

Poster Session I

LYMPHOMA: BIOLOGICAL ASPECTS AND PROGNOSIS

001 CD10 AND BCL-2 EXPRESSION COMBINED WITH THE INTERNATIONAL PROGNOSTIC INDEX CAN IDENTIFY SUBGROUPS OF PATIENTS WITH DIFFUSE LARGE-CELL LYMPHOMA WITH VERY LOW AND VERY HIGH RISK

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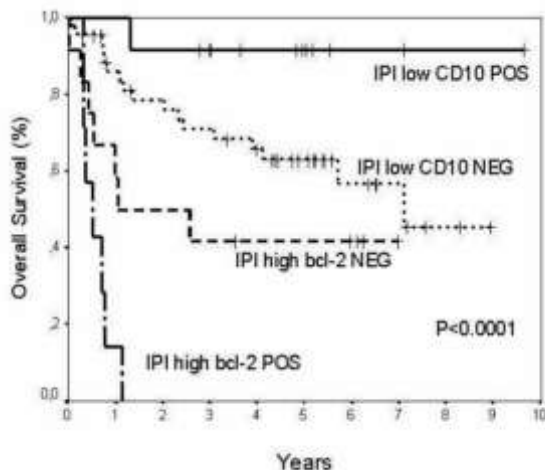
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Background and aim: Diffuse large B-cell lymphoma (DLBCL) is characterized by marked biologic heterogeneity. The identification of simple and reproducible parameters that can be combined with the International Prognostic Index to better predict outcome could lead to the development of effective risk-adapted strategies.

Methods: Bcl-2 and CD10 expression were determined by standard immunohistochemistry. The impact of the positivity on survival was evaluated in combination with the IPI in 86 patients with a confirmed diagnosis of DLCL who had been adequately treated with anthracyclin-containing regimens. Patients were divided according to the IPI in low-risk (0-2 factors) or high-risk (3-5 factors) groups.

Results: Positivity rates were 24% for CD10 and 41% for bcl-2. In a Cox regression analysis, IPI (hazard ratio=5.98, $p<0.001$) and bcl-2 (HR=2.43, $p=0.02$) were independent poor prognostic factors and CD10 (HR=0.41, $p=0.052$) predicted a favorable outcome. Among patients in the low-risk IPI group, CD10 positivity was associated with excellent 7-year overall survival (92% vs. 45%, $p=0.06$). In the high-risk IPI group, bcl-2 positivity identified a group of patients with invariably fatal disease (2-year overall survival 50% vs. 0%, $p=0.02$). **Summary:** The expression of CD10 in the low-risk IPI group, and the expression of bcl-2 in the high-risk IPI group can identify two subgroups of patients who might benefit from a new risk-adapted treatment approach.



Caption 1: Overall survival according to IPI, bcl-2 and CD10

002 COMPARISON OF GENE EXPRESSION PROFILING BETWEEN MANTLE CELL LYMPHOMA IN LEUKEMIC PHASE AND NORMAL NA VE B-CELLS WITH OLIGONUCLEOTIDE MICROARRAYS

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Mantle cell lymphoma (MCL) is a distinctive subtype of B-cell lymphoma characterized by recurrent association with the t(11;14)(q13;32) and

