherapy combination of oral fludarabine and cyclophosphamide with rituximab is a salvage regimen with excellent efficacy and tolerability in patients with relapsed/refractory low-grade lymphoproliferative disorders. Moreover, oral forms of both cytostatics offer convenient administration at home.

1152**BASIC FIBROBLAST GROWTH FACTOR (bFGF) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ARE ELEVATED IN PATIENTS WITH EARLY STAGE B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA**L. Smolej, C. Andrys, P. Zak, D. Belada, O. Siroky, J. Maly
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Background. B-cell chronic lymphocytic leukemia is a disease with an extremely variable clinical course. New prognostic factors such as IgVH mutation status or genetic aberrations detected by fluorescent in situ hybridization can identify patients with high risk disease. It has been shown that angiogenesis is increased in B-CLL and might play a major role in the pathogenesis of this disease. **Aims.** To find out whether peripheral blood plasma angiogenic activators are elevated in Rai stage 0 B-CLL. **Methods.** In our study we measured peripheral blood plasma concentrations of VEGF and bFGF using commercially available sandwich enzyme-linked immunosorbent assay (Human VEGF and bFGF Quantikine kit, RD Systems, USA) in 37 untreated patients with Rai stage 0 B-CLL and 20 healthy donors. **Results.** We found a statistically significant increase of both VEGF (median 100.2 vs. 57.5 ng/mL, 95% CI 64.3-172.4 vs. 27.7-136.4 ng/mL, p=0.0485) and bFGF (median 201.0 vs. 9.3 ng/mL, 95% CI 121.2 -388.0 vs. 8.7-11.8 ng/mL, p<0.0001) in patients with B-CLL compared to the control group. **Summary.**

We show that angiogenic factors are elevated even in Rai 0 B-CLL. Larger study is planned for multivariate analysis to determine the prognostic significance of plasma VEGF and bFGF on patients' clinical course. Supported by grant No. 8373-3 from Ministry of Health of Czech Republic.

1153**NUMERICAL ABERRATIONS OF THE MLL GENE IN ACUTE NON-LYMPHOBLASTIC LEUKEMIA: A CLINICAL EVALUATION**S.I. Papadhimitriou¹, E. Goumakou², A. Leivada², E. Kritikou-Griva², A. Galanopoulos², A. Davea², G. Paterakis², A. Filiotou³, I. Savvidou², N.I. Anagnostopoulos², M. Georgiakaki²¹G. Gennimatas' Athens Regional General H, ATHENS, Greece;²G. Gennimatas' Athens Regional General H, ATHENS, Greece;³Dept of Medicine II, Athens Un, ATHENS, Greece

Background. Acute non-lymphoblastic leukemia (ANLL) with rearrangements of the MLL gene has well-defined biological and clinical features and has been recognised as a distinct form of the disease according to the WHO classification for the hematological neoplasms. Yet, other chromosomal lesions affecting the 11q23 region and resulting in under- or over-representation of the unrearranged MLL gene are frequently detected in ANLL, but their biological and clinical correlates have not been fully investigated. In this study, we have attempted to clarify whether such MLL aberrations can be of value in the initial prognostic stratification of the patients. **Methods.** The study included 200 adult patients with ANLL. In all cases, conventional cytogenetic analysis (CCA) was performed on unstimulated bone marrow cultures. The diagnostic marrow smears were studied with fluorescence in-situ hybridization (FISH), using a commercially available probe set for the detection of MLL rearrangements ('break-apart'), simultaneously with a chromosome 11 centromeric probe, directly labelled with a third fluorochrome. In case the FISH signal pattern could not be reliably interpreted in the light of CCA findings, additional FISH study was carried out using appropriate probes for other regions of chromosome 11 short and long arm. **Results.** Evidence for MLL rearrangement was obtained in 9 cases. Numerical abnormalities of the MLL gene were detected in 23 cases (11.5%). These included: i. 5 cases of MLL deletion, all due to 11q-, ii. 8 cases in which 3 copies of the non-rearranged MLL were detected (4 of them due to trisomy 11, 2 due to isochromosome 11q and the remaining 2 on the basis of near-triploid karyotype with trisomy 11), iii. 6 cases with 4 copies of the non-rearranged MLL (5 on the basis of a near-tetraploid karyotype with tetrasomy 11 and one due to an additional isochromosome 11), and iv. 4 cases with multiple copies of the non-rearranged MLL, due to a 3- to 10-fold gene amplification at the DNA level. CCA showed a complex karyotype in 18 of the 23 cases. Except for the M3, all other FAB subtypes were represented. 9 cases were classified as 'ANLL with multilineage dysplasia' according to the WHO guidelines. The median age of the patients was 75 years (range: 44-92) and 6 of them had a documented history of myelodysplasia. A short-lived remission after standard induction chemotherapy was achieved in only 3 cases. The median overall survival was 5 months (range: 0-14+). **Conclusions.** In ANLL, numerical abnormalities of the MLL gene may be found on the basis of a heterogeneous cytogenetic background, but they are uniformly associated with poor outcome. Since they usually concern elderly patients with a complex karyotype or disease with multilineage dysplasia, it is unlikely that they represent an independent prognostic factor. However, because they can be easily detected during routine molecular screening for MLL rearrangements at no additional cost, they may be useful as a surrogate marker of adverse prognosis in ANLL.

1154**ADULT BIPHENOTYPIC ACUTE LEUKEMIAS - SINGLE CENTRE EXPERIENCE**

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Background. Acute leukaemias (AL) classification is based on expression of myeloid or lymphoid lineage antigens on the blast cells. In the French-American-British (FAB) classification combination of morphology and cytochemical staining was sufficient for majority of AL. Introducing immunological markers and flow cytometric immunophenotyping has shown some AL of ambiguous lineage. Biphenotypic AL (BAL) is characterised by blasts which coexpress myeloid and T or B lineage antigens. BAL must be distinguished from acute lymphoblastic leukaemia and acute myeloid leukaemia with one or two aberrant markers. The European Group for the Immunological Characterisation of Leukemias (EGIL) introduced good scoring system for BAL classification. **Aims.** In this study we describe the biological, clinical characteristics and outcome of 24 adult BAL patients seen in our centre. **Materials and Methods.** We have analyzed a group of 480 patients with AL diagnosed and treated in Dept. of Haematology, Blood Neoplasms and Bone Marrow Transplantation, Wroclaw Medical University, Poland between 1999 and 2004. There was EGIL scoring system employed to define BAL. **Results.** 24 (5%) patients fulfilled the EGIL criteria of BAL (M/F-10/14, median age-60, range from 25 to 84). Most cases (23) were primarily diagnosed AL and one patient developed AL from myelodysplastic syndrome. We found a co-expression of myeloid and T, B or both T and B lymphoid lineage in 15, 4 and 5 cases respectively. Before treatment: mean WBC was 19,15±26,69 (range 0,7-89,9) median 5,4 G/L; mean percentage of bone marrow blasts was 57,17±24,12 (range 23,5-98,0) median 61,5%; mean percentage of blood blasts was 35,65±32,57 (range 0-93,0) median 23,0%. Twelve patients had a normal karyotype and twelve had chromosome abnormalities. There were 3 cases (12,5%) of solid tumours in this group (lung, ovarian and colorectal carcinoma) before BAL diagnosis. 22 patients were treated with conventional chemotherapy (Polish Acute Leukaemia Group protocols for: AL <60 yrs and > 60 yrs) and in two cases allogeneic bone marrow transplantation from matched unrelated donors (MUD alloBMT) was performed. 12 patients achieved complete remission (50%). Ten patients died due to the disease progression (no response after conventional therapy) and in one case an extramedullary relapse after MUD alloBMT was a cause of death. OS was 40%. **Conclusions.** In our centre BAL represents a minority of AL (5%). We have noticed high frequency (12,5%) of solid tumours before BAL diagnosis. Patients with BAL have poor outcome: remission induction was difficult and overall survival was low. It seems that due to the rarity of BAL and bad treatment results multi-centre collaboration with larger numbers of patients for analysis is required.

1155**BCR-ABL E6A2 TRANSCRIPT VARIANT - ANY SIGNIFICANT ROLE FOR IMATINIB RESISTANCE?**

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Background. Chronic myeloid leukaemia (CML) is often characterised by the presence of the Philadelphia chromosome, a Bcr-Abl fusion transcript. The major breakpoint(M-bcr) on Bcr exon 13 (e13) and/or e14, the minor breakpoint(m-Bcr) on e1 and the micro breakpoint(u-Bcr) on e19 are well known breakpoints. Recently, a breakpoint on Bcr e8 has been proposed to

represent a fourth breakpoint 1. Other breakpoints are rarely described. Only, four patients with CML are reported to harbour an e6a2 Bcr-Abl transcript 2-5. The different transcripts seem to affect the progression of the disease. Short transcripts, like e1a2, e6a2 and e8a2 have been associated with more progressive disease than with the longer transcripts e13/ e14a2, and 19a2. **Aims.** The aims of the study are to describe a rare Bcr-Abl transcript found in two CML patients and to investigate their resistance to imatinib. **Methods.** The rare Bcr-Abl transcripts have been investigated by FISH, qualitative - and quantitative RT-PCR (real-time PCR). The sequence analyses have been performed to detect the transcript and to search for mutations in the ATP binding region of the Abl gene. **Results.** Both patients have been identified as Philadelphia chromosome positive CML. Quantitative RT-PCR analysis conducted with primers for the e1a2 and e13a2 transcript has been negative in both cases. A qualitative multiplex RT-PCR analysis showed a faint gel band close to the BCR housekeeping gene signal for both subjects. Sequence analysis of the fragment revealed an e6a2 Bcr-Abl transcript. Clinically, both patients developed resistance to imatinib after a year / 6 weeks of treatment and both had to be transplanted. Relative quantitation of the e6a2 transcript showed a suboptimal molecular response in both cases. Analysis performed at time of resistance did not uncover imatinib resistance associated mutations. **Discussion.** Widely used multiplex PCR assays for Bcr-Abl transcript detection do not always detect the e6a2 transcript 3 because of low reproducibility with the risk of being under-diagnosed. Moreover, the clinical history of our two patients, and three of four earlier described cases point out that the e6a2 CML is more aggressive than ordinary CML disease. In addition, our patients developed resistance to imatinib. We did not find evidence for the main resistance mechanism (mutations in the ATP binding region) and claim therefore that(i) alternative resistance mechanisms are of importance in these cases or (ii) the e6a2 fusion protein is primary resistant to imatinib. Further molecular analysis of the e6a2 transcript is needed to examine this hypothesis. **Summary/Conclusions.** We describe the fourth and fifth case of CML patients with e6a2 Bcr-Abl transcript. Our clinical and molecular findings indicate that the transcript subtype may have implications for resistance to imatinib.

1. Demehri et al., *Leukemia*, 2005.

2. Hochhaus et al., *Blood*, 1996.

3. Schultheis et al, *Leukemia* 2003.

4. Colla et al., *Haematologica*, 2004.

5. Dupont et al., *Leukemia*, 2000.

1156**ZAP-70 MRNA QUANTIFICATION IN B-CELL CHRONIC LYMPHOBLASTIC LEUKAEMIA**

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Background. The mutational status of the IgVH gene in the leukaemic cells of B-cell chronic lymphocytic leukaemia (B-CLL) identifies two subgroups of patients with significantly different outcomes. Until recently, with the introduction of a multiplex PCR method1 using BIOMED-2 protocols, the detection of mutational status did not lend itself readily to routine use in most laboratories. This study investigated the reported association of ZAP-70 expression with IgVH mutational status in B-CLL by quantifying ZAP-70 mRNA by RT-PCR to evaluate its use as a surrogate marker for the gold standard of IgVH mutational status. **Aims.** To develop a RT-PCR assay for the detection of ZAP-70 expression in a group of patients whose mutational status had been determined previously. **Methods.** Quantitative RT-PCR was used to analyse ZAP-70 expression in B cells in peripheral blood from 41 B-CLL patients whose mutational status had been determined previously. B cells were purified using CD19 magnetic bead AutoMACS system (Miltenyi Biotec) and total RNA was isolated using the TRIzol (Invitrogen) method.

Quantitative PCR was performed using Taqman PCR with 18S rRNA as an internal endogenous control (Applied Biosystems). **Results.** Twenty-two patients had mutated and nineteen had unmutated IgVH genes. Mutated subjects had significantly higher (Δ CT=23, range 20-28) values which are associated with a low level of ZAP-70 expression compared to low (Δ CT=16, range 13-20) values in unmutated cases which indicate a high level of ZAP-70 expression ($p < 0.001$). In 4 patients (9.7%), ZAP-70 expression and IgVH mutational status were discordant: 3 Ig-unmutated CLLs had low ZAP-70 expression (Δ CT =20) and 1 Ig-mutated CLL had high ZAP-70 expression (Δ CT =19). In 5 subjects ZAP-70 levels were compared in purified B cells and non-purified cells containing B, T and NK cells. ZAP-70 levels were significantly higher in non-purified cells. **Conclusions.** This paper describes the development of a reverse-transcriptase-PCR assay for the detection of ZAP-70 expression in which the purification of B cells are required. The results confirm that IgVH unmutated CLL cells have a high expression of ZAP-70 in comparison to IgVH mutated CLL. This robust RT-PCR method acts as a surrogate marker for IgVH mutational status albeit with less than 100% concordance. However it does provide better concordance with IgVH mutational status than that reported using flow cytometry and gives important prognostic information for patients with CLL.

1157**EFFICACY AND SAFETY OF EPOETIN BETA (NEORECORMON®) 30 000 IU ONCE WEEKLY IN ANAEMIC PATIENTS WITH NON-MYELOID MALIGNANCIES RECEIVING CHEMOTHERAPY**

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Background. Once weekly (QW) epoetin beta (NeoRecormon®) (30 000 IU) rapidly increases haemoglobin (Hb) levels and prevents transfusions in patients with lymphoid malignancies (Cazzola et al 2003). The aim of this study was to evaluate the efficacy and safety of epoetin beta 30 000 IU QW in patients with non-myeloid malignancies. **Methods.** Adult anaemic patients with non-myeloid malignancies (Hb 8–12 g/dL), WHO performance status 0–2 and who were scheduled to receive chemotherapy (CT) entered into this multicentre, open-labelled, single-arm study. Patients received epoetin beta 30 000 IU QW for 16 weeks. Primary efficacy endpoint was the percentage of Hb responders during epoetin beta therapy. Hb response was defined to account for the Hb level at baseline: Hb level of 13 g/dL achieved in patients with baseline Hb 11–12 g/dL or Hb increase of 2 g/dL and/or Hb level of 12 g/dL achieved in those with baseline Hb <11 g/dL. **Results.** This analysis reports data from the first 98 patients (mean age 63.4 [+/- 12.4] years; 61% solid, 39% haematological malignancies). Overall, 67% of patients responded to epoetin beta treatment, with a greater number of patients with haematological malignancies (78.9%) obtaining a Hb response. Median time to response was 43 days. Mean Hb level at baseline was 10.1 (+/- 1.04) g/dL. Hb levels increased to a mean of 12.8 (+/- 1.75) g/dL over a median treatment period of 14 weeks with epoetin beta. Median Hb increases of 1.5 g/dL and 2 g/dL at 7 and 10 weeks, respectively, were recorded. A greater number of patients receiving non-platinum (Pt)-based CT responded (73.5%) than those receiving Pt-based CT (51.7%). At least one thromboembolic event (TEE) was observed in 5% of the patients. Only one TEE was considered to be related to the study treatment. **Conclusions.** Epoetin beta 30 000 IU QW is an effective and safe treatment of anaemia in patients with non-myeloid malignancies, regardless of chemotherapy type and appears particularly effective in patients not receiving platinum-based CT or with haematological malignancies.

1158**SERUM THYMIDINE KINASE LEVELS ARE SIGNIFICANTLY ELEVATED, AT DIAGNOSIS, IN BINET STAGE A B-CLL PATIENTS WITH UNMUTATED IGVH GENES OR CYTOGENETIC ABERRATIONS ASSOCIATED WITH POORER OUTCOMES**

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Background. B-CLL patients with unmutated IgVH genes have significantly shorter survivals than do patients with mutated IgVH genes. Additionally, cytogenetic aberrations such as 17p13 deletions or mutations and del11q23 have been associated with particularly poor outcomes, trisomy12 with intermediate outcomes and patients with del13q14 or no detectable abnormality usually have indolent disease. Recently, we have identified a further chromosomal aberration, del14q32, which appears to be associated with poorer prognosis. Thymidine kinase (TK) is a cellular enzyme involved in DNA synthesis by a salvage pathway, where it catalyses the conversion of deoxythymidine to deoxythymidinemonophosphate. Serum TK levels are thought to reflect the proliferative activity of the tumour in patients with cancer. Its measurement has been proposed as a prognostic marker in Binet stage A B-CLL patients, with levels over 7.0 U/L identifying those previously untreated Binet Stage A patients with significantly shorter survivals. AIMS This study was designed to investigate possible associations between serum TK levels at diagnosis and IgVH mutational status or cytogenetic aberrations. **Methods.** Sixty recently diagnosed, previously untreated, B-CLL patients (all Binet Stage A) were studied. IgVH mutational status and gene usage investigations were performed using BIOMED-2 standardised primers and protocol, and the sequences analysed using the Ig blast database. FISH analysis was carried out using CLL Set and t(11;14) FISH probes (Abbott Diagnostics). Serum TK levels were determined by batch analysis using a radio-enzyme assay (DiaSorin Inc, Stillwater, MN55082). Statistical analysis (Mann Whitney) was performed using SPSS. **Results.** Highly significant differences were found between serum TK levels in patients with mutated (N=34, TK 8.69 U/L) and unmutated (N=26, TK 26.78 U/L) IgVH genes ($p < 0.001$). Twenty-one of 26 patients with unmutated IgVH genes, and 11 of 34 patients with mutated IgVH genes had serum TK levels >7 U/L. Serum TK levels were significantly higher in patients with intermediate or poor prognosis cytogenetic aberrations (del17p13, del11q23, del14q32 or trisomy12) (25.54 U/L) than patients with del13q14 or no detectable abnormality (8.06 U/L) ($p 0.003$). No associations were found between serum TK levels and absolute lymphocyte count. **Conclusions.** These findings identify a significantly higher proliferative activity, measured by serum TK levels, in Binet Stage A B-CLL patients, at diagnosis, with unmutated, compared to mutated, IgVH genes and in patients with cytogenetic aberrations associated with poorer outcomes. Furthermore, the finding that all but five patients with unmutated IgVH genes had TK levels >7 U/L is in keeping with previous reports that a level >7 U/L, in newly diagnosed Binet Stage A patients was associated with progressive disease. This study highlights the potential prognostic value of TK measurements at diagnosis, which should be confirmed in a prospective study.

1159**ANALYSIS OF EXPRESSION OF THE DEOXYCYTIDINE KINASE AND CN-II NUCLEOTIDASE GENES BY QUANTITATIVE REAL-TIME PCR IN ACUTE PROMYELOCYTIC LEUKEMIA (APL)**

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Ara-C is one of the most effective drugs used in the therapy of Acute Myeloid Leukemia (AML). However, in Acute Promyelocytic Leukemia (APL), this drug neither appears to significantly

affect disease outcome nor does it seem to provide better results than those obtained with other chemotherapeutic agents such as anthracyclines (Head et al, 1985, Sanz MA et al, 1999). Deoxycytidine kinase (dCK) is the key enzyme necessary to metabolize ara-C to its pharmacologically active form, ara-CTP (ara-C triphosphate). On the other hand, catabolism of monophosphate forms of ara-C through 5' nucleotidases decreases the amount of activated ara-C forms. Some authors have reported alterations in the expression of the dCK gene and/or cN-II nucleotidase gene as one of the mechanisms responsible for clinical resistance to ara-C (Schirmer et al, 1998, Veuger et al, 2001). A previous gene expression profiling analysis, carried out by our group, revealed a low level of expression of the dCK gene in 53 newly diagnosed patients with APL. To elucidate the molecular and clinical role of dCK and cN-II genes in APL, we analyzed mRNA expression of both genes by real-time polymerase chain reaction (PCR) in 39 patients diagnosed of APL (17M/22F; median age: 40 yr (range: 3-90)). In addition, 14 patients diagnosed of AML with either t(8;21) (n=8) or inv (16) (n=6), known to have particular good response to treatment with high-dose ara-C, (6M/8F; median age: 18 yr (range: 3-73)), and 16 CD34+ cell samples from healthy donors were included in the study to compare expression levels. Expression analysis was performed using ABI PRISM 7300 Sequence Detection Instrument and software (Applied Biosystems). Specific oligonucleotides and TaqMan probes for each selected gene were purchased from Applied Biosystems (Assay-on-Demand Gene Expression Products). All experiments were normalized to the housekeeping gene GAPDH as control for mRNA recovery and retrotranscriptase PCR efficiency. Preliminary results showed that dCK gene appeared significantly underexpressed in APL samples respect to CD34+ cell samples (p = 0.006). Also, the APL cases showed a trend to dCK underexpression with respect to AML samples with t(8;21) or inv (16), although the difference did not reach statistical significance (p = 0.057). By contrast, cN-II gene was underexpressed in APL samples with respect to AML with t(8;21) or inv (16) and to CD34+ cell samples (p<0.001 and p<0.001 respectively), providing a more complex scenario in this issue. In conclusion, our results show that dCK gene is underexpressed in APL, suggesting that the reduced expression of this gene may contribute to the marginal role that ara-C plays in the treatment of APL.

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IGVH MUTATIONAL STATUS, GENE USAGE AND CHROMOSOMAL ABERRATIONS IN IGG B-CLL PATIENTS

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Background. The leukaemic cells from most patients with B-CLL show IgM or IgM+D immunoglobulin heavy chain restriction, whatever the IgVH mutational status. However, a subgroup of patients expresses IgG on the leukaemic cells and forms an interesting subcategory of disease. Recent advances in knowledge of the pre or post germinal centre origin of B-CLL cells, and the resulting effect on mutational status and prognosis, have refocused interest on the IgG positive patients. The assumption has been that, as these cells have undergone Ig class switch, they will be of post-germinal centre origin, should have mutated IgVH genes and thus be in the good prognosis subcategory of B-CLL. **Aims.** This study was designed to identify IgG positive B-CLL patients in a cohort of 114 patients. The hypothesis tested was that these patients would have mutated IgVH genes. Furthermore, gene usage was determined to study if it was restricted in these patients, which would support the concept of a common aetiological factor. Finally, chromosomal aberrations were studied to determine if associations between IgG heavy chain restriction and acquired chromosomal abnormalities occur. **Methods.** IgH class was determined by the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique on cytopspins from 114 B-CLL patients using antibodies against IgG and IgM (DakoCytomation Ltd). IgVH mutational status

and gene usage investigations were performed using BIOMED-2 standardised primers and protocol, and the sequences analysed using the Ig blast database. A cut-off at 98% homology to the germ-line sequence was used to classify patients as IgVH mutated or unmutated. Chromosomal aberrations were identified by FISH analysis using CLL Set and t(11;14) probes (Abbott Diagnostics). **Results.** Of 114 patients studied, 76 expressed IgM, 28 expressed IgG, whilst 4 expressed IgM+IgG and 6 expressed IgA or IgD alone. Twenty of the 28 IgG positive patients had mutated IgVH genes, whilst 8 were unmutated. In the IgM subgroup of patients equal numbers (N=38) had mutated as had unmutated IgVH genes. IgG patients had significantly fewer poor prognosis chromosomal aberrations than the IgM group (p 0.041) and no apparent associations with gene usage were identified. **Conclusions.** The hypothesis that IgG positive B-CLL cells would have mutated IgVH genes was not proven, as 10 out of 28 patients (36%) had unmutated genes. The reasons for this are not clear. However, synchrony may not always exist between Ig class switch and IgVH mutation and asynchrony between IgVH mutation and Ig class switch may occur quite frequently in B-CLL. Furthermore, antigen presentation outside germinal centres may be adequate to initiate class switch but not IgVH mutations. These findings are not unexpected as recent studies have shown that some mantle cell lymphoma/leukaemia patients have mutated IgVH genes whilst some lymphoma with splenic lymphoma with circulating villous lymphocytes have unmutated IgVH genes. These disease categories had been assumed to arise in pre and post germinal centre cells, respectively. It remains to be seen if this asynchrony between IgH class switch and IgVH mutational status identifies subcategories of disease with a significantly different prognosis.

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THE COMPARISON OF THE SENSITIVITY OF NORMAL AND LEUKEMIC CFU-GM AND BFU-E TO 2-CHLORODEOXYADENOSINE USED IN COMBINATION WITH EITHER INTERFERON ALPHA OR IMATINIB MESYLATE IN CULTURES IN VITRO

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For the last few years interferon alpha (IFN α) used alone or in combination with arabinoside cytosine (Ara-C) has been the standard treatment in patients in chronic phase of chronic myelogenous leukemia (CML). Recently, another drug, imatinib mesylate (STI571) has been also found to demonstrate significant efficacy against this disease. Nevertheless, in order to further improve the effectiveness of the drugs, the interactions between IFN α or STI571 and other antileukemic drugs are being investigated. Therapeutic activity in CML of still another drug, 2-chlorodeoxyadenosine (2-CdA) has been also demonstrated in some studies. The aim of our study was to compare the sensitivity of normal and CML granulocyte-macrophage (CFU-GM) as well as erythrocyte progenitors (BFU-E) to 2-CdA (Biodribin, Bioton, Poland) in combinations with either IFN α (Intron A, Schering Co, New Jersey, USA) or STI571 (Glivec, Novartis Pharmaceuticals, Switzerland) in semisolid culture *in vitro*. Additionally, we estimated the type of interaction between the above drugs on CFU-GM and BFU-E progenitors. For CFU-GM and BFU-E cultures the commercially available media containing methylcellulose fetal calf serum and growth factors were used. The drugs were added to the cultures at the following combinations of concentrations: 5 nM 2-CdA with either 102 U/mL IFN α or 0.5 mM STI571, 10 nM 2-CdA with 103 U/mL IFN α or 1.0mM STI571 and 20nM 2-CdA with 104 U/mL IFN α or 2.0mM STI571. We demonstrated that all the combinations of drugs concentrations inhibited the growth of both types of CFU-GM and BFU-E progenitors in a dose dependent-manner. The combinations of two higher concentrations of 2-CdA with IFN α showed significant differences between CML and normal CFU-GM (p= 0.0009, p=0.0007, respectively). However, only the combination of the highest concentrations showed statistically significant differences in the colony growth inhibition between normal and CML BFU-E (p=0.0005). For the combina-

tions of these drugs an additive effect on either normal CFU-GM or on both types of CML colonies was observed, whereas the combination of these drugs on the normal BFU-E showed a sub-additive effect (CI=0.99; 1.11; 0.82; 1.75; respectively). In case of all the combinations of 2-CdA with STI571 a significant inhibition of colony growth of CML CFU-GM, as compared to the normal progenitors was observed. The combinations of two higher concentrations of these drugs significantly inhibited the growth of CML BFU-E, as compared to their normal counterparts. For the combinations of these drugs a subadditive effect on normal CFU-GM, and additive effects on CML CFU-GM as well as on both BFU-E were shown (CI= 1.62; 1.17; 0.83; 1.14, respectively). In conclusion we suggest that there are no significant differences between either of the combinations of these drugs concerning their influence on both normal and leukemic hematopoietic precursors.

Supported by Grant of Medical University of Lodz No 502-11-209 and by the Foundation for Development of Diagnostic and Therapy (Warsaw).

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CLINIC-PRONOSTIC VALUE OF THE DISCREPANCIES OF MICROSATELLITE DNA REGIONS BETWEEN RECIPIENT AND DONOR AFTER ALLOGENEIC IDENTICAL MHC TRANSPLANTATION

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Introduction. Detection of disparity in microsatellite DNA regions (STR - Short Tandem Repeats) between recipient and donor allows for sensitive and specific monitorization of the degree of haematopoietic chimerism. It is well known that disparities between donor and recipient in various polymorphic systems (mainly MHC) are associated with an increased incidence of graft-versus-host disease (GVHD). However, it is still unknown whether or not STR disparities could have a similar biological effect. **Aims.** To study the relationship between STR disparities and frequency of GVHD, overall survival (OS) and event free survival (EFS) in patients who have received allogeneic transplantation. **Patients.** 167 consecutive transplantations with peripheral blood stem cells from identical MHC sibling donor at a single center were included in the study. Their characteristics were: median age 45 (18-69); Male/Female: 87/64; Sex disparity: 47%; Diagnosis: 34 AML, 22 ALL, 22 MDS, 19 MM, 17 CML, 15 NHL, 10 CLL, 9 HD, 1 CMPD, 1 CLL+HD, and 1 Hypereosinophilic Syndrome. The conditioning regimen was of reduced intensity in 87 patients and myeloablative in 80. All 167 patients engrafted and were valuable for acute GVHD (aGVHD). 134 were included in the analysis for chronic GVHD (cGVHD), according to the follow-up (534 days; 17-3148). **Methods.** After genomic DNA extraction, PowerPlex@16 System kit (Promega Corporation, Madison, WI) was used to amplify 16 STR regions (15 plus gender marker, Amilogenin). The amplified products were analysed using GeneScan 2.1 (Applied Biosystems, Foster City, CA) after electrophoresis in the ABIPrism 377 (Applied Biosystems). The chi-square and t-Student tests were used for statistical analysis. Log-rank analysis was applied for comparing differences in survival. Multivariate analysis was carried out using the Cox model. **Results.** The number of STR disparities between recipient and donor ranged from 4 to 15 (median: 10). This number did not differ according to diagnosis, sex, age, conditioning regimen or pre-transplant status. The number of loci discrepancies was not related to any clinical parameter included in the analysis (aGVHD, cGVHD, EFS y OS). However, when patients were grouped according to STR mismatches (≤ 10 disparities and > 10 , n=116 and 35, respectively), patients differing > 10 STR displayed a higher frequency and severity of GVHD, although differences did not reach statistical significance. A shorter OS and EFS was associated in patients with > 10 disparities (p=0.0139, p=0.0040 respectively). Regarding individual STR regions,

CSF1PO discordances were associated with a more severe aGVHD (p<0.05). Discrepancies in vWA and D16S539 were associated with a more frequent and severe cGVHD, although differences did not reach statistical significance. Discordances in D13S317 were associated with shorter OS (p=0.0221). In the multivariate analysis, the presence of > 10 disparities were associated with shorter OS, EFS and higher TRM (p<0.001). D13S317 discordances were associated with shorter OS (p<0.01). **Conclusions.** The presence of STR disparities could be associated with the prognosis and the development of complications during sibling allogeneic transplantation, including presentation of aGVHD. However, these data must be considered as preliminary and should be confirmed in homogeneous series of patients.

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PROTHROMBOTIC RISK FACTORS IN PATIENTS WITH NON-ARTERITIC ANTERIOR ISCHEMIC OPTIC NEUROPATHY (NAION)

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Background. Non-arteritic anterior ischemic optic neuropathy (NAION) is the most common (incidence: 2-3/100.000 annually) acute optic neuropathy in subjects older than 50 years of age. **Aims.** The aim of this study was to evaluate the prothrombotic risk factors in patients affected by NAION. **Methods.** We investigated 16 consecutive, unselected patients affected by NAION: 11 males, 5 females, median age 58 years (range 34-72). The control population consisted of 15 healthy subjects: 7 males, 8 females, median age 55 years (range 38-82), sex and age-matched. Patients and controls were not on antithrombotic therapy. All patients and healthy subjects underwent comprehensive tests for congenital and acquired thrombophilic disorders. **Results.** No patients had a deficiency of the Antithrombin, Protein C, one patient presented mild deficiency of Protein S (55 U/dL). The determination of lupus anticoagulant (KCT, DRVVT) was negative in all patients. In one patient, we found the presence of FV Leiden and in another the mutation G20210A of prothrombin. The heterozygous C 677T MTHFR genotype was found in 7 patients; the homozygous A1298C MTHFR genotype was found in 4 patients, heterozygous A1298C in 8 patients. Plasmatic hyperhomocysteinemia was found in 8 patients. In the control group, no alterations of congenital and acquired thrombophilic tests were found. The heterozygous C677T MTHFR genotype was found in 7 controls and homozygous in 1 control. The heterozygous A1298C MTHFR genotype was found in 10 controls. Plasmatic hyperhomocysteinemia was found in 3 control subjects. Mean plasma homocysteine levels were significantly higher in the patients than in the control population (OR 8.80; 95% CI=1.35-67.28). **Conclusions.** This study suggests that the congenital thrombophilia, the prothrombotic polymorphisms and the presence of lupus anticoagulant were not related to the pathogenesis of NAION. In our patients, we found a significantly increase of plasmatic homocysteine levels respect to a control population. The homocysteine levels were independent from MTHFR mutations. It is assumed that by reducing the plasmatic homocysteine, the probability of vascular occlusion will be reduced.

1164**TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA WITH CHEMOTHERAPY WITH OR WITHOUT STEM CELL TRANSPLANTATION- LONG TIME FOLLOW UP**

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Background. Overall long-term survival rate for adults with acute lymphoblastic leukemia (ALL) is 35-50%. **Aims.** To present our results of treatment adults with ALL. **Patients and Methods.** In the Clinic of hematology, Military Medical Academy, since January 1989. till January 2005, 75 patients (pts) with ALL were treated, among them 53 were males, 22 were females, average age was 30,6 years (16-75). Cytomorphological varieties were L1 28, L2 46 and L3 1 pt. 15 pts had T- and 28 B-immunophenotype (determined in 43 pts) and cytogenetic analysis have succeeded in 21 pts. At the time of initial presentation 22 pts had Le count $> 30 \times 10^9/L$, 6 pts had CNS and 11 pts had mediastinal involvement. Pts were treated with induction, consolidation and maintenance therapy under modified YU-ALL regimen for 'high-risk' (57 pts), LALA 94 (5), BFM, CHOP, DAOP etc. (12 pts). Medicament prevention (without radiotherapy) of CNS disease was applied to every pts younger than 50 years. **Results.** Complete remission (CR) was achieved in 67 pts (delayed CR in 14). Resistant to therapy were 4 pts (5,8%). 5 pts have died (2 early deaths, 2 before evaluation of remission and 1 after achieving the remission during the intensification due to infective complications). Maintenance therapy was applied for 24-36 months. Hematopoietic stem cell transplantation (HSCT) was done in 19 pts- allogeneic in 11 pts (6 pts in second CR) and autologous in 8 pts (5 pts in second CR). 43 pts have relapsed with an average time to relapse of 10,8 months. Frequency of relapses were higher in pts on maintenance therapy (35/45-74,5%) comparing to the transplanted pts (8/19-42,1%). Long-time survival without relapses (approximately 65 months) had 18/65 pts (27,7%), i.e. only 8 pts (12,3%) on maintenance therapy. Impact of varies prognostic factors on the survival/relapses in adult pts with ALL were tested. **Conclusions.** Our preliminary results were similar to the others and confirms that treatment of adult ALL is still unsatisfactory. It is necessary to definite precise prognostic factors, to stratify pts according to them and to use intensive chemotherapy with or without HSCT in due time.

1165**EVALUATION OF IL-1- μ , IL-2 AND IL-4 LEVELS IN PATIENTS WITH THE MALIGNANT HEMATOLOGIC AND ONCOLOGIC PATHOLOGY**

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Background. Abnormal regulation of cytokines system is the important factor in mechanisms of development of many pathological processes. Interleukines (IL) take part in regulation of all parts of the system and local immune answer at tumoral process. The change in interleukines system in case of malignancy can be shown by disbalance of their (IL) production. **Aims.** Optimization of prognostic and efficiency of treatment criteria for patients with different malignant pathology on the ground of IL-1- β , IL-2 and IL-4 monitoring. **Methods.** 24 patients (4-60 years) with different malignant pathology (acute leukemia (AL)-8, HD-4, MM - 8, solid tumours - 4) were investigated, 19 from them have received autoPBCST. For patients undergoing autoPBCST the investigation carried out at the following stages: before conditioning treatment, after autoPBCST in time of restoration of hemopoiesis, through +3 - +17 months after transplantation. For patients with AL (3-20 years old), receiving just

only conventional chemotherapy, investigation executed in the acute period and in the remission. The evaluation of spontaneous IL-1 β , IL-2, IL-4 production in supernatants of peripheral blood mononuclear cells (PBMNC) daily cultures were measured by ELISA (Diacdone, France). The control group consisted of 20 healthy persons. **Results.** In while, IL-1 β level was lower in patients group then in control group (205,7+64,6 pg/mL). Secretion of IL-1 β at autoPBCST recipients has wavy character - before transplantation the cytokine level fluctuated within 8-37 pg/mL limits, in the early posttransplant period at the majority of patients it raised up to 55-133 pg/mL and in late posttransplant period (the maximal observation - 17 months after SCT) was again reduced up to reference values. It is noticed, that at the patients, died in term up to +5 months after SCT (n=3), IL-1 β in the early posttransplant period was much higher, than at the others and changed within 268,3-500,2 pg/mL limits. IL-2 and IL-4 levels in PBSCT recipients group did not differ from those in control in all points of observation. In AL group significant decrease in IL-1 β levels also is revealed in comparison with normal values. The prominent tendency to increase of IL-1 β level is observed in remission in comparison with the acute period of AL. Besides, in acute period reliable increase of IL-2 (16,0 \pm 0,37 μ g/mL) and decrease in IL-4 (0,66 \pm 0,01 μ g/mL) is revealed in comparison with those parameters in remission (11,88 \pm 2,33 pg/mL and 0,8 \pm 0,06 pg/mL respectively). **Summary.** The obtained data testify to presence of distinctions in IL-1 β , IL-2, IL-4 levels in view of pathology, course of disease and outcome. Future investigation in this field can promote optimization of prognostic criteria and treatment effectiveness for patients with the specified pathology.

1166**AUTOLOGOUS STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA - A 17 YEAR SINGLE CENTER EXPERIENCE**

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Autologous stem cell transplantation is a standard treatment approach in patient with AML without HLA identical donor younger than 60 year of age. Here we present the results of treatment outcome with autologous transplantation for AML patients in a 17-year period. **Patients and Methods.** From April 1988 to January 2005 142 (69 male and 73 female) patients with AML were treated with intensive therapy followed by reinfusion of autologous stem cell. From December 1996 adult patients with AML in whom CR have been obtained were consecutively submitted to PBSCT. 116 (81,7%) of them were in first complete remission. The age of transplant recipients was between 2-61 years (median 38 year). The conditioning regimen consisted of busulfan 16 mg/kg BW and cyclophosphamide 120 mg/kg BW; 18 (12,68%) patients received fractionated TBI without lung shielding instead of busulfan. 54 pts (38%), received PBSCT, 71 pts (50%) BM, and 17 (12%) BM+PBSC. **Results.** The probability of 5-year overall survival for all patients, irrespectively of their pretransplant disease status (1st CR vs >1st CR) was 45,6% (1st CR 49,7%, >1st CR 22,29%; p=0,0002). Disease free survival for the AML patients in 1st CR was 49,9% and for patients >1st CR 23,2% (p=0,0002). Relapse rate was significantly lower in patents autografted in 1st CR compared to patients autografted in advanced stage of disease (44,9% vs. 58,3%, p=0,0029). There was no difference in OS, DFS, and relapse rate between the patients receiving PBSC or BM. Transplant related mortality was 9,9% for all group. **Conclusions.** Autologous stem cell transplantation is a standard postremission treatment for AML patients without HLA identical donor. Relapse rate was significantly lower in patients autografted in 1st CR compared to patients received autologous stem cells in >1st CR.

1167**ADDITION OF HYDROXYUREA IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA RESISTANT TO IMATINIB ALONE**R. Latagliata¹, M. Breccia², I. Carosino², M. D'Andrea², M. Mancini², D. Diverio², S.G. Morano², F. Gentilini², G. Alimena²¹University 'La Sapienza', ROME, Italy; ²University 'La Sapienza', ROME, Italy

The introduction of Imatinib in the treatment of Chronic Myelogenous Leukemia (CML) in chronic phase (CP) has led to the achievement of Complete Cytogenetic Response (CCR) in 50 to over 80% of patients; however, in a portion of patients Ph+ cells still persist also after standard (400 mg/day) or higher (600 mg/day) doses of Imatinib. These patients are thus considered 'resistant', and their management is presently not yet defined. From 11/2002 to 10/2004, we treated by adding Hydroxyurea (HU) to Imatinib 11 CP patients (7 M and 4 F, median age 52.5 yrs, range 29 - 68), who were persistently 100% Ph+ (10 patients) or BCR/ABL+ (1 patient with Ph- BCR/ABL+ CML) after Imatinib at standard dose (for at least 6 mos) followed by increased 600 mg/day dose (for at least 3 mos). Eight patients had been treated with IFN before Imatinib; median times from diagnosis and from Imatinib treatment to HU addition were 51 mos (range 23-151) and 14 mos (range 10-31), respectively. HU was given according to WBC count: patients with WBC $\geq 10 \times 10^9/L$ started HU at the dose of 1 g/day, patients with WBC $> 10 \times 10^9/L$ received a initial dose of 1.5 g/day. Imatinib was continued at the previous dosage of 600 mg/day in 6 patients and of 400 mg/day in 5 patients, who had hematological toxicity. Two patients achieved a CCR after 3 and 12 months, respectively, and a third patient (Ph- BCR/ABL+ at diagnosis) showed undetectable molecular transcript at RT-nested-PCR analysis after 9 months. Two patients achieved a major CR (Ph+ < 33%) after 3 and 9 months, respectively. Of the 5 responding patients, 3 (2 CCR, 1 PCR) relapsed with 100% Ph+ cells after 4, 5 and 12 mo.s, respectively. The remaining 6 patients did not respond to the drug combination, and showed persisting 100% Ph+ cells. Toxicity was mild and only 1 patient discontinued therapy for 2 weeks due to transient thrombocytopenia; no extra-hematological side effects were recorded. After a median follow-up of 19 mo.s (range 3-27), 2 patients (1 resistant and 1 relapsed after CCR) evolved to Blast Crisis (BC), 7 patients are in stable CP with 100% Ph+ cell and 2 patients are still in response (1 in major CR at +3 mo.s and 1 in molecular CR at +12 mo.s, respectively). In conclusion, the association of HU with Imatinib can induce CCR in near one third of patients resistant to Imatinib alone, with minimal toxicity, thus showing a possible synergistic effect *in vivo*; however, these responses are mostly of short duration. Study of molecular mutations possibly involved in the resistance mechanism might help to explain different responses among patients, while longer follow-up is required to evaluate the real usefulness of combining HU and Imatinib.

1168**COMPLICATIONS AND CAUSES OF DEATH IN ELDERLY PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA MANAGED WITH PALLIATIVE TREATMENTS**R. Latagliata¹, M. Breccia², I. Carosino², F. Biondo², V. Bongarzone³, A. Spadea⁴, E. Russo², S. Mecarocci², F. Mandelli², G. Alimena²¹University 'La Sapienza', ROME, Italy; ²University 'La Sapienza', ROME, Italy; ³Hematology - Osp S. Giovanni, ROME, Italy; ⁴Hematology-Regina Elena, ROME, Italy

Elderly AML patients not eligible for intensive chemotherapy (CT) represent a frequent problem for haematologists: for them, supportive care is probably less accurate and prompt than for patients who are enrolled into controlled trials. We retrospectively evaluated 244 consecutive elderly AML patients (M/F 143/101, median age 72 years, range 60-90) diagnosed at our Institution from January 1989 to December 1998 and not eligi-

ble for intensive CT. Median overall survival was 179 days (1-3278) and 177 patients (72.5%) required palliative CT to control the disease. As to infections, 69 patients (28.3%) did not have any documented infective episode; 52 patients (21.3%) presented one or more episodes of fever of unknown origin (FUO), defined as a fever $\geq 38^\circ$ lasting at least 48 hours without any other clinical, microbiological or radiologic sign of infection. Documented infections were observed in the remaining 123 patients (50.4%): of them, 94 (76.4%) had a single episode, and 29 (23.6%) had ≥ 2 episodes. Among bacterial infections, 54 were broncopneumonias, 45 abscesses, 23 sepsis and 21 local infections (synusitis, cystitis, colecystitis and phlebitis); a documented fungal infection was recorded only in 5 patients (4 broncopneumonias and 1 sepsis). Haemorrhages (WHO ≥ 2) were observed in 96/244 patients (39.3%), with 23 of them presenting ≥ 2 episodes in different sites. The most common events were gingival and nasal bleedings (48 patients), followed by gastro-intestinal (33 episodes) and cerebral (26 episodes) haemorrhages. A cardiac impairment (WHO ≥ 2) developed during the AML course in 46 patients (18.9%), 22 of whom (48%) did not refer any previous cardiologic disease. After a minimum observation period of 5 years from the last included patient, no patient is alive, 12 (4.9%) were lost to follow-up and 232 (95.1%) died; of these, 22 (9.5%) died from unknown causes, 110 (47.4%) died from disease progression and 100 (43.1%) died while in stable disease (from infections 23 patients, from haemorrhages 27 patients, from heart impairment 27 patients, from other organ failures 21 patients and 2 patients from a second neoplasia). As expected, a variety of complications was observed in our patients, which were often not directly related to the disease itself: in fact, about one half of the patients died from complications while being in stable leukemic phase. This observation points to the need of better defining the 'gold standard' of the supportive care also for this neglected patient subset, as the identification of more effective measures to prevent and manage microbiological, haemorrhagic and cardiologic complications could possibly lead to a better quality of life as well as to a longer survival.

1169**REFRACTORY TRANSFORMED MYCOSIS FUNGOIDES RESPONDING TO ALLOGENEIC IMMUNE EFFECT**G. Anghel¹, L. De Rosa¹, M. Montanaro², D. Remotti¹, G. Cruciani³, A. Locasciulli¹, I. Majolino¹¹S. Camillo-Forlanini, ROME, Italy; ²Hematology Unit, VITERBO, Italy; ³S. Camillo-Forlanini Hospital, ROME, Italy

A 36 year-old man presented in October 2000 with a year-long history of chronic dermatitis with a 2 cm major diameter erythematous plaque on the left side of the neck. A skin biopsy showed large (> 25% of ANC) and intermediate-sized atypical lymphoid cells, in part with hyperchromatic cerebriform nuclei, localized in the dermo-epidermal layer. Morphologic and immunophenotypic features were consistent with mycosis fungoides (MF) transformed to a large T-cell cutaneous lymphoma. A comprehensive work-up did not reveal other disease localizations. The patient received 12 weekly courses of MACOP-B, followed by involved-field radiotherapy (30 Gy) attaining a 2 months partial remission (PR) of the neck skin lesion. In May 2001, he presented with a new nodular plaque on the upper external side of the right leg, adding to the previous one, and a second-line chemotherapy with 2 cycles of DHAP was started without effect. The patient was shifted to the IGEV combination, attaining a PR. During the second of three courses of IGEV, peripheral blood stem cells (PBSC) were collected. In December 2002, after high-dose (180 mg/m²) melphalan the patient was rescued with his PBSC. Engraftment was rapid and complete, but no disease response was obtained, as previously described cutaneous lesions remained unmodified. Since the patient had an HLA-identical sister, in February 2003, he was conditioned with anti-CD52 MoAb (Campath-1H) 30mg/day for 3 days, followed by thiothepa 10 mg/kg (day -6), cyclophosphamide 30 mg/kg (dd -4, -3) and fludarabine 30 mg/m² (dd -4, -3). As graft

he received $8.9 \times 10^6/\text{kg}$ CD34+ cells from his sister peripheral blood. GVHD prophylaxis consisted of CSA 2 mg/kg iv from day -1 to +12, followed by CSA 4 mg/kg orally from day 12. PMN ($>0.5 \times 10^9/\text{L}$) and platelet ($>20 \times 10^9/\text{L}$) engraftment occurred on day 10 and 11, respectively. Skin lesions however, remained unmodified during the following 3 months, and CSA was therefore tapered and withdrawn on day +42. Since the skin lesions still persisted unmodified, and there was no evidence of GVHD, the patient received a treatment course with his donor lymphocyte infusions (DLI). One DLI was performed on day +84, with 1×10^6 CD3+ T cells/kg. Acute cutaneous and mucous GVHD was observed 2 months after lymphocyte infusion (+140). Shortly after, a complete regression of the hematologic picture, confirmed by skin biopsy was attained. GVHD was effectively controlled by low-doses methyl-prednisolone (0.2 mg/kg) tapered and then withdrawn 3 months after. Late infective complications consisted in 3 episodes of broncho-pneumonia occurred in the last 6 months. He has been followed as outpatient and at the present time he is disease free, at 22 months after allogeneic BMT, and 38 months from onset, respectively. Our case showed a positive stable response to allogeneic transplantation. Clinical and histological data support the hypothesis that response of neoplastic lymphoproliferation to the transplant was immunologically based on graft-vs-malignancy and GVHD combined effects.

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SPONTANEOUS CFU-MK AND BFU-E IN HYPERCELLULAR MYELOFIBROSIS WITH MYELOID METHAPLASIA (MMM): COMPARATIVE RESULTS FROM BONE MARROW AND PERIPHERAL BLOOD

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Background. In vitro formation of spontaneous megakaryocytic (sCFU-Mk) and erythroblastic (sBFU-E) colonies represents one of the biological markers in MMM. It is well known that in advanced fibrosclerotic stage of MMM, hematopoietic stem cells are mobilized and migrate from the bone marrow to the bloodstream, which consequently enhanced number of spontaneous *in vitro* colonies from peripheral blood (PB) in comparison with those from bone marrow (BM). However, it is less known about *in vitro* proliferation of sCFU-Mk and sBFU-E in early, prefibrotic, ie hypercellular stage of MMM. **Aims.** The aim of our study was to analyse the number and frequency of *in vitro* formed sCFU-Mk and sBFU-E from BM and PB in MMM patients in hypercellular phase of disease. **Methods.** We performed a prospective study in 14 previously untreated patients (pts) with median age of 56 years and f/m ratio 8/6, with hypercellular MMM (ECF criteria). The mean number of hematological parameters in PB were as follows: platelets were $600 \times 10^9/\text{L}$, WBCs were $20 \times 10^9/\text{L}$ and hemoglobin value was 133 g/L. sCFU-Mk and sBFU-E in BM and PB cultures were performed on methylcellulose (Iscove et al, 1974. and Messner et al, 1982.), without adding growth factors. **Results.** were compared with control group (K), consisted of healthy volunteers. **Results.** sCFU-Mk were detected with higher frequency in PB (50% of pts), than in BM (25% of pts) cultures. Similarly, sBFU-E was more frequent in PB, than in the BM (79% of pts vs. 58% of pts). Consecutively, sCFU-Mk and/or sBFU-E were detected in higher frequency in PB, than in BM (86% of pts vs. 75% of pts). Beside that, the average number of spontaneous colonies formed in PB cultures was greater than those from BM (5 sCFU-Mk colonies in PB vs. 0.5 sCFU-Mk colonies in BM, and 171 sBFU-E colonies in PB vs. 42 sBFU-E colonies in BM). **DISCUSSION AND Conclusions.** On the basis of our results PB cultures have greater diagnostic value than BM cultures in detection of sCFU-Mk and sBFU-E in MMM pts, even in early prefibrotic phase. It could be speculated that in spite of absence of marked fibrosis in BM in early prefibrotic phase, higher frequency and number of spontaneous colonies in PB compared with BM, might be the result of some other, not yet well defined mechanism. Nevertheless, the standard diagnostic procedure in all pts

with MMM, included hypercellular, prefibrotic phase, should be completed with *in vitro* detection of circulating spontaneous colonies. Assessment of the both type of spontaneous colonies (sCFU-Mk and sBFU-E) enhances the test sensitivity and diagnostic usefulness of *in vitro* colony assay in MMM.

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IMATINIB RESISTANCE IN CHRONIC MYELOID LEUKAEMIA (CML) PATIENTS

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Background. The Tyrosine Kinase inhibitor Imatinib Mesylate induces better haematologic and cytogenetic responses than conventional chemotherapy (alpha-interferon, Cytarabine, Hydroxyurea) in CML patients. However, there are patients who do not respond to Imatinib or, after an initial response, develop resistance. There are several resistance mechanisms that can either be BCR-ABL dependent (genomic amplification and point mutations in BCR-ABL fusion gene) or BCR-ABL independent (acquisition of additional cytogenetic abnormalities and aberrant expression of other genes, for example, WT1). BCR-ABL point mutations are the main mechanism of acquired resistance, being known, until now, more than 30 different mutations (1). Some of them appear to be Imatinib dose dependent while others, namely those located in P-loop, appear to be independent from the dose escalation. **Aims.** To identify the mutation frequency and mutation pattern in fourteen patients and report the adapted therapy. **Methods.** BCR-ABL quantification was done according to EAC protocol. Mutation screening was done by automatic sequencing of a RT-PCR-amplified segment including the BCR-ABL Tyrosine Kinase domain. Karyotype was performed according to standard procedures. Fourteen CML patients filling criteria for acquired resistance were studied. **Results.** a) Five patients (36%) had six point mutations-five already published (E255K, F317L, M351T/G250E and F486S) (1) and one-E435K-once described previously (J. Kaeda, personal communication). b) Four patients (28%) showed a complex variant or additional cytogenetic abnormalities: two at the time of diagnosis {46,XX,t(1;9;22) (q42;q34;q11)[9]/ 46,XX[11] and 46,XX,del(3) (p11p21),t(9;22) (q34;q11)} and two during the course of disease {46,XX,t(5;12),t(9;22) (q34;q11)[8]/ 46,XX,t(9;22) (q34;q11)[12] and 46,XY, t(6;11;12),t(9;22) (q34;q11)[3]/46,XY,t(9;22) (q34;q11)[17]}. None of these represented constitutional alterations. c) In five patients (36%) no alteration was identified. **Conclusions.** There is no evidence based therapy to treat patients with Imatinib resistance. However, in those having BCR-ABL point mutations, the existing data justify the adaptation of therapy (higher dose, cessation of Imatinib or combined therapies) according to the type of mutation. A higher Imatinib dose (600 or 800 mg/day) was given to patients having the mutations F317L, F486S, E453K and the patient with apparently two resistant clones (M351T and G250E). The patient with E255K died some time after detection of the mutation. Concerning the patients with complex karyotypes, no Imatinib dose adjustment was proposed. In the group without point mutations or additional cytogenetic abnormalities, alternative genes (for example WT1 and IPITM1) should be investigated to identify patients who become insensitive to Imatinib and could, therefore, be candidates for interferon administration or other treatment modalities.

Acknowledgements: Some of the studies were possible with support from Novartis Oncologia Portugal.

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GRANULOCYTIC SARCOMA: 32 CASES AND REVIEW OF THE LITERATURES. Paydas¹, S. Zorludemir¹, M. Ergin²¹Cukurova University, ADANA, Turkey; ²Melek, ADANA, Turkey

Thirty two cases with granulocytic sarcoma (GS) have been presented here. The female/male ratio was 1 (16/16), age range was from 16 to 70 years (mean 39.75±14.91). GS accompanied to AML in 13 cases, ALL (My+) in one case, CML in 11 cases (6 in chronic-3 in accelerated and 2 in blastic phase) and MDS in 2 cases. In 3 cases GS was isolated. In 14 cases, GS was detected after the diagnosis of the underlying disease. The interval between the antecedent hematologic disease and GS was 5 to 60 months (mean 17.47±14.14). GS was diagnosed simultaneously with leukemia in 5 cases. In 8 cases, GS predated the leukemia by 0,5 to 24 months (mean 5.19±7.78). GS was unifocal in 14 patients, and multifocal in 18 cases. Lymph node and soft tissue involvement were the most commonly detected localizations and found in 10 and 8 cases, respectively, followed by bone and pleural involvement (each in 4 cases). Other organs involved by GS were ovary, uterus, pelvic mass, testis, bladder, kidney, abdominal mass, eye, orbita, gingiva, nasal mass, breast, stomach, mediastinum, pericardium and surrenal. The diameters of these tumors varied between 2cm and 20cm and some "giant" masses caused compression signs and also severe pain according to their localisation. GS was diagnosed by light microscopy in all, except one case with orbito-ocular GS associated with CML. GS was the first diagnosis in 19 cases. Initially 7 cases had been diagnosed as NHL. Histopathologically blastic variant was found in 11 samples, immature variant in 9 samples and mature variant in 11 samples. Overall survival after GS diagnosis was 8.57±12.76, 7.71±8.04 and 24.71±33.15 months for blastic, immature and mature types, respectively. Myeloperoxidase was found to be positive in 30 of 30 cases tested for this stain. Ly was positive in 24 cases; Pan T and Pan B antigens were screened in cases who had suspected NHL and CD68 and CD43 were used for confirmation of GS diagnosis in a few cases. Local excision and local radiation were performed in one case with localised femur involvement. Local radiation was given to 5 cases. Surgery was performed for mass excision in 3 cases. Fluid drainage was done in cases with pleural and pericardiac involvement. Systemic chemotherapy was given to all AML, CML and MDS cases. The clinical outcome of the patients after the diagnosis of GS was poor and the majority died in a short time (median 9.51±19.38 months). However some cases lived longer. Seven cases survived more than one year and one case lived more than 96 months.

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CLONALITY ANALYSIS IN ESSENTIAL THROMBOCYTHEMIA (ET): DIFFERENCES BETWEEN ADULTS AND CHILDREN

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Background. While in the past ET was always considered to be a clonal disease, recently many ET polyclonal cases have been described. Moreover, it is usually considered that clonal patients have a higher thrombotic risk when compared with monoclonal cases. The tests currently in use for clonality, which evaluate the X gene inactivation pattern, can be informative only in females younger than 65, strongly reducing the number of evaluable patients but being significant in pediatric age. Finally, some evidences suggest that pediatric ET may be a variant of that in adults. **Aims.** To evaluate the clonality status of children ET and its correlation with thrombo/hemorrhagic risk. **Methods.** We studied 20 young females diagnosed to be affected with ET in agreement with the PVSG criteria. 9 cases were diagnosed in pediatric age (11±4 years) and 11 were older than 30 years (43±9). The median follow-up was 5.8 years (3.7 in children and 7 years in adults). The occurrence of thrombo/hem-

orrhagic event was registered. The HUMARA assay was performed according to standard method in genomic DNA extracted from both granulocytes and lymphocytes. If homozygous HUMARA was found, PGK gene was evaluated. **Results.** The homozygous cases for HUMARA were 4 (20%). One of them, an adult, was excluded because homozygous in both evaluated genes. In 4 other adults skewing results were obtained. Therefore, the informative patients were 15 (75%) (all children and 6 adults). One child resulted monoclonal while the other 8 (88%) were polyclonal. In contrast, 4 (40%) adults were monoclonal and 2 (20%) were polyclonal. One polyclonal child had a supra-hepatic vein thrombosis while 1 portal, 1 supra-hepatic vein and 1 cerebral sinus thrombosis plus 1 major gastro-intestinal haemorrhage were registered in adults. All of them were monoclonal. The incidence of hemostatic events in monoclonal adult cases was significantly higher than in polyclonal cases (p=0.002). All pediatric ET cases resulted informative concerning clonality status, in contrast with 60% of adults. **Conclusions.** We conclude that 1) the use of two X genes improves the clonality assays; 2) all but one of our children are polyclonal and 3) monoclonality in adults but not in children correlates with a thrombo/hemorrhagic risk.

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INTERIM ANALYSIS OF EFFICACY OF FLUDARABINE VS FLUDARABINE AND CYCLOPHOSPHAMIDE REGIMENS IN PATIENTS WITH CLL-THE EXPERIENCE OF UKRAINIAN STUDY GROUP ON CLL

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The aim of our study was to evaluate the efficacy of fludarabine and combination fludarabine with cyclophosphamide in the treatment of CLL patients. **Material and methods.** Between October 2000 and July 2004 159 CLL patients were enrolled in the study. Prior to the study 121 patient had received from one to five different treatment regimens, while 38 had no prior treatment. 55 patients received fludarabine intravenously (IV) 25 mg/m²/day on 1-5 days every 28 days and 104 received IV fludarabine 25 mg/m²/day on 1-3 days plus cyclophosphamide 300 mg/m²/day on 1-3 days every 28 days. **Results.** In the 129 patients valuable to response, the OR was 82,2% including CR 38,7%, PR 43,5%. There was no significant difference in response rate between untreated (n=35) and pretreated patients (n=94) with an OR 85,7% and 79,8% respectively. It was found that patients treated with fludarabine and cyclophosphamide (n=94) had significantly higher OR comparing with patients who received fludarabine alone (n=35) (86,1% and 71,4% respectively, p<0,05) and CR (44,7% è 22,8% respectively, p<0,05). After a median observation time of 15 months the median progression free survival was 14.5 months (21.5 months in patients with CR and 12.5 months in patients with PR). The most common adverse event was myelosuppression (in 58,8% after treatment with fludarabine alone and 69,5% after combination treatment) and infection 44,1% and 46,3% respectively). **Conclusions.** Fludarabine and fludarabine plus cyclophosphamide are effective for patients with CLL as treatment option for both untreated and previously treated CLL patients. The response rate of combined regimen with fludarabine and cyclophosphamide is higher as well as CR rate. Fludarabine based treatment is well tolerated with more frequent myelosuppression after fludarabine and cyclophosphamide combination regimen.

1175**THE REGISTRO ITALIANO TROMBOCITEMIA (RIT) AS TOOL OF EPIDEMIOLOGICAL, CLINICAL AND BIOLOGICAL RESEARCH. A GIMEMA PROJECT**

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The Registro Italiano Trombocitemia (RIT) is a project approved and supported by the GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) Foundation. The RIT is electronically structured and besides a general section open to everyone (public area), comprehends a database of the Essential Thrombocythemia (ET) patients diagnosed in Italy since January 2004. The ET patient registration will be done, respecting the privacy rules, by the Haematological Centres belonging to the GIMEMA Group and subsequently by the other Centres of Internal Medicine, Blood Transfusion, Angiology, Cardiology, Paediatrics, Gynecology, etc. The epidemiological, clinical and biological data will be subject to validation and analysis by various Expert Subcommittees on Cellular and Molecular Biology, Genetics, Haemostasis, Histopathology, Pregnancy, Pediatric and Familial ET, Conventional and Experimental Drugs. Specific objectives of the RIT are: - to check the ET diagnostic criteria utilised in the Italian Centres, with the aim to improve the appropriateness and the homogeneity of the diagnostic approach, with particular interest in the Bone Marrow Histopathology. - to identify the therapeutic approach adopted by the Centres and their compliance to the therapeutical Guide Lines of SIE, SIES, GITMO. - to monitor the ET patient, particularly those receiving experimental molecules as Interferon alpha and Anagrelide (observational study with annual updates). - to evaluate the incidence and the diagnostic-therapeutic approach of Pregnancy, Paediatric Age and Familiarity in ET. - to identify the prognostic values of the new biological parameters. - to obtain information useful to design new clinical and biological studies. The RIT, coordinated by the Haematology Unit of Reggio Emilia, has been active since March 2005.

1176**HIGH RESPONSE RATE IN PRETREATED PATIENTS AFFECTED BY CHRONIC LYMPHOBLASTIC LEUKEMIA WITH LOW-DOSE OF ALEMTUZUMAB THERAPY**

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Background. Several studies reported the efficacy of alemtuzumab in previously treated CLL, at a dose of 30 mg three times weekly, but not data were published regarding the use of low-dose alemtuzumab. *Aims.* To assess efficacy, hematological and extrahematological side effects, infectious disease rate, CMV reactivation and immunological recovery after low-dose alemtuzumab in relapsed or refractory CLL. *Methods.* twelve patients were included (8 males, 4 females; median age of 61.5 years), 7 were in relapse and 5 were refractory to previous treatment. All patients received alemtuzumab at 10 mg as target dose, 3 times weekly, for a maximum of 10 weeks. At study entry, one patient was in stage A/progressive, five in stage B/II and six in stage C/IV. Seven patients had unmutated IgVH genes, 4 had mutated IgVH and 1 patient was not evaluable. *Results.* Two patients obtained a CR (16%) and 3 patients (25%) achieved a PR. When Binet/Rai stage was taken into account, the OR was 83% in stage A-progressive/B-II compared to 0% in stage C/IV (p=0.01). With respect to VH gene mutational status, OR was 50% in patients with mutated VH genes and 29% in patients with unmutated VH genes (p=ns). None of refractory patients achieved response to alemtuzumab, of seven

patients treated in relapse, five responded. At this time, 2 patients are in CR without detectable MRD at 15 and 10 months. Among patients achieving PR, one is in continuous PR at 11 months, one died 7 months from the end of treatment in PR for idiopathic pneumonia and one is alive at 24 months with disease progression. The median response duration was 10 months. Two patients showed grade IV neutropenia and required G-CSF during the treatment. Grade III anemia was noticed in three patients, one patient received 1 RBC unit and grade III thrombocytopenia occurred in 2 patients. Mild adverse infusion related events (grade I/II) occurred during the first week of treatment. Alemtuzumab was generally well tolerated with no episodes of febrile neutropenia or bacterial infection during treatment. The incidence of CMV reactivation (Ag and/or CMV DNA) was 66% even if no evidence of CMV disease was noticed. All patients were successfully treated with oral ganciclovir 1000 mg tid for a median of 14 days. Immunological recovery was markedly delayed during and after alemtuzumab therapy. Before treatment all lymphoid subsets, except for total lymphocytes and NK cells, showed median values into the normal range. Total lymphocytes, T-helper and T-suppressor cells rapidly decreased, achieved a minimum level after 2 months, and thereafter showed a mild trend toward recovery. However, their median values were still below the normal range at the end of the study. *Conclusions.* low-dose alemtuzumab induced significant responses in these CLL patients, with mild hematological and extrahematological side effects and low risk of infections, even in the presence of long-lasting severe immunosuppression. Among these patients we detected a particular subgroup (relapsed CLL, A-progressive/B-II stage, mutated-IgVH status) in which low-dose alemtuzumab offered a higher OS and response rate.

1177**THE RESULTS OF TREATMENT OF CHILDRENS CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA WITH INTRON A ALMATY, KAZAKHSTAN**

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Background. ITP- the most frequent acquired hemorrhagic diseases in children. Despite of the relatively favorable prognosis for disease in children the optimal methods of treatment in various course of disease have not worked up yet. *Aims.* 26 patients with chronic ITP were examined and treated with intron A. *Methods.* We use the scheme of therapy which lasted from 4 to 16 weeks depending of the respond during the first 4 weeks. During the induction therapy (1-8 weeks) administrated dose of intron A was 3 UE three times a week. Accordingly the scheme of supportive therapy the administrated dose was 2 UE three times a week during the 9-12 weeks and 1 UE three times a week during the last 13-16 weeks. There are four types of response: complete response when the platelet count of 150000/mm³ or higher during the first 4 weeks and remission lasted not less than 12 weeks; partial response - platelet counts in the range 30 to 150000/mm³ during the first 4 weeks and remission lasted not less than 6 weeks; and late effect when the platelet counts in range 30000 to 150000/mm³ only after 4 weeks of treatment and such type of response lasted not less than 6 weeks and non-response type when the platelet count remains decreased. The most frequent side effects of treatment - in influenza syndrome was treated by administration of paracetamol. *Results.* 16 girls and 10 boys age from 4 to 18 with the duration of diseases from 6 weeks to 18 years were treated. All patients had been treated with steroids before administration of intron A. Hemorrhagic syndrome includes cutaneous bleeding and mucous membrane hemorrhage, in 19 cases - epistaxis and gingivitis, in 3 cases - menorrhagia. The platelet counts was from 30000/mm³ and less. The myelogram match the diagnosis of ITP. Complete response reached in 5 patients (19,2%); partial response - in 9 (34,6%) patients; in 3 patients the clinical-hematological response was temporal and non-response group of patients were 9 patients (34,7%). In summary, in treatment of 26 patients with intron A the clinical-hematological

response was reached in 17 patients - 65,3%. The platelet counts in what group increased from 8000-42000 to 170000-280000 /mm³. Among the patients with complete and partial response the initial platelet count was more than 20000/mm³. In non-response cases the initial platelet count was below 20000/mm³. **Conclusions.** In treatment of chronic ITP with intron A the clinical-hematological remission reached in 65,3% cases; complete response for treatment was reached in 19,2% patients; the results of treatment

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COMPARATIVE ANALYSIS OF CLL IN PERSONS WHO SUFFERED AFTER CHERNOBYL ACCIDENT AND IN UNEXPOSED CLL PATIENTS

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CLL after Hiroshima is considered not to be radiation-induced. However our preliminary data showed that CLL after Chernobyl accident affected more younger people than in population and has some features. The aim of our study was to find the differences of CLL between patients who suffered after Chernobyl nuclear power plant accident in 1986 and unexposed CLL patients. 34 exposed CLL patients (group I) were compared with 34 unexposed CLL (group II) of the same age and gender. In all cases the diagnosis was confirmed using morphology, histology and immunophenotyping of peripheral blood/bone marrow. The treatment of CLL patients was standard with alkylating agent (17/34, group I and 20/34 group II) and purine analogs (17/34, group I and 14/34 group II). **Results.** There were no significant differences between these groups in staging: at the diagnosis 2/0, 29/28 and 3/6 patients were at stage A, B or C according to Binet classification in the first and second groups respectively. CLL/PLL morphology was more frequent in the first group of patients (5 vs 2 in the second group). The most of the exposed patients had a hyperplastic syndrome and leukocytes number until $30 \times 10^9/L$ in peripheral blood. Time to progression in the exposed CLL patients was shorter Time to progression in the exposed CLL patients was shorter than in unexposed patients (11,05±5,9 versus 27,7±9,4 months respectively). There was no significant difference in overall response to fludarabine-based treatment in unexposed and exposed CLL patients (12/14 and 12/17 patients respectively, $p > 0,05$), but relapse rate was significantly higher in exposed (9/12 and 4/12 respectively, $p < 0,05$). **Conclusions.** our preliminary data demonstrate some differences in CLL course in exposed and unexposed. CLL in patients who suffered from Chernobyl accident is characterized by more aggressive course with rapid progression and resistance to standard treatment regimens.

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CLINICAL UTILITY OF SERUM OSTEOPROTEGERIN LEVEL IN PATIENTS WITH PH-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background. Myelofibrosis with myeloid metaplasia (MMM), polycythemia vera (PV) and essential thrombocythemia (ET) belong to the group of Philadelphia (Ph)-negative chronic myeloproliferative disorders (CMD). All these three disorders have an elevated risk of vascular complications. Distinctive biological features of MMM include, in addition to bone marrow fibrosis and myeloid metaplasia, also osteosclerosis, which is not found in PV and ET. Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor (TNFR) superfamily that acts as a decoy

receptor for the receptor activator of nuclear factor- κ B ligand (RANKL), inhibiting RANK/RANKL interaction on osteoclasts and osteoclastogenesis. Murine models indicate that osteosclerosis predominantly occurs via an up-regulation of OPG in thrombopoietin-induced myelofibrosis (Blood 2003;101:2983-9). Recent clinical studies have also reported a correlation of OPG with vascular diseases. **Aims.** To prospectively assess the clinical utility of serum OPG levels in 166 patients with Philadelphia (Ph)-negative CMD. Patients and **Methods.** From February to November 2004, blood samples from 166 consecutive patients with Ph-negative CMD were collected. In detail, 35 patients (10 at clinical onset and 25 at follow-up; 18 under treatment and 17 out of therapy) had MMM, 71 patients (10 at clinical onset and 61 at follow-up; 39 under treatment and 32 out of therapy) had PV; 60 patients (16 at clinical onset and 44 at follow-up; 31 under treatment and 29 out of therapy) had ET. At the time of serum OPG measurement, thrombotic complications were observed in 11 patients with MMM, 22 with PV and 28 with ET. Osteoprotegerin was measured in a blind manner by enzyme immunoassay (Biomedica Gruppe, Wien) following the manufacture instructions. Bone marrow biopsies from 45 patients (21 at clinical onset; 24 at follow-up) were evaluated in a blind manner at the time of OPG measurement to assess bone marrow fibrosis and osteosclerosis. **Results.** Median serum OPG levels were 5.23 pg/mL (range 1.91-20.61) in MMM, 5.6 pg/mL (range 0.99-21.13) in PV, 5.44 pg/mL (range 0.4-28.23) in ET and 4.48 pg/mL (range 2.8-8.5) in age-matched healthy control. Kruskal-Wallis ANOVA did not show a significant difference in serum OPG levels between different patient groups. Non-parametric Mann-Whitney test did not reveal any significant correlation between serum OPG levels and degree of osteosclerosis and bone marrow fibrosis. A general linear model showed that serum OPG levels correlate with history of thrombosis ($p=0.023$) and age ($p=0.008$). **Conclusions.** Serum OPG is not a surrogate marker of osteosclerosis and does not allow MMM to be distinguished from PV or ET. The relationship with history of thrombosis rather indicates that OPG might be useful to predict vascular complications in patient with Ph-negative CMD.

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TELOMERASE ACTIVITY IN MULTIPLE MYELOMA

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Background. Telomerase activity is associated with most malignant tumors. **Aim.** The aim of this trial was to evaluate telomerase activity in multiple myeloma (MM) including prognostic potential of this marker. **Methods.** CD138+ myeloma cells were isolated from bone marrow patients with active MM (28 new diagnosis; 5 relapse) using magnetic activated cell sorting Miltenyi. **Results.** Ten patients with monoclonal gammopathy under significance (MGUS) represent control group with low activity of disease. Median follow-up of MM patients was 715 days, over all survival was 630 days. The analyses of telomerase activity were undertaken on 34 samples with high purity of CD 138+ myeloma cells with median 85.7% (range 50,0-100,0%). There was heterogeneity in telomerase activity. Index for telomerase activity in MM patients was 0.92 for CD138+ myeloma cells and 0.68 for CD138- cells ($p=0,238$). The corresponding indexes for newly diagnosed MM and relapse only were 1.18 (CD138+) and 0.81 (CD138-). In control group of (MGUS), patients reflecting low activity index of telomerase activity was only 0.20 ($p=0,054$) with very low activity of telomerase activity detectable in 2 of 10 patients only. No significant correlations with standard prognostic factors were observed.