consequent ectopic overexpression of cyclin D1 in the tumor cells. Clinically, the disease is predominantly disseminated at diagnosis and a frank leukemic phase is detected in one fourth of patients. Median survival of MCL is short (about 36 months) and to date no specific treatment is available. Ontogenetically, MCL is considered the malignant counterpart of pregerminal center nave B-cells. Although the overexpression of cyclin D1 plays a pivotal role on the pathogenesis of MCL, studies with transgenic mice have shown that this is not sufficient, by itself, to cause lymphoma. In addition, a growing number of distinct genetic and biological alterations have been associated with MCL and a better understanding of the molecular genetics of this disease may provide insights towards a potentially curable therapy. To address this issue, we compared the gene expression profile of MCL and nave B-cells using oligonucleotide microarrays representing 10,000 genes. MCL cells and normal nave B-cells (IgD+CD38CD27-) were highly purified, by magnetic activated cell sorting, from the peripheral blood of patients with MCL in leukemic phase and from tonsils, respectively (purity > 95% in all samples). Three patients were selected from each group and experiments were performed in replicates using the Amersham CodeLink Human UniSet 1 Bioarray. Microarrays were scanned on GenePix 4000B Array Scanner. Spot finding, data extraction and normalization were performed with the CodeLink Expression Analysis software and data mining with Vector Xpression, Informax. After filtering for poor spots, data from replicates were averaged and genes displaying at least three-fold differences in expression between MCL and nave B-cells, in all nine possible comparisons, were identified. According to these criteria, a total of 106 differentially expressed genes were found in MCL cells: 63 overexpressed and 43 underexpressed. Gene Ontology classification of the 63 overexpressed genes in MCL originated 185 records, with nucleic acid metabolism (9%), signal transduction (8%), DNA binding (7%), cell proliferation (5%) and transmembrane receptor activity (4%) as the most common categories after unclassified genes (17%). The 43 underexpressed genes originated 133 records, with signal transduction (16%), response to biotic stimulus (11%), nucleic acid metabolism (8%) and response to pathogen (7%) as the most common categories after unclassified genes (23%). For validation purposes, differential expression of seven selected genes was confirmed by TaqMan real-time RT-PCR in non-purified peripheral blood samples from 25 patients with MCL in leukemic phase, compared with purified tonsil nave B-cells, purified peripheral blood B-cells and total peripheral blood. Data on large scale gene expression of MCL have been obtained mainly with cDNA microarray studies of tumor lymph nodes and results have favored a predominant expression of proliferation genes. The present study represents a different strategy which emphasizes disease at the cellular level, revealing that the nucleic acid metabolism and signal transduction categories of genes are also significantly modulated in MCL.

003 FISH IS THE BEST METHOD TO DETECT BCL2/IGH TRANSLOCATION IN FOLLICULAR LYMPHOMA AT DIAGNOSIS. A COMPARATIVE STUDY WITH CONVENTIONAL CYTOGENETICS, FISH AND PCR TECHNIQUES USING BIOMED-2 PRIMERS

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BACKGROUND

Follicular lymphoma (FL) is a B-cell neoplasm characterized by the presence of the t(14;18)(q32;q21) in nearly 85% of cases. The t(14;18) relocates the BCL2 gene from its normal position on chromosome 18 and juxtaposes it to IgH sequences on chromosome 14. Thereby, BCL2 gene expression is deregulated, and its overexpression inhibits programmed cell death and prevents cells from entering the cell cycle. There are different laboratory techniques to detect such translocation. The aim of the present study was to test the efficiency of three different techniques: conventional cytogenetics, FISH and PCR, to detect t(14;18) in FL at diagnosis.

PATIENTS AND METHODS

Twenty-two samples from FL were analyzed at diagnosis: 1 from peripheral blood, 6 from bone marrow and 15 from lymph node. Conventional cytogenetics cultures were established for 72 hours with tetraphorbol myristate acetate (TPA) and chromosomes were identified by G-banding pattern. FISH was performed with BCL2/IgH dual

color-dual fusion locus specific probes (VYSIS). PCR was designed using the BIOMED-2 primers covering the MBR, 3MBR and mcr regions for the BCL2 gene and the consensus JH primer for the IgH gene (1).

RESULTS

Seven (32%) of 22 cases had cytogenetic aberrations, in three t(14;18) was found and in one t(18;22) was detected, always in a complex karyotype. FISH was performed in 19/22 patients and 14/19 (74%) were positive for BCL2/IgH translocation. PCR technique was applied to 21 cases, and positivity was detected in 10/21 (48%) samples (six cases positive for MBR region, three cases for mcr region and one case for 3MBR region). In five cases, FISH showed positivity not detected by PCR and in one case, PCR showed positivity not detected neither by FISH nor by conventional cytogenetics. In three (14%) of 22 cases, BCL2/IgH was not detected by any of the three tested techniques. Bone marrow was the least rendible tissue at diagnosis to obtain positivity to BCL2/IgH translocation.

CONCLUSIONS

- In our experience, with a limited number of cases, FISH becomes the most effective technique to detect IgH/BCL2 at diagnosis, probably because BCL2 locus specific FISH probe covers all the possible breakpoints in BCL2 gene.

- In bone marrow samples, PCR is the most sensible technique due to the heterogeneous infiltration. However, it is the tissue with the least positive results obtained by conventional cytogenetics and FISH.

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ACKNOWLEDGMENTS

Partially supported by Special Action SAF-2001-4947 and Red de Grupos G03/179 from the Spanish Ministries of Science and Technology and Health.