Similar was true for parameters of survival. *Conclusions.* The results have shown that telomerase activity has trend to correlate with activity of MM. Unfortunately telomerase activity in marrow of multiple myeloma patients is not specific for CD138+ myeloma cells. This fact limits usefulness of telomerase activity for prognostic purpose in this diagnosis.

Supported by grant IGA MZ-R 7475-3.

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PERIPHERAL BLOOD STEM CELL COLLECTION IN PATIENTS WITH CML ACHIEVING COMPLETE CYTOGENETIC REMISSION ON IMATINIB

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Background. Autologous stem cell transplant is a recognised therapeutic option for patients with CML. Low levels of residual disease in the harvest product have been correlated with better outcome. *Aims.* Feasibility of harvesting patients who achieved complete cytogenetic remission (CCyR) on imatinib. *Methods.* Patients in CCyR on Imatinib were mobilized with daily G-CSF (10µg/kg or 16µg/kg according to physician preference) for 5 consecutive days. On day +5 a peripheral blood CD34 count was performed and if it was $5 \times 10^3/\text{mm}^3$ aphaeresis was done. We aimed to harvest $2 \times 10^6 \text{CD}34/\text{kg}$ body weight or $10 \times 10^6 \text{MNC}/\text{kg}$ body weight. Aphaeresis was repeated daily until the target dose was reached. Imatinib was continued throughout this period. In patients who failed to mobilize, imatinib was interrupted 4 to 6 weeks prior to the second harvest. We performed 110 attempts of mobilization in 82 patients who achieved CCyR on imatinib. The median age was 50 years (range 21-73 years), 39 males and 41 females. The median time from diagnosis to start of imatinib was 1.93 years (range 0 to 10.3 years). 74 patients were in chronic phase at the moment of starting imatinib and 8 met criteria for accelerated phase. Sokal risk group distribution of the patients were 24 (30%), 26 (32.5%) and 29 (37.5%) in the low, intermediate and high risk groups respectively. 51 patients previously failed to achieve CCyR on IFN- α and 29 patients were started on imatinib shortly after diagnosis (in chronic phase). At the moment of the harvest, Imatinib was continued at the dose patient was taking - 400mg in 75 (68%) and 600mg or more in 25 (23%) occasions. In 10 instances imatinib was interrupted. The intended dose of G-CSF was 10µg/kg and 16µg/kg in 72 and 28 patients respectively. *Results.* The CD34 count in peripheral blood was lower than $5 \times 10^3/\text{mm}^3$ on 38 occasions and the harvest was not attempted. On 72 occasions when harvest was performed, the median CD34 yield was $1.26 \times 10^6/\text{kg}$ (range 0.1-13.8) and the median MNC yield was $7.66 \times 10^6/\text{kg}$ (range 1.38-19.74). Of the 110 attempts of mobilization, the target yield was achieved on 56 occasions (50.9%) which is 78% of the instances where harvesting was actually attempted (72 attempts). The level of residual disease in the harvest product and in the patients was comparable (BCR-ABL/ABL ratios of 0.24 and 0.27% respectively). Univariate analysis was performed in order to identify predictive factors for a successful harvest and the only significant variable newly diagnosed versus interferon failure (RR=2.4, p=0.04). Interestingly, the dose of G-CSF did not seem to influence the outcome. In 7 out of 10 cases where imatinib was interrupted due to previous failed mobilization, the patients achieved the targeted yield. *Summary.* 51% of CML patients in CCyR on Imatinib had successful PBSC collection using our protocol. Discontinuation of Imatinib seems to facilitate harvest of patients who previously failed to mobilise without significant increment in the BCR-ABL/ABL ratio.

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EXPRESSION OF MULTI-DRUG RESISTANCE GENES IN CHILDREN WITH B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Modern treatment protocols lead to complete remission in a high proportion of patients with childhood acute lymphoblastic leukemia (ALL), but a large number of them show a relapse of the disease. Treatment failure in these patients is mainly attributable to *de novo* or acquired resistance to a wide variety of cytotoxic drugs, which is called multi drug resistance (MDR). Expression of multi drug resistance 1 gene (MDR1), multiple resistance protein (MRP) and lung resistance protein (LRP) gene, is implicated in the drug-resistance mechanism. The aim of this prospective study was to investigate the expression of MDR1, MRP and LRP genes and their correlation with clinical and laboratory parameters and outcome in children with B-cell ALL. *Methods.* Study population was 15 children with B-cell ALL with an excess of 90% bone marrow infiltration with leukemic blasts at initial diagnosis. All patients were treated according to the BFM95 chemotherapy protocol. Total RNA was isolated from bone marrow samples at initial diagnosis and on the 33rd day, a time point when the response rate to chemotherapy is evaluated. The expression of MDR1, MRP, LRP and the housekeeping β -actin gene was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) using the appropriate primers. *Results.* According to our results, MRP and LRP gene expression was detected in all the studied cases at initial diagnosis, while different expression was detected in the bone marrow samples on the 33rd day. However, expression of MDR1 gene was only detected in 4 (26%) patients. One of these patients, experienced relapse of the disease, while another patient didn't have complete remission in the bone marrow on the 33rd day. The co-expression of MRP and LRP genes did not significantly influence the response rate to the induction therapy. Significant correlation was detected between the initial WBC count ($>50 \times 10^9/\text{L}$) and the expression of MDR1 gene. *Conclusions.* The results so far, show that MRP and LRP genes are expressed in B-cell ALL cases. The prevalence of MDR1 expression in our cases, is comparable with other published reports. The determination of MDR1 gene expression might contribute to early identification of patients at risk of treatment failure.

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IMATINIB IN THE TREATMENT OF ELDERLY PATIENTS WITH PH+ CML IN THE LATE CHRONIC PHASE

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CML is involving all the age groups of patients with the median 55 years at diagnosis. According to published data 54-63% of patients are older than 60 years. Previously if the patient was not a candidate for allogeneic hematopoietic cell transplantation the only possibility of treatment was the use of hydroxyurea or interferon alfa (IFN), which was limited by rather frequent adverse events. When imatinib mesylate using the mechanism of inhibition of tyrosine-kinase activity of BCR-ABL gene was introduced to clinical treatment, a change of therapeutic approach has appeared. So far the results of clinical studies with imatinib show huge efficacy concerning both haematological and cytogenetic response. The aim of our study was to show imatinib efficacy in the treatment of elderly Ph+ CML patients verifying its tolerability and adverse toxicity in this group. *Methods.* 37 patients (13 male, 24 female) with the median age 63 years (60-74) were included in the study. All of them were in the

chronic phase of the disease but resistant to long-term treatment of IFN. The used daily dose of imatinibe was 400-600 mg/day. We evaluated the frequency to achieve complete haematological remission (CHR) and both complete (CCR) and major (MCR) cytogenetic response. We used the classical karyotype examination together with FISH and in the majority of patients the amount of BCR-ABL transcripts by quantitative RT-PCR was measured. The toxicity was evaluated by NCI/NIH criteria. *Results.* 37 patients were evaluated in the 1st chronic phase. The median of the time duration from the diagnosis to imatinib treatment was 33 months (6-140). The drug was started after previous IFN treatment in the patients who either had not achieved cytogenetic response to IFN or IFN had not been tolerated. The median duration of IFN treatment was 27 months (6-68). Imatinib was administrated 6-39 months (median 22). The criteria for CHR was fulfilled by 94% patients (35/37). Cytogenetic response was achieved in 67% patients (25/37), MCR in 51% and CCR in 27%. In 14% of patients (5/37) there was a cytogenetic progression. In the time of the study 11% (4/37) of the patients died, all of them from the CML progression. Quantitative RT-PCR BCR-ABL monitoring showed 2-3 log decline in 30% of patients. The number of achieved responses was negatively influenced by the prior disease duration and intensity and length of IFN pretreatment. Haematological toxicity grade 3 (cytopenia) led only to the dose reduction. Non haematological toxicity was seen in 25% of patients (9/37), mainly as swellings, weigh gain, muscle pain and cramps. The achieved intensity of this toxicity was only grade 1 to 2 and was relieved by symptomatic treatment. *Conclusions.* The study demonstrates that imatinib is significantly effective in the treatment of elderly patients with CML Ph+, with the achievement of CHR 94%, MCR 51% and CCR 27% and allows a good long-term survival without serious adverse events with good quality of life. Supported with the grant 5902-3 IGA MZ CR.

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IMATINIB MESYLATE FOR TREATMENT OF PATIENTS WHO RELAPSE AFTER STEM CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKEMIA

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Introduction: Allogeneic stem cell transplantation (alloSCT) is the only curative treatment for patients (pts) with chronic myeloid leukemia (CML). Relapse is the major cause of treatment failure. Imatinib mesylate, a drug that inhibits the Abl tyrosine kinase and thus blocks the proliferation of CML cell lines and clonogenic CML progenitor cells is a new treatment for pts with CML in relapse after alloSCT. Patients and *Methods.* From November 2002 to November 2004 nine pts were assigned to receive imatinib mesylate for CML relapse after alloSCT. Six had failed previous response to donor lymphocyte infusions (DLI), 1 had extensive chronic graft versus host disease (GVHD), 1 with no disponsible donor for DLI and 1 as first line therapy. Their median age was 38 years old (range: 28 - 52), being 6 male and 3 female. Disease status was respectively 2 pts in haematological relapse, 4 in cytogenetic relapse and three in molecular relapse. Median time between relapse and imatinib therapy was 313 days (range:20-1661). Imatinib was administered at a dose of 400 mg daily. Definition of CML response and toxicities were according respectively IBMTR and NCI-CTC criteria. Bcr/Abl was monitored by using real-time quantitative PCR. *Results.* With a median therapy time of 317 days (range: 30 - 706), 8 pts (88%) achieved complete molecular remission. At a median follow-up of 464 days (range: 73-800), 8 pts (88%) remain in complete molecular remission. Only two cases of grade 3-4 toxicities were found, one haematological and one digestive. *Conclusions.* 1) Imatinib mesylate is a highly effective option to induce molecular remission in patients with CML relapsed after alloSCT. 2) Imatinib mesylate is well tolerated

with few toxicities compared with DLI. 3) It is possible that imatinib mesylate and DLI (simultaneous or sequential) can be beneficial in some cases, thus reducing the need for higher doses of DLI and reducing the risk for complications.

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CYTOGENETIC FINDINGS IN 45 CASES OF MANTLE CELL LYMPHOMA

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Background. Mantle cell lymphoma (MCL)-malignant lymphoid tumor, composed of CD5+ B-cells immunophenotypically consistent with mantle B-cells and carrying specific cytogenetic hallmark-t(11;14) (q13;q32). Clinical behavior of MCL is aggressive with high rate of blastoid transformation and median overall survival (OS) near 3 years. Nevertheless clinical presentations of the disease are heterogeneous and survival varies in wide limits. *Aims.* To define the type and frequency of secondary chromosome changes in MCL, its possible role in pathogenesis and correlation with clinical course and prognosis. *Methods.* In 45 t(11;14) (q13;q31)-positive patients, 34 men and 12 women (3:1), middle age 53 years (21-79), 30 - small cell morphology, 6 with *de novo* blastoid variant and 9 with blastoid transformation we performed conventional cytogenetic assay (CCA) and FISH using 5 DNA probes (11q23/ATM, 13q14, 17p13/p53, 9p21/p16 and centromere-12 specific probes). *Results.* Secondary chromosome abnormalities were found in 39 of 45 patients. Most frequent aberrations are listed in Table 1.

Table 1. Most frequent chromosome aberrations.

Aberration	Frequency Cases (%)	Method
del(11)(q25)	23 (51%)	CCA+FISH
del(9)(p21)	17 (37%)	CCA+FISH
-13del(13)(q14)	18 (39%)	CCA+FISH
-17del(17)(p13)	10 (22%)	CCA+FISH
del(8q)	10 (22%)	CCA
del(1)(p1q)	10 (22%)	CCA
+3q1q	8 (17%)	CCA
-20	5 (11%)	CCA
+12t12q	4 (8%)	CCA+FISH
11(18)(p13)	3 (7%)	CCA

Frequency of chromosome aberrations detected by FISH in various histological types and clinical phases of the disease

Cell type and disease status (N)	del11q N (%)	del13q N (%)	+12 N (%)	del9p N (%)	del17p N (%)
Small cell primary (11)	7 (37%)	6 (32%)	2 (10%)	4 (21%)	4 (21%)
Small cell relapsed (11)	6 (55%)	2 (18%)	-	3 (27%)	-
De novo blastoid (7)	3 (50%)	3 (50%)	1 (17%)	4 (67%)	1 (17%)
Blastoid transformation (9)	7 (78%)	5 (56%)	1 (11%)	6 (67%)	5 (56%)

Del11q23/ATM was detected half as much again in relapse and with double frequency in *de novo* blastoid variant. Del9p21/p16 and del13q14 were markers of large cell tumors. Del17p13/p53 was revealed more than in half cases of blastoid transformation, at the same time 4 of 19 (21%) patients with small cell variant were found out to have p53 deletion. In all 10 cases this aberration was accompanied by other abnormalities, most frequently 11q23, 13q14 and 9p21 deletions. The major clinical feature of patients with del6q was bulky lymph nodes involvement. Median OS in patients with del11q23 and/or del13q14 and/or del9p21 and/or del17p13 was 31 months versus 42 months in patients without these aberrations ($p < 0.05$). Number of cytogenetical abnormalities did not influence OS in our sample. Summary. The most frequent secondary chromosomal aberration was del11q23. Mean number of cytogenetic abnormalities in ATM-deficient cases was twice higher than in cases without it (7, v. 4,3) and it can be indirect evidence that cases with del11q23 are characterized by impaired or absent reparational delay of G1/S transition. Del9p21/p16 was observed in 37% of patients, most often in transformed and *de novo* blastoid cases. Del13q14 was found in 35% of cases and in contrast to smoldering B-CLL was always accompanied by del11q23 and/or del9p21 and/or del17p13 and/or other alterations. Del17p13/p53 was detected not only in transformed or *de novo* blastoid cases as it had been described. This coarse aberration is usually the mark of terminal tumor progression and is associated with refractoriness to numerous cytostatic agents and radiotherapy. MCL is known to be chemoresistant and have shortest time-to-progression among all lymphomas. May be in part it is due to p53 deficiency, which can be discovered even in primary patients. All 10 patients with this aberration did not respond to treatment including high-dose schemes and immunochemotherapy, so this abnormality must be revealed by FISH together with t(11;14) (q13;q31) in the very beginning of the disease.

1186

EXPRESSION OF ONCOGENES AND IPS LIKE PROGNOSTIC FACTORS IN HODGKIN`S LYMPHOMA - OUR EXPERIENCE

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Background. Hodgkin`s lymphoma (HL) is a curable malignant disease. The International Prognostic Score (IPS) is currently the accepted prognostic score for patients with HL. However, oncogenes with important role in etiopathology of HL was not used as an independent variable in prognosis of patients with HL. **Aims.** The aim of the present study was to assess and compare the prognostic significance of the immunohistochemistry expression of proapoptotic genes p53 and retinoblastoma (Rb) and conventional prognostic factor-IPS. Patients and **Methods.** Our series included 40 patients with HL (M/F ratio 24/16) (median age 30,2 yr, range: 15-68 yr), diagnosed in a single institution between october 2001 and june 2003. In each patient the relationship among IPS, response to therapy and survival was investigate. Also, we evaluated the expression of p53 and Rb gene with an immunohistochemical technique. The expression of investigated genes was assessed as percentage of positive Reed Sternberg cells (RCS) in relation to all slightly RSC on slides. **Results.** Average value of p53 and Rb expression were respectively 2.9% and 21.8%. Complete response (CR) rate to systemic chemotherapy was not correlated with p53 and Rb expression. Patient with expression Rb >20% had significantly better overall survival (29.8 months) than patients with expression $\leq 20\%$ (18.7 months) while p53 expression significantly not influenced to overall survival. In our group, on presentation, 50% of pts had low risk for disease progression (IPS value 0-2). Kaplan-Meier analysis of survival showed that IPS value turned out to be of prognostic value for overall survival but IPS significantly correlate with event free survival: pts from group of low risk had significantly longer survival to relapse (32.2 months)

vice versa pts with intermediate and high risk (21.8 months). **Conclusions.** Further studies with large numbers of samples and homogenous group of HL are needed to determine the prognostic value of proapoptotic genes, p53 and Rb in HL. According to our investigation, IPS is still main predictor for prognosis in pts with HL.

1187

ADRIAMYCIN ANTILEUKEMIC ACTIVITY IS ENHANCED BY OCTREOTIDE IN VITRO AND IN VIVO: UP-REGULATION OF THE TOPOISOMERASE II-ALPHA AND TOPOISOMERASE I TARGET MOLECULES

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Background. Anthracycline antibiotics, such as adriamycin (DOX) are essential components of first-line chemotherapy in the treatment of a variety of solid and hematopoietic tumors. Nuclear targets of DOX include primarily the topoisomerase II-alpha (Topo IIalpha) inhibition through ternary complex stabilization and catalytic inhibition, and topoisomerase I (TopoI) in much less magnitude. Among the mechanisms of resistance qualitative and quantitative alterations of topoisomerase II have been identified, where Topo II activity was significantly less in resistant cells than in sensitive cells. Octreotide (OCT) is an eight amino-acid peptide, which attains its biological effects on target cells by binding preferentially to sst2 and, to a lesser extent, to sst3 and sst5 somatostatin receptors (SS-Rs). The presence of SS-Rs in human lymphoid leukaemia cell lines, in malignant lymphomas and in lymphoproliferative diseases is clearly detected. **Aims.** The effect of OCT on Topo IIalpha and I expression in leukemic cells has not yet been investigated. We studied the *in vitro* effect of OCT alone or in combination with DOX on the Topo IIalpha and I expression in two lymphoid leukemia cell lines as well as the *in vitro* and *in vivo* antileukemic activity of ADR alone or in combination with OCT. **Methods.** The *in vitro* effect of OCT alone or in combination with DOX on the Topo IIalpha and I expression was estimated in Jurkat and MOLT-4 human lymphoblastic leukemia cell lines by the detection of Topo IIalpha and I mRNAs. RNA was extracted from cells by the RNazol method. cDNA amplification was carried out by RT-PCR and the evaluation of mRNA expression was accomplished by quantification of the electrophoresed specific PCR products with Molecular Imager FX. The *in vitro* cytostatic and cytotoxic effects of DOX alone or in combination with OCT on Jurkat and MOLT-4 human lymphoid leukemia cell lines and on P388 murine lymphocytic leukemia were evaluated with the MTT colormetric metabolic assay. The respective *in vivo* antileukemic activity was estimated against BDF1 male mice bearing P388 murine lymphocytic leukemia. **Results.** OCT directly and significantly up-regulates the expression of Topo IIalpha, whereas Topo IIalpha is depleted under the continuous DOX effect. Moreover upon the addition of OCT Topo IIalpha expression is clearly detected. OCT did not influence the TopoI expression. However, when cells were treated with OCT in combination with DOX the TopoI expression was significantly enhanced. These effects were dose dependent. OCT induced no antileukemic activity *in vitro* and *in vivo*. When DOX was combined with OCT, DOX antileukemic activity was significantly augmented *in vitro* (>25% reduce of DOX IG50, TGI and IC50, $p < 0.001$) and *in vivo* (>2 folds lifespan increase, $p < 0.001$). **Conclusions.** OCT significantly enhances the ADR antileukemic activity *in vitro* and *in vivo* by up-regulating the Topo IIalpha expression, the major nuclear target of DOX. Up-regulation of TopoI expression induced by OCT is less potent for DOX activity, however this effect requires the simultaneous cytotoxic activity of DOX. Further investigation in the molecular basis of chemo-sensitizing neoplastic cells to anthracyclines is required.

1188**MITOXANTRONE, FLUDARABINE, CYTARABINE, CISPLATIN AND DEXAMETASONE AS SALVAGE CHEMOTHERAPY IN RELAPSING AND REFRACTORY AGGRESSIVE LYMPHOMA**

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Background. The therapy of patients with prognostically unfavorable recurrent and refractory aggressive non-Hodgkin lymphoma is still unsatisfactorily. **Aims.** The aim of the study was to evaluate the feasibility and efficacy of the combination of mitoxantrone, fludarabine, cytarabine, and cisplatin in patients with prognostically unfavorable recurrent and refractory lymphoma. **Methods.** Forty-two patients (median age 39 years, range 18-61) with relapsed (71,42%) or refractory (28,57%) malignant lymphoma were enrolled (low-grade/transformed LMNH, 40,47%; high-grade LMNH, 59,53%). A total of 27 patients (64,28%) showed multiply relapsed diseases with duration of prior remission of < 12 months and had lymphoma being resistant to prior chemotherapy. The therapy consisted of fludarabine (15 mg/m², q. 12 h, day 1-4), cytarabine (50 mg/m² by continuous infusion (CI), day 2-4), cisplatin (100 mg/m² by CI over 24 h, day 1), mitoxantrone (4 mg/m², day 2-5) and dexametasone (40 mg, day 1-4). **Results.** Ten patients (23,8%) achieved complete remission (CR) and 15 patients (35,7%) partial remission (PR), for an overall response (OR) rate of 59,5%. After a median follow-up of 12 months only 8 (19,04%) patients are in continuous CR. The median duration of event-free survival (EFS) and overall survival (OS) were 6.5 and 13.3 months, respectively. Probabilities of EFS and OS after 2 years were 19% and 40%. Unfavorable prognostic factors for EFS by univariate analysis were refractory lymphoma (Chi2= 4,35 p > 0,056) and the presence of B-symptoms (Chi2= 4,75 p > 0,048). Significant prognostic factors for OS were refractory lymphoma, B-symptoms, and bone marrow involvement. The major toxicities were granulocytopenia and thrombocytopenia of the World Health Organization (WHO) grade IV in nearly all courses. In contrast, non-hematological side effects were moderate, predominantly of WHO grades I and II. Treatment-related mortality with this regime was 7,14% (three patients with septicemia). **Conclusions.** This regime is an effective salvage protocol for patients with poor-risk recurrent or refractory lymphoma. The observed toxicity seems to be acceptable considering the unfavorable prognosis and intensive pretreatment. The response rate was acceptable, the duration of response is unsatisfactory, but the results can be ameliorated by undergoing HDT with autologous stem cell support in responding patients.

1189**FLUDARABINE, ARABYNSIL CYTOSINE AND IDARUBICIN FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA**

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Background. The addition of idarubicine to fludarabine and intermediate dosage Ara-C (FLAI) generate a synergistic regime that might reduce the multidrug resistance (MDR), one of the most important causes of treatment failure in acute myeloid leukemia (AML). **Aims.** The aim of the study was to evaluate the feasibility and efficacy of this combination in our patients with *de novo* AML. **Methods.** The induction regimen included five days of fludarabine (30 mg/m²), followed four hours later by a 2-hour infusion of Ara-C (2 g/m²) and by a 30 minute infusion of idarubicin (10 mg/m²) on days 1,3,5. High dose Ara-C and idarubicin consolidation or another FLAI was planned for all patients in first CR with good clinical conditions. Fifteen consecutive patients (8 males, 7 females) with *de novo* and untreated non M3 AML (FAB M0 3, M1 5, M2 4, M4-M5 3) entered the study. The mean age of patients was 38 (range 24-52). Seven patients had intermediate prognosis cytogenetic alterations; poor prognosis karyotypes have been detected in 2 patients. **Results.** The

median time of granulocyte recovery (ANC > 1.000/mL) was 26 days (range 15-55) and a platelet count > 50.000/mL was reached after a median of 24 days (range 15-80). The non hematological toxicity was mild and the most common side effects were nausea and vomiting (WHO grade I or II). In particular only 2/15 patients developed a grade 3 or 4 gastro-intestinal toxicity. During the neutropenic phase Gram+ or Gram- bacteria were isolated from the blood in 5 patients. In addition, fever of unknown significance was observed in 8 patients. Ten (77%) patients were evaluated for response. Three patients died during induction of sepsis (1 patient) and gastrointestinal (1) and cerebral (1) haemorrhage and were not evaluated for response. Complete response was achieved in 8/10 patients (80%) whereas 2/12 patients had resistant disease (20%). After a mean follow up of 7 months, 9 patients are alive, 5 have died (3 during induction, the two other of disease progression). One patient has relapsed 5 months after the completion of induction therapy. **Conclusions.** In spite of very small number of patients our results suggest that the FLAI regimen is a good first-line induction therapy for patients with newly diagnosed AML and FLAI regimen can be useful to overcome primary MDR-Pgp mediated.

1190**DETECTION OF BLOOD COAGULATION FACTOR XIII IN DE NOVO B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA**

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Background. Most acute lymphoblastic leukemia cases belong to the precursor B-cell ALL. The detection of minimal residual disease (MRD) is extremely important after the first remission in order to predict the prognosis of the disease. The most valuable marker in the investigation of MRD with flow cytometry is the cyTdT-CD10-CD19 triple labeling. Since these markers are expressed during normal B cell maturation, a new marker which does not appear on normal B cells would be a valuable tool in the detection of MRD. Blood coagulation factor XIII (FXIII) is a protransglutaminase circulating as a tetramer built up from two subunits (A2B2). It crosslinks fibrin chains in the terminal step of blood clotting. The intracellular form of FXIII (A2) is present in platelets, megakaryocytes, monocytes and macrophages. The A2 subunit has been detected in monocytic leukemias. **Aims.** The aim of our study was to investigate FXIII-A expression in *de novo* ALL bone marrow samples. **Methods.** Between January 1. 2001 and December 31. 2004 we examined 47 *de novo* ALL cases of B cell origin by triple colour labeling with flow cytometry. An extra tube (FXIII-A-CD34-CD45) was added to our acute leukemia panel which contained a newly developed, anti FXIII-A monoclonal antibody conjugated to FITC. Labelling was done after cell permeabilization. In selected cases FXIII-A was detected on cytospin slides prepared from blasts and visualized with fluorescent microscope and in two cases with confocal laser scanning microscope. FXIII-A antigen was measured with a highly sensitive ELISA method developed in our department recently. FXIII-A was also detected by Western blot analysis using a polyclonal anti FXIII-A antibody. **Results.** 3 cases were proB ALL, 29 cALL, 13 myeloid positive ALL and 1 mature B cell ALL. 17 of them was found to be FXIII-A positive (64%±21%). Antigen concentration was 3.3±1.2 fg/blast, while mature lymphocytes did not contain FXIII-A. Western blotting analysis showed a single band at 83 kD corresponding to FXIII-A. Confocal laser scanning microscopic examination revealed FXIII-A presence both in the cytoplasm and the nucleus of lymphoblasts. **Conclusions.** We demonstrated the presence of FXIII-A in 21 *de novo* ALL cases in the last 4 years. It is suggested to include FXIII-A labeling in leukemia immunophenotyping since this aberrant immunophenotype is a valuable diagnostic tool in MRD detection. A prospective study is going on in order to determine the prognosis and survival rate of FXIII positive ALL cases compared to FXIII negative ones.

1191**LATE CARDIAC EFFECTS OF DOXORUBICIN THERAPY FOR LYMPHOMA IN ADULTS**

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Background. Doxorubicin is a major drug for the treatment of lymphoma but its use is limited by the severe cardiotoxicity it may cause usually after cumulative doses greater than 550mg/m². **AIM:** To estimate the incidence of late clinical and subclinical cardiomyopathy in adult patients with lymphoma who had received treatment regimens including doxorubicin in the past. **Methods.** We analyzed a group of patients with lymphoma who had previously received doxorubicin-based chemotherapy at a cumulative dose greater than 250mg/m². Cardiac evaluation was performed at least 5 years after therapy was completed. Left ventricular end-diastolic and end-systolic dimensions, left ventricular fractional shortening (FS) and left ventricular ejection fraction (LVEF) were measured by echocardiography. Clinical cardiomyopathy was diagnosed by the presence of clinical signs of heart failure. Subclinical cardiomyopathy was defined by the decrease of FS (FS<25% or FS<28% and LVEF<50%). We also estimated by Doppler a novel index, known as Tei index, which combines systolic and diastolic myocardial time intervals. Tei index is calculated from the equation (a-b)/b, where 'a' is the interval between cessation and onset of the mitral inflow and 'b' is the left ventricular ejection time (both in msec). This index allows assessment of cardiac reserve and enables earlier detection of myocardial dysfunction. It was considered indicative of cardiomyopathy when it was above 0.45. Treatment with cyclophosphamide, cumulative dose of doxorubicin and total dose of cyclophosphamide were evaluated as potential risk factors for the development of cardiac dysfunction. **Results.** We studied 43 patients, 24 male and 19 female with median age 58 years (range 22-79). Fourteen patients had Hodgkin's and 29 non-Hodgkin's lymphoma. They had received a median total dose of doxorubicin 300mg/m² (range: 250-600mg/m²), 5 to 22 years before the evaluation. Thirty patients had received cyclophosphamide and two of them had also received mediastinal radiotherapy. One of the 43 patients (2.3%) developed clinical signs of heart failure (NYHA Class II). Two patients (5%) had subclinical cardiomyopathy according to the FS. However abnormal Tei index was found in 15 patients (35%). Comparing patients with normal or abnormal Tei index, therapy with cyclophosphamide (Pearson's χ^2 , p=0.85), total dose of doxorubicin (Mann-Whitney test, p=0.84) and total dose of cyclophosphamide (Mann-Whitney test, p=0.64) were not found to be risk factor for the development of cardiomyopathy. **Conclusions.** Patients who have been treated with doxorubicin may have subclinical cardiac abnormalities many years after treatment. Myocardial performance index (Tei index) seems to be a very sensitive parameter for the detection of subclinical cardiomyopathy and may be used for monitoring left ventricular dysfunction in anthracycline-treated patients.

1192**IMATINIB MESYLAT IN TREATMENT OF CHRONIC PHASE CML AFTER IFN- ∞ FAILURE**

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Background. Imatinib mesylate is a selective inhibitor of Bcr-Abl tyrosine kinase activity. Its effectivity for CML treatment, including cases after failure IFN- ∞ treatment, was proved by numerous investigations. But several issues are still unanswered. Until current time common prognostic factors for survival of patients with chronic phase (CP) CML are absent. Total amount of patients with complete cytogenetic response (CCR) is sustained or even increased with long-term follow-up. As for infor-

mation about its stability it is not published anywhere. The question about the progression tempo while imatinib treatment is still unclear: will it remain at following-up period on as low level as at the beginning or not? **Aims.** To estimate effectiveness of Imatinib in CP CML after IFN- ∞ failure. **Methods.** 79 pts with CP CML post IFN- ∞ failure were analyzed. Male/female 41:38. Mean age-44 (21-66) years. Distribution of risk profile according to Sokal: low-54%, intermediate-29%, high risk-17%, according to the EURO score: low -72%, intermediate-19%, high risk-9%. The median duration of chronic phase (CML diagnosis to start of imatinib-therapy) was 79 (7-202) months. The median follow-up time-44 (37-50) monts. Imatinib was given 400 mg daily. Cytogenetic studies were performed every 6 months. Survival was calculated from the treatment start until death or last follow-up. **Results.** The CHR rate was 97%. The cytogenetic response rate was 85%, the major cytogenetic response (MCR) rate-57%, CCR-48%. The majority of CCR was achieved in first 12 months, late only 19% of pts was achieved CCR. The 4-year survival rates is 91%, progression-free survival-80%. Annual mortality was not increased during 4 years: 2,6, 3,9, 2,6, 1,3%. Reliable distinctions in survival and progression-free survival in Sokal and EURO prognostic groups are not found. The reliable worse rate of progression-free survival was found at patients with 3 or more risk factors at diagnosis moment (splenomegaly ≥ 5 sm, Hb < 100 g/L, blasts in PB $\geq 3\%$, eosinophils in PB $\geq 4\%$): 44% vs 86% for low (p < 0,025) and 80% for intermediate risk group (p < 0,05). Univariate analysis did not find statistically significant correlations of CCR with: sex, disease duration, chemotherapy duration, IFN dosage (standard/low), spleen size, initial blood analysis (WBC, platelets, Hb, blasts, basophiles, eosinophils at therapy start period). The important factor for survival is a cytogenetic response: 4-years survival rates were 100% for pts achieved CCR, 98% for pts achieved minor, 86% - partial CR, vs 58% for pts not achieved CR. While CCR stability estimation a very significant fact was discovered. Only at 16% pts with CCR Ph-positive metaphases is constantly absent. As for 84% pts it was sometimes found. **Conclusions.** Probably the complete Ph⁺-positive clone eradication in late chronic phase with the imatinib as a second-line monotherapy is not achievable. Even at CCR achievement necessary to continue imatinib treatment because of cytogenetic relapse danger.

1193**EFFICACY OF THALIDOMIDE AND DEXAMETHASONE AS SALVAGE REGIMEN FOR MYELOMA PATIENTS RELAPSING AFTER AUTOLOGOUS TRANSPLANT**P. Falco¹, M.T. Ambrosini¹, T. Caravita², P. Musto², M.T. Petrucci², P. Pregno², I. Avonto¹, A. Bertola¹, S. Bringhen¹, F. Cavallo¹, G. Ciccone¹, M. Boccadoro¹, A. Palumbo¹¹A.S.O. S.Giovanni Battista-Osp.Molinette, TORINO, Italy; ²Multiple Myeloma Study Group, Italy

Background. High-dose therapy followed by autologous stem cell transplantation (AT) is the standard treatment for newly diagnosed multiple myeloma (MM) patients aged 65 or less. Despite improvement in survival and quality of life, relapse always occurs and no definitive data are available to define the best treatment option for MM patients relapsing after AT. **AIMS** Compare efficacy of three different salvage regimens for patients relapsing after autologous transplantation: association of Thalidomide and Dexamethasone (TD), AT and conventional chemotherapy (CC). The end points of the study were response, PFS and OS from first relapse. **Methods.** Between January 1999 and September 2003, 90 multiple myeloma patients (median age 61) received first salvage treatment after AT performed at diagnosis. Forty-three patients were treated with TD (Thalidomide 100 mg/day associated with DEX 40 mg on days 1-4 each month), 19 patients were treated with CC (84% Doxorubicin and/or Cyclophosphamide containing regimens) and 28 patients repeated AT at first relapse (97% conditioned with Melphalan 100 mg/mq). Patients were treated at first relapse after a median time from diagnosis of 32 months for TD and CC and after a median time from diagnosis of 29 months for AT. A retro-

spective analysis was conducted after a median follow-up from the start of salvage TD of 34 months (range 4.5-45), from the start of salvage AT of 18 months (range 3.5-24) and from the start of salvage CC of 21 months (range 2-19.5). **Results.** Response was defined according to the EBMT/IBMTR criteria. Response rate was significantly lower for patients receiving salvage CC in comparison with AT and TD ($p < 0.001$). In details, response rate at first relapse among patients who received TD was: 19% near CR (nCR) (absence of M protein detected by electrophoresis), 28% partial remission (PR) (M-protein reduction 50-99%), 35% stable disease (SD) (M-protein reduction 0-49%) and 19% progressive disease (PD). The response rate at first relapse after AT was 11% nCR, 71% PR, 11% SD and 7% PD; after CC was: 16% PR, 32% SD and 53% PD. Response rate after TD was slightly lower than after AT but the time to progression was significantly prolonged. Median PFS from relapse for TD was 20.3 months vs 9 months for AT and 4.5 months for CC - $p < 0.001$ -. Moreover 20% of patients treated with TD showed a longer PFS at relapse than at diagnosis. TD also improved OS from relapse (median OS from first relapse was not reached for patients receiving TD; it was 15 months after AT and 27.5 months after CC - $p = 0.008$ -). No difference in patients characteristics were observed among different group. TD, Beta2microglobulin and age were the only independent risk factors associated with improved outcome on a multivariate analysis. **CONCLUSIONS** TD is effective as first salvage approach for MM patients relapsing after AT: it significantly prolongs PFS and OS from first relapse.

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DUAL APPROACH OF THALIDOMIDE-BASED FIRST-LINE THERAPY IN PREPARATION FOR AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PREVIOUSLY UNTREATED MULTIPLE MYELOMA: PRELIMINARY DATA

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Background. and Objectives: In the treatment of multiple myeloma, autologous peripheral blood stem cell (PBSC)-supported high-dose melphalan is now considered standard therapy. New immunomodulatory drug, thalidomide, has efficacy in advanced and refractory myeloma. We reported preliminary data that the patients with previously untreated multiple myeloma were treated with combined thalidomide and dexamethasone (TD) or cyclophosphamide, thalidomide, and dexamethasone (CTD) as primary therapy and were performed autologous stem cell transplantation (SCT) after high-dose melphalan. Design and **Methods.** Previously untreated 39 patients who had a confirmed diagnosis of multiple myeloma from sep. 2003 enrolled and were treated with TD or CTD repeatedly before collection of peripheral stem cell and performed subsequent autologous transplantation. We divided two different arms. First arm was consisted of thalidomide 200 mg p.o. daily and dexamethasone 20mg/m² p.o. or i.v. on days 1-4, 9-12, and 17-20 in odd cycles and on days 1-4 in even cycles with low-dose prophylactic warfarin. Second arm was thalidomide 400 mg p.o. on days 1-5, 15-19, cyclophosphamide 150 mg/m² p.o. on days 1-4, and dexamethasone 20 mg/m² p.o. or i.v. on days 1-5, 15-19. Response to therapy based on EBMT criteria was estimated after 4 cycles of therapy. Patients who emerged to complete remission (CR) or partial remission (PR) proceeded to autologous PBSC collection received intermediate-dose cyclophosphamide (3g/m²) and granulocyte colony-stimulating factor (10 ug/kg/day) and followed by high-dose melphalan therapy. **Results.** Twenty-five patients were estimated response and toxicities after 4 cycles of TD or CTD. Median age was a 64 years (range: 39-75) and overall response after thalidomide-based therapy was 76%, including 12 patients (48%) achieved a CR/near CR, 7 patients (28%) showed a PR on an intent-to-treat analysis. Grade 3 or 4 neutropenia occurred in 5 patients, neurotoxicity in 2 patients and deep vein thrombosis in 2 patients. Other side effects were mild and tolerated. 9 patients who were achieved more than PR performed autologous SCT. Median

number of infused CD 34+ dose was 1.69 X 10⁶/kg (range: 0.5-18.5) and median follow-up duration was 6.5 months (range: 0.8-12). 2 patients relapsed after SCT: one died on 7.1 months and one was followed by non-myceloablative stem cell transplantation.. Although the results of this presentation was just a preliminary data, but the combination of TD or CTD was an effective and relatively well tolerated induction regimen and did not adversely affect stem cell mobilization. More studies are ongoing and definitive conclusions will be made after clinical trials.

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ANALYSIS OF THE PATTERN AND FREQUENCY OF EXPRESSION OF FMC7 MEMBRANE GLYCOPROTEIN IN CHRONIC B-CELL LYMPHOPROLIFERATIVE DISORDERS

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Background. The FMC7 membrane antigen (Ag) is 105 kDa glycoprotein expressed with variable density in a subset of mature B-cells of normal adults. It was shown that FMC7+ B-cells respond *in vitro* to mitogens and antigens. On the other side, the expression of FMC7 Ag is also found in different chronic mature B-cell malignancies. However, in the majority of patients (pts) with chronic lymphocytic leukemia (CLL) this Ag is not expressed. **Aims.** The aim of our study was to analyze the FMC7 expression in a group of 156 pts with newly diagnosed B-CLL and non-Hodgkin's lymphoma (NHL) in leukemic phase and to compare it with the expression of other pan B-cell or activating Ags. The frequency and pattern of FMC7 Ag expression were also analyzed in pts with different scores according to scoring system proposed for B-CLL. **Methods.** The immunophenotyping was performed on peripheral blood mononuclear cells, using two- and three-color standard antibody panels and the level of fluorescence was analyzed by Flow cytometer. Intensity of Ag expression was scored semi-quantitatively using isotypic controls and internal positive and negative populations as standards. Positivity for Ags was defined as more than 30% of labeled cells. **Results.** Using scoring system for B-CLL, it was found that score 4-5 have 134/156 pts (86%) and lower scores (0-3) have 22/156 pts (14%). The frequency of FMC7 expression in the whole group of pts was 21% (33/156 pts). The mean level of FMC7 expression was 64% of labeled cells. Two different patterns of FMC7 expression were found: dim (20/33, 61% pts) and bright(13/33, 39% pts). All pts with FMC7 expression were CD19+, CD20+ and CD22+, although some other Ags showed variability in expression: CD5+ (26/33 pts, 79%), CD79b+ (11/16 pts, 69%), CD23+ (21/33 pts, 64%), CD38+ (13/28 pts, 46%), kappa+ (18/33 pts, 55%) and lambda+ (14/33 pts, 42%). FMC7+ pts were distributed in all score categories except score 5, but with different frequencies: score 4 (12/33 pts, 36%), score 3 (4/33 pts, 12%), score 2 (5/33 pts, 15%), score 1 (9/33 pts, 27%) and score 0 (3/33 pts, 9%). All FMC7+ pts with score 4 were diagnosed as typical B-CLL according to the morphological, histological and clinical findings. FMC7+ pts with score 0-3 (21/33 pts) were diagnosed as atypical B-CLL (7/21 pts, 33%) and low or intermediate grade B-NHL (14/21 pts, 67%). **Conclusions.** The FMC7 Ag expression is well correlated with bright pattern of expression of CD20 and CD79b Ags. The dim pattern of FMC7 Ag expression is correlated with typical B-CLL cases. The bright pattern of FMC7 Ag expression is correlated with NHL pts and atypical variants of B-CLL. The bright pattern of FMC7 Ag expression may have potential value in excluding typical cases of B-CLL.

1196**DIRECT DESIGNATION OF IMMUNOPHENOTYPE, CELL CYCLE AND CHROMOSOME TRANSLOCATIONS IN ACUTE LEUKEMIA**

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Background. The classification of acute leukemia according to the World Health Organization has been based on the morphology, the immunophenotype, cytogenetics and molecular genetics of the blast cells. Despite the wide range of morphologic variation, strict criteria to define the subgroups have only recently been proposed. Precise diagnosis and classification are essential to successful treatment, clinical behaviour and biologic study of acute leukemias. Aim The aim of this study is to present a novel direct method of analysis of the antigenic profile, the DNA ploidy levels and the genomic abnormalities in the bone marrow. **Methods.** We analyzed bone marrow cells from patients at diagnosis by morphology, flow cytometry, and molecular genetics. The blast cells separated from the bone marrow of patients with acute leukemias by means of a density-gradient technique. Cell-surface antigens were analyzed by flow cytometry using a panel of monoclonal antibodies (MAbs). The assessment of cell cycle state was performed using the Cycle test(DNA Reagent kit Becton Dickinson) and the appropriate Becton Dickinson's programme Cell Q. The molecular detection for chromosomal translocations was performed by transcription-polymerase chain reaction (RT-PCR) analysis and Split-Out PCR, (Hemavision DTM∞ Technology A/S Aarhus C, Denmark) This technique can detect 28 different translocations or chromosomal rearrangements, found to be specific for particular subtypes of acute leukemias (table 1).

Table 1. Translocations in acute leukemias and the involved genes.

t(11;11)(q23;q23)	MLL/AF16	t(9;9)(q34;q34)	SETD3/AN
t(11;11)(q21;q27)	MLL/AF14	t(10;11)(p12;q27)	MLL/AF19
t(11;19)(q23;p13) t(3;21)(p26;q22)	E2A/PBX1 AML/EBP1/MLL/AF11	t(11;17)(q23;q21) t(11;17)(q23;q21)	MLL/AF17 PLZF/RARA
t(3;5)(q25;q34) t(4;11)(q21;q23)	NPM/MLF1 MLL/AF4	t(11;11)(q23;q17.1) t(11;11)(q23;q17.1)	MLL/ELL MLL/ENL
t(5;12)(q33;p13) t(5;17)(q25;q21)	TEL/VD-GRB TFE3/BAF3a	t(12;21)(p13;q22) t(12;22)(p13;q21)	TEL/AML1 TEL/ML1
t(6;11)(q27;q25) t(8;9)(p22;q24)	MLL/AF6 DER/CAF	t(15;17)(q22;q21) t(16;21)(q11;q22)	FML/ KASa TLS/ERG
t(8;21)(q22;q22) t(9;11)(q22;q27)	AML1/MZF2 MLL/AF9	t(17;15)(q22;p13) t(16;13)(q13;q22)	E1A/HEP CBF/MYH11
t(9;12)(p24;p13) t(9;22)(q34;q11)	TEL/ABL FIC1/ABL	t(2;11)(q17;q27) TAL1/ctb1/crip3/4)	MLL/AFK TEL/TAL1

Results. Indicative, we represent three patients which were completely analysed with the above techniques in only 72 hours. Specifically, the immunophenotype analysis and the cycle test can classify the leukemia in the first 8 hours, and the genetic identity comes after 64 hours. The immunophenotype of the first patient, a 16 years old man, revealed a pathologic cell population which express CD 19, CD22, CD34, HLA-DR, TdT, CD10, CD33, and CD13. The cell cycle test did not find any aberration in the DNA ploidy. The RT-PCR indicated a chromosomal rearrangement t(12;21) (p13;q22). Those data conducted in short time and with high precision the haematologists to the classification of c-Acute Lymphoblastic Leukemia. In the second patient, a 17 years old woman, the immunophenotype

revealed a pathologic cell population which express CD33, CD7, CD34, HLA-DR, *PO, CD117, CD13 and CD15. The cycle test did not find aneuploidy as well as the RT-PCR did not find any chromosomal translocation. The haematologists diagnosed Acute Myeloblastic Leukemia (M2). The diagnosis for the third patient, a 73 years old man, was Acute Undifferent Leukemia. The immunophenotype revealed a pathologic cell population which express CD34, CD133 and HLA-DR. The cycle test designated tetraploidy and the RT-PCR a chromosomal translocation t((X;11) (q13;q23). **Conclusions.** A combination of immunophenotypic, and molecular analyses consists a time and cost sparing tool for definite classification and diagnosis to be rendered in new cases of leukemia.

1197**SAFE PREGNANCY IN PATIENTS WITH THE ESSENTIAL THROMBOCYTAEMLIA TREATED WITH INTERFERON-ALPHA**

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Pregnancy (PR) in women with essential thrombocythemia (Et) is dramatic due to the risk of the placenta vessels thrombosis, leading to fetal dystrophy and death. Use of Interferon-alpha-2b (IFN-α), which reduces the number of megakariocytes, hyperproduction of platelets and decreases the risk of fetal death. Five patients (26-35 years) with ET at the beginning of the disease had hyperthrombocytosis of 2000–2500 x10⁹ /L. All 5 received aspirine 0,3 and the effective IFN-α, 9-21 ME/wk, for 1-2,5 years without adverse events. Total disease duration at PR diagnosis was 7-56 mths. in 4 patients, in 1 ET and PR were diagnosed at the same time. The platelets level in all patients had lowered to <500x10⁹/L after 4-17 weeks of the IFN-α treatment. In 4 pts therapy by IFN-α was continued at the PR. 1 patient in remission after 8 months of IFN-α discontinuation has become pregnant. She was maintained on clopidogrel without complications until the Cesarean section at week 38. She has delivered a health boy, which is now 38 months old. The others 4 have delivered healthy infants, which are now 6-24 months old. One woman at the week 39 had the placental separation and required the application of obstetrical forceps. In 2 patients we have found lupus anticoagulant or high titer of antiphospholipid antibodies and in 1 was heterozygous for methylentetrahydrofolatereductase deficiency, that required plasmapheresis (in combination with low-molecular weight heparin and folates). Thus, pregnant women with hyperthrombocytosis requires treatment with IFN-α and aspirin regardless of ... The search for the wide-spread thrombophilia causes is also required, because it makes possible the adequate prophylaxis of thromboembolism.

1198**CONSERVATIVE TREATMENT OF PATIENTS WITH PRIMARY DIFFUSE LARGE B-CELL GASTRIC LYMPHOMA**

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Background. The optimal treatment of primary large B-cell lymphoma of the stomach remains controversial. **Aims.** Efficacy of chemotherapy and radiotherapy of primary diffuse large B-cell gastric lymphoma are to be clearly defined. **Methods.** We have own experience of chemo- and radiotherapy of 19 patients (16 women and 3 men from 19 to 71 years; means 50 years) with diffuse large B-cell gastric lymphoma modified Ann-Arbor stages IE and IIE. All patients were treated between 1995 and 2005 years in our center and other clinics of Moscow. There were 10 patients in group A (treated only chemotherapy) and 9 patients in group B (treated chemotherapy and radiotherapy). All clinicopathologic features were similar between the two groups of patients. All patients were treated six courses standard

CHOP and CHOP-like regimens of chemotherapy. Additionally, in group B patients were treated by radiotherapy with gastric and paragastric irradiation (40 Gy). **Results.** in group A there were 9 complete remissions at median follow-up 50 months (range 13-119 months) and 1 woman died from sepsis after third course; in group B there were 7 complete remissions at median follow-up 37 months (range 22-47 months) and 2 women died from relapses and progression of lymphoma after 6 month of stopping radiotherapy. **Conclusions.** our data suggest that systemic chemotherapy alone is very curable treatment for stage III large B-cell gastric lymphoma. The additional advantages of radiotherapy need to be clearly re-estimated.

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CORRELATION ANALYSIS DIFFERENTIATION STATUS OF MEGAKARYOCYTES AND PLATELETS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by ineffective hematopoiesis, frequently the development of acute myeloid leukemias [Bennett J.M, et al., 1982] and abnormal megakaryocytic proliferation and differentiation [Hofmann W.-K., et al., 2000]. **Methods.** In this study we analyzed correlation between of degree differentiation, functional activity of bone marrow megakaryocytes and count of blood platelet in the control group and group of MDS patients (n=26, 10 - with refractory anemia (RA), 16 - with unclassified - refractory cytopenia without excess blasts (MDS-u)), 80% of MDS patients had different degree of thrombocytopenia. Correlation analysis was performed by program STATISTICA-55, correlation coefficient are significant at $p < 0,05$. **Results.** In the control group platelet count was positive associated with total number of functional megakaryocytes ($r=0.77$), polychromatophilic megakaryocytes with platelet formation ($r=0.69$) and negative associated with 1-lobulated megakaryocytes ($r=-0.80$). The number of polychromatophilic megakaryocytes with platelet formation correlate with polychromatophilic megakaryocytes ($r=0.71$) and total quantity of megakaryocytes with platelet formation ($r=0.89$). These data confirm that the most functional active population of megakaryocytes are polychromatophilic cells in the control group. Hyperlobulated megakaryocytes correlated with count of megakaryoblast ($r=0.83$) and 3-lobulated megakaryocytes ($r=0.68$) that suggest stimulation of megakaryocytopoiesis. In the group of MDS patients the platelet number was associated with: total number of functional megakaryocytes ($r=0.51$) (the same in the control group), basophilic megakaryocytes with platelet formation ($r=0.41$) and negative correlate with polychromatophilic megakaryocytes ($r=-0.48$), megakaryocytes with 4-7 lobulated nuclei of megakaryocytes ($r=-0.45$) that significant different from the control group. The megakaryocytes with 4-7 lobulated nuclei are most numerical population of megakaryocytic cells in the control group. These cells positive correlate with polychromatophilic megakaryocytes ($r=0.57$) and negative correlate with micromegakaryocytes ($r=-0.44$), dysplastic megakaryocytes ($r=-0.79$), megakaryoblasts ($r=-0.6$) in the group of MDS patients. The number of megakaryocytes with fragmented lobul of nuclei associated with oxyphilic megakaryocytes with platelet formation ($r=0.44$), hypersegmentated megakaryocytes correlated with count of polychromatophilic megakaryocytes with platelet formation ($r=0.48$) and oxyphilic megakaryocytes with platelet formation ($r=0.43$) that's why these cells associate with stimulation of megakaryocytopoiesis. In the same time basophilic megakaryocytes correlate with: promegakaryocytes ($r=0.59$), total quantity of megakaryocytes with platelet formation ($r=0.51$), basophilic megakaryocytes with platelet formation ($r=0.82$). Total number of megakaryocytes with platelet formation associate to: promegakaryocytes ($r=0.46$), basophilic megakaryocytes with platelet formation ($r=0.66$), polychromatophilic megakaryocytes with platelet formation ($r=0.82$), oxyphilic megakaryocytes with platelet formation ($r=0.44$),

micromegakaryocytes ($r=0.6$), naked nuclei of megakaryocytes ($r=0.51$) to distinguish from control group. **Conclusions.** According to our study in the group of the patients with myelodysplastic syndromes to platelet creation involved basophilic, polychromatophilic and oxyphilic megakaryocytes to distinguish from the control group with platelet associated to polychromatophilic megakaryocytes. Besides them, quantity of naked nuclei, promegakaryocytes and micromegakaryocytes correlate with megakaryocytes with platelet formation suggest active stimulation to platelet production and abundans grow old and destruction of megakaryocytic cells. These alteration of megakaryocytic cells associate with different degree of thrombocytopenia.

1200

FIRST PRELIMINARY EVALUATION OF ESCALATED BEACOPP CHEMOTHERAPY FOR ADVANCED HODGKIN'S DISEASE (PROSPECTIVE STUDY ABOUT 30 TUNISIAN PATIENTS)

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This study was designed to assess the efficacy and safety of a BEACOPP regimen four escalated cycles followed by four at baseline cycle in the treatment of advanced stage Hodgkin's disease (HD): stage IIB (bulky), III and IV. From June 2002 to June 2004, 30 patients who had newly diagnosed HD in unfavorable stage were treated with eight cycles of BEACOPP (four escalated + four baseline), with haematological growth factor support, non radiation is planned. There were 22 males and 8 females with a median age of 39yr (17-56yr); 24 (80%) had nodular sclerosis HD; 7 (24%) had bulky disease (≥ 10 cm); 2 (6%) were in stage IIB, 7 (24%) in stage III and 21 (70%) in stage IV, all patients had ≥ 3 prognostic factors according to the international prognostic score. The tumor stage was reevaluated after four and eight cycles by means of a clinical examination and imaging or biopsy methods appropriate to the site of initial involvement. Of the 30 patients we enrolled, 3 patients were not assessed because 2 died before restaging therapy and 1 died of acute toxicity during the third course. At the first complete evaluation after the fourth cycle of chemotherapy, 19 (71%) patients achieved a complete remission (CR), 1 (3%) achieved a partial response (PR), 6 (23%) had stable disease (SD) and 1 (3%) had progressive disease (PD). Eight patients with PR, SD or PD received salvage therapy, 6 had chemotherapy only and 2 had chemotherapy and autologous hematopoietic stem cell transplantation. Among the 27 patients, 19 received the intended eight cycles of treatment; progression occurred in 2 patients up to 2 months after treatment and 1 patient died 2 months after treatment from non HD related cause. With a mean follow-up period of 29 months, actuarial 18 months survival rate is 30,36%, and the rate of early progression was 11%. The acute toxicity of BEACOPP was severe but manageable, grade 3-4 leukopenia and neutropenia occurred in 16 (53%) of the patients, respectively whereas in 12 (40%) of the patients infections was observed; 1 death was recorded as complication of first line treatment. The results of the present study are preliminary, and slightly inferior compared with the German studies. Further follow-up time is required to evaluate disease free survival and a long term toxicity.

1201

SEQUENTIAL QUANTITATIVE ANALYSIS OF BCR-ABL FUSION GENE IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS AFTER ALLOGENIC STEM CELL TRANSPLANTATION BY REAL-TIME PCR

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Introduction: Allogenic stem cell transplantation (SCT) is the most important curative treatment for patients with CML.

Detection of minimal residual disease (MRD) in these patients has prognostic value after SCT. We have attempted to assess the clinical significance and predictive value of monitoring of BCR-ABL transcripts by quantitative RT-PCR assay after SCT, in order to identify patients who are at high risk of relapse and may require additional therapeutic intervention. **Material & Method:** Sequential Peripheral blood samples from 73 CML patients underwent allogeneic SCT were collected every 3 months for the presence of BCR-ABL transcript by using a double step nested RT-PCR. If a sample was found positive by RT-PCR technique, relative quantification was performed by Real-time PCR. To assess the quantity and quality of the RNA, G6PDH was used as a control gene to standardize our criteria. **Results.** During 6 months after SCT, BCR-ABL levels was high and detectable in almost all cases. After 6-12 months, BCR-ABL levels were decreased slowly and became undetectable in about 50% of the cases. However, some patients remained persistently detectable for prolong periods of time without evidence of hematological relapse. The level of detectable BCR-ABL transcripts in these patients was usually very low and the patients were destined to remain in remission. On the other hand, those patients who had increasing or persistently high levels of BCR-ABL transcripts on sequential analysis were usually destined to relapse. The relapse could be predicted often several months before the clinical relapse. Three patients who were treated with donor lymphocyte infusions (DLI) at 5-6 years after SCT regained molecular remission. **Conclusions.** The kinetics of disappearance of MRD in CML patients is slow and BCR-ABL transcripts remain detectable at low levels in considerable number of patients in long term complete remission. Therefore, non-quantitative RT-PCR positivity are of limited value, the results of serial Real-time quantitative PCR studies are highly informative and can effectively predict an impending relapse while the patient is still in hematological remission.

1202

MULTIPLEX STR-PCR FOR SEMI QUANTITATIVE ANALYSIS OF CHIMERISM AFTER STEM CELL TRANSPLANTATION

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Background. Monitoring of hematopoietic donor/recipient chimerism is of major significance in detecting of early marrow engraftment, disease relapse, and graft rejection. STRs, which are standard tools for genotyping in parentage testing and forensic human identity testing, provide an excellent tool for this purpose because of their high degree of polymorphism and relatively short length. **Aims.** The aims of this study was to analyse the chimeric status of peripheral blood leukocytes in recipients of allogeneic bone marrow transplantation (BMT) with the use of short tandem repeats (STR) microsatellite markers for monitoring the efficacy of BMT and donor leukocyte infusions (DLIs). **Methods.** We have investigated 120 allogeneic stem cell transplantation suffering from different types of leukemia (n=80) or non-malignant hematologic disorders (n=40) by close molecular monitoring during 15 days to 12 months after transplantation. A STR-PCR was performed on DNA isolated from whole WBC, PMNs, MNCs, and in some cases on isolated T-cell of recipient patients. A set of twelve STR markers were used in Multiplex PCR with a highly discriminative capacity between individuals. **Results.** The multiplex PCR showed a sensitivity of 1-2% when analyzed on polyacrylamide gel. Of the 100 patients, 85 patients (71%) exhibited full donor chimerism, 23 Patients (19%) had mixed chimerism and 12 patients (10%) had lower chimerism and reject the transplant. Some patients with full donor chimerism switched back to mix chimerism, and some showed relapse, however, with periodic and frequent monitoring of patients we could detect the patient clinical out-

come and need for DLI. **Conclusions.** STR analysis using a multiplex PCR can provide an accurate, rapid and quantitative assessment of chimerism in patients with post-allogeneic BMT. These studies also showed that the analysis of mixed chimerism in leukocyte subsets is more informative than analysis of whole blood cells only and may reveal clinically important variation in the origin. Such information could be useful for patients in guiding early implementation of additional treatment designed to circumvent graft failure or suppression of relapse.

1203

EFFECT OF BORTEZOMIB ON TOLL-LIKE RECEPTOR FUNCTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF HEALTHY DONORS

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Background. Toll-like receptors (TLRs) are a new family of receptors of central importance to immunity and inflammation. TLRs have been identified by virtue of their ability to recognize various structures of pathogens, such as bacteria and viruses. Eleven members of this family with different specificities have now been described in mammals. TLRs have been shown to form functional pairs that can change their specificity or influence their signaling ability. TLRs are mainly expressed in cell types involved in the first line of defense, such as macrophages, dendritic cells, neutrophils, mast cells, mucosal epithelial cells and endothelial cells, and fibroblasts. Bortezomib is a novel anti-neoplastic agent that has been approved for the treatment of relapsed multiple myeloma. It is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome, a large protein complex that degrades ubiquitinated proteins. **Aims.** The aim of the study was to evaluate the effect of bortezomib on the response of peripheral blood mononuclear cells (PBMCs) from healthy donors to TLR ligands. **Methods.** PBMCs of two healthy donors were cultured in 96-well plates at 2×10^6 cells/well, in triplicates. Cells were pretreated with bortezomib at a range of 1 nM-10 μ M for 30 min and then stimulated for 20h with the following TLR ligands: lipopolysaccharide (LPS), peptidoglycan, loxorubin and unmethylated DNA. Supernatants of the cultures were collected and levels of tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) were assessed by ELISA method (BD Biosciences Pharmingen, San Diego, CA, USA). **Results.** All results are expressed as a per cent change of the levels of the respective cytokines vs. their levels in bortezomib-treated PBMCs that were not exposed to TLR ligands. Bortezomib at certain doses abrogated the production of TNF- α and IL-6 in response to bacterial LPS and peptidoglycan without significant toxic effects on the viability of the cells at concentrations up to 100 nM. More specifically, LPS-induced TNF- α production was inhibited 0-80% at a 1 nM of bortezomib, 81-96% at 10 nM, and more at higher concentrations. Peptidoglycan-induced TNF- α was inhibited 0-11% at 1 nM, 53-87% at 10 nM and more at higher concentrations. Similarly, LPS-induced IL-6 production was inhibited 0-30% at 1 nM, 58-83% at 10 nM and more at higher concentrations. Peptidoglycan-induced IL-6 was inhibited 0-48% at 1 nM, 35-83% at 10 nM, 90-99% at 100 nM and more at higher concentrations. Responses of PBMCs to loxorubin and unmethylated DNA were very weak for the effects of bortezomib to be assessed accurately. However, in one donor responding to loxorubin, bortezomib also inhibited loxorubin-induced TNF- α almost completely (99%) at doses higher than 10 nM. **Conclusions.** Our data suggest that bortezomib has profound effects in TLR-mediated responses in healthy donors. Whether this *in vitro* finding corresponds to clinical symptoms may be related to the concentration of bortezomib and the time of exposure to the drug. Nevertheless this observation may open up the way for using bortezomib as a potent anti-inflammatory drug in the clinical practice.

1204**MULTIDRUG RESISTANT GENE EXPRESSION IN RESISTANT CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB MESYLATE IN THE ABSENCE OF BCR/ABL MUTATIONS: CAN IT BE A NOVEL MECHANISM OF TREATMENT FAILURE?**I. Bendit¹, M. Conchon¹, M.N.Y. Novaes¹, A.C.M.F. Souza¹, G.H. Fonseca¹, M. Novaretti², L.C.S. Dias¹, C. Linardi¹, W.K. Neto³, M. Bendit³, D.A.F. Chamone¹, P.E. Dorhliac-Llacer¹¹Hospital Das Clinicas, SAO PAULO, Brazil; ²Fundacao Pro-Sangue HSP, SAO PAULO, Brazil; ³Ciencias Farmaceuticas USP, SAO PAULO, Brazil

Background. Imatinib mesylate is molecularly targeted drug specific for BCR-ABL and has been very successful in the treatment of chronic myeloid leukemia (CML) patients. However, resistance to imatinib is an emerging problem where point mutations in abl kinase domain are one of the major causes. Nevertheless, other mechanisms could be responsible for the emergence of resistance to imatinib. One of the most important mechanisms of drug resistance is the overexpression of ATP-binding-cassette (ABC) transporters that functions as a drug efflux pumps, and the best characterized member of this family is the P-glycoprotein (PGP) which is the product of the MDR1 gene. Moreover, the chemical structure of imatinib suggests lipophilic properties, making the PGP a likely candidate to imatinib resistance. **Objective:** The aim of the present study was to determine the incidence of mutations in those patients who did not achieve CCR and the presence of overexpression of MDR1 gene throughout Real Time PCR (QPCR) in those where no mutations were detected. **Methods.** We investigated 24 CML (12 CP and 12 AP) patients at time of resistance or relapse, M/F = 3/1, age: 47 (26-70). RNA was extracted from BM and analyzed for BCR-ABL point mutations using a seminested reverse transcriptase-polymerase chain reaction (RT-PCR) followed by direct sequencing. The same RT-PCR product was studied for MDR1 expression by Q-PCR and the reference gene ACTIN was used as an internal control for RNA quality and cDNA quantity. RNA from CML patients at diagnosis was also studied for MDR1 expression. **Results.** We identified one point mutation in RNA from 6 patients at 5 different positions (2 M351T; F317L; Y253F; L359C; Q355G), and one patient had 2 different mutations at the P-loop domain (G250Q; G255K). Four patients in CP (33%) and 2 in AP (16%) presented mutations. All patients with BCR-ABL mutations regardless the site of the genetic abnormality are still alive in complete hematological response, 4 are receiving imatinib 800 mg/day, 1 is receiving imatinib 300 mg plus Ara-C (M351T) and 1 is taking hydrea (L359C). The overexpression of MDR1 was present in 7 patients (4 CP, 3AP) where only one out of 7 presented both abnormalities, MDR1 overexpression and Y253F mutation. The group of patients with resistance to imatinib presented expression of MDR1 significantly higher (Wilcoxon test, $p < 0.0001$) when compared to the ones from the same group and also when compared with those patients with CML at diagnosis. There was no difference in expression between the 2 groups without MDR1 expression (Wilcoxon test, $p = 0,5$). **Conclusions.** We concluded that 1) mutations occur frequently among patients with acquired resistance to imatinib (30%); 2) P-loop mutations may not be associated with worse outcome compared to other mutations; 3) there was no correlation between the presence of mutations and MDR1 overexpression; and 4) overexpression of MDR1 could be considered a mechanism involved in resistance to imatinib and the use of drugs as cyclosporine A could be a strategy to modulate the product of MDR1 gene.

1205**THE RESULTS OF MONITORING OF MINIMAL RESIDUAL DISEASE FOR THE PATIENTS WITH CHRONIC MYELOID LEUKEMIA RECEIVING INTERFERON ALPHA AND GLIVEC THERAPY**

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Background. During the last years it became possible for the patients with chronic myeloid leukemia to achieve not only complete cytogenetic remission receiving the conservative therapy (interferon alpha and Glivec) but also to get complete molecular remissions. **Aims.** The aim of our investigation was to estimate the frequency of complete cytogenetic remission for the patients with chronic phase chronic myeloid leukemia receiving conservative therapy (interferon alpha or Glivec). **Methods.** We have used the qualitative two-step nested PCR to estimate the residual disease. The sensitivity of the method in our laboratory was nearly 10⁻⁵. We observed a group of 18 patients with complete cytogenetic remission receiving interferon alpha and a group of 16 patients with complete cytogenetic remission receiving Glivec. The median of observation in the 1st group was 54 months (range 6-72 months). The median of observation in the 2nd group was 12 months (range 6-24 months). Peripheral blood and bone marrow were studied every 6-12 months. **Results.** The majority of the studied samples were positive by nested PCR. In the group of patients receiving interferon alpha therapy the frequency of complete molecular remissions correlated with the terms of therapy. 14 of 16 samples analysed in terms before the 24 month of treatment were positive. In 3 cases the PCR positivity was detected simultaneously with the transitoral loss of complete cytogenetic remission. In terms from 24 to 48 months 25 samples from 18 patients were analysed. 14 of them were positive, 11 were negative by nested PCR. This tendency also was observed for the patients on the late terms of the interferon alpha therapy. After 48 months of therapy 6 of 11 analysed samples were positive, 5 were negative. In the group of patients receiving Glivec therapy complete molecular remissions were detected on earlier terms of the therapy. In terms before 12 month of treatment 13 samples were analysed, 6 of them were positive, 7 were negative. In terms from 12 month to 24 month of treatment 6 of 8 analysed samples were negative. 6 samples showered false-negativity due to the low sample volume and NA degradation. **Summary.** The majority of the studied samples were positive by nested PCR. In both groups of patients the frequency of complete molecular remissions correlated with the terms of therapy. The frequency of complete molecular remissions was higher in the group of patients who received Glivec therapy. Also we observed the achievement of complete molecular remissions in earlier terms for the patients on Glivec therapy comparetevely with the patients receiving alpha-interferon therapy.

1206**GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY: TWO NEW MUTATIONS CAUSING CHRONIC HAEMOLYSIS IN PORTUGUESE PATIENTS**L. Manco¹, P. Gonçalves¹, P. Melo², C. Seabra³, M.C. Bento¹, M.L. Ribeiro¹¹Centro Hospitalar de Coimbra, COIMBRA, Portugal; ²Hospital Figueira Foz, FIGUEIRA DA FOZ, Portugal; ³Hospital Infante D. Pedro, AVEIRO, Portugal

Background. A small number of glucose-6-phosphate dehydrogenase (G6PD) deficient individuals have mutations encoding class I variants associated with chronic non-spherocytic haemolytic anaemia. G6PD class I variants are mainly due 1) to missense mutations in and around exon 10 of G6PD gene, in Xq28, with the corresponding aminoacids sited within or close to the dimer interface of the active enzyme, or 2) to mutations occurring involving conserved aminoacids. **Aims.** To identify the

chronic haemolytic anaemia pathophysiology in three patients with G6PD deficiency. Patients: Case 1. A 25-years-old Portuguese male presented with asthenia, jaundice and dark urine during the last 3 days. His spleen was palpable 15cm below the left costal margin. He had neonatal jaundice, needing phototherapy, chronic yellowish colour of the sclera and a severe haemolytic episode in infancy. Hb 70g/L, MCV 101fL, reticulocytes 12%, serum bilirubin: total 68.4 micromol/L (N:<17), unconjugated 58.1 micromol/L (N:3-20); LDH 1404U/L (N:420-750); peripheral blood smear showed macrocytes, polycromasia and some erythrocytes with oxidative stress. Out of the haemolytic episodes, he has a just palpable spleen, moderate macrocytic anaemia, reticulocytes 7.0% and G6PD activity 0.9% of normal. Case 2. A 3-years-old Portuguese boy presented in a febrile episode, with jaundice, no palpable spleen; Hb 86g/L, serum bilirubin: total 55.7 micromol/L, unconjugated 51 micromol/L. He had a history of neonatal jaundice, needing phototherapy, but no other important haemolytic episode. In steady state he has jaundice, macrocytosis, reticulocytes 5.2% and G6PD activity 2.8% of normal. Family studies revealed a maternal uncle (Case 3) with chronic jaundice, a palpable spleen and several haemolytic episodes. G6PD activity was 1.2% of normal; Hb 137g/L, MCV 110fL, reticulocytes 4.9%. The most common causes of non-spherocytic haemolytic anaemia were screened. *Methods.* After informed consent, genomic DNA was extracted from EDTA peripheral blood samples and G6PD gene was studied by PCR, SSCP and sequencing analysis. *Results.* Patient 1 G6PD gene PCR fragment spanning exon 10 showed an SSCP mobility shift and automatic sequencing revealed the new mutation 1205C>A, predicting the aminoacid change 402Thr>Asn. Structural analysis using the crystal structure of *Leuconostoc mesenteroides* showed that T402 in beta sheet M of the polypeptide chain is located within the protein dimer interface. Patient 2 and 3 G6PD gene DNA sequencing revealed a new mutation in exon 12, 1366G>A substitution, predicting the aminoacid change 456Asp>His. Structural analysis showed that D456 in alpha helix N is at protein surface, far away from dimer interface. Both mutations were confirmed by restriction enzyme digestion and no other mutations were detected in the remainder exons and adjacent regions of G6PD gene. The screening of 100 alleles from a control group failed to detect these mutations. *Summary/conclusions:* We described two new G6PD missense mutations causing chronic haemolysis. One affects a variable aminoacid within the dimer interface, following the most common genetic profile for the class I mutations; the second is a nonconservative aminoacid substitution concerning a highly conserved residue at the protein surface. The new variants T402N and D456H were named G6PD 'Covão-do-Lobo' and G6PD 'Figueira-da-Foz', respectively.

1207

IMATINIB MESYLATE TREATMENT OF PATIENTS WITH CHRONIC MYELOID LEUKAEMIA RELAPSING FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Stem cell transplantation (SCT) is associated with long-term disease-free survival in patients transplanted for chronic myeloid leukaemia. However a proportion of patients continue to have haematological, cytogenetic and molecular relapses up to 15 years following SCT. Donor lymphocyte infusions given post SCT relapse are associated with long-term molecular remissions and disease-free survival but depend on the availability of the original donor. In addition the use of DLI can be complicated by both Graft vs Host Disease (GvHD) and bone marrow aplasia. *Methods.* We have treated 12 patients (pts) who have relapsed following SCT with Imatinib Mesylate (IM). 4/12 pts had previously received DLI for haematological relapse. The propositus was unable to receive DLI because his donor had died, hence the initial impetus for the study. IM was given at a

dose of 300-600mg/day and was well tolerated in all patients. Response to the treatment was evaluated by bone marrow analysis, karyotypic and molecular analyses. Molecular analysis for BCR-ABL was performed by nested RT-PCR and by real time RQ-PCR using TaqMan probes for BCR-ABL and ABL transcripts in serial samples following treatment with IM. *Results.* 68 samples from the 12 pts (median 4, range 2-13) were analysed by PCR analysis. Time points ranged from 1-38 months from commencement of IM therapy (median 11 months). The cohort was comprised of 8 males and 4 females. Median age: 44 (21-57 years). 11 pts received bone marrow as the source of stem cells; one received peripheral blood stem cells: 10 pts from a histocompatible sibling donor, 1 unrelated and 1 related phenotypically identical donor. Median time to first relapse was 36 months (range 5 -120 months). 5 of the 12 patients were treated with IM at the time of haematological relapse (n=3) or cytogenetic relapse (n=2). 4/5 responded with a cytogenetic remission. BCR-ABL transcripts became undetectable in 2/5 (end point sensitivity <10⁻⁵). One patient was in accelerated phase (AP), failed to respond and died. The remaining 7 patients were treated following molecular relapse. 5/7 had BCR-ABL transcripts undetectable at 3 months post IM therapy. Of the remainder one has falling BCR-ABL transcripts. The other pt was transplanted in AP and had progressive disease. No patient experienced GvHD following IM treatment. 10/12 patients are alive and have responded to IM 3-13 years following their original SCT. In five patients, IM was discontinued and in 3 of these, BCR-ABL transcripts became detectable again. *Conclusions.* IM induces cytogenetic and molecular responses in patients treated for relapse of CML post SCT without the side effects of GvHD and bone marrow aplasia. However the optimum length of therapy and the durability of responses remain unknown. A comparison of DLI versus IM in patients relapsing more than one year after transplant is warranted.