004 ABSENCE OF MALT1 TRANSLOCATIONS IN MALT LYMPHOMAS OF THE SKIN

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BACKGROUND

Mucosa-associated lymphoid tissue (MALT) lymphomas include low-grade B-cell lymphomas from a number of extranodal sites, including both mucosal organs such as the salivary gland, lung, thyroid, conjunctiva, and liver and nonmucosal organs such as the orbit and the skin. These organs are devoid of any native lymphoid tissue, and the lymphoma arises from the MALT acquired as a result of a chronic inflammatory or autoimmune disorder. Genetically, only 5% of MALT lymphoma show t(1;14)(p22;q32), that causes BCL10 deregulation, which specifically links the antigen receptor signaling to the NFkB pathway. However, the most frequent structural abnormality in MALT lymphoma is t(11;18)(q21;q21), that juxtaposes the API2 gene to the MALT1 gene. This abnormality has been detected in 30% of gastric MALT lymphoma, and also in other localizations at marked variable frequencies. Recently, t(14;18)(q32;q21) involving IgH and MALT1 genes has been described (1). In MALT lymphoma of the skin, there is a controversy regarding the existence of MALT1 translocations (1,2). The aim of the study was to determine if MALT lymphomas of the skin show MALT1 translocations.

PATIENTS AND METHODS

Eighteen samples were included in the study. FISH was performed using a MALT1 (18q21) dual color break apart translocation probe (VYSIS, Downers Grove, IL) on 14 paraffin-embedded cutaneous samples from patients affected with MALT lymphoma of the skin, to detect MALT1 translocations. Among these cases, 12 showed bcl-10 nuclear positivity and two of them were negative for nuclear bcl-10 expression. FISH was analyzed in parallel sections from those studied for immunohistochemistry, and bcl-10 positive areas were then relocalized for FISH observation. One hundred nuclei were scored by two different observers. In addition, four peripheral blood samples were tested with the same FISH probe as controls.

RESULTS

FISH technique was successful in eleven of 14 paraffin-embedded skin samples, and in all four peripheral blood controls. MALT1 translocations

were not detected in the analyzed cases. However, in two cases with MALT lymphoma of the skin, some areas showed three copies of the MALT1 gene.

COMMENTS

- We have analyzed fourteen cases of cutaneous MALT lymphoma and we have not detected MALT1 translocations.

- Streubel et al. (1) described 3 (27%) of 11 cases with MALT lymphoma of the skin showing t(14;18)(q32;q21) MALT1/IgH translocation by FISH.

- Ye et al. (2) analyzed the existence of t(11;18)(q21;q21) API2/MALT1 translocation by PCR and the MALT1 translocations by FISH and among 27 cases with cutaneous MALT lymphoma, no MALT1 translocations were detected.

- More studies are warranted in order to determine the real incidence of MALT1 translocations in cutaneous MALT lymphomas.

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ACKNOWLEDGMENTS

Partially supported by Special Action SAF-2001-4947 and Red de Grupos G03/179 from the Spanish Ministries of Science and Technology and Health.

005 IMPROVED MOLECULAR DIAGNOSIS OF T(14;18) IN FOLLICULAR LYMPHOMAS: ROUTINE USE OF LONG-DISTANCE POLYMERASE CHAIN REACTION

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Background: The t(14;18)(q32;q21) translocation involving the BCL2 gene and junctional segments (JH) of the immunoglobulin heavy chain gene (IgH) is present in 70-90% of follicular lymphoma (FL) cases at diagnosis. Although the breakpoints in BCL2 are largely clustered within the major breakpoint region (MBR) and minor cluster region (mcr), it is known that some breakpoints map away from these regions resulting in negative amplification of standard polymerase chain reaction (PCR). To circumvent this problem we applied a novel PCR technology for long DNA targets long-distance PCR (LD-PCR) to the detection of BCL2/IgH fusion genes.

Aim: To improve molecular detection of the t(14;18) in bone marrow (BM) with a new LD-PCR protocol and compare it with a standard qualitative PCR method.

Methods: Bone marrow specimens from 26 patients with histologically confirmed FL were analyzed. 24 patients were examined at the diagnosis and 2 patients in relapse. Median age was 51 (range 40-74), M:F ratio was 8:18; 5 patients were in stage I/II and 21 in stage III/IV. Immunophenotyping was performed in all patients, lymphoma cells in BM were confirmed in 14 of them (54%). For determination of breakpoints in and outside MBR and mcr regions of BCL2 gene we used LD-PCR method with a set of five primers covering 25 kb long stretch.

Results: The results of immunophenotyping divided our cohort of patients into two groups. The first group of 14 patients exhibited cells of FL in bone marrow (BM+) while 12 patients were negative for presence FL cells (BM-). Using standard PCR 4/14 BM+ and 3/12 BM- patients were positive for BCL2/IgH rearrangement. Total 7 (27%) patients were PCR positive. Using LD-PCR 9/14 BM+ and 4/12 BM- patients were PCR positive. Total 13 (50%) patients were positive by LD-PCR. The completion of standard PCR by LD-PCR in FL improved the detection of BCL2/IgH rearrangement from 27% to 50%.

Summary: The LD-PCR is a highly sensitive, reliable and easy-to-use method for molecular detection of t(14;18). It helps to identify patients with atypical breakpoints and proves useful for improving primary diagnosis of FL and monitoring of minimal residual disease.

006 COMBINED ELEVATION OF IL-10, TNF-alpha- AND VEGF PLASMA LEVELS DISCRIMINATE SEVERAL GROUPS OF DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS WITH A MARKEDLY DIFFERENT OUTCOME

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Intoduction: The aim of the study was to assess the combined effect of elevated levels of tumour necrosis factor- (TNFα), interleukin-10 (IL-10),

and vascular endothelial growth factor (VEGF) for clinical characteristics and survival of lymphoma patients.

Methods: One hundred fifty-seven newly diagnosed patients with diffuse large B-cell non-Hodgkins lymphoma (DLBCL) treated in the Department of Hematology, Centre Hospitalier Lyon-Sud, Pierre Benite (France) were analyzed. Plasma samples were collected before treatment initiation and tested by enzyme-linked immunoabsorbent assay (ELISA) to determine cytokines levels.

Results: The plasma IL-10 levels were undetectable in 122 (78%) and elevated to greater than 5 pg/mL in 35 (22%) of DLBCL patients. The median of IL-10 detectable value was 35 pg/mL (range, 5-2480 pg/mL). Plasma TNF α values were determined in 108 lymphoma patients. Median TNF α was 32 pg/mL (range, 3-1836 pg/mL). Plasma VEGF concentrations among 157 DLBCL patients ranged from undetectable (in 40 patients) to 1454 pg/mL (median, 85 pg/mL; mean 150 pg/mL). One fourth of the patients had VEGF greater than or equal to 171 pg/mL (the highest quartile).

The presence of detectable IL-10 levels at diagnosis were correlated with ECOG status ≥ 2 , age > 60 years, elevated serum lactate dehydrogenase (LDH) and $\beta 2$ -microglobulin levels, bulky tumor, B symptoms, anemia and low serum albumin levels. TNF α plasma levels greater than median value were associated with ECOG status ≥ 2 , stage III/IV, elevated LDH and $\beta 2$ -microglobulin levels, bulky tumor, B symptoms, anemia and low serum albumin levels. Elevated VEGF levels were correlated with ECOG status ≥ 2 , stage III/IV, ≥ 2 involved extranodal sites, elevated $\beta 2$ -microglobulin levels as well with B symptoms, anemia and low serum albumin levels. Elevated IL-10, TNF α and VEGF predicted shorter freedom from progression (FFP), $p=.002$, $p<.00001$, $p=.015$ respectively. Shorter overall survival (OS) was associated with elevated plasma levels of IL-10 and TNF α ; $p=.0002$ and $p<.00001$, respectively.

The pattern of association of elevated plasma levels of IL-10, TNF α and VEGF with numerous adverse prognostic factors allowed us to create three distinct risk groups of patients depending on the pre-treatment plasma levels of the cytokines. The low-risk group ($n=43$) compared patients with low levels of IL-10, TNF α and VEGF, the intermediate-risk group ($n=56$) consisted of patients with one or two elevated parameters and to the high-risk group ($n=7$) belonged patients with elevated levels of all three cytokines. Intermediate- and high-risk groups of patients were associated with ECOG status ≥ 2 , stage III/IV, elevated LDH and $\beta 2$ -microglobulin levels, bulky tumour, B symptoms, and low serum albumin levels. FFP survival rates for the low-, intermediate-, and high-risk groups were 84%, 45%, and 14%, respectively (log-rank, $p=.00001$). OS rates in these groups were 82%, 46%, and 28% (log-rank, $p=.00004$). **Conclusions:** Our data suggest the possible use of cytokine-index based on pretreatment levels of IL-10, TNF α and VEGF for monitoring prognosis and clinical outcome in DLBCL patients.