1208

FLUORESCENCE IN SITU HYBRIDISATION (FISH) CYTOGENETIC ANALYSIS OF B-CHRONIC LYMPHOBLASTIC LEUKEMIA (B-CLL), SMALL LYMPHOBLASTIC LYMPHOMA (SLL) AND WALDENSTROM'S MACROGLOBULINEMIA (WM)

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Background: Chromosomal abnormalities in B-CLL are considered as potent prognostic factors. The cytogenetic abnormalities of SLL, the tissue counterpart of B-CLL have not been studied separately and MW, a closely related small lymphocytic disorder, has not been extensively studied and various results have been reported. *Aims.* The purpose of the present study is to identify any potential differences in chromosomal abnormalities between B-CLL, SLL and MW Patients and *Methods.* We studied 44 consecutive B-CLL, 17 SLL and 9 MW patients diagnosed in our Unit. Diagnosis was based on standard morphologic and immunophenotypic criteria for B-CLL and SLL patients. Cases with absolute lymphocyte count $\leq 5 \times 10^9/L$ were considered as SLL. The diagnosis of MW was established in the presence of serum monoclonal IgM $\geq 2 g/L$ and lymphoplasmacytic infiltration of bone marrow. Separated lymphocytes from peripheral blood (B-CLL) or bone marrow (SLL and MW) were fixed and FISH analysis for trisomy 12 and 13q deletion was performed using standard techniques. 200 intact interphase nuclei were scored for each sample. Cut-off levels were defined by the mean value plus 3x standard deviation of the frequency of the abnormalities in 10 normal controls. *Results.* 11/44 B-CLL cases were positive for trisomy 12 (25%) versus 7/17 (41%) SLL and 0/9 (0%) MW cases. Del13q was found in 19/42 (45%) of B-CLL, versus 3/14 (21%) of SLL and 0/9 (0%) of MW patients. Biallel-

ic 13q deletion was observed in 5/19 B-CLL cases, while it was not observed in any SLL patient. Only 1/42 B-CLL patient had del13q and trisomy 12 as concurrent abnormalities, whereas 2/14 SLL patients had both abnormalities. *Conclusions.* We found a difference in the cytogenetic profile between B-CLL and SLL, two entities that are biologically closely related but demonstrate a different tissue localization pattern. Thus trisomy 12 was more frequently observed in SLL, whereas the opposite was true for del13q. In addition none of these two chromosomal abnormalities was encountered in MW, a finding that could potentially serve as an additional tool for the differential diagnosis between these related disorders. Our findings need to be confirmed in a larger series of patients and their biologic significance should be further investigated.

1209

IMMUNOPHENOTYPIC ANALYSIS OF SPLENIC MARGINAL ZONE B-CELL LYMPHOMAS (SMZL)

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Background: The diagnosis of SMZL is based on a combination of clinical, hematologic, morphologic immunophenotypic and molecular findings. The immunophenotype is usually CD5 (-) and CD23 (-). However there are no typical surface or molecular markers for this type of lymphoma. Therefore differential diagnosis from other forms of splenic B-lymphoproliferative disorders is not easy in the absence of circulating villous lymphocytes or histologic confirmation. *Aims.* The purpose of this study is the immunophenotypic analysis of SMZL. *Patients and Methods.* 28 patients with splenic B-cell lymphomas diagnosed in our unit were analysed. Blood morphology was reviewed by two different observers. Blood and/or bone marrow immunophenotype included the following antigens: CD19, CD20, CD22, CD23, CD5, sIg, CD79b, FMC-7, CD11c, HLA-DR, CD103 (Becton Dickinson). Molecular analysis (PCR) for the detection of bcl-1/IgVH and bcl-2/IgVH rearrangements was performed in 11 patients. *Results.* Patients' median age was 63 yrs (45-79) and 10 of them were male. All patients had splenomegaly at diagnosis (27/28) or during follow-up (1/28). Only 3/24 had palpable small size lymph nodes. Leukocytosis was found in 10 patients, lymphocytosis in 18 and leucopenia in 6. Villous lymphocytes (>20% of lymphocytes) were observed in 16 patients. 15 patients had anemia, three of them autoimmune hemolysis and 8/28 thrombocytopenia. Serum monoclonal immunoglobulin was found in eight patients (IgM in five and IgG in three). Splenectomy was performed in 10 patients. Spleen biopsy was consistent with SMZL in seven patients, while in three it revealed infiltration by a low grade B-cell lymphoproliferative disease without further specification. Three patients were positive for bcl-1/IgVH and one for bcl-2/IgVH and were not included in the immunophenotypic analysis. All 24 SMZL patients were CD5 (-) and 18/24 patients were CD23 (+). The expression of CD23 by the malignant B-lymphocytes was characterized by a relatively low mean fluorescence intensity in contrast to the uniform strong expression of CD23 that is usually observed in B-Chronic Lymphocytic Leukemia (B-CLL). All patients were CD20 (+) with intermediate or strong expression, 23/23 were CD79b (+) (4/19 with weak expression), 22/22 were FMC-7 (+), 23/23 were CD22 strongly positive and 8/24 had intermediate/strong surface light chain (sIg) expression. 14/14 studied patients were CD103 (-), 15/23 CD11c (+) and 4/18 CD25 (+). ^aatutes score was 1 in 12/23

patients 2 in 7/23, 3 in 2/23 and 0 in 2/23. FISH analysis for trisomy 12 was performed in 12 CD23 (+) patients. None of them had trisomy 12, thus excluding the possibility of atypical B-CLL. *Conclusions.* In the present series of 24 SMZL patients immunophenotype was CD5 (-), with intermediate/strong CD20 expression and ^aatutes score usually was less than 3. In contrast to what is usually reported in the literature, in a significant number of cases (75%) CD23 was positive with a relatively weak expression.

1210

HEREDITARY FUNCTIONAL PROTEIN C AND PROTEIN S DEFICIENCY IN A COHORT OF THROMBOPHILIC PATIENTS

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Background. The activated protein C-protein S-thrombomodulin complex is the main anticoagulant system in the normal hemostasis, its action reduces the activity of activated factors V and VIII. *Aims.* We wanted to determine the functional hereditary deficiencies of protein C and S in a strictly selected cohort of thrombophilic patients, but also the incidence of this deficiency, clinical manifestations and the evolution of this patients. *Methods.* We determined the functional activity of activated protein C and S in a cohort of strictly selected patients suspected of hereditary thrombophilia. Hereditary thrombophilic disease was differentiated from an acquired one by the thromboembolic episodes at an early age (15-45 years), familial distribution, occurrence in unusual sites (mesenteric, cerebral and axillary veins), occurrence in the same site or in separate sites with or without triggers despite a adequate therapy with warfarin. We studied a group of 38 patients examined in the Hematology Department of the City Clinical Hospital, Timisoara within four years. The selection criteria were: age under 50, recurrent thrombosis without an apparent cause, all of them having no clinical, imagistic or laboratory signs of paraneoplastic, inflammatory or infectious activity. Global plasma activity of protein C and S were determined using an ACL 2000 nephelometric centrifugal analyzer and IL Test kits provided by Instrumentation Laboratory. We analysed the incidence of protein C and S deficiency in this strictly selected cohort, clinical manifestations, thromboembolic events and the evolution of this patients. *Results.* The studied cohort included 28 male patients and 10 female patients with recurrent thrombotic events in personal and family history. Five patients presented functional protein C deficiency, 6 patients presented functional protein S deficiency and one patient was diagnosed with associated deficiency of protein C and protein S. The most of them had thrombosis of the inferior member veins 25 patients (65,79%), followed by pulmonary thromboembolism 5 patients (13,15%), thrombosis of axillary vein 5 patients (13,15%), followed by mesenteric thrombosis, inferior cave vein thrombosis, renal artery thrombosis. The evolution of this patients was favourable, 5 patients presented 2 recurrences, 3 patients presented only one recurrence. *Conclusions.* We can say that a strict selection of thrombophilic patients increases the possibility of detection of protein C and S deficiency, this tests on strictly selected cohorts are necessary and efficient.

1211

MEGACHEMOTHERAPY AND AUTOGENEIC BONE MARROW TRANSPLANTATION INFLUENCE ON GAMMA-DELTA T-CELL RECEPTOR (TCR $\gamma\delta$) EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTE POPULATION IN MULTIPLE MYELOMA (MM) PATIENTS

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Background. Gamma-delta T lymphocytes constitute average

4% of all T lymphocytes population in peripheral blood. \square T cells CD4-CD8- appear a immune response to acute lymphoblastic leukemia following allogenic BMT, resultating in improved relapse-free survival for those patients, who survive 100 days from transplant. Activated \square T cells express antigens CD 25+ (late activator) and CD69+ (early activator) on their surface. *Aims.* Megachemotherapy and autogeneic bone marrow transplantation (mega-chmt+BMT) influence on \square T cells mean percentage (%) in peripheral blood in MM patients was investigated. *Material and Methods.* 6 MM patients (pts) treated in Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation of Wrocław Medical University between 2002-2004 and 14 healthy controls were included into analysis. 2 pts were in II, 3 in III stages, 1 pts was MM non-secretorius, according to Durie-Salomon classification. 4 pts were classified to A and 2 to B groups. Samples of MM pts blood were taken before and after mega-chmt+BMT. \square T cells were estimated by flow-cytometry (FACS), using a fluorescence-activated cell sorter and monoclonal antibodies (MoAbs: Ab-anti TCRgamma1-FITC (Becton-Dickinson), Ab-anti CD14-PE/CD45-FITC [Leukogate], CD2-PE, CD3-PE, CD4-PE, CD8-PE, CD25-PE, CD56-PE and CD69-PE). *Results.* Mean% of total T \square lymphocytes in peripheral blood before mega-chmt+BMT was 1,68% of all MM pts. It was lower than in control group (1,68 vs 5,63%). Lymphocytes immunophenotyping was CD2+CD3+CD4-CD8-CD25-CD56-CD69- in all MM pts. \square T cells mean% increased after mega-chmt+BMT to 2,04%, compared with mean% before this treatment (1,68% vs 2,04%), but was also lower than in health volunteers (2,04% vs 5,63%). All MM pts (100%) stayed in RC 6 months after mega-chmt+BMT. *Conclusions.* This study shows a favorable trend, that megachemotherapy and autogeneic bone marrow transplantation, used in Myeloma multiplex patients, causes the increase of gamma-delta T lymphocytes population in peripheral blood. More over, this increase is strictly connected with improved relapse-free survival, so may be considered as better prognostic factor for MM patients, treated megachemotherapy and autogeneic bone marrow transplantation. Our MM patients group is small, so it needs a further examination.

1212

CHEMOTHERAPY INFLUENCE ON GAMMA-DELTA T-CELL RECEPTOR (TCR γ) EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTE POPULATION IN MULTIPLE MYELOMA (MM) PATIENTS

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Background. Gamma-delta T lymphocytes constitute average 4% of all T lymphocytes population in peripheral blood. Most of them are known as 'double negative' lymphocytes (CD3+CD4-CD8-). They appear to possess intrinsic cytolytic activity against tumour cells in carcinomas and lymphomas. *Aims.* chemotherapy influence on \square T cells mean percentage (%) in peripheral blood in MM patients was investigated. *Material and Methods.* 28 MM patients (pts) treated in Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation of Wrocław Medical University between 2002-2004 and 14 healthy controls were included into analysis. 5 pts were in I, 1 pt in I/II, 2 in II and 20 in III stages. 75% pts was classified to A and 25% to B groups. Samples of blood were taken at MM diagnosis and at the time of efficacy therapy evaluation (after 3 chemotherapy cycles). \square T cells were estimated by flow-cytometry (FACS), using a fluorescence-activated cell sorter and monoclonal antibodies (MoAbs: Ab-anti TCRgamma1-FITC (Becton-Dickinson), Ab-anti CD14-PE/CD45-FITC [Leukogate], CD2-PE, CD3-PE, CD4-PE, CD8-PE, CD25-PE, CD56-PE, CD69-PE). *Results.* The most of pts (54%) were treated by VA (E)D (vincristine-adriamycin (epirubicin)-dexamethason) cycle. MP (melfalan-prednisone) cycle was the second main treatment (14% pts). Complete remission or disease stabilization was achieved in 54% of pts. \square T lymphocytes immunophenotyping was CD2+CD3+CD4-CD8-CD25-

CD56-CD69- in most of MM cases. Mean% of total \square T lymphocytes in peripheral blood at the diagnosis was 4,42% in all MM pts and was lower than in control group (4,42% vs 5,63%) ($p > 0,05$). It decreased after treatment to 2,33% vs 4,42% ($p < 0,05$). Mean% were at diagnosis: 5,89% and 3,82% in I+II and in III stages, respectively and decreased after therapy to: 4,65% and 2,33% in I+II and in III stages, respectively. Mean% of \square T cells decreased in both A and B groups after chemotherapy (A: 4,74% vs 2,25% ($p < 0,05$) and B 6,4% vs 2,76% ($p > 0,05$)). \square T cells mean% in pts, treated VA (E)D was 4,26% at diagnosis and decreased to 2,31% after treatment ($p < 0,05$), in pts treated MP was 6,65% in recognition and decreased to 5,50% after treatment ($p > 0,05$). The decrease of \square T cells mean% after therapy was observed: from 4,09% to 3,22% ($p > 0,05$) and from 5,82% to 2,01% ($p > 0,05$) in pts, achieved RC or disease stabilization and in pts with disease progression, respectively. *Conclusions.* This study shows, that chemotherapy, used in Myeloma multiplex, causes the reduction of gamma-delta T lymphocytes population in peripheral blood. It is more notice in advanced MM, in which often additional therapy (plasmaferesis, dialysis) is necessary. Higher \square T cells mean percentage in MM patients, stayed in RC or disease stabilization, than in MM progression patients may confirm the significant role of gamma-delta T lymphocytes in immune response to myeloma multiplex and demonstrates their impact on better clinical outcomes in myeloma multiplex patients.

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AUTOLOGOUS STEM CELL TRANSPLANTATION FOR HEMATOLOGICAL MALIGNANCIES - A NINE-YEAR SINGLE INSTITUTION EXPERIENCE

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Background. High-dose therapy followed by autologous peripheral blood stem cell transplant (autoPBSCT) is frequently used in the treatment of eligible patients (pts) with hematological malignancies: Hodgkin's disease (HD), non-Hodgkin's lymphoma (NHL), multiple myeloma (MM) and acute myeloid leukaemia (AML), resulting in better disease-free survival (DFS) for chemosensitive disease. AIM: To characterize the procedures, complications and outcome associated to autoPBSCT in hematological malignancies in our Centre. *Methods.* Between June 1996 and December 2003, 90 pts with haematological malignancies (30 NHL, 27 MM, 17 HD, 16 AML) were submitted to autoPBSCT. Median ages were 40, 48, 26, 39 years (y), respectively. Eligible criteria included age < 60 y (< 70 for MM), absence of comorbidities and complete remission or good partial remission for AML, NHL, HD and a reduction of at least 75% of M component in MM. Mobilization was performed in all pts with G-CSF +/- chemotherapy (CT). Median number of apheresis procedures was 2. Quantification of CD34+ cells was performed by flow cytometry. PBSCT were not submitted to any selection procedure. Conditioning regimens were BEAM for HD/NHL, Melphalan 140mg/m² (2 pts) and 200mg/m² (25 pts) for MM and BuCy for AML. All pts received growth factor support after autoPBSCT and oral Co-trimoxazole, Itraconazole and Acyclovir prophylaxis. Median number of CD34+ cells infused was 4,9x10⁶/Kg. Conditioning regimens and autoPBSCT were done in HEPA-positive pressure rooms. *Results.* During apheresis procedure, paresthesias were observed in 4 pts (4,4%), easily reversible with oral calcium gluconate. There was no statistically significant difference between the number of CD34+ cells collected in the studied pathologies. Minor cardiovascular incidents were observed during infusion in 19% pts. Engraftment was obtained in all cases. Median duration of neutrophils $< 0,5 \times 10^6/L$: 7 days; median duration of platelets $< 20 \times 10^6/L$: 6 days. All pts received platelet transfusions (median 17 units/patient) and erythrocyte transfusions were reported in 63% (median 2 units/patient). Febrile neutropenia was observed in 53 pts (59%) and infection was microbiologically documented in 45%. Pathogens more frequently identified were gram-positive bacteria in 60% of the infectious episodes (Staphylo-

coccus epidermidis in most cases). Infections caused by gram-negative were mainly due to *E. Coli*. One patient presented with a disseminated form of cutaneous herpes simplex virus. No fungal infection was documented. Gastrointestinal (GI) toxicity NCI grade III/IV was reported in 21 cases (23%). Two pts died in the first 100 days (grade IV GI toxicity with severe hydro-electrolytic disturbance and probable DMSO cardiac toxicity). DFS was 64,7; 31,2; 64,7; and 52,9 months (m) for NHL; MM; HD; and AML, respectively. Median overall survival (OS) was 162,6m (NHL), 70,8m (MM); 89,5m (HD); and 62,8m (AML). **Conclusions.** Non-hematological transplant-related (TR) toxicity, mainly GI, was acceptable and easily reversible. TR mortality was low (2,2%). We obtained a better DFS than it would be expected only with conventional CT, as published in the literature. However, median OS is not significantly different from that described with high-dose CT without cellular support.

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EXTRACORPOREAL PHOTOPHERESIS FOR THE TREATMENT OF GRAFT VERSUS HOST DISEASE

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Background. Graft versus Host Disease (GvHD) remains an important cause of morbidity and mortality on allogeneic transplantation. Has also a significant impact in the quality of life of transplanted patients, and enormous time spent in hospital related both to infections and the toxicity of immunosuppressive drugs. The growing number of unrelated donors, more HLA disparity and older patients are all increasing the dimension of GvHD problem. Extracorporeal photopheresis (ECP) has been used in the transplantation setting since 1992, both for the treatment of GvHD and graft rejection prevention. **Aims.** To analyze ECP impact on the treatment of GvHD. **Methods.** Since 2000 we started using ECP for the treatment of GvHD and we present the results of the first 22 patients. **Results.** 15 males and 7 females were treated with ECP. Only 2 patients were below 18 years old. The original diagnosis was chronic myeloid leukemia in 10, acute lymphoblastic leukemia in 6, acute myeloid leukemia in 2 and one case of each aplastic anemia, Fanconi anemia, chronic lymphocytic leukemia and non-Hodgkin's lymphoma. Seven patients have been grafted with bone marrow and the others with PBPCs. Two cases were grafted with non related donors and in four it was the second transplant of the same donor. In one case the patient was treated before with donor lymphocytes infusions. Clinical manifestation of GvHD at ECP start were skin involvement in 18, oral and ocular symptoms in 16, liver function tests abnormalities in 9, waisting syndrome in 9, arthralgia in 4, bronchiolitis obliterans in 4 and GI tract symptoms in 3. The large majority of patients were under 2 or more immunosuppressive drugs. The median for ECP starting was day + 450 (76-3960), and the average number of treatments was 18. Ten patients are still under ECP. Eight patients have complete responses; they are out of ECP and without any immunosuppressive drug. Six patients achieved a partial response (clinical improvement but still under immunosuppressive drugs or ECP), two with stable disease (they became dependent of ECP) and two with refractory disease. Four patients are not evaluable because they have less than three months of treatment. The responses by organ were different. All the four patients with arthralgia are free of symptoms. The oral complaints and the scleroderma manifestations respond also very well. Waisting syndrome has a very unpredictable course and lung involvement was not affected by ECP. Only two patients relapse of malignancy and we haven't observed any excess in infections. **Conclusions.** ECP is an effective treatment in chronic GvHD, with very low toxicity. Its role in prophylaxis of GvHD and the mechanism of action remains to be clarified.

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THE MARKERS OF THE BONE METABOLISM IN PATIENTS WITH MULTIPLE MYELOMA AFTER CHEMOTHERAPY AND CLODRONATE TREATMENT

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Background. Osteoblasts activity markers are: C-terminal propeptide of type I collagen (PICP), bone alkaline phosphatase (BAP); osteoclasts: C-terminal Telopeptide of type I collagen (ICTP). **Aims.** The aim of this study was to demarcate the effects of clodronate treatment as a supportive care on bone metabolism in MM patients. **Material and Methods.** 44 patients with MM were studied (31 women and 13 men) aged 35 to 84 years (m=59) treated: 32 - standard VAD chemotherapy treatment and auto-PBSCT and the rest -conventional chemotherapy. Patients with renal failure were excluded of the study. Patients were in the stage of the clinical progression of the disease on base of Durie-Salmon scale: 11-IA, 10-IIA, 3-IIB, 20-IIIA. In 33 patients bone change were detected in X-ray examination. All the patients were also treated as supportive care with clodronate-900 mg iv. every 4 weeks. Serum BAP, PICP, ICTP was elevated twice-before and after the treatment(chemotherapy and clodronate). Control group was 20 healthy volunteers. Serum PICP and ICTP concentrations were analyzed by radioimmunoassays, BAP by ELISA method. All of the results have been statistically tested by using ANOVA test for the independent groups and Spearman correlation. For statistically significant results were $p < 0.05$. **Results.** Median concentration of PICP before treatment-115.3 $\mu\text{g/L}$ (SD 71.67 $\mu\text{g/L}$), after treatment-207.32 $\mu\text{g/L}$ (SD 131.72 $\mu\text{g/L}$). In control group median-133.6 $\mu\text{g/L}$ (SD 42.42 $\mu\text{g/L}$). Concentration of PICP after treatment were elevated in comparison with those before the treatment($p=0.001$), and in comparison with control group ($p=0.011$). Concentration of PICP negatively correlation with clodronate dosage ($p=0.027$, $r = -0.43$). Negative correlation between concentration of PICP and concentration of ICTP ($p=0.012$; $r = -0.98$), and positive correlation between concentration of PICP and calcium secretion in urine ($p=0.049$; $r=0.95$), LDH serum level ($p=0.026$; $r=0.42$), total serum protein level ($p=0.09$; $r=0.32$), and plasmocyte percentage in bone marrow ($p=0.06$; $r=0.16$). Median concentration of BAP before treatment-37.8 $\mu\text{g/L}$ (SD 19.82 $\mu\text{g/L}$), after treatment-38.65 $\mu\text{g/L}$ (SD 18.4 $\mu\text{g/L}$). In control group median-20.73 $\mu\text{g/L}$ (SD 15.05 $\mu\text{g/L}$). Concentration of BAP both before and after treatment were higher than in control group ($p=0.003$ and $p=0.018$ respectively). Positive correlation between BAP concentration and β 2-microglobulin level ($p=0.05$; $r=0.37$), and negative correlation between BAP serum concentration and IL-6 level ($p=0.016$; $r = -0.38$), and TNF-alpha ($p=0.025$; $r = -0.97$). Median concentration ICTP before the treatment-3.76 $\mu\text{g/L}$ (SD 10.06 $\mu\text{g/L}$), after the treatment-6.85 $\mu\text{g/L}$ (SD 14.27 $\mu\text{g/L}$). In control group median-4.72 $\mu\text{g/L}$ (SD 2.43 $\mu\text{g/L}$). Concentration of ICTP were higher in comparison to control group ($p < 0.05$). Positive correlation between ICTP serum concentration and levels of phosphates ions ($p=0.032$; $r=0.96$), daily calcium secretion in urine ($p=0.043$; $r=0.95$), and β 2-microglobuline ($p=0.006$; $r=0.35$). The concentrations of ICTP negatively correlates with sIL-6R levels ($p=0.019$; $r = -0.98$), and daily protein secretion to urine ($p=0.006$; $r=0.99$). **Results.** Increasing PICP concentration can be the result of osteoclasts activate and stabilization activity of ICTP concentration may be the result of restraining osteoclasts activity followed by clodronate treatment

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TELOMERASE ACTIVITY OF BONE MARROW CELLS IN MYELODYSPLASTIC SYNDROMES

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Myelodysplastic syndromes (MDS) are heterogeneous clonal disorders of multipotent stem cells characterized by ineffective myelopoiesis, leading to acute leukemia in about 30% of patients. The molecular background of MDS pathogenesis and MDS progress to leukemia is still not fully elucidated. Upregulation of telomerase activity, enzyme responsible for synthesis of chromosome termini and thus karyotype stability, was observed in MDS and more frequent in leukemic transformation. The aim of the study was to evaluate telomerase activity in different subtypes of MDS at diagnosis and to correlate it with cytogenetic and clinical parameters. Telomerase activity was determined using semi-quantitative TeloTTAGG Telomerase PCR ELISA kit (Roche) and normalized to the activity measured in 500-cell equivalent of K562 cell line. Bone marrow mononuclear cells were obtained from 40 non-treated patients at diagnosis. Patients were classified according to the FAB and WHO criteria and divided to FAB subgroups: refractory anemia (RA) - 20 cases, RA with excess of blasts (RAEB)- 12, RA with ringed sideroblasts (RARS) - 2, RAEB in transformation (RAEB-t) - 4 and chronic myelomonocytic leukemia (CMML) - 2. Telomerase activity was detected in 50% of bone marrows. Highest activity was observed in RAEB-t and RARS subgroups, lowest in RA. Differences of activity between FAB subgroups were significant ($p=0.012$, Kruskal-Wallis ANOVA). There was no correlation of telomerase activity with patient's age or degree of anemia. Enzyme activity correlated weakly with percentage of blasts in bone marrow ($R=0.31$, $p=0.049$). Telomerase activity was higher in 18 patients bearing (according to international prognostic scoring system for MDS, IPSS) unfavorable/intermediate chromosome aberrations than in 22 patients with normal/favorable karyotype (mean relative telomerase activity: 0.345 ± 0.281 vs. 0.152 ± 0.144 , $p=0.028$, respectively). Analysis of risk factors with relation to telomerase did not show significant differences in enzyme activity between different IPSS groups. In conclusion, elevated telomerase activity seems to be related to MDS with high-risk cytogenetic aberrations and accompanying MDS in transformation.

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SURFACE IGG EXPRESSION IS COMMON IN B-CELL CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL) AND IDENTIFIES DIFFERENT CLL SUBSETS

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Background. A characteristic diagnostic feature of B-cell chronic lymphocytic leukemia (CLL) is the weak surface expression of immunoglobulin (sIg) of the IgM/D isotypes. The expression of other sIg isotypes is believed to be a rare phenomenon in CLL. Occasional reports have suggested that IgG expression in CLL is frequent and may correlate with stage of disease (1, 2). **Aims.** A systematic analysis of surface IgG expression in CLL and characterization of the features of sIgG-expressing CLL. **Methods.** 105 unselected, consecutive cases of CLL were analysed. Flow cytometry was performed using fresh whole blood and red cell lysis (Immunoprep, Beckman Coulter). Anti human Ig antibodies (Dako Ltd), CD38PE (Immunotech) and anti ZAP70 (Upstate Ltd) were used. Positivity by flow cytometry was established at a 30% threshold for sIg. Immunofluorescence on slide-fixed cells was also performed. **Results.** Table 1 shows the 105 cases categorized according to their sIgG and sIgM expression. 44% (46/105) of all CLL cases displayed sIgG, with 18 being sIgG+ alone and 28 dual sIgG+, sIgM+.

Dual expression was confirmed in all cases to be due to double staining on the same cells and not to the presence of two clones. As expected, for all 105 cases, less than 10% had a 'CLL score' (3) of 3 or less, but there was a marked increase of such cases in the IgG+ IgM- group (6/18, 33%; $p = 0.0006$). Thus, the IgG+ IgM- group included 2/3 of all cases with a low CLL score. This group also showed marked variation from the whole population with respect to the reduced incidence of ZAP70 positivity (20 v. 39%), while CD38 positivity was increased (56 v. 39%). The available cytogenetic data at the time of writing showed that the IgG+ IgM- group had a high frequency of adverse abnormalities (17p-, trisomy 12, biallelic 13q deletion)-9/11 (81%). Only 2 patients had a favourable cytogenetic result without any additional adverse abnormalities (both 'normal'). Summary and conclusions: Surface expression of IgG is frequent in CLL, which contrasts with the dogma that CLL is characterised almost exclusively by sIgM/D. Some early studies reported frequent sIgG expression (1, 2) and suggested that sIgG+ CLL correlates with an earlier clinical stage of disease. IgG+ IgM- CLL appears to be a unique subset of CLL, characterised by low CLL scores, with paradoxical results suggesting both good risk (low frequency of ZAP70+) and poor risk features (CD38+ and cytogenetics). An updated and completed data set will be presented, including IgVH mutation.

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Table 1: Features of 105 consecutive cases of CLL grouped according to surface IgG and IgM expression

	Cases n (%)	CLL score ≤3 n (%)	x ± s.d.	ZAP70+ n (%)	CD38+ n (%)
IgM+ IgG-	48 (46)	1 (2)	1.0	19/41 (48)	18/45 (42)
IgM- IgG+	18 (17)	6 (33)	6.8	3/16 (20)	10/16 (56)
IgM+ IgG+	28 (27)	2 (7)	1.3	8/22 (38)	6/24 (25)
IgM- IgG-	11 (10)	1 (9)	6.8	3/8 (37)	3/27 (11)
Total	105	10/105 (10)	-	33/86 (38)	38/88 (43)

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PROCALCITONIN, C-REACTIVE PROTEIN AND PROINFLAMMATORY (IL-6, TNF-α) AND ANTIINFLAMMATORY (IL-10) CYTOKINES IN FEBRILE CHILDREN WITH CANCER DURING NEUTROPENIA

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Introduction: Despite improvement in oncological treatment a part of children still die due to inflammatory complications. There is an opinion that the used so far laboratory markers of infection appear to be insufficient. In neutropenic patients fever is often the only sign of infection and other clinical inflammatory symptoms may be absent. Now we are looking for new markers of infection: procalcitonin and proinflammatory and antiinflammatory cytokines. The aim of this study was an assessment of the activity of CRP, PCT interleukin 6 (IL-6), tumor necrosis factor - alpha (TNF-α), interleukin 10 (IL-10) in febrile children with cancer during myelosuppression. Patients and **Methods.** 81 children with cancer were examined. Those patients divided on: group A (febrile patients with cancer), B (afebrile patients with neutropenia), C (patients with new diagnosed cancer). CRP was determined with immunoturbidic

methods, PCT was determined by an immunoluminometric assay, cytokins by PharMingen OptEIA ELISA. *Results.* The highest levels of PCT was observed in group A (fever patient with neutropenia) $x=2,61\text{ng/mL}$ ($Me = 0,92\text{ng/mL}$) and was significantly higher than in group B (patient with neutropenia without fever) $x=0,3i2\text{ng/mL}$ ($Me=0,27\text{ng/mL}$; $p < 0,03$) i C (patient with new diagnosed cancer) $x=0,39$ ($Me=0,36\text{ng/mL}$). There wasn't any significantly difference between group B and C. The highest levels of CRP was in group A ($x=70,7\text{mg/L}$; $Me=51,4\text{mg/L}$) and C ($x=47,9\text{mg/L}$; $Me=50,4\text{mg/L}$). In group B the mean CRP level was ($x=17,9\text{mg/L}$; $Me=8,4\text{mg/L}$). Significantly higher levels observed in G (-) bacteremiae. Mean levels of IL-6 $x=110,89$ pg/mL; $Me=39,36$ pg/mL, TNF- α ($x=18,2$ pg/mL; $Me=15,42$ pg/mL) and IL-10 ($x=17,3$ pg/mL; $Me=11,89$ pg/mL) were significantly higher in group A (febrile patients with neutropenia) than in group B (patients with neutropenia without fever) and C (patients with newly diagnosed cancer). Higher mean levels of IL-6 and TNF- α were found in G (-) bacteremiae than G (+). For IL-10 there wasn't statistical differences between G (-) and G (+) bacteremiae. *Conclusions.* 1. Procalcitonin is a new and more useful inflammatory marker in febrile neutropenic children than CRP. 2. Neither malignant disease nor neutropenia has any effect on the activity of procalcitonin. 3. Concentration of proinflammatory cytokins was significantly higher in febrile neutropenic children than in both control groups. 4. Significantly higher concentrations of proinflammatory cytokins were found in G (-) bacteremiae.

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PREGNANCY IN TYPE I GAUCHER'S DISEASE

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Background. Gaucher disease is an autosomal recessive genetic disorder in which there is a deficiency in the lysosomal enzyme glucocerebrosidase and an accumulation of glycosphingolipids in the spleen, liver and bone marrow with resultant organ dysfunction and variable clinical symptomatology. The most prevalent form of the disorder is the type 1, chronic non-neuronopathic, whose incidence is estimated at 1/200,000 in the non-Jewish population and 1/1,000 in the Ashkenazi Jewish group. The type 1 patients show significant clinical variability in age of clinical presentation and severity of symptoms. Pregnancy and delivery for patients with non-neuronopathic Gaucher disease can be complicated and require several considerations. *Methods.* We describe 6 patients carried 8 pregnancies to term, including one with two early pregnancy losses before enzyme replacement therapy (ERT). In 6 pregnancies, the patients did not interrupt ERT and in two, the ERT was interrupted during the three first months. The patients in whom ERT was interrupted had bone pain, liver increase, platelet count decrease and anemia. All the other 6 pregnancies were uneventful and all 8 pregnancies resulted in healthy newborns (now, ages 9 months to 5 years). *Conclusions.* The ERT had a remarkable impact on the improvement of Gaucher disease-associated symptoms, prevention of progressive manifestations of the disease and improvement in the patient's quality of life. Intravenous infusion every two weeks is an effective and safe procedure, with rare development of antibodies or immune-mediated reactions. Pregnancy is not contraindicated for Gaucher patients and the treatment should not be interrupted because the clinical improvement due to ERT is important in preventing complications during pregnancy and delivery and its use has any adverse effect on fetal development.

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HEMATOPOIETIC STEM CELL TRANSPLANTATION IN HIGH RISK MYELODYSPLASTIC SYNDROME AND SECONDARY ACUTE MYELOGENOUS LEUKEMIA

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Objective. Patients (pts) with high risk myelodysplastic syndrome (MDS) and secondary acute myelogenous leukemia (secAML) have poor prognosis. Allogeneic hematopoietic stem cell transplantation (alloHSCT) remains currently the only curative therapy for this group. *Material and methods.* We present the outcome of 17 pts (median age 46, range 19-65) with MDS (10) and secAML (7) treated with alloHSCT from identical sibling donors. Time from diagnosis to transplantation was 11.5 (range 4-58) months. Three pts proceeded to HSCT without previous treatment, 14 pts received induction chemotherapy. Six pts were conditioned with ablative therapy BuCy2 (busulphan, cyclophosphamide) and transplanted with bone marrow stem cells. Graft versus host disease (GVHD) prophylaxis consisted of cyclosporin (CsA) and metotrexate. Eleven pts received non-myeloablative fludarabine-based treatment and were grafted with peripheral blood stem cells. GVHD prophylaxis consisted of CsA alone. Transplanted material contained 4 (range 3-7.5) $\times 10^6/\text{kg}$ CD34+ cells. *Results.* Myeloid (ANC>0.5 G/L) and platelet >20G/L engraftment was reached in all pts at median time of 14 (range 11-23) and 16 (range 11-35) days, respectively. Acute GVHD occurred in 8 (47%) pts, including 5 (29%) pts with grade 3 or 4. Extensive chronic GVHD was seen in 1 patient. With a median follow up of 13 (range 2-91) months, the overall survival estimated by Kaplan-Meier method was 0.26 \pm 0.12. Survival was better for pts younger than 40 yrs (0.53 \pm 0.24 vs 0.19 \pm 0.12; ns). Six (35%) pts died during first 100 days. Primary causes of death were acute GVHD (5) and sinusoidal obstruction syndrome (1). Deaths after +100 day were caused by relapse in 4 (23%) pts and ischaemic cerebral insult(1). *Conclusions.* AlloHSCT gives opportunity to cure pts with MDS/secAML, especially younger than 40 yrs. Acute GVHD and relapse remain the main causes of death.

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MOBILIZATION OF HAEMATOPOIETIC AND MESENCHYMAL STEM CELLS INTO PERIPHERAL BLOOD AND ACTIVATION OF CIRCULATING T LYMPHOCYTES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Background. Experimental and clinical data suggest that intracoronary transplantation of peripheral blood or bone marrow derived adult stem cells after myocardial infarction (MI) can reduce a postinfarction scar, attenuate pathologic remodeling and improve cardiac function. The optimal source and collection time of stem cells is not known as well as the relation with activation of circulating T lymphocytes which is thought to play the key role in pathogenesis of acute coronary syndrome. Aim of the study: Analysis of the mobilization dynamics of CD34+ and CD133+ stem cells into peripheral blood and its correlation with lymphocytes T activation in patients with acute MI. *Material and Methods.* A total of 18 patients (12 males, 6 females) (median aged 62, range 48-77 years) with first ST-segment-elevation acute MI treated with primary percutaneous coronary intervention (PCI) were enrolled to the study. Blood samples were drawn immediately after admission on day 0 and then on day 2, 4 and 7 of acute phase of MI. The percentage and the number of CD34+ stem cells, CD133+ stem cells, CD3+/HLA-DR+ and CD3+/CD69+ lymphocytes was assessed by use of a flow cytometer. Troponin I was detected as a marker of myocardial injury. *Results.* The number of CD34+ stem cells on day 0 positively correlated with troponin I level measured on admis-

sion ($p < 0,05$) A significant increase of percentage of CD34+ stem cells was found on the day 2, 4 and 7 ($p < 0,05$) in comparison with the day 0. The number of CD34+ stem cells and the percentage of CD133+ increased on day 4 ($p < 0,05$). The number of CD133+ stem cells did not change significantly. The percentage of CD3+/CD69+ lymphocytes showed a positive correlation with the percentage of CD34+ and CD133+ stem cells on the day 4 ($p < 0,05$). The percentage and the number of stem cells are presented in table. **Conclusions.** Activation of circulating T lymphocytes and stem cells mobilization into peripheral blood was observed after MI. On the fourth day of acute phase of MI a maximal increase of mobilized stem cells was found. The positive correlation between lymphocyte T activation and stem cell mobilization suggests that inflammation process is an important factor influencing this mobilization. .

Table. Stem cells mobilization into PB in acute MI.

	Day0	Day3	Day4	Day7
	(n=44+SD)	(n=44+SD)	(n=44+SD)	(n=44+SD)
CD34+ cells	0,024±0,004	0,034±0,004	0,044±0,004	0,054±0,004
CD34+ stem cells	2,8 ± 1,2 n=1063	3,8 ± 1,2 n=1165	4,8 ± 1,2 n=1267	5,8 ± 1,2 n=1369
CD34+ CD69+ cells	0,034±0,004	0,044±0,004	0,054±0,004	0,064±0,004
CD133+ cells	2,8 ± 1,2 n=1063	3,8 ± 1,2 n=1165	4,8 ± 1,2 n=1267	5,8 ± 1,2 n=1369

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A PILOT STUDY OF REDUCED INTENSITY CONDITIONING (RIC) WITH LOW DOSE ATG IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-SCT)

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Background. Several different RIC regimens have been established in an attempt to reduce toxicity in patients not eligible for standard myeloablative allo-SCT (either by age or co-morbidity). Whereas hematopoietic engraftment can be accomplished with minimal toxicity in these patients after RIC allo-SCT, high rates of morbidity and mortality from acute and chronic graft versus host disease (GvHD) still remain a major problem to be resolved. Anti-thymocyte globulin (ATG), in addition to its dominant action as a T cell depleting agent, has been implicated to bear the capacity to beneficially influence GvHD by depleting host antigen presenting cells. **Aims.** To evaluate the effect of low dose rabbit ATG (5 mg/kg) on the balance between the anti-neoplastic effect of donor-derived cells and the morbidity and mortality due to GvHD which exceedingly affects the efficiency of allo-SCT following RIC. **Patients and Methods.** Sixteen adult patients (8 AML, 3 CML, 2 CLL, 3 NHL), median age 48 years (range 21-64) underwent RIC allo-SCT. As a conditioning regimen they received Fludarabine 30mg/m² (3-5 consecutive days; with the addition of Cyclophosphamide 300mg/m² for 3 consecutive days in case of NHL) and fractionated total body irradiation (fTBI, 200-400 cGy). GvHD prophylaxis consisted of cyclosporin A (1,5 mg/kg twice daily starting at day -1), mycophenolate mofetil (15 mg/kg twice daily starting 6h after SCT) and rabbit ATG (5 mg/kg at days -4 to -1, Fresenius, Bad Homburg, Germany). Eleven patients (69%) had matched unrelated donors, 3 patients (19%) matched related and 2 patients (12%) mismatched unrelated donors. Acute and chronic GvHD were graded according to standard Seattle criteria after allo-SCT. **Results.** After a median follow up of 493 days 8 of 16 patients (50%) were alive. The overall survival at d100 was 81%, after 1 year 50%. One patient (6%) developed acute GvHD grade I-II and 4 patients (25%) acute GvHD grade III-IV. De-novo limited chronic GvHD showed 1 patient (9%). Extensive chronic GvHD was not present at all. The transplant related mortality was 19%, 1 patient died in consequence of acute GvHD grade IV and 2 patients due to infections. Five patients

(31%) died due to relaps and/or progressive disease. **Conclusions.** RIC including low dose (5 mg/kg) rabbit ATG resulted in a considerably low incidence of extensive chronic GvHD in this pilot study. The preferential dose and type of ATG are currently under investigation in many transplant centers. A prospective randomized trial is needed to define the role of low dose ATG during RIC allo-SCT.

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CHROMOSOMAL ABNORMALITIES IN PHILADELPHIA NEGATIVE METAPHASES IN A PHILADELPHIA POSITIVE CHRONIC MYELOID LEUKEMIA PATIENT DURING IMATINIB THERAPY

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Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder characterized by a specific cytogenetic abnormality, the Philadelphia Chromosome (Ph), that results from the reciprocal chromosomal translocation t(9;22) (q34;q11). The emergence of additional cytogenetic abnormalities in Ph positive cells, known as clonal evolution, is associated with disease progression. On the other hand, the development of chromosomal abnormalities, as trisomy 8 and monosomy 7, in Ph negative cells during the treatment with Interferon alpha (INFa), and more recently with Imatinib, has been reported. However, their pathogenesis and clinical significance is still unclear. In this work the authors report a case of a 48-year-old Ph positive CML female patient, without an HLA-matched related donor, that developed cytogenetic abnormalities during Imatinib therapy. The patient was initially treated with INFa, later discontinued as a result of neurological toxicity, started Imatinib at a 400 mg daily dose. Due to myelosuppression a dose reduction to 300 mg daily was required. Six months later a major cytogenetic response was observed (9/30 metaphases). However, other cytogenetic abnormalities in Ph negative cells had arisen: 12/30 metaphases showed a partial deletion of chromosome 7 (-7) and 9/30 metaphases had trisomy 8 (+8). Three months later definitive suspension of Imatinib was necessary due to toxic hepatitis. Then the disease progressed to a blastic phase, but a complete response to a cytarabine and anthracycline based regimen was achieved. As the patient refused unrelated donor allogeneic transplantation, she was maintained for the last 12 months in second chronic phase, under low dose subcutaneous cytarabine therapy. No evidence of the chromosomal abnormalities (-7, +8) was detected in the cytogenetic evaluations performed immediately after Imatinib suspension, in blastic phase and during the second chronic phase. This case highlights the necessity for close monitoring and long-term follow-up of CML patients under Imatinib treatment, to contribute to the elucidation of the clinical significance of these cytogenetic abnormalities.

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ABCB1 (MDR1) GENE HAPLOTYPES AND SUSCEPTIBILITY TO MULTIPLE MYELOMA

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Background. Inherited genetic variations in mechanisms responsible for protection of normal tissues against exposure to environmental toxicants may affect susceptibility to cancer including multiple myeloma (MM). ABCB1 gene (also known as MDR1) encodes for P-glycoprotein, an ABC family transporter, involved in transport of wide spectrum of xenobiotics. **OBJECTIVES:** The aim of this study was to compare allele and haplotype frequency of three common, partially-linked, single nucleotide polymorphism (SNP) in ABCB1 gene, namely C1236T, G2677T/A and C3435T SNPs, between 100 MM

patients and 100 healthy controls of Caucasian origin. *Methods.* Genotyping was performed by automated sequencing and RFLP method. Haplotypes were inferred using Bayesian algorithm. *Results.* The genotype data of 95 MM patients and 70 healthy controls were included in the preliminary analysis. Allele and genotype frequencies of all 3 studied SNPs were comparable in patients and controls ($p > 0.05$). However, significant differences between tested cohorts were observed when haplotype data were analysed. The frequency of carriers of haplotype containing all mutant alleles (1236T-2677T/A-3435T haplotype) was found to be increased in MM patients ($p < 0.001$), while carriers of haplotype 1236C-2677T/A-3435T were more prevalent among healthy individuals ($p < 0.001$). *Conclusions.* The preliminary results of our study indicate that ABCB1 gene haplotypes may play a role in genetic predisposition to MM.

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DETECTION OF ACTIVATED PROTEIN C RESISTANCE IN A COHORT OF THROMBOPHILIC PATIENTS

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Background. The activated protein C - protein S - thrombomodulin complex is the main anticoagulant system in the normal hemostasis. The activated protein C has serin protease activity and will inactivate activated factors V and VIII, this action is amplified by its binding with free protein S. The activated factor V resistance at activated protein C proteolytic cleavage is related with factor V gene mutation. The activated protein C resistance in the presence of mutant Leiden factor V has a high prevalence and it is the most important risk factor for hereditary thrombophilia. *Aims.* The aim of this study was to establish the incidence of activated protein C resistance in a cohort of strictly selected patients suspected of hereditary thrombophilia. *Methods.* We analysed a cohort of 26 patients, 13 males and 13 females with ages between 18-50 years which were examined in the Hematology Department of the City Clinical Hospital of Timisoara in the last four years, for thromboembolic events. The patients presented personal and family history of recurrent thromboembolic disease with no sign of paraneoplastic, inflammatory or infectious disease. Activated protein C resistance was determined by coagulometric methods using an ACL 2000 nephelometric centrifugal analyzer with Instrumentation Laboratory kits. We analysed clinical, biological and imaging evocative data presented by the patients suspected of hereditary thrombophilia. *Results.* Activated protein C resistance was found in 6 patients (23%). Positive results were confirmed at 6 months retesting in the same conditions. Clinical manifestation: 15 patients with superficial inferior members thrombosis, 3 patients with deep thrombosis of the inferior member, 3 patients with retinal artery thrombosis, 2 patients with repeated abortions, one patient with pulmonary embolism, one patient with port vein thrombosis and one patient with axillary vein thrombosis. *Conclusions.* Activated protein C resistance is important to be determined in all patients with a prothrombotic state suspected of an hereditary thrombophilia. The high incidence of this states in patients with hereditary anomaly is making these tests cost-efficient. First degree relatives of patients with activated protein C resistance should be examined to determine whether they should receive primary prophylaxis.

Study supported by grant no. 1166 of CNCSIS (Romanian National Council of Scientific Research).

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LIPOPROTEIN LIPASE ASSOCIATED GENE EXPRESSION SIGNATURE IN B-CELL CHRONIC LYMPHOBLASTIC LEUKAEMIA

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Background. Gene expression profiling has introduced a new dimension into our understanding of molecular mechanisms underlying B-cell chronic lymphocytic leukemia (B-CLL). Although microarray analysis has identified a number of potential prognostic or therapeutic targets, only a few have been validated for their clinical importance. One of the genes most closely related to IgVH mutational status is lipoprotein lipase (LPL). High expression of this central enzyme of lipid metabolism was associated with unmutated B-CLL in almost all profiling studies, regardless of methodology or B-cell selection process. We and others have recently shown that mRNA expression of LPL predicts treatment free as well as overall survival in B-CLL patients (Heintel et al., Leukemia 2005, in press). *Aims.* We reasoned that microarray analysis of B-CLL patient cells differing greatly in their LPL expression should reveal gene patterns not only associated with prognosis but also with biology, particularly in regard to LPL expressing tissues. *Methods.* To test this hypothesis we determined large-scale gene expression profiles using Affymetrix U133A GeneChips interrogating >20,000 genes in 2 groups of unsorted B-CLLs selected for either high ('LPL+'; n=10) or low ('LPL-'; n=10) LPL mRNA expression as determined by real-time PCR. The median LPL mRNA expression between these 2 groups differed by more than 2 logs (269 vs 0,9 as compared to normal PBMC). Selected genes were further RT-PCR verified in an extended, well characterized patient cohort (n=42). *Results.* Using this approach we were able to identify 110 genes differentially expressed more than 1.5-fold between LPL+ and LPL-. This 'LPL-associated gene signature' contains several known as well as novel genes significantly related to immune/defense response, muscle development and lipid metabolism as determined by database searches for gene function. Among the top discriminating genes we identified several candidates which had previously been described in B-CLL, including dystrophin, gravin (AKAP12), PEG10, BCL7A, FGL2, PNMA2, SORL1 or ZNF288. Other genes included septin 10, DUSP1, SIAH1, SAT, or sarcoglycan α (SGCE). Furthermore, using mRNA from adipose and muscle tissue we provide experimental evidence for an intriguing relationship in gene expression with LPL+ B-CLLs. To evaluate the prognostic power of the most prominent factors evolving from our 'LPL-associated gene signature' we performed real time PCR using mRNA from a well defined cohort of 42 B-CLL patients. The top 3 genes associated with time-to-treatment included dystrophin, septin 10 and gravin ($p < 0,01$). We have also evaluated the expression pattern of these genes in other hematologic malignancies. *Conclusions.* Besides the intriguing biological questions regarding origin, regulation, or plasticity of B-CLL cells raised in this study, we provide solid evidence for the clinical importance of some of the discriminatory genes associated with LPL expression.

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ASSESSMENT OF ARSENICTRIOXIDE (AS203) EFFECT ON TELOMERASE ACTIVITY IN APL PATIENTS AND NB4 CELL LINE

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Introduction: Telomerase the patching up enzyme that extends shortened telomeric repeats of chromosomal ends is differentially expressed in cancer cells and in normal cells.

Telomerase is activated in more than 85% of malignant tumors; therefore it might be as an important target for cancer therapy strategies. Telomerase inactivation is considered as a key reason of apoptotic and anticancer effects of As₂O₃ in Acute promyelocytic leukemia (APL) patients. To investigate anti telomerase effect of As₂O₃, we evaluated APL patients and NB4 cell line as a model of APL pre- and post- As₂O₃ treatment. *Methods.* Arsenic was used with dosage of 0.15 mg/kg/day as a routine treatment for APL patients at shariati hospital. Sequential peripheral blood samples were collected pre- and post- As₂O₃ treatment from 25 patients. The human NB4 cell line was cultured in presence of different concentration of As₂O₃ (0, 0.25, 0.5, 0.75, 1.0 and 2.0 μM). Telomerase activity was assayed using TRAP-ELISA procedure and gel electrophoresis in both APL patient and NB4 samples. *Results.* and conclusion: Establishment of quantitative assays for telomerase activity in APL patients had shown more than 80% elevated levels of telomerase activity in comparison with normal individuals and decreased after remission to normal level. Telomerase activity was shown down regulating in NB4 cell line after 24-48h as a result of As₂O₃ therapy. Decreasing in telomerase activity in time was intensively correlated with increasing in As₂O₃ treatment doses in NB4 model. These data indicate that telomerase can be a potent target for cancer therapy in APL treatment with As₂O₃ and to monitoring disease condition.

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CROSSING OVER EVENTS AFFECTING LEUKOCYTE RECEPTOR CLUSTER (LRC, 19Q13.4) ARE FREQUENT IN LEUKEMIA PATIENTS

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Leukocyte Receptor Cluster (LRC) region on chromosome 19q13.42 contains genes of immunoglobulin-like receptors involved in the regulation of natural killer (NK) cells (KIR, LAIR, NKp46) and of antigen presenting cells (ILT other names: LIR, MIR, LILRA/B). KIR and ILT genes are polymorphic, in addition the number of KIR genes is variable. Mendelian inheritance predicts that 25% of sibling pairs shares parental haplotypes of LRC. Microsatellite Short Tandem Repeat (STR) markers were used to ascertain haplotype segregation of LRC in 42 patient families referred to our centre for BMT. The underlying hematological diseases were: AML (12 cases), ALL (13), Aplastic Anaemia (9), CML (5), NHL (3). All patients were studied with both parents and at least one healthy sibling. Ten STR markers flanking LRC from centromeric (D19S572, D19S589, D19S924, D19S927) and telomeric side (D19S926, D19S418, D19S605, D19S880, D19S210, D19S88) were chosen on the basis of proximity to LRC, high heterozygosity and numbers of alleles. DNA fragments containing the repeat sequences were amplified by PCR with fluorescent-labelled primers. The PCR products were run in denaturing gel (BMA Rockland, USA) on ABI Prism 377 sequencer (Applied Biosystems, USA). The variability of products lengths stems from variable numbers of repeats and the lengths represent alleles. The analysis revealed discrepant segregation of parental STR alleles into haplotypes on the basis of one as compared to other sibling (s). Such discrepancies result from crossing over (c-o). 21 c-o events were detected in the studied group of 42 families. Remarkably 13 of them mapped to the space between the telomeric and centromeric markers groups, thus to the LRC region. The events accumulated in leukemia patients families (5/12 AML, 5/13 ALL, 2/5 CML) but not in aplastic anemia or lymphoma (1/9 AA, 0/3 NHL). In 4 families more than two siblings could be tested, in all of them c-o occurred during patient's, but not the healthy sibling's meiosis. Control group consisted of 12 healthy families of which one showed c-o within the LRC. Genetic recombination maps (Genethon, Marshfield and DeCode) indicate 5 cM distance between the centromeric and telomeric markers, the expected number of c-o is thus 5 per 100 meioses (5%). In line with this we observed c-o within the LRC in 1 out of 12 healthy sibling pairs (1/24). The frequency of c-o in the studied group of sib-

lings is 16% (13/80) and could reach 31% if in all the cases the patient's haplotypes were recombined. Interestingly the events accumulated in families of leukemia patients (12/60 siblings-20%) raising the possibility that 40% (12/30 patients) of leukemia patients inherited recombined haplotype of LRC. LRC genes characterize with high homology and dense alignment making it likely that unique haplotypes of KIR and/or ILT genes are created as a result of unequal c-o, such events have already been reported. We hypothesize that c-o affecting LRC region contributes to the pathogenesis of leukemia, possibly by influencing immune-surveillance capacity of NK cells.

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COMBINED ANTIFUNGAL TREATMENT FOR INVASIVE ASPERGILLOSIS IN CHILDREN WITH ALL-REPORT OF TWO CASES

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Invasive fungal infections are the leading cause of mortality due to infectious complications in the patients with hematological malignancies. The magnitude of this problem in pediatric hematology units is not precisely known, although some data indicates that is less frequent than in adult patients. Low sensitivity of conventional diagnostic methods is the major reason for scarce epidemiological data. This paper reviews clinical and radiological presentation, as well as treatment and outcome of two pediatric patients with invasive aspergillosis. Case 1: Fourteen-year-old girl was referred to our hematology ward for investigation of pancytopenia. According to the bone marrow morphology and immunophenotype diagnosis of preB ALL was established. In the third week of induction treatment she suffered pneumonia accompanied with high fever, unresponsive to broad spectrum antimicrobials. Initially, fluconazole was introduced, but after isolation of *Aspergillus flavus* from sputum culture on two occasions, therapy was changed to liposomal amphotericin B (4 mg/kg for two weeks than 2 mg/kg for four weeks) and voriconazole IV. Although she became afebrile in next two days, clinical course was complicated with pericardial effusion and severe pulmonary hemorrhage. Antifungal therapy lasted for 6 weeks, with further intermittent use of oral voriconazole during periods of neutropenia. Complete hematological remission was obtained despite interrupted induction, and she is now on maintenance treatment. Case 2: Six-year-old girl with preB ALL on maintenance treatment suffered high fever during period of prolonged neutropenia. As the fever did not subside on broad spectrum antimicrobials, further evaluation revealed pulmonary infiltrates and *Aspergillus flavus* was isolated from hemocultures. She was treated with liposomal amphotericin B (4 mg/kg in the first week, than 2 mg/kg next week) and voriconazole IV for two weeks, with good response. She was considered to be in a low risk of recurrence of fungemia having a complete hematological remission and fast recovery of absolute neutrophil count. Although invasive fungal infections seems to be a rare event in children treated for ALL, empirical antifungal treatment is mandatory in patients in neutropenia with prolonged fever unresponsive to broad spectrum antimicrobials. Combined antifungal treatment with liposomal amphotericin B and voriconazole for proven invasive aspergillosis was safe and effective in our patients.

1230**OSTEONECROSIS OF THE JAW ASSOCIATED WITH THE USE OF PAMIDRONATE IN MULTIPLE MYELOMA**M. Fisis¹, D. Barbarousi¹, A. Mylonas², V. Markos¹, D. Presvelos¹, K. Petraki², S. Savva¹, G. Morphopoulos¹, H. Kokka¹, K. Stefanoudaki-Sofianatou¹¹Amalia Fleming General Hospital, Athens, MELISIA, Greece; ²Metro-politan Hospital, ATHENS, Greece

Biphosphonates, pamidronate (Aredia) and zoledronate (Zometa) are widely used in the management of multiple myeloma and metastatic bone disease. They are nonmetabolized analogues of pyrophosphate, capable to localize to bone and inhibit osteoclastic function (which is in excess activation by cytokines produced by the neoplasm) causing disruption of osteoclast-mediated resorption. It has been demonstrated that they affect bone turnover by several mechanisms such as inhibition of osteoclast development from monocytes, increased osteoclast apoptosis, stimulation of osteoclast inhibitory factor, prevention of osteoclast development from bone marrow precursors, reduction of osteoclast activity and downregulation of matrix metalloproteinases. However the function of osteoclasts in bone resorption and remodeling is essential in maintaining normal bone homeostasis, which when interrupted results in the inhibition of new bone formation. It consequently affects the quality of bone during growth and fracture healing leading to bone necrosis. In addition the antiangiogenic properties of the biphosphonates can explain the apparent ischemic changes observed in patients bones. In the past few years a number of patients who received chronic biphosphonate therapy, presented with necrotic lesions of the maxilla, mandible or both. The lesions were refractory to surgical debridement, antibiotic therapy or other treatment. A female patient 69 years old with IgG- κ multiple myeloma received pamidronate for 30 months, in combination with chemotherapy and interferon. Two months after the withdrawal of pamidronate she presented with a loose left 3rd upper molar and periodontal inflammation, which gradually spread to the neighbouring molars leading to their removal. Nine months later the patient presented with a left oroantral fistula and symptoms of ipsilateral chronic sinusitis. Surgical debridement and closure of the oroantral communication were ineffective, leading to the relatively soon reemergence of oroantral communication symptoms. The patient was admitted with a clinical appearance of osteomyelitis in the left molar maxillary region on a ground of oroantral communication and concomitant ipsilateral chronic sinusitis, confirmed by axial and coronal CT scan imaging examination. The histopathologic examination revealed bone necrosis, neutrophil infiltration and mucosal inflammation. Taking into account both the serious general condition of the patient and the possible poor results of surgical intervention mentioned in the literature, it was decided that no further management was indicated. Osteonecrosis associated with biphosphonates is an extremely rare complication. The exclusive appearance in the jaws can be attributed to the presence of the teeth, through which the jaws are exposed to the external environment. More than 2.500.000 pts have been given biphosphonates and only about 100 have been reported as having osteonecrosis of the jaws. However taking into account the continuously increasing numbers of cases that have been reported and the broadening indications for the use of biphosphonates, it becomes imperative that a complete dental evaluation is performed before the commencement of treatment. In conclusion although biphosphonates remain an effective agent in the treatment of osteolytic bone lesions, the clinician should be aware of the possibility of this complication.

1231**SUCCESSFUL TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA IN A HIV AND HCV INFECTED PATIENT**

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In the literature four patients affected by APL and HIV infection are described, two of them being alive and in CCR. Here, we report the occurrence of Acute Promyelocytic Leukemia (APL) in a patient affected by HIV and HCV infection, treated with Idarubicine and ATRA containing therapy, HAART therapy, Interferon and ribavirine, and now alive and disease free at 4 years after the diagnosis of leukemia. A 38 y.o. woman was admitted to the Infectious Disease Department of our hospital in December 2000, with a several weeks history of progressive pancytopenia, a 6 years history of HIV infection, in treatment with HAART therapy, and a several years history of HCV chronic hepatitis, treated with interferon through June 2000. She was heterosexual, and she had no history of drug abuse. At the admission to the hospital the pancytopenia was thought to be related to the HAART treatment, and no more diagnostic evaluation was performed. She had no hemorrhagic diathesis and the coagulation was normal. HAART treatment was stopped, but after three weeks no changes in blood counts were observed. In January 2001 scattered blasts were observed in the peripheral blood, and a bone marrow evaluation was performed, that showed 10% myeloid blasts, and 55% atypical promyelocytes with Auer Rods. Chromosomal analysis identified a translocation (15;17) in 13 out of 20 metaphases, and molecular biology confirmed the presence of pml-rar-alfa transcript. A diagnosis of APL was done. Due to the very good clinical conditions of the patient, we treated her according to the schedule of italian protocol Gimema AIDA2000. She was treated outside of the protocol, because she was not eligible due to the two concurrent infections. The patient received one course of induction chemotherapy with Idarubicine 12 mg/m²/day for 4 days and ATRA for 30 days, followed by three consolidation courses, respectively with Idarubicine 5 mg/m²/day for 4 days, Mitoxantrone 10 mg/m²/day for 5 days, and Idarubicine 12 mg/m²/day for 1 day, all of them combined with ATRA for 15 days. She obtained a hematological and cytogenetical remission at the end of the induction, and a molecular remission at the end of the consolidation courses. She thereafter maintained a complete molecular remission with ATRA, taken 15 days every 3 months, through July 2003. Due to elevated transaminases at the end of consolidation, she did not receive Metotrexate and Mercaptopurine as maintenance. She was able to continue the maintenance treatment with ATRA without toxicities. She continued to receive PEG-interferon and ribavirine, through January 2003, and Efavirenz, Lopinavir-Ritonavir and Abacavir. Liver enzymes progressively improved over time, and HIV-RNA was undetectable through all 2004. Since July 2003 she is no more taking any maintenance treatment for APL. The last bone marrow evaluation, of December 2004, shows an absence of pml-rar-alfa. This case points out that even high risk patients, who are frequently excluded from intensive chemotherapy because of expected major liver toxicities and infectious complications, can tolerate with success a complete course of antileukemia treatment, and maintain a long lasting complete remission.

1232**THE INFLUENCE OF PLASMA AND CONDITIONED MEDIA FROM PATIENTS WITH NON-HODGKIN'S LYMPHOMAS (NHL) AND GASTRIC CANCER (GC) ON ADHESIVENESS OF HUVECS IN COMPARISON TO EFFECT OF RHTGF β 1**

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TGF β 1 and fibrinogen are some of the key regulators of endothelial cells (EC) interactions in malignancy which can modify the EC properties increasing their adhesiveness to tumour cells. The aim of our investigation was to compare the changes of EC adhesive properties under influence of plasma as well as conditioned media of malignant cells cultures (CM) from patients (pts) with NHL (39 pts) and GC (21 pts) and to reveal a possible role both of endogenous or secreted TGF β 1 (en/secr TGF β 1) and fibrinogen concentration (FC) in this process. *Methods.* We used plasma of all pts with malignancy and CM of mononuclear cells from peripheral blood (MNPB), affected lymph nodes (MNLN) or spleen (MNS) in NHL pts as well as of tumour cells in GC pts obtained by disaggregation with collagenase and DNase. The attachment of K562, L1210, L929 cell lines labeled by 3H-thymidine to monolayer of HUVECs under influence of rhTGF β 1 and patient's material was estimated as an index of adhesion (IA). En/secrTGF β 1 level was quantified by bioassay using CCL64 cells line as target. The FC was measured by conventional method. *Results.* Plasma as well as CM from MNS, MNLN of NHL pts showed comparatively strong influence on adhesive activity of HUVECs to K562 and L1210 (IA=1,90 \pm 0,36 and 2,19 \pm 0,24, respectively). CM from MNPB of NHL pts induced attachment both of L1210 and K562 to HUVECs more slowly (IA=1,62 \pm 0,11). The attachment of L929 to HUVECs was more strong after stimulation by CM from poorly differentiated GC tumours (IA=1,67 \pm 0,18), than by well differentiated ones (IA=1,20 \pm 0,04). The concentration of TGF β 1 in plasma (3,65 \pm 0,73 ng/mL) as well as in CM of MNS (6,78 \pm 0,23 ng/mL) from NHL pts were two-fold higher, than in GC pts (1,58 \pm 0,26 ng/mL) or healthy donors (2,11 \pm 0,38 ng/mL). The levels of secrTGF β 1 in samples of CM from poorly and well differentiated GC were 2,30 \pm 0,40 and 2,00 \pm 0,40 ng/mL, respectively, similarly to healthy donors. Elevated FC was found both in GC and NHL pts (7,20 \pm 0,60 and 6,63 \pm 0,48 mg/mL, respectively) in comparison with healthy persons (4,00 \pm 1,00; p<0,02) contrary to normal FC in pts with splenomegaly of non-malignant origin. We found that low concentrations (0,01 up to 2,40 ng/mL) of rhTGF β 1 stimulated significantly the adhesiveness of HUVECs. RhTGF β 1 in the ranges from 3,60 ng/mL up to 8,30 ng/mL inhibited adhesion of K562 cells (IA<1,00), but in the higher doses it stimulated adhesive properties of HUVECs again (IA>1,00). *Conclusions.* RhTGF β 1 *in vitro* influences on adhesive properties of HUVECs in a dose-dependent manner. EnTGF β 1 and FC are elevated both in NHL and GC pts and may influence on metastatic spread of these tumors. EnTGF β 1 may play a different role in NHL and GC pts. Plasma and CM in NHL and GC pts stimulate adhesiveness of HUVECs. But we didn't observe any correlation between en/secrTGF β 1 concentrations in plasma as well as in CM both from NHL and GC pts and their influence on adhesive activity of HUVECs.

1233**THE EXPRESSION OF MESSENGER RNA OF SURVIVIN IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA**

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Background. Apoptosis, an active suicidal response to different physiological or pathological stimuli, plays an important role in a variety of biological events, including morphogenesis, homeostatic maintenance of different tissues and removal of

harmful cells. Aberrations in the mechanism of apoptosis lead to autoimmune diseases, neurodegenerative disorders and carcinogenesis. Survivin is a recently described member of the family of inhibition of apoptosis proteins. It inhibits apoptosis induced by chemotherapeutic agents, Fas, and caspases. It is shown that it is overexpressed in the most common human cancers. *Aims.* In this study, we aimed to investigate the expression of survivin mRNA in the bone marrow blasts in childhood ALL by using RT-PCR method. *Methods.* In the study, 25 cases with ALL at the ages of 2-14 years are included, the average age was 7,7 years in the patients. The age distribution of the control group varied 1-19 years, the average age was 8,4 years consisted of 13 children. *Results.* The average of survivin mRNA expression was 652,2 copy/100000 cells at the diagnostic aspiration material from bone marrow samples of the patients. The statistical comparison of the average values of the ALL patients and control group have shown important difference statistically (p=0,013). Survivin mRNA expression value was found to be important for the expected survival time. *Summary/conclusions:* Survivin is thought to be an important prognostic parameter in children with ALL.

1234**PRAME EXPRESSION IN CHRONIC LEUKEMIAS**

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Background and Aim. PRAME (preferentially expressed antigen of melanoma) is a tumor antigen expressed in various malignant tumors including solid tumors and hemopoietic neoplasias but no or weak expression in normal tissues. PRAME is recognized by cytolytic T cells and with this property it may be a good candidate for immunotherapy and also this antigen may be a good marker to determine the minimal residual disease in tumors expressing this antigen. The aim of this study is to determine the frequency and the clinical importance of the expression of this antigen in chronic leukemias. *Patients and Methods.* PRAME mRNA was measured by real time RT-PCR. Samples taken from 88 cases with chronic leukemia and 42 controls were used as study material. Seventy cases had chronic myelocytic leukemia/chronic myeloproliferative disorder (CML/CMPD) [56 had chronic phase-14 had accelerated/blastic phase disease] and 18 cases had chronic lymphoid leukemia (CLL) [11 had early phase and 7 had late phase disease]. Mann Whitney U, Wilcoxon and Kruskal Wallis tests were used for statistical analysis. *Results.* Twenty four of 70 cases with CML/CMPD and 5 of 18 cases with CLL showed PRAME expression. However only 2 controls showed weak PRAME expression (which was 2 copy /10.000 cell: cutoff level is less than 2.5 copy / 10.000 cell). There was an important difference for PRAME expression between the chronic leukemias and controls (p: 0.000). There was not an important difference between PRAME (+) and PRAME (-) cases for age, Hb, Hct, WBC count, platelet count, disease stage and response to therapy. PRAME was monitored in 8 cases during follow-up: In 3 cases PRAME was negative at chronic phase and expression developed at the accelerated/blastic phase. PRAME was positive in 4 cases (3 CML-1CLL) and expression disappeared after chemotherapy. There was important increases and decreases in PRAME levels in a case with CML-BP during chemotherapy and relapses. *Conclusions.* PRAME is expressed in approximately one third of the cases with chronic leukemias. PRAME changes may be detected during the progression of these disorders and/or after therapy. PRAME may be a useful marker to detect the minimal residual disease.

1235**GRANULOCYTE-COLONY STIMULATING FACTOR AFFECTS CD62L EXPRESSION ON LYMPHOCYTES AND NK CELL SUBPOPULATIONS PRESENT ON PBPC GRAFTS**I.L. Barbosa¹, P.A. Benevides², F.I. Gomes³, C.C. Sousa², A.P. Correia³, A. Avila², S.R. Sousa², A. Campos², C.P. Vaz², F. Campilho², P. Pimentel², A. Carvalhais³¹IPO CROP SA -Porto, PORTO, Portugal; ²IPO CROP SA, PORTO, Portugal; ³Ipo Crop SA, PORTO, Portugal

Granulocyte-colony stimulating factor (G-CSF) can be used to mobilise peripheral blood progenitor cells (PBPC). It has been shown that G-CSF mobilization leads to a down-modulation of adhesion molecules, namely L-selectin on haematopoietic cells. The aim of this study was to determine if G-CSF affects adhesion properties of lymphocytes and NK cells present in PBPC grafts. Thus, the expression of a L-selectin (CD62L) and an integrin (CD11a) was evaluated on both peripheral blood (PB) prior to mobilization and PBPC grafts from healthy donors (n=27) and patients with haematologic malignancies candidates to autologous transplantation (n=26). For both groups of individuals blood samples were collected prior to the beginning of mobilization and PBPC were collected by apheresis started on the 5th day of mobilization. Lymphocytes and NK cells were identified using four colour fluorescence labelling together with a lyse and wash technique. Based on the expression of CD56, NK cells were sub-divided in two major sub-populations: NK bright cells (CD56++) and NK dim cells (CD56+). All the results are presented as median values of percentage of positive cells. In the present study, both donor and patient PB lymphocytes and NK cells were CD11a+ (median values >95%) and CD62L was expressed on the majority of lymphocytes (60% and 55% for donors and patients respectively) and CD56++NK cells (86% and 93% for donors and patients respectively). Only a small number of CD56+ NK cells from donors (25%) and patients (18%) express CD62L. After G-CSF mobilisation there was a significant increase in PB leukocyte counts, reflected in the number of PBPC collected by apheresis. In PBPC grafts there were no significant changes in CD11a expression in the studied cell types. For both groups there was a significant decrease in the percentage of lymphocytes CD62L+ (6% and 18% for donors and patients respectively) and CD56+ NK cells CD62L+ (12 and 14% for donors and patients respectively), in comparison with the same cells prior to mobilization. Both for donors and patients the majority of CD56++ NK cells from PBPC grafts continue to express CD62L. In summary, the G-CSF mobilization lead to a similar down modulation of CD62L expression on lymphocytes and CD56+ NK cells. Furthermore, this mobilization procedure did not have an effect on the expression of CD11a on lymphocytes and NK cells present on PBPC grafts as compared to premobilization PB. However, in this study and independently from the CD62L expression, both NK cell subpopulations migrated to the blood in response to G-CSF stimuli. CD62L might not be down regulated from CD56++NK cells or have a different time response to the G-CSF in comparison with other cells. Thus, G-CSF mobilisation seems to induce selective changes on CD62L expression on lymphocytes and a NK cell subpopulation present on PBPC Integrins and selectins may have a different role in mobilisation and the effect of these changes in engraftment following haematopoietic transplantation deserves further investigations.

1236**PREVENTION OF NEUTROPENIC FEVER DURING ADMINISTRATION OF HIGH DOSE VP-16 PLUS G-CSF FOR MOBILIZATION OF PBSC-THE EFFICIENCY OF PROPHYLACTIC ANTIBIOTIC TREATMENT**

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Neutropenia after high -dose VP-16 plus G-CSF, as mobilization chemotherapy regimen prior autologous setting in patients with hematological malignancies, is common finding, although infectious complications have not been previously described. In the attempt to reduce infective complications and the higher incidence of hospitalization for neutropenic, fever prophylactic antibiotic regimen was administered for patients receiving this regimen. We evaluated 35 patients with lymphoproliferative malignancies (NHL13, HD 6, MM 12, and ALL 4) treated with HDT/ASCT at Department of hematology, Skopje, Macedonia. The patients were mobilized with VP-16 (2 gr/m²) and G-CSF 10mcg/kg starting from day 5 of chemotherapy regimen. The regimen was effective in the progenitor cell mobilization and almost 84% of analyzed patients reached at least 2x10⁶/kg CD34+ cells with median 3 (ranges 1-6) apheresis procedures. Only two patients with HD and one AML failed mobilization. The patients were divided in two groups:1) no specific antibiotic prophylaxis (n=7); (2) vancomycin i.v., cefixime p.o, (n=13); (3) amoxicillin/ clavulonic acid and ciprofloxacin p.o. (n= 15). The first group of patients revealed higher incidence of need for hospitalization (67%) due to neutropenic fever, versus second (28%) and third group (15%) of patients respectively (p<0,001 between the first and the other two groups). At the end we conclude that VP-16+G-CSF mobilization schedule revealed significant high incidence of neutropenic fever that can be substantially reduced by a vigorous antimicrobial prophylactic program.

1237**A PRELIMINARY REPORT OF BORTEZOMIB PLUS DEXAMETHAZONE IN 17 RELAPSED OR REFRACTORY MULTIPLE MYELOMA PATIENTS**

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Introduction. Multiple myeloma is an incurable haematological malignancy of B-cell origin. Although patients generally respond to front-line chemotherapy, eventually the majority either relapse or become refractory. Clinical studies suggest that bortezomib, a proteasome inhibitor, is effective in patients with refractory or relapsed multiple myeloma. The addition of dexamethasone may offer some additional response effect and is suggestive of possible synergy. Purpose of this study is to report the preliminary results of clinical evaluation of efficacy and toxicities of bortezomib in combination with dexamethasone in 17 refractory or relapsed multiple myeloma patients. *Patients and Method.* Seventeen patients aged 58-80 years, 10 men and 7 women, who had either refractory or relapsed multiple myeloma after front and second-line chemotherapy, were designed to receive bortezomib 1.3 mg/m² intravenous bolus twice weekly for 2 weeks (days 1, 4, 8 and 11) of a 21-d cycle for up to eight cycles, in combination with dexamethasone 20mg daily on days 1,2 of each cycle. Bortezomib was withheld from patients with drug-related grade 3 non-haematological or grade 4 haematological toxicity and then resumed at 1.0mg/m² or 0.7 mg/m². Evaluation of response was designed to perform between days 11-18 of cycles 2, 4 and 6, and following cycle 8. Responses were assessed using the European Group for Blood and Marrow Transplantation (EBMT) response criteria. Adverse events were assessed at each visit and graded according to the World Health Organization Common Toxicity Criteria. *Results.* After completion of 3-5 cycles of treatment, 1 patient experienced CR, 7 patients experienced PR and 8 patients had evidence of stable

disease. One patient died of pneumonia within 5 d after the last administration of bortezomib on cycle 1. Dose reduction was necessary in 4 patients because of an adverse event. Overall, the most commonly reported adverse events of any grade in this study were diarrhoea in 1 patient, pyrexia in 2 patients, paralytic ileus in 2 patients, peripheral neuropathy in 3 patients, thrombocytopenia in 16 patients, rash in 1 patient and upper respiratory tract infection in 2 patients. The most common grade 3 and 4 adverse events related to the study drug were peripheral neuropathy, diarrhoea and thrombocytopenia. Upper respiratory tract infection was probably an unrelated toxicity to the study drug. Peripheral neuropathy was reported on the baseline evaluation of 2 patients, who later developed worsening of symptoms. Three patients discontinued because of adverse events, two patients due to peripheral neuropathy and one patient due to severe thrombocytopenia which led to subdural hematoma after trauma. Conclusions. The preliminary findings in this study demonstrate that bortezomib in combination with dexamethazone has synergistic therapeutic activity in patients with relapsed or refractory multiple myeloma after front-line treatment. The efficacy and toxicity results are also favourable relative to other standard cytotoxic therapies used for salvage therapy after front-line treatment.

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MULTI-DAY INFUSION OF AUTOLOGOUS CRYOPRESERVED PERIPHERAL BLOOD PROGENITOR CELLS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES- THE INFLUENCE ON ENGRAFTMENT AND TOXICITY

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Peripheral blood progenitor cells (PBPC) are increasingly used as source of stem cells in both autologous and allogeneic settings for patients with hematological malignancies. Based on previously non-randomized studies for enhanced engraftment during multi-day infusion of cryopreserved PBPC in the autologous transplantation, some transplant centers infuse harvests over 3 days. To evaluate the benefit of fractionated infusions of PBPC we included 37 patients with hematological malignancies (AML 11, NHL 9, HD 9, MM 6, ALL 2) treated with high-dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) at Department of hematology, Skopje, Macedonia. The patients were randomized to receive cryopreserved PBPC concentrates divided over 1, 2 or more than 3 days. PBPC were mobilized with high-dose VP16 2g/m² for AML patients, intermediate-dose cyclophosphamide 1-2 g/m² for lymphoproliferative malignancies and/or G-CSF 5microgr/kg alone. PBPC concentrates were cryopreserved with 5% DMSO solutions using controlled rate freezing procedures (Nicole plus PC Espace 330). Patients received daily G-CSF 10microgr/kg i.v over 30 min beginning 4h after the infusion of the first aliquot of PBPCs. The median amount of infused PBPC solution was 430 ml (240-780ml). Engraftment was registered for Ne>0.5x10⁹/L on day +10 (8-14) and for Plt>20x10⁹/L on day +13 (8-20) with no significant difference between groups that received 1, 2 or more than 3 days infusions of PBPCs. Transfusion requirements were for Er 2 doses (0-4) and Plt 14 doses (0-33). The statistical data revealed that infusion related toxicity was similar for all groups of patients. At the end we can conclude that multi-day infusion of PBPC harvests does not influence the engraftment or reduce toxicity.

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THIRD ALLOGENEIC SIBLING TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA: A CASE REPORT

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The most frequent therapeutic dilemma after leukemia relapse is allowing any further attempt at leukemia eradication, or whether to offer only palliative treatment. Remission induction for AML relapsing after HSCT can be achieved in about 40% of patients. We present a case of 34 years female patient with AML FAB M2 with no cytogenetic abnormalities diagnosed in April 1998. After two induction chemotherapy cycles (ARA-C 100mg/m² days 1-7 and Doxorubicin 50mg/m² days 1,3,5) and consolidation with one cycle of high dose chemotherapy (ARA-C 2x500 mg/m² days 1-6 and Doxorubicin 50mg/m² days 4-6) complete remission was achieved. First allogeneic sibling transplantation was performed from fresh bone marrow as source of stem cells and Bu-Cy conditioning, followed by conventional GVHD prophylaxis with MTX+ Cyclosporine. After 24 months disease relapse was registered. The patient was treated with induction chemotherapy with two more cycles of DAE regimen followed by second allogeneic transplant from the same donor with peripheral blood progenitors (PBPC). Two years after the second transplant third relapse was registered. Remission was achieved with L6 chemotherapy regimen with low dose cytarabine and thioguanine. After that, third allogeneic sibling transplantation was performed with Bu-Cy conditioning, PBPC as source and no GVHD prophylaxis. The patient is still +180 days in CR after the third allogeneic transplantation with good quality of life. At the end we can conclude that leukemia relapse after stem cell transplantation still remains a significant cause of treatment failure in patients with acute leukemia. The success of multiple transplants depend very much on the time interval from the first transplantation, the intensity of prior therapy, the risk of high mortality rate due to increased regimen toxicity (RRT) as well as undelaying disease and patient performance status prior transplantation.

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GRANULOCYTE-COLONY-STIMULATING FACTOR (FILGRASTIM) IS SAFE AND USEFUL TO IMPROVE NEUTROPENIA INDUCED BY IMATINIB THERAPY IN PATIENT WITH CHRONIC-PHASE CHRONIC MYELOGENOUS LEUKAEMIA

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Background. Hematological toxicity during imatinib therapy in patients with chronic myelogenous leukaemia (CML) is common occurring in about 30-50% of cases. Grade 3 or 4 neutropenia occurs in 35-45% of patients with CML in chronic phase who receive imatinib. Often myelosuppression results in treatment interruption or dose reduction producing a negative effect on the efficacy of therapy. *Aims.* To reverse neutropenia induced by imatinib therapy in a patient with chronic-phase CML we used granulocyte-colony-stimulating factor (filgrastim) evaluating the response and the safety. *Methods.* A chronic-phase CML Philadelphia positive was diagnosed in a 42 years old woman presenting with elevated thrombocytosis (platelets 1082x10⁹/L), splenomegaly and mild leucocytosis (white cells 10.8 x10⁹/L). Cytogenetic analysis showed a typical bcl-abl rearrangement in 100% of mitoses. Hematological and clinical parameters were used to obtain a mild Sokal risk score (0.9). Imatinib was started at standard dose of 400 mg/day producing a partial cytogenetic response (bcl-abl in 30% of mitoses). After 8 weeks of therapy the patient showed a rapid decrease of

platelets number to normal level but presented a grade 2 anemia (haemoglobin 9.6 gr/dL) and a grade 3 neutropenia ($760 \times 10^9/L$). The imatinib dose was first reduced to 300 mg/day without leucocytes number recovery. The therapy was then stopped and reintroduced at a dose of 200 mg/day after increase of total leucocytes number but the hematologic toxicity reappeared. A cytogenetic analysis performed after five months by the start of therapy showed a progression of leukemic clone (bcl-abl in 75% of mitoses). Granulocyte-colony-stimulating factor (filgrastim) was then introduced with a schedule of 300 microgr every week. **Results.** The granulocyte-colony-stimulating factor therapy was well tolerated and the patient showed a disappearance of haematological toxicity after only 3 weeks. Neutrophils number was ever superior to $1.5 \times 10^9/L$ and haemoglobin level remained at a value of 11-11.5 gr/dL. The absence of hematologic toxicity permitted to increase the imatinib therapy at a standard dose of 400 mg/day. The patient is now under bone marrow evaluation to test the cytogenetic response. **Conclusions.** The probability of response to imatinib is decreased for patients who developed myelosuppression and that needed a dose reduction of therapy. The use of granulocyte-colony-stimulating factor to reverse neutropenia represents a safe and efficacy method to contrast the hematologic toxicity and to permit an optimal imatinib therapeutic schedule.

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EFFICACY AND TOLERABILITY OF ALEMTUZUMAB (CAMPATH-1H) IN THE SALVAGE TREATMENT OF PROLYMPHOCYTOID TRANSFORMATION OF B-CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL) AND B-PROLYMPHOBLASTIC LEUKEMIA (PLL)

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Background. Alemtuzumab (Campath-1H) is a monoclonal antibody against human CD52. It is used for the therapy of refractory or relapsed B-chronic lymphocytic leukemia (CLL) patients. The treatment of patients with prolymphocytoid transformation of CLL or prolymphocytic leukemia (PLL) remains problematic and the prognosis of these patients is very poor. **Aim** We report on the response rate, tolerability and treatment-related problems observed with the Campath-1H treatment in a series of heavily pre-treated patients suffering from prolymphocytoid transformation of CLL and PLL. **Patients and Methods.** Six patients with prolymphocytoid transformation of CLL and two patients with PLL were included in this study. The patients (7 males and 1 female) presented the following characteristics: [median (range)] age 53.5 years (42-80), WBC $55.6 \times 10^9/L$ (38-507), Hgb 8.6 g/dL (5.6-10.6), Plt $68.6 \times 10^9/L$ (15.4-90). The patients were heavily pre-treated with a median of 3 previous treatment(2-4). All patients received 3, 10 and 30 mg of Campath-1H on sequential days, and then were subsequently scheduled for 30 mg/i.v./3 times weekly. Campath-1H started being used 36 months from diagnosis (10-104). Duration of treatment ranged from 4 to 12 weeks, with a median dose of 650 mg. Allopurinol 300 mg/day was given for tumor lysis syndrome prophylaxis. All patients received standard prophylaxis was oral cotrimoxazole twice daily/2 days/week and oral acyclovir 400 mg daily, both through the whole period of the treatment and after completion of treatment till the CD4+ lymphocytes were $\geq 200/mL$. CMV antigen in blood test was done weekly to detect any reactivation. **Results.** Two out 8 patients (25%) responded achieving partial remission (PR) according to NCI criteria. Four patients (50%) had stable disease (SD), two (25%) had progressive disease (PD). From start of Campath-1H treatment, the median overall survival was 8 (4-19) months, the median time to progression was 6 (1-10) months. Two out 8 patients are currently alive with a median follow-up time of 13 (12-14) months. There were six deaths, all due to progressive disease. Most non-hematological toxicities were grade 2 WHO or lower and infusion-related. Most ematological toxicities were grade 3 neutropenia with infections and grade 2 thrombocytopenia. After therapy with Campath-1H, hemoglobin and platelets increased in 5/8 (62%) patients. CMV reactivation occurred in 4/8 (50%)

patients, but CMV disease was prevented through treatment with oral ganciclovir (oGCV). Reactivation of hepatitis B virus (HBV) was observed in one HBs Ag positive patient. Treatment with oral lamivudine (oL) achieved a decrease of viral load and no abnormalities of liver parameters were noticed during Campath-1H. **Conclusions.** The treatment with Campath-1H has been feasible, with an acceptable safety profile in a group of poor prognosis patients with PLL and prolymphocytoid transformation of CLL. The clinical activity has had a short duration. A pre-emptive treatment with oGCV or oL according to the reactivation has been able to prevent the clinical manifestations of CMV or HBV in the same group.

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THE DIAGNOSTIC VALUE OF MPV AND THE LEVEL OF THE EOSINOPHILES IN THE ACUTE CORONARY SYNDROME

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Background/Aims. The aim of this project is to identify, except cardiac enzymes and troponin, new indexes that would be counted easily and quickly but also reliable to estimate angina. Mean Platelet Volume (MPV) is an easily countable index that shows the activity of the platelets. The eosinophiles as white blood cells is likely to participate in the progress that leads to rupture of the plaque. **Methods.** 116 patients (82 men and 34 women middle age 64 ± 7 years) with acute coronary syndrome were examined. 64 of them had an acute myocardial infarction and 52 of them had unstable angina pectoris. These patients compared to 63 healthy subjects of the same age approximately (control group). The import in the hospital was about 3 hours after the benigning of the pain. They were all undergone blood examinations (full blood test and cardiac enzymes) at the import time. **Results.** We noticed that MPV was remarkable increased in patients with acute myocardial infarction. Medium price 10.92 ± 1.6 for patients with acute myocardial infarction, and 8.6 ± 2.8 for the control group. ($p < 0.0001$). A reduction of the percentage of the eosinophiles was recorded in patients with acute myocardial infarction (medium price 1.1 ± 1.2) in comparison with the control group (medium price 2.2 ± 1.6), ($p < 0.0002$). However we did not observe a reduction of the percentage of the eosinophiles in patients with unstable angina pectoris (medium price 2.5 ± 2.2) ($p < 0.0001$ between patients with acute myocardial infarction and patients with unstable angina pectoris). **Conclusions.** We could report that MPV and the percentage of the eosinophiles are beneficial and helpful indexes in diagnostic evaluation of patients with acute chest pain providing quickly some useful information about the progress of the chest pain. The percentage of the eosinophiles may be estimated in the differential diagnosis between acute myocardial infarction and unstable angina pectoris.

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AVASCULAR JAW OSTEONECROSIS ASSOCIATED WITH THE USE OF BISPHOSPHONATES IN MULTIPLE MYELOMA (MM)

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Background. Bisphosphonates, powerful inhibitors of bone resorption, are successfully used in the treatment of skeletal-related complications associated with multiple myeloma (MM). Nevertheless nitrogen-containing bisphosphonates such as pamidronate and zoledronate seem play a role in avascular jaw osteonecrosis (JON). **Aim** We report a series of five MM patients in an advanced phase of disease with an avascular JON that appeared after treatment with bisphosphonates. **Patients and methods** All five patients, two males and three females with a 66 year median age (range 44-80), were affected by MM. The

diagnosis and the stage of MM (4 patients/stage III A; 1 patient/III B), established according to Durie and Salmon criteria, was made from 17 to 130 months (mean 74,2) before the onset of JON. All patients had received treatments for MM (monthly pulses of oral melphalan/prednisone (MP), preceded in one case by local radiotherapy on the sacrum, in four cases; intra venous (i.v.) chemotherapeutic combination VAD followed by a high-dose melphalan with autologous stem cell transplantation in one case. All patients were responsive to the up-front therapy achieving a partial remission. On the onset of JON, three of them had already been treated (oral MP, i.v. cyclophosphamide/dexamethasone, oral thalidomide respectively) for relapsed MM. Four patients had received pamidronate (60mg/i.v. at monthly intervals) of whom three had been switched to zoledronate (4mg/i.v. at monthly intervals). One patient had received only zoledronate. The duration of the bisphosphonate therapy ranged from 10 to 60 months (mean 34). The total doses of bisphosphonates ranged from 300 to 3600 mg (mean 1740) regarding pamidronate and from 40 to 76 mg (mean 55) regarding zoledronate. On the onset of JON, all cases had an orthopantomograph of the jaws. All patients underwent a biopsy in an area of the jawbone selected on the basis of the radiographic images, and in the overlying mucosa. **Results.** The clinical presentation was characterized of local pain in all cases and lower lip numbness restricted to the distribution of the distal trigeminal nerve in three cases. All patients had been repeatedly evaluated by their dentists and treated with antibiotics for recurrent dental abscesses before going to a tooth extraction. The persistence of non-healing extraction socket and the exposed bone represented a significant cause for their ill-healing with superficial contamination from oral microorganisms. The biopsy of the exposed jawbone and overlying mucosa showed respectively necrotic bone and inflammatory infiltration of polyclonal lymphocytes and plasmacells, without evidence of myelomatous involvement. In two out of five cases, the laboratory examination showed necrotic bone with evidence of filamentous bacteria consistent with an actinomycotic osteomyelitis. One of these two patients developed orofacial communications and cutaneous fistula to the neck with suppuration. The patients had persistent bone necrosis despite they had suspended the treatment with bisphosphonates. **Conclusions.** Our observation could contribute to alert hematologists involved in the treatment of MM patients to consider and early diagnose this potential complication associated with the use of bisphosphonates. sf.capalbo@ematba.uniba.it

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REDUCED ASPIRIN SENSITIVITY OF PLATELETS IN TYPE 2 DIABETICS WITH NON ST ELEVATION ACUTE CORONARY SYNDROMES

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Background. Type 2 diabetic patients have worse prognosis than non-diabetics suffered from an acute coronary syndromes without ST segment elevation (NSTACS). However, there are limited data concerning to the possible difference in platelets sensitivity to aspirin between type 2 diabetics or non-diabetics in this setting. **Methods.** Closure time (CT) was estimated in 502 consecutive patients who admitted in our institute because of NSTACS. Determination of CT was made by PFA-100. Measurements were made upon admission for those who receive aspirin before the current event and 6 hours after aspirin administration in those who did not receive such therapy. **Results.** There were 162 type 2 diabetics (32.3%). Type 2 diabetics had significantly lower CT values than non-diabetics (212±82sec vs 254±79sec; p=0.001). After adjustment for baseline differences between the 2 groups, type 2 diabetes was an independent predictor of reduced platelets sensitivity to aspirin (reduced CT) (p=0.007). **Conclusions.** Type 2 diabetes mellitus is an independent predictor of reduced platelets sensitivity to aspirin in patients with NSTACS. This phenomenon may contribute to the worse prognosis of type 2 diabetics in this setting.

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USING SECRETORY LYSOSOMES FOR LOCAL DELIVERY OF CYTOKINES OR CYTOKINE RECEPTORS

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Immunological responses are tightly controlled through a balance between stimulatory and inhibitory cytokines. This is used therapeutically in chronic inflammatory diseases, where anti-cytokine therapy has proven to be useful. Furthermore, stimulatory cytokines are used in treatment of some malignant diseases. However, both anti-cytokine therapy and cytokine therapy are systemic treatments with severe side-effects: thus local delivery of the treatment may improve the therapeutic efficacy and reduce the systemic side-effects. We therefore propose the concept of local delivery using secretory lysosomes of hematopoietic cells as vehicles. A secretory lysosome is a compartment with a dual function of storage and degradation found in secretory cells, e.g. in neutrophils and lymphocytes. The secretory lysosomes are released by degranulation at sites of inflammation or infection. Therapeutically-active protein could be induced for expression and storage in secretory lysosomes and thereby be locally released by degranulation resulting in local delivery of therapeutically active protein. In support of this concept, we have shown that: I) gene transfer and granule loading can be achieved using the soluble TNF- α receptor (sTNFR1) after cDNA expression in hematopoietic cell lines and hematopoietic progenitor cells; II) endoplasmic reticulum export can be facilitated by the addition of a transmembrane domain; III) constitutive secretion can be prevented by incorporating a cytosol-sorting signal resulting in secretory lysosome targeting; IV) the sTNFR1 is released from the transmembrane domain into the secretory lysosome by proteolytic cleavage; V) regulated sTNFR1-secretion can be triggered by activation or calcium signal. *In vivo* investigations are currently determining the feasibility of local protein delivery at sites of inflammation or malignancy.

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AGING OF BONE MARROW MESENCHYMAL STEM CELL

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Background. Recently much attention has been focused on mesenchymal stem cell (MSC) in treatment of various diseases. The low frequency of MSCs in bone marrow (BM) necessitates their isolation by *in vitro* expansion prior to clinical use. **Aims.** In this study we evaluated the effect of long term culture on the senescence of these cells. **Method.** Ten ml BM cells were taken from the bag of 11 normal BM transplant donors with mean age of 25 year. Mononuclear cells were isolated and cultured in T-75 flasks with 21×10^6 cell/ flask in DMEM-LG medium, consisting of 10% FBS. Flasks were incubated at CO₂ incubator and were fed every 4 day with total culture medium change. When the confluence of MSC reached over 90%, they were detached, counted, and re-seeded at 21×10^4 cell/flask. This was repeated for 120 day. In different passages MSC were examined for different aging indicators. Including; telomere length assay, differentiation ability to adipocyte and osteocyte, immunophenotyping of CD13, CD44 and CD34 antigens, determination of cumulative population doublings (CPDS), and study of morphological characteristics of MSC cultures. **Results.** All 11 BM produced MSC in the culture. The mean long term culture was 118 day (range: 63-164) and the mean passage number was 9. The average number of PD in primary culture was about 7.7 which decreased to 1.2 in the 10th passage. The mean telomere length in the first passage from 9.19 Kbp decreased to 8.7 kbp in the 9th passage. Differentiation potential to adipocyte

and osteocyte dropped from the 6th passage on. This variation accompanied by morphological abnormalities was typical of the Hayflick model of cellular aging from day 80 (passage 6) on. We did not observed appreciable differences in CD markers. *Conclusions.* we believe that MSC, like all other cells, enter senescence almost undetectably from the moment of *in vitro* culturing. Simultaneously these cells are losing their stem cell characteristics (self-renewal, differentiation potential) just as slowly. Therefore, it is a much better alternative to consider these cells for cell therapy and gene therapy early on (the younger, the better). We believe the best time is between the 2nd and 4th passage, this is because they have the highest PD rate, lowest confluence time, 100% differentiation potential to adipocyte and osteocytes, and they have a reasonable cell population yield.

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COGNITIVE DECLINE IN PATIENTS WITH NON-HODGKIN LYMPHOMA DURING CHOP THERAPY VERSUS R-CHOP

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Background. Many authors emphasized that cytotoxic therapy had great influence on cognitive functions. *Aims.* To determine cognitive functions in patients (pts) with non-Hodgkin lymphoma (NHL) during therapy, compared those who received CHOP versus R-CHOP protocol. *Patient and Methods.* In a total of 22 pts with NHL (15 on CHOP, and 7 on R-CHOP protocol) cognitive abilities were measured before and after every cycle of chemotherapy (in total 64 applications of CHOP and 20 R-CHOP therapy). Assessment of cognition (simple psychomotor reaction on visual signals, simple psychomotor reaction on hearing signals, complex psychomotor reaction, convergent inductive thinking, diffuse attention and spatial visualisation) was performed by Complex Reactimeter Drenovac (CRD). *Results.* We found cognitive decline after every single application of chemotherapy in both groups. Statistical analysis showed significantly less cognitive deterioration in pts receiving R-CHOP then CHOP protocol alone (p 0,05). *Conclusions.* Our data suggests that when combined with Rituximab, CHOP protocol causes less cognitive impairment than CHOP alone. Future researches on a larger number of pts are needed to confirm and explain if Rituximab in some way preserve cognitive abilities in NHL pts.

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PROTEASOME INHIBITORS MAY CIRCUMVENT RESISTANCE TO CHEMOTHERAPY IN ACUTE LYMPHOBLASTIC LEUKAEMIA CELLS IN CULTURE

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Resistance to chemotherapy and low efficiency of chemotherapeutic agents are responsible for therapy failure and relapse in patients with neoplastic malignancies. Several lines of evidence suggest that alterations in the non-lysosomal proteolytic pathway, known as ubiquitin-proteasome system, are involved in tumour progression, drug resistance and angiogenesis. Recent studies have shown that proteasome degrade proteins responsible for cell cycle progression and cell death control. Bax, p53, NFκB inhibitor, IκB, and tBid are examples of proteins degraded by this pathway. It was also shown that proteasome inhibitors induce apoptosis mainly in cancer cells with lesser effect in normal cells. So, therapeutics regimes that manip-

ulate proteasomal activity could restore normal cellular homeostasis in some cancer patients. Our previous work shown that proteasome inhibitors induce apoptosis in T Acute Lymphoblastic Leukemia (T-ALL) cell line, sensitive and resistant to azaguanine. The aim of this work is to evaluate the potential therapeutic of proteasome inhibitors, and mechanisms involved, in acute leukemia refractory to first line chemotherapy. For this purpose human T acute lymphoblastic leukaemia cell lines sensitive (CEM) and resistant to daunorubicin (CEM/DNR) and vincristin (CEM/VCR), were incubated with increasing concentrations of proteasome inhibitors, a boronic acid derivative, MG262, and a peptidyl aldehyde, PSI, as single agents, or in combination with DNR and VCR, respectively. Cell morphology was evaluated by light microscopy examination of May-Grünwald Giemsa stained cells and cell viability was estimated by trypan blue exclusion. Cell death was evaluated by annexin V/propidium iodide incorporation and detected by flow cytometry. The expression of the proteins involved in apoptosis regulation, Bax, Bcl-2 and p53, as well as polyubiquitinated proteins were determined by flow cytometry using monoclonal antibodies. Our results show that proteasome inhibitors, MG262 and PSI, decrease cell growth and induce apoptosis in both sensitive and resistant acute leukaemia cells, in a dose and time dependent manner. However a lesser effect was observed in CEM/VCR cells compared with the others cells, which may suggest cross-resistance between these proteasome inhibitors and VCR. However other mechanism may be present and may be specific to this cytostatic. On other hand, unlike other studies, we didn't observe a synergistic effect when we treated the cells with proteasome inhibitors combined with VCR or DNR, which suggest that these new anticarcinogenic drugs may be used as single agent for treatment of ALL patients. Besides the observed apoptotic effect, the mechanisms involved in the cytotoxicity is cell type dependent. In CEM/VCR we observe an increase in Bax and p53 expression, two pro-apoptotic proteins as well as in ubiquitinated proteins, as previous expected. In CEM/DNR other mechanisms not studied yet, as NF-κB/IκB levels or others, may be related with the observed cytotoxic effect. These results suggest that proteasome inhibitors may be used as a single potential therapeutic approach in T-ALL, namely in the presence of resistance to conventional therapy, providing an alternative strategy to overcome resistance to cytotoxic drugs in refractory or relapsed acute lymphoblastic leukaemia. This work is supported by CIMAGO.

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MESENCHYMAL STEM CELL THERAPY FOR MULTIPLE SCLEROSIS

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Background & Aims. Human mesenchymal stem cell (MSC) can be isolated from bone marrow (BM) and differentiate into multiple cell types including neurons, oligodendrocytes, astrocytes, and etc... *in vitro* and *in vivo*. These properties make them promising tools in cell and gene therapy. Injection of MSC into EB-X rats have demonstrated successful regeneration of the damaged myelin sheath. Up to now, no definite therapeutic intervention for late stage of multiple sclerosis (MS) has been found. We decided to inject MS patient with autologous expanded MSC to evaluated, 1-safety of this injection, 2-degree of demyelinated region repair in humans. *Methods.* until now five patients (3female, 2 male) with the mean age of 31 years participated in this ongoing study with their consent, and approval of the ethical committee of Tehran university of medical sciences. Patients were injected intrathecally with the culture expanded BM MSCs. The mean number and volume of cells was 7x10⁶ and 5.5 ml respectively. The included criteria were; Expanded Disability Status Scale (EDSS) no greater than 6, progressive from of MS not responding to IVIg, bet interferons and novatrone. Patients were followed monthly for their

clinical status and every 3 months regarding their magnetic resonance imaging (MRI). *Results.* On the day cells were injected, 4 of 5 patients had a slight headache, 3 got better without prescription but one used 3 doses of analgesic. The fifth one got meningitis 48 hours after injection, but she was treated for a week and released by the end of 7 days without serious problems. During 5 months follow up, the sensory function of one patient improved by 2 scores and the cerebellar function of another patient improved by 1 score. The functional condition of 2 patient have not changed till now and the fifth has just been injected. The first MRI findings of patients (every 3 month MRI was done) showed no changes neither in size nor in number of lesions. *Conclusions.* This is only a primary report, up to this point we can claim that the injection of expanded MSC intrathecally is a safe procedure and the side effects are similar to any regular intrathecal injection, therefore not related to MSC injection in particular. But to find out about the role of these cells in myelin repair, patients should be followed for at least one year and a larger sample is required in order to draw a definite conclusion.

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BEAM-LIKE CONDITIONING REGIMEN WITHOUT CRYOPRESERVATION IN NON-HODGKIN LYMPHOMA AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction: One of the most important factors that affect the outcome of HSCT in non-Hodgkin lymphoma patients is the given conditioning regimen. 50% of patient died due to weakness of regimen to eradicate malignant cells. *Aims.* This study was conducted to assess the curability of a BEAM-LIKE conditioning regimen and their toxicity. *Methods.* Twenty two (16 males and 6 females) non-Hodgkin lymphoma patients received a BEAM-LIKE regiment (CEAM) followed by infusion of bone marrow (n=2) or peripheral blood (n=20) stem cell transplantation. Median age was 32 (ranged 13 to 61). 11 patients were in complete remission 2 (CR2), 7 in CR1, 2 in CR3, and 2 patients were in primary induction failure (PIF) status. No cryopreservation and radiotherapy was applied to the conditioning regimen. The BEAM-LIKE conditioning regimen was as follows: CCNU was administered as 200 mg/m²/po for one day, VP16 as 300 mg/m²/IV for 2 days, Arac-c as 300mg/m²/BID/IV for 2 days and melphalan 140 mg /m²/IV for one day (modified BEAM) in the conditioning regimen. The patients were assessed for possible side-effect toxicities (hematological, gastrointestinal, pulmonary, renal and cardiac) post-treatment follow up from October 2002 to January 2005. *Results.* Median time of ANC recovery was 17 days (ranged 1-45 days) and platelet recovery was 19 day (ranged 9-130 days). Detected toxicities were as follows: Pulmonary in 2, gastrointestinal in 22, and renal in 6 patients, respectively. No cardiac toxicity was seen. Out of 4 relapsed patients one died PI failures. The three others are still alive and under treatment. There were two other death records: one due to encephalitis and the other one because of non engraftment. Overall survival and disease free survival (DFS) at 300 days median follow up were 84.2% and 87.7%, respectively. As an overall result of conditioning regimens, all patients had graded thrombocytopenia, anemia, and granulocytopenia. *Conclusions.* CEAM conditioning regimen seems promising and useful for treatment of non-Hodgkin lymphoma and its possible toxicities are reversible

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SIGNIFICANT DIFFERENCE IN PROGNOSIS BETWEEN PATIENTS WHO REACHED AND DID NOT REACH AUTOLOGOUS TRANSPLANTATION (AT)

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Background. In our first trial 4W total of 246 newly diagnosed pts. were enrolled from whom 185 pts. were undergo AT. In the last analysis (August 31, 2004), with median follow up of 63.3 months median of overall survival (OS) for group of pts. randomized (R) after AT (185) was 77.1 months. Almost 25% (61/246) have been lost during induction part of the treatment due to several reasons. Prognosis of this cohort of pts. was very pure and our data confirmed, that AT is key factor for prognosis of pts. *Methods.* and *Results.* Two groups of pts. enrolled to the trial 4W were compared: randomized pts. who underwent AT as required (4W-R; n=185) vs. non-randomized pts. (4W-nonR; n=61) who were dropped out from the trial before transplantation. Median follow-up of was 65.3 vs. 69.6 for 4W-nonR group. From standard parameters only age (53.4 vs. 57 for 4W-nonR; p=0.01), renal failure (5.4% vs. 23% for 4W-nonR; p<0.001) and LDH (5.3 vs. 7.0 for 4W-nonR; p=0.003) changed significantly and beta2M (4.3 vs. 6.4; p=0.073) had trend reflecting higher number of pts. with renal failure in 4W-nonR group. Distribution of pts. based on Durie-Salmon v.s. International Staging System (stage 1: 43% vs. 29%; stage 2: 36% vs. 35%; stage 3: 21% vs. 35%) did not reach significance despite trend to higher frequency of stage 3 ISS in 4W-nonR group. Distribution of response on VAD therapy was significantly different (p=0.01) due to 6% of progressions in 4W-nonR group. From total of 61 pts. 90% were lost during VAD induction treatment, 7% during stimulation and graft collection. Main reasons of dropping out from the trial were early death due to complication or resistance on therapy during VAD treatment (23% pts.; 14/61). Also deep venous thrombosis or pulmonary embolism were significant factors for contraindication of AT. Finally EFS and OS were compared. For all pts. median of EFS was 40.6 and OS was 64.4 moths. 4W-R group has median of EFS 33.9 and OS 77.1 moths but for 4W-nonR group median of OS was dramatically lower (12.8 moths). Based on the statistic modeling was suggested that the decrease number of pts. who did not reach AT from 25% to 20% will prolong OS of all group for 6 months, respectively decrease from 25% to 12% will represent almost one year benefit of OS for all group. *Conclusions.* There is a significant difference in prognosis between pts. who reached and did not reach AT if this treatment is indicated. The analysis of the reasons for the loss the pts. concluded that better supportive care and especially earlier diagnosis and more safe induction treatment of MM is essential in order to bring the benefit of AT to more pts.

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PBOX-15, A POTENT PYRROLO-1,5-BENZOXAZEPINE, INDUCES APOPTOSIS IN B-CHRONIC LYMPHOBLASTIC LEUKAEMIA

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Background. B-chronic lymphocytic leukaemia (CLL) is characterised by a clonal expansion of mature long-lived B cells that are highly resistant to apoptosis. CLL is considered to be primarily due to defects in apoptosis and not cell growth as circulating CLL cells are arrested in the G0G1 phase of the cell cycle and are therefore not dividing. As such CLL is a good model to study novel apoptosis inducing agents independent of effects on the cell cycle and cell growth. *Aims.* The aim of this study was

to assess the ability of a small organic molecule PBOX-15 to induce apoptosis in CLL cells *ex vivo*. *Methods*. Pyrrolo-1,5-benzoxazepines (PBOX) are a family of novel agents which we have shown induce apoptosis in leukaemia, breast and prostate cancer cell lines. To assess the activity of a potent member of this family, PBOX-15, in CLL, we exposed peripheral blood mononuclear cells from CLL patients (n=30) to varying concentrations of this agent (3nM-10uM). *Results*. PBOX-15 exhibited cytotoxicity in CLL cells in a dose dependant manner, with IC50 values of less than 1uM (mean IC50 of 0.55uM). PBOX-15 induced apoptosis in CLL cells was demonstrated by flow cytometry analysis of an annexinV/propidium iodide assay and by PARP cleavage. PBOX-15 induced apoptosis in CLL cells was partially inhibited by the caspase inhibitor z-VAD-FMK and the MAP kinase JNK inhibitor SP600125. Bcl-2 is an anti-apoptotic protein that has been implicated in CLL cell resistance to apoptosis. No change in bcl-2 was observed prior to PBOX-15 induced apoptosis. This mechanism of action is different from what we have observed for PBOX-15 induced apoptosis in the chronic myeloid leukaemia cell line K562, which is JNK activation dependent and is preceded by bcl-2 phosphorylation. PBOX-15 had marked selective cytotoxicity when compared with normal blood mononuclear cells (IC50 > 3uM, n=5). Inhibition of the proliferative capacity of normal bone marrow progenitor cells taken from normal donors at bone marrow harvest was minimal after incubation with 1uM PBOX-15 (n = 5), as determined using the colony forming unit granulocyte macrophage assay. *Summary and Conclusions*. CLL is an incurable disease and the need for novel treatments is acute, especially for the subgroup of patients with poor clinical outcome. Our research programme has allowed us to identify novel agents with significant activity *ex vivo* against CLL cells. PBOX compounds will be further investigated for their anti-cancer potential and may, in addition, provide insights into the molecular mechanisms resulting in the aberrant apoptosis that is characteristic of CLL.

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COMPARISON OF THE DETECTION OF MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKEMIA USING CLONOTYPIC AND ALLELE SPECIFIC OLIGONUCLEOTIDE PCR ASSAY

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Background. Immunoglobulin chains (Ig) and T-cell receptor (TCR) gene rearrangements are excellent patient specific PCR targets for MRD detection in complete remission (CR) after about 4 weeks of chemotherapy with ASO-PCR and clonotypic techniques. MRD monitoring in acute lymphoblastic leukemia patients has demonstrated high prognostic value for predicting subsequent relapse. *Aims*. To evaluate the kinetics of molecular response achievement with use ASO-PCR and Clonotypic techniques in ALL patients after therapy or BMT. *Materials and Methods*. PB samples were obtained at the time of diagnosis, after treatments, and in CR every month for up to 18 months. The presence of clonality in leukemia blasts is searched in PB sample of 30 patients obtained at diagnosis, using different combinations of FR1 family-specific primers and J primer flanking the unique CDR3 region. When clonal IgH or TCR- β gene rearrangements were present in leukemia cells, one-sized PCR signal was obtained which was used to monitor MRD with these primers (Clonotypic method). For ASO-PCR method, PCR products from 10 patients were sequenced and ASO primer for the CDR3 region of each specific clone was designed. The ASO primer was then used in conjunction with FR1 primer in a PCR amplification to detect leukemia-specific rearrangements in subsequent samples obtained from the same patient and then compares residual disease levels estimated by ASO-PCR and Clonotypic. *Results*. Monitoring the kinetics of MRD during the first year of therapy permitted identification of two MRD-based risk groups of patients with low (stable or falling MRD) and high risk (rising or

persistently high level of MRD) of relapse. We compared two methods with periodic and frequent monitoring of MRD level and we could anticipate the patient's clinical outcome. The ASO-PCR (with sensitivity of 10⁻⁴ to 10⁻⁶) and Clonotypic (with sensitivity of 10⁻² to 10⁻³) methods were able to detect residual malignant cells with the same pattern in PB samples from patients who were considered to be in CR. *Conclusions*. In comparison of residual disease levels estimated by ASO-PCR and Clonotypic, the application of the ASO-PCR method of immunoglobulin heavy chain (IgH) and T-cell receptor (TCR) gene rearrangement regions provides a sensitive and highly specific detection with potential clinical relevance of MRD in ALL patients. Monitoring the kinetics of MRD with these techniques provide important prognostic information on the *in vivo* effectiveness of treatment in ALL, and with the increase of the MRD level enable to anticipate impending relapse.

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ALLOGENEIC STEM CELL TRANSPLANTATION FOR THE TREATMENT OF AML PATIENTS -A SINGLE CENTER RETROSPECTIVE ANALYSIS FOR 10 YEARS PERIOD (1994-2003)

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Allogeneic stem cell transplantation is a standard treatment approach for younger AML patients having an HLA identical donor (except acute promyelocytic leukemia patients). The experience of Zagreb transplant center in 10 years period (1994.-2003) is presented. *Methods*. 53 AML patients, (23 male and 30 female), median age of 32 years (2-51) having an HLA identical donor underwent allogeneic stem cell transplantation. All of the patients received induction and consolidation therapy according to the EORTC protocols for the treatment of acute myeloid leukemia (EORTC No 06931 and No 06991). In 42 out of 53 (79.2%) patients allogeneic transplantation was has been performed in first complete remission (1st CR) and 11 patients (20.8%) were transplanted in advanced phase of the disease. 49 patients (92,4%) received the conditioning regimen consisted of busulfan 16 mg/kg body weight in 16 doses and cyclophosphamide 120 mg/kg body weight during two days and 4 patients received total body irradiation and cyclophosphamide. Cyclosporine and short courses of methotrexate were given for GvHD prophylaxis. *Results*. The probability of 5 year overall survival for patients transplanted in 1st CR is 61.3%, with a median of 19.5 months (1-116 months), while the probability of OS for patients who received stem cell transplant in advanced phase of the disease was 18.2%, with median of 7 months (2-109 months), p=0.0008. Disease free survival (DFS) for the AML patients transplanted in 1st CR was 59.9%, with a median of 16.5 months (1-116 months) vs. 18.2%, median 4 months (2-109 months) for patients in >1st CR, (p=0.0004). Relapse rates for patients in 1st CR were 25.3% compared to 59.1% (p=NS) for the other group. Time to relapse for patients transplanted in 1st CR ranged from 7-67 months (median 20.5 months), while for those transplanted in more advanced phase was 2-13 months, with median of 3.50 months Transplant related mortality (TRM) was also lower for patients in 1st CR than for those transplanted in advanced phase of the disease, 45.6% vs. 18.3% (p=0.056). *Conclusions*. Allogeneic stem cell transplantation is a standard post-remission treatment for AML patients offering significantly longer overall survival and disease free survival with lower transplant related mortality and lower relapse rate when performed in first complete remission than in more advanced phases of the disease.

1256**THROMBOTIC THROMBOCYTOPENIC PURPURA-A REPORT OF TWELVE CASES**

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Thrombotic Thrombocytopenic Purpura (TTP) is a rare disease characterized by microangiopathic haemolytic anaemia with direct antiglobulin test (DAT) negative, thrombocytopenia and fever, often accompanied by organ dysfunction, particularly brain and kidney. The precise aetiology of TTP is unknown. Recently TTP has been associated with a deficiency of a protease usually involved in the ultra-large von Willebrand factor (vWf) multimers cleavage in plasma. The vWf cleaving protease was identified as a new member of the ADAMTS family of metalloproteases and designated ADAMTS 13. The deficiency of a vWf cleaving protease favours the presence of ultralarge hyperactive vWf that easily adhere to the vessels wall and promotes platelet adhesion and aggregation in terminal arterioles and capillaries, leading to the characteristic clinical picture. The deficiency of ADAMTS 13 in TTP can be congenital or acquired. The acquired form, the most frequent, is caused by autoantibodies that neutralize ADAMTS 13 activity or bind to the protein causing its accelerated plasma clearance. It can be idiopathic or secondary to autoimmune diseases, pregnancy, metastatic cancer, drugs, infections, sepsis or transplantation (particularly allogenic bone marrow and solid organs). The diagnostic relevance of ADAMTS 13 levels is actuality questioned. TTP was a rapid fatal disease, with less than 3% of surviving patients, until the use of plasma exchange treatment that probably removes some harmful substances from circulation and replaces the deficient factor. *Patients and Methods.* Between May 1992 and January 2005 twelve patients with TTP, two men and ten women, age range 5-60 years, were diagnosed and treated in our Centre. The TTP diagnosis was made based on thrombocytopenia, microangiopathic haemolytic anaemia (morphologic criteria) DAT negative, normal coagulation screening tests and high serum lactate dehydrogenase levels, associated or not with fever and organ dysfunction. All the cases were treated with fresh frozen plasma infusion, plasma exchange and nine cases received adjuvant therapy with steroids and other immunosuppressant drugs. In two patients, requiring surgical procedures and invasive diagnostic manoeuvres, activated recombinant factor seven was successfully used. *Results.* TTP was idiopathic in six cases and secondary in other six (autoimmune diseases in 2; drugs in 1; pregnancy in 1; cancer in 2). TTP was the early manifestation of gastric cancer in two patients that died within two months. Six patients, four secondary and two idiopathic, achieved complete remission; four idiopathic patients had several relapses. The follow-up ranges between 17 days to 156 months. Discussion TTP can be a catastrophic disease if a plasma exchange treatment is not performed on time, which creates the requirement for a prompt and simple diagnosis that has to be based on clinical, biochemical and morphological criteria. There are no randomized clinical trials to provide data for management protocols. In our experience plasma exchange is the most effective treatment compared with other therapies.

1257**THE GOLD STANDARD FOR TREATING HODGKIN'S DISEASE PATIENTS: WHERE DOES ABVD ACQUIRE ITS EVIDENT SUPERIORITY?**

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Different treatment approaches, regarding chemotherapy and radiotherapy, were available and utilized over different time periods for patients with Hodgkin's disease (HD). Even today, different centers and schools have adopted different treatment modalities as standard. Efforts are directed towards tailoring the optimal treatment method for each patient. Nevertheless, in

the segment of chemotherapy, most researchers accept the ABVD regimen as the present 'gold' standard for treating all stages and types of HD, in combination or without adjuvant radiotherapy. In a single-center study, on a population of 400 HD patients, treated at our Department over a 25-year period, we attempted to define points where ABVD has shown superiority over earlier chemotherapy option (s), the sustainability of prognostic models for HD patients when analyzed separately according to treatment protocol, as well as the benefit it implies, evaluated by patient longevity and quality of life. Univariate statistical analyses were performed on a number of patient characteristics, disease manifestations and treatment modalities and their outcomes, and compared in patients grouped according to treatment protocol, meaning also according to the period when they were observed and/or treated at our Department. Special attention was placed on the subgroup of HD patients in whom the ABVD chemotherapy protocol was the principal one. Multivariate analyses were performed in order to define whether known predictive factors, certainly the ones incorporated in the IPI, and others, sustain prognostic significance within the more recently observed subpopulation of HD patients, treated initially with the present gold chemotherapy standard. The most obvious advantage of ABVD treated patients was observed in the field of life expectancy. Overall survival was above 85% at 10 years, reaching its plateau shortly after year 2, as opposed to a near 55% for MOPP-based treated patients. EFS followed this line of conclusions. Chance for relapse was also markedly low. Patients treated with the hybrid MOPP/ABV (D) were not valuable for the analyses, since when this treatment option first became available, it was widely used for poor risk HD patients, thus diminishing their chances for a privileged outcome. In the segment of univariate statistical analyses, most of the parameters maintained their prognostic significance, although with slightly higher p-values. This applies to the IPI parameters also, both with the original settings, as well as under our dichotomization criteria (slightly altered points derived from population and disease characteristics). Nevertheless, in multivariate analyses, ABVD treated patients manifest no outcome affection by the known predictive parameters, except for a couple: one patient characteristic - age, and one disease manifestation - symptom presence. Therefore, HD patients treated with ABVD chemotherapy as their underlying regimen, have significantly greater outcome expectations and very high reliability for achieving definite disease control. In that process not many patient characteristics, nor disease manifestations can influence their favorable prognosis, which is why this chemotherapy protocol is applicable to patients with different age and gender, with disease of different type or extent, with comparably similar favorable effect. The evidently lower incidence of undesired late effects is also a fact deserving respect and a confidence vote.

1258**ALEMTUZUMAB (CAMPATH-1H) S.C. IS EFFECTIVE IN PATIENTS WITH B-CLL WITH ADVERSE CHROMOSOMAL ABERRATIONS**

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Background. Several studies have shown that alemtuzumab is an effective therapy for CLL patients, also in cases with adverse chromosomal aberrations and resistance to chemotherapy. *Aims.* To evaluate the efficacy of alemtuzumab in patients with chemorefractory disease and with del (17p), del (11q) or trisomy 12. *Methods.* Thirteen consecutive B-CLL patients with resistance to chemotherapy (mostly alkylating agents and fludarabine) and with adverse chromosomal aberrations detected by FISH, were treated with subcutaneous alemtuzumab 30 mg three days weekly during a treatment period of 3-18 weeks. *Results.* A clinical response was seen in all patients. Complete remission in blood and normalized hemoglobin and thrombocyte counts was obtained in ten patients (5/7 with del (17p), 3/4 with del (11q) and 2/2 with trisomy 12). Regression of lymphadenopathy was seen in 4/6 patients with enlarged nodes.

Subcutaneous alemtuzumab was well tolerated, but limited local reaction in the thigh and fever was observed in a few patients during the first week of injections. Eight patients had a CMV reactivation and in six mild clinical symptoms were seen, but no cases had pneumonitis or other serious complications. One patient had an uncomplicated pulmonary aspergillus infection. The duration of response was more than one year in seven patients. During a median follow up of 18 months (range 5-26 months) one patient died. *Conclusions.* In this retrospective series of CLL patients with adverse chromosomal aberrations subcutaneous alemtuzumab showed a high efficacy without serious side effects.

1259**HYPOCROMIA AND MICROCYTOSIS WITH NORMAL Hb A2 LEVELS - GENOTYPE STUDIES IN A GROUP OF PORTUGUESE INDIVIDUALS**

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Hypochromia and microcytosis with normal Hb A2 level, excluded iron deficiency, can be due to alpha-thalassemia (thal), some Hb variants, deltabeta-thal or beta-thal in association with delta-thal. Differential diagnosis has to be done by molecular biology techniques. In Portugal the most frequent alpha-thal mutations are the 3.7 and 4.2 Kb deletions with sporadic cases of alpha⁰-thal (-MED e -20.5) and non-deletional mutations. The aim of our study was to characterise at molecular level samples with hypochromia, microcytosis and normal Hb A2 levels. *Methods.* 213 subjects (adults and children) with MCV and MCH below the normal values attending to the age, normal Hb A2 (<3.5%), Hb F (<2%) and normal iron status were studied. According to the hematological parameters, obtained by standard methods, samples were divided into two groups: those with borderline MCV and MCH values (Group I) and those with lower MCV and MCH (Group II). The two groups were screened for the most common alpha-deletions (-alpha3.7, -alpha4.2; -MED and -20.5) using Gap-PCR (Tan et al, 2001). Beta-globin genes were screened for mutations by SSCP and sequencing. Samples from Group II that tested negative for the common alpha-thal mutations were studied by other techniques: Southern-blot hybridisation was used to test rare alpha-globin gene deletions and alpha-globin gene sequencing was used to detect non-deletional mutations. Delta-globin gene sequencing was performed on samples with beta-thal mutations. *Results.* In Group I, with 133 samples, 96 were heterozygous -alpha3.7 and 15 were heterozygous -alpha4.2; in the remaining 22 samples we could not find any alpha- or beta-globin gene mutations. In Group II, with 80 samples, 50 were homozygous -alpha3.7, 5 compound heterozygous -alpha3.7/-alpha4.2 and one heterozygous -alpha3.7. One individual was heterozygous for Hb Plasencia (alpha2 (125)CTG-CGG); one was heterozygous for a [alpha2-IVSII-1 (-G)] deletion; one was a compound heterozygous for an alpha1 deletion (CDs 82-84) /insertion (5 nts) and a large deletion not yet identified. Beta-gene analysis of remaining samples detected: two heterozygous beta IVSI-6 (T-C); one beta IVSI-1 (G-A) associated in trans with delta-7.2 kb Corfu deletion and one beta IVSI-1 (G-A) associated in trans with a delta Cd29 GGC-GCC. In 17 samples we couldn't identify any alpha- or beta- alteration. Further investigation of this group of patients is ongoing. *Conclusions.* As expected, in this study of 213 samples with hypochromia and microcytosis with normal Hb A2 levels the most common mutation found was the -alpha3.7 Kb deletion (202 / 266 alleles), in heterozygous and homozygous state. The 4.2 Kb alpha deletion was detected in 20 alleles; the genotype -alpha3.7/-alpha4.2 was detected in 5 cases. In group II, with lower MCV and MCH values, three mutations not previously described were found: one alpha2-IVSII-1 (-G) deletion, one alpha1 deletion (CDs 82-84) /insertion (5 nts) and one delta Cd29 GGC-GCC.

1260**ISOLATION OF THE CD34+ HEMOPOIETIC PROGENITOR CELLS IN MYELODYSPLASTIC SYNDROMES WITH VARIOUS CHROMOSOMAL ABNORMALITIES: EVIDENCE OF INVOLVEMENT IN THE TRANSFORMED CLONE**

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Although myelodysplastic syndromes (MDS) are considered disorders of the pluripotent hemopoietic progenitor cell, there are reports raising the suggestion that the lymphoid lineage is spared at list in some cases. The aim of this study was to investigate whether specific chromosomal abnormalities observed in MDS cells are associated with CD34+ cell involvement in the transformed clone and whether this could be used as prognostic marker. Thirty two MDS patients with various chromosomal aberrations, detected by FISH, were studied. Purified cell populations were obtained from the bone marrow and/or peripheral blood cells consisted of CD34+, CD34+/CD14, CD34+/CD33+, CD34+/CD19+ using magnetic cell sorter. Single cell clonogenic assay as well as long term cultures were used to investigate caryotyping abnormalities in isolated cell populations. In 30 patients the chromosomal abnormalities were detected in all the studied populations. In two patients CD33+ and CCD14+ cells were positive for the chromosomal defect but bone marrow CD34+ cells and peripheral CD19+ cells were negative. Furthermore, the positive colonies after single cell clonogenic assays, were significantly lower than the positive cells in the starting population. This finding suggest an *in vitro* growth disadvantage of the transformed cd43+ cells against normal cells. This disadvantage could be of benefit in cases of autologous CD34+ cells transplantation in MDS without selection of untransformed cells since the existing sorting techniques do not permit such a selection, at the moment

1261**CEREBRAL INVOLVEMENT IN CHRONIC LYMPHOBLASTIC LEUKEMIA**

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Background. Central nervous system (CNS) involvement is an extremely rare complication of chronic lymphocytic leukemia (CLL). *Aims.* We reviewed 26 autopsy cases of CLL with special attention on pathological findings in CNS. *Patients:* The study group consisted of 18 males and 8 females aged 45-75 years (average 62 years). The diagnosis of CLL was obtained on review of the hematoxylin and eosin-stained slides and immunophenotype data. *Results.* Leukemic infiltration of CNS was evident clinically and grossly in two cases. The first patient was 53-years-old man with CLL of 3 years' duration, who developed a sensorimotor aphasia with right-sided hemiplegia six weeks prior to death. At autopsy, a large (6x6x4cm) ill-defined tumor mass was found in the temporo-occipital region of the left cerebral hemisphere with marked perifocal edema and temporal herniation. Histologically, tumor tissue was very cellular, composed of large cells with vesicular nuclei, prominent nucleoli and occasionally giant and Reed Sternberg-like cells admixed with a small number of mature lymphocytes. Mitoses were numerous. Immunohistochemically, both types of malignant proliferation (small and large cells) stained positively for CD20 (pan-B marker). The diagnosis of cerebral diffuse large cell lymphoma in CLL (Richter syndrome) was obtained. The second patient was 70-years-old man with CLL of one year's duration, who developed a polyradiculoneuropathy one month prior to death. At autopsy, infiltration of spinal roots and cranial nerves as well as diffuse leptomeningeal dissemination ('leukemic meningitis') were found. Histologically, peri- and endoneural

leukemic infiltration, leukostasis and perivascular leukemic infiltration (prevalent in the brainstem and spinal cord) and meningitis were presented. Histopathological findings (without evidence of a mass lesion) in 2/26 CLL cases without neurological signs were in the form of petechial hemorrhages with leukostasis and perivascular leukemic infiltration, localized in the white matter of the cerebral hemispheres. In all other cases of CLL (22/26, 84,6%) cerebral leukemic infiltration was not present. *Conclusions.* The clinicopathological features of these cases indicate that, despite of rarity of CNS involvement in CLL, any neurological manifestation in CLL patients should be aware of the possibility of this complication.

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GRAFT-VERSUS-HOST DISEASE AFTER NONMYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Nonmyeloablative stem cell transplantation (NST) has been used widely in the hope of decreasing GVHD especially in old age patient population. However, GVHD following NST continues to be an obstacle for successful outcome. *Aims.* To evaluate the incidence, severity and response to therapy of GVHD following NST in Korea. *Methods.* We evaluated fifty-eight patients transplanted using the fludarabine based conditioning regimen between Oct 2000 and Feb 2004; GVHD prophylaxis was done using cyclosporine (5 mg/kg- 3 mg/kg iv or po from day -1 to +90). Patients underwent NST because of old age, comorbidities or advanced stage of the disease at the time of transplant. There were 37 males and 21 females with an age range of 17-66 years (median 48). *Results.* Incidence of grades II-IV acute GVHD and chronic GVHD was 34% and 70%, respectively. Grade 1-2 and 3-4 acute GVHD was observed in 4 and 12 patients, respectively. Chronic GVHD was observed in 23 patients. 11 (70%) and 5 (21%) patients of acute and chronic GVHD showed no response despite treatment. Cumulative incidence rates of death with manifestations of GVHD under treatment were 50% at 15 months. With a median follow-up of 195 days, the 3-year estimates for overall survival were 32%. *Conclusions.* The incidence of grade III acute GVHD in Korea may be less than in Western countries. But acute GVHD responded poorly and GVHD related death rates were fairly high. Further studies are needed to improve the eventual outcome not only of acute but also of chronic GVHD following NST.

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MOLECULAR MONITORING OF CHRONIC MYELOID LEUKEMIA BY REAL-TIME PCR USING BCR-ABL FUSION GENE

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Chronic myeloid leukaemia (CML) is a myeloproliferative disease consistently associated with a characteristic cytogenetic abnormality, the translocation t(9;22), that results in Philadelphia chromosome (Ph). This translocation fuses sequences of the BCR gene on chromosome 22 with regions of ABL gene on chromosome 9. The site of breakpoint in BCR gene may influence the phenotype of the disease. In CML this breakpoint is almost always in the Major breakpoint cluster region (M-BCR) generating fusion transcripts with a b2a2 or b3a2 junction. Although good correlations have been established between PCR quantification of the Bcr-Abl transcripts and the evolution of the disease, regarding the chosen therapy, this technique cannot

identify the presence of Ph-positive clones that may be responsible for relapse after suspension of treatment. The aim of this work is the evaluation of the response to therapy in CML patients by molecular monitoring of the levels of the BCR-ABL fusion transcripts compared with the related gene. For this purpose a total of 234 samples from 36 CML patients (20 males-55,6%, 16 females-44,4%) were analysed. Six of those patients (16,7%) are under imatinib therapy, 13 (36,1%) receive imatinib as 2nd line therapy and 17 (47,2%) are treated with INF-alfa. Patients total RNA and genomic DNA were extracted from peripheral blood samples and total RNA was reverse transcribed into cDNA. Real-time PCR absolute quantification with taqman probes was used to measure the fusion transcripts levels and Sybr®Green I dye were used to measure the fusion gene levels. Our results show a greater incidence (69,4%) of b3a2 fusion transcripts (24 of 36 patients) compared with the incidence of b2a2 (about 30,6% - 11 of 36 patients). However the molecular evolution of the disease seems to be independent of the type of fusion transcripts. On other hand, the levels of fusion transcripts are more stable in patients receiving imatinib than in those undergoing IFN-alfa therapy. Besides the low levels of fusion transcripts observed in response to therapy, an increase in BCR-ABL gene levels was observed, namely in those patients with discontinuous imatinib. Accordingly with other authors our results show that the quantification of the fusion transcripts by real-time PCR is a good technique to evaluate the response to therapy. However our data suggests that a residual leukemic cell population is mainly detected by BCR/ABL gene quantification.

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INCREASED LEVELS OF GALECTIN-3 IN PATIENTS WITH ACUTE GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Galectins, members of endogenous mammalian leptin, have been documented as main regulators of immune cell homeostasis and inflammatory processes. Among these, galectin-3 is a chimera-type galectin with anti-apoptotic activity, and it had been reported that increased galectin-3 expression correlated with defective T-cell apoptosis in patients with some immune disorders. Here, we evaluated the level of galectin-3 in patients receiving allogeneic hematopoietic stem transplantation (HSCT) in the context of the presence of acute graft-versus-host disease (GVHD). *Material:* Fifty-five patients receiving allogeneic HSCT for acute myelogenous leukemia (AML) were enrolled. We determined the level of galectin-3 in serum of enrolled patients at three different times of sampling (day-14, day 0, and day+14) by enzyme-linked immunosorbent assay (ELISA). *Results.* Among 55 patients, 20 patients showed acute GVHD more than grade II. The patients were divided into two groups according to the presence of acute GVHD. Patients with grade II-IV acute GVHD had significantly higher level of galectin-3 at time of day+14 after HSCT than those without (mean 78 ng/mL vs 44 ng/mL, P = 0.042). The levels of galectin-3 were not significantly different in both groups at day-14 (pre-transplantation period), but were slightly higher than those without acute GVHD at day of transplantation without statistical significance (mean 50 ng/mL vs 39 ng/mL, P = 0.086). In addition, the patients with acute GVHD showed the statistically significant increment of the galectin-3 level after transplantation (P = 0.03). *Conclusions.* The level of galectin-3 is higher in patients of acute GVHD than those without after HSCT for AML, and also higher after transplantation than before or at transplantation day in these patients. These findings suggests that the galectin-3 might be one of significant molecules in pathogenesis of acute GVHD, and the successive evaluation of galectin-3 levels might be one of informative tests predicting the occurrence of acute GVHD. The clinical significance of this study should be further evaluated for the large number of patients.

1265**COMPARATIVE ANALYSIS OF KIR-POSITIVE NATURAL KILLER AND T CELL CONTENTS BETWEEN GRANULOCYTE COLONY-STIMULATING FACTOR-MOBILIZED LEUKAPHERESIS PRODUCT AND BONE MARROW HARVESTED FROM HEALTHY DONORS**

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Killer cell immunoglobulin-like receptors (KIR) are a family of inhibitory and activating receptors that are expressed by most natural killer (NK) cells and by small subpopulations of T cells. It has been shown that KIR regulates NK cell and T cell function concerned with the control of graft-versus-host disease (GVHD) and graft-versus-leukemia effect after allogeneic hematopoietic stem cell transplantation (HSCT). However, the comparative enumeration of these cell populations in the allograft between the bone marrow and granulocyte colony-stimulating factor (G-CSF)-mobilized leukapheresis product has been lacking in human setting. We prospectively conducted a comparative study to evaluate the levels of NK cells expressing KIR2DL2, KIR3DL1 (p70), KIR NKAT2, and CD94/NKG2A in the bone marrow and the G-CSF-mobilized leukapheresis products, both were harvested from healthy volunteers. Flow cytometric analysis revealed that the proportion of CD56+ cells expressing KIR2DL2, KIR3DL1, KIR NKAT2, or CD94/NKG2A antigen was not different between the G-CSF-mobilized leukapheresis and bone marrow product. However, the absolute number of CD94/NKG2A-positive CD56+ cells was significantly higher in leukapheresis product compared to bone marrow ($4.5 \pm 1.7 \times 10^7$ /kg vs $1.3 \pm 1.1 \times 10^7$ /kg, $P < 0.05$). There was no difference in the proportion of KIR2DL2+ CD8+ cells, KIR3DL1+ CD8+ cells, KIR NKAT2+ CD8+ cells, and CD94/NKG2A+ CD8+ cells between G-CSF-mobilized leukapheresis and bone marrow product. The absolute number of CD8+ T-cells expressing CD94/NKG2A receptor was also significantly higher in leukapheresis product compared to bone marrow ($12.6 \pm 3.3 \times 10^7$ vs $4.1 \pm 2.0 \times 10^7$, $P < 0.05$). Sixteen patients (AML, 9 cases; ALL, 7 cases) received a myeloablative preparative regimen with cyclosporine/short-course methotrexate as GVHD prophylaxis. The difference in the incidence of acute GVHD was not observed between the patients who had received the blood stem cells and bone marrow. There was no correlation between the number of infused KIR2DL2+, KIR3DL1+, KIR NKAT2+, or CD94/NKG2A+ NK cells and the development of acute GVHD. Very similar findings were observed in the settings of CD8+ T-cells. Although the difference in the incidence of GVHD was not demonstrated in this small number of patients, the clinical implication of this finding should be further evaluated for the large number of patients and on the basis of cell dose parameter and graft-versus-leukemia effect.

1266**MCL-1 GENE PROMOTER INSERTIONS DO NOT CORRELATE WITH DISEASE OUTCOME, STAGE OR VH GENE MUTATION STATUS IN CHRONIC LYMPHOBLASTIC LEUKEMIA**G. Tobin¹, A. Skogsberg¹, U. Thunberg¹, A. Laurell¹, A. Aleskog¹, M. Merup², C. Sundström¹, G. Roos³, K. Nilsson¹, R. Rosenquist¹¹Uppsala University, UPPSALA, Sweden; ²Huddinge University Hospital, STOCKHOLM, Sweden; ³Umeå University, UMEÅ, Sweden

Chronic lymphocytic leukemia (CLL) is characterised by defects in apoptosis resulting in long-lived neoplastic B-cells. High levels of the anti-apoptotic protein Mcl-1 has been shown to correlate with disease progression and cytostatic drug resistance in CLL. Recently, the presence of insertions within the mcl-1 gene promoter, either 6 or 18 nucleotides, was associated with worse survival in CLL. These insertions were only

detected in CLL cells but not in controls. To analyse this further, we investigated a large cohort of 173 CLL cases and 105 age-matched controls for the presence of the mcl-1 promoter insertions utilising fragment analysis. The immunoglobulin variable heavy chain (IgVH) gene mutation status was also assessed in 168 CLL cases. The mcl-1 promoter insertions were present in 61.4% of CLLs and 68.6% of the control material, thus substantially higher than the previously reported frequency in CLL (30%) and controls. When analysing for prognostic impact, the mcl-1 promoter insertions showed no significant difference between CLL cases with either the 6 or 18 nucleotide insertion and cases without insertions for predicting overall survival. Furthermore, no association with VH mutation status or Binet staging was indicated in our CLL cohort. We suggest that the mcl-1 promoter insertions instead represent a new insertional polymorphism with unknown functional significance.

1267**DELAYS IN THE DIAGNOSTIC AND THERAPEUTIC PATHWAYS FOR GAUCHER DISEASE PATIENTS: THE RESULTS OF A U.S. PATIENT SURVEY**

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Purpose: Gaucher disease (GD) is a progressive, potentially disabling and life-threatening disease. The spectrum of clinical presentations, with or without neurological manifestations, is remarkably heterogeneous and ranges from early onset, severe disease to absence of symptoms in elderly adults. Early diagnosis and adequate treatment are important to prevent irreversible damage. Recognizing GD in an individual patient may be challenging, especially if medical specialists are relatively unfamiliar with the disease. The purpose of this research was to better understand the specifics and timing of disease presentation and diagnosis. *Methods.* Genzyme Corporation, via its U.S. patient services group Genzyme Treatment Support, mailed the survey to 135 patients with a confirmed diagnosis of GD who started Cerezyme® (imiglucerase) treatment in the period Q2 2002 - Q1 2004. The survey included questions on the onset of first symptoms, physicians' referrals, diagnosis and initiation of Ceredase® or Cerezyme, the former having been available commercially in the U.S. since 1991. *Results.* 77 responses were received; 49% from male and 51% from female patients. Patients were aged <18 years (27%), 18-44 (25%) or 45+ (48%). For 43 patients, required data with regard to age of onset, diagnosis and ERT initiation were available (see table). Comparing the results to previous surveys, the average of the gap between symptom onset and first visit to a physician seems to be decreasing (30 months, compared to 34 months in 1999-2002 survey). Also the gaps between first visit to diagnosis (20 months; was 38 months in 1999-2002 survey) and diagnosis to initiation of Cerezyme® therapy have diminished (94 months; was 100 months in 1999-2002 survey). Initial symptoms most frequently present included easy bruising or bleeding (48%), enlarged abdomen (44%), fatigue (33%), pain in bone/joints, or bone fractures (30%), and delayed growth (15%).

Onset first GD symptoms	mean age of 24.3 years
Initial presentation to a physician with symptom(s)	mean age of 26.8 years
Diagnosis of GD	mean age of 28.5 years
Patients initiated on Cerezyme® therapy	mean age of 36.3 years

These symptoms prompted the patient to seek medical attention in 28%, 35%, 22%, 23%, and 8% of the cases, respectively. The average number of physicians seen prior to diagnosis (2.8 medical specialists) remained fairly constant compared to previous surveys. Medical specialists first seen included family physician/internal medicine (40%), pediatrician (29%), and haematologist/oncologist (13%). As a second consulted physician, the haematologist/oncologist was seen most frequently (44%), while the geneticist was consulted in only 6% of the cases. *Conclusions.* The survey results suggest that patient and doctor delays, between onset of first Gaucher symptoms, presentation to medical specialist, diagnosis, and initiation on Cerezyme, have only slightly decreased over the last decade. Indeed, inadequate and/or delayed action by medical specialists is still a concern. Awareness of Gaucher disease among the medical community and patients is apparently increasing but still needs further improvement. For the future, the main challenge is to reduce the delay of 12 years between onset of first symptoms and initiation of Cerezyme, as observed in this U.S. survey.

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STUDY OF CD28 EXPRESSION ON CD4 & CD8 T LYMPHOCYTES & INFECTIOUS SENSITIVITY IN BETA THALASSEMIA MAJOR PATIENTS AS IRON OVERLOAD MODEL

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Background. The CD28 expression on T lymphocyte has a costimulator function in order to induce and sustain immune responsiveness with APC and B lymphocytes. In addition, the CD28 expression is necessary for T-cell differentiation and proliferation. It has been stated that in a pathological situation such as iron overload the cell marker expression can be affected. Beta thalassemia major patients, were chosen to evaluate the CD28 expression on T-cell subpopulation as a iron overload model. *Aims.* Evaluation of CD28 expression on T lymphocytes in iron overload conditions and its comparison with normal conditions. *Methods.* PBMC from beta thalassemia patients were taken and their lymphocytes were isolated by using Ficoll-Hypaque technique. Then the lymphocyte were treated with mouse anti CD28 antibody conjugated with Phyco-Erythrin anti CD28-mouse-Serotec, UK) and also with anti CD4 and anti CD8 conjugated with FITC. After 1-2 hour incubation times in dark room, the CD28, CD4 and CD8 were assayed using flow

cytometer (Becton Dickinson). *Results.* The CD4+ lymphocytes were 48.3±5.3% (including 43.7±4.39% for CD28+ and 4.6±1.34% for CD28-) in control group and 44.2±4.9% (including 38.7±2.98% for CD28+ and 5.5±1.52% for CD28-) in patient group. The CD8+ lymphocytes were 25.04±3.25% (including 19.44±2.41% for CD28+ and 5.6±1.42% for CD28-) in healthy group and 21.8±2.41% (including 12.03±3.6 for CD28+ and 9.77±1.5% for CD28-) in patient group. *Conclusions.* In this model, the results show that, the expression of CD28 molecules on CD4+ T cell lymphocytes did not significantly altered in iron overload patients. But the CD28 expression on CD8+ T lymphocytes decreased significantly. So the down regulation of CD28 expression on the surface of CD8+ lymphocytes may cause impair of CD8+ lymphocyte function and the system could be more sensitive to infected agents.

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DIFFERENTIATION OF UMBILICAL CORD BLOOD CD14+ CELLS INTO ENDOTHELIAL LIKE CELLS

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It has been shown that circulating human non-adherent CD34+ cells co-expression vascular endothelial growth factor (VEGF-R2) and AC133 have the capacity to differentiate into adherent mature endothelial cells. However, prior studies have demonstrated that a much bigger subset of primary adherent mononuclear cells can also form endothelial progenitor cells (EPC). So, this study initiated to evaluate the capacity of CD14+ as monocyte-macrophages to differentiate into an endothelial phenotype. CD14+ cells were isolated from umbilical cord blood by adherence separation and magnetic bead selection (Purity>90%) by antibody against CD14+ and culture on fibronectin-Coated plastic dishes in DMEM medium supplemented with VEGF (10ng/mL), bFGF (2ng/mL) and 20% FBS. After 2 week of culture, using fluorescence activated cell analysis we observed expression of the endothelial markers included Von Willebrand Factor (vWF), PECAM1 (CD31). The proportion of cell expressing these markers further increased after 4 weeks (96 and 92.5% vs. in 65 and 18% of cells, respectively). The present study data indicate that under angiogenic stimulation, Cord blood macrophages develop to endothelial phenotype with expression of specific surface markers and suggesting that these cells population may be recruited for vasculogenesis and my use for cell therapy in future.

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