0007 INCREASED VASCULARIZATION PREDICTS FAVORABLE OUTCOME IN FOLLICULAR LYMPHOMA, AND IS NOT MEDIATED BY VEGF OR RELATED TO PROLIFERATION

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Background

Recently we demonstrated that in follicular lymphoma (FL) increased vascularization is associated with improved progression-free and overall survival. This is in contrast with the notion that in most solid and hematological malignancies angiogenesis is related to more aggressive clinical behavior or adverse outcome. Possible explanations of these unexpected findings are that the vasculature in itself does not cause this effect but that it is a reflection of underlying biological features such as proliferation rate and that the mechanisms of angiogenesis in FL might differ from those in solid tumors leading to functionally different blood vessels.

Aim

The aim of this study is to assess the influence of proliferation on the vascularization and to investigate the role of VEGF in angiogenesis in FL.

Methods

Sections of pre-treatment lymph node biopsies of 46 previously untreated patients were immunohistochemically stained with anti-CD34 in order to highlight blood vessels. Microvessel density (MVD) was assessed interactively at 200x magnification in three separate fields located in different vascular hot spots. In 39 cases immunohistochemistry was performed for Mib-1, a marker of proliferation. Scoring was performed by assessing 200 individual cells per follicle in 5 follicles; the number of Mib-1 positive cells

was expressed as a percentage of all assessed cells. Ten sections were selected for VEGF RNA in situ hybridization. This selection was based on the observed MVD: five sections on both extremes of the MVD range were selected. The patients were treated with 8 cycles of cyclophosphamide, vincristine and prednisone in combination with interferon alfa-2b, followed by interferon maintenance therapy in responding patients.

Results

MVD ranged from 10 to 70. The median progression-free survival was 47 months in patients with MVD in the highest tertile and only 13 months in patients with lower microvessel density ($p = 0.02$). Overall survival in patients with low vessel density was 59 months, while in patients with high vessel density median overall survival was not reached ($p = 0.03$). The association of high MVD with improved outcome was independent of the IPI. The percentage of Mib-1 positive cells ranged from 3.8% to 49.2%. There was no correlation between MVD and the percentage of Mib-1 positive cells ($r = -0.257$, $p = 0.129$). There was almost a complete lack of expression of VEGF RNA in the lymphoma specimens, both in sections with low MVD and high MVD.

Summary and Conclusions

In this population of patients with FL, increased vascularization is associated with improved outcome both in terms of progression-free survival and overall survival. Apart from hypoxia, pro-angiogenic molecules derived from proliferating malignant cells themselves can be stimulators of angiogenesis. VEGF, however, seems not to be involved in angiogenesis in FL. Also, the proliferation rate of the lymphomas appears of no influence on tumor vasculature in FL. This points to different pathways of angiogenesis in FL compared to most solid tumors. This could result in functional differences between the vascular bed in FL and solid tumors, responsible for the observed effect on patient outcome.

0008 ABERRANT SOMATIC HYPERMUTATION IS INVOLVED IN THE MOLECULAR PATHOGENESIS OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS

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Background: Post-transplant lymphoproliferative disorders (PTLD) are a heterogeneous group of lymphoproliferations arising in solid organ transplant recipients. The molecular pathogenesis of PTLD has been elucidated only in part. It has been recently shown in diffuse large B-cell lymphoma (DLBCL) of the immunocompetent host and HIV-non-Hodgkin lymphoma that aberrant somatic hypermutation (SHM) activity can affect multiple genetic loci, including the proto-oncogenes PAX-5, Rho/TTF, PIM-1 and c-MYC. Mutations involve 5' untranslated regions as well as coding sequences, are independent of chromosomal translocations to the immunoglobulin (Ig) genes and display features and distribution typical of IgV SHM, suggesting that this process is malfunctioning.

Aim: To investigate whether aberrant hypermutation is associated with PTLD, we performed a mutational analysis of the four genes throughout a spectrum of 25 monoclonal B-cell PTLD. **Methods:** The tumor panel included 4 polymorphic PTLD (P-PTLD), 18 DLBCL and 3 Burkitt lymphoma (BL). Mutational analysis was performed by amplification and direct sequencing of a region spanning up to 1.5 Kb from the transcription start site and previously shown to harbor over 90% of the mutations.

Results: Mutations targeting at least one of these 4 genes were found in 7/25 (28%) PTLD, namely 6/18 (33%) DLBCL, 1/3 BL (not considering c-MYC, which is rearranged to the Ig genes in 100% of the cases) and 0/4 P-PTLD. None of the 7 mutated cases harbored mutations in more than one gene (not considering BL). Three of the 4 genes are altered in a fraction of PTLD. PAX-5 was mutated in 4/25 (16%), c-MYC (not considering BL) was mutated in 1/25 (4%), Rho/TTF was mutated in 1/25 (4%) while PIM-1 was not mutated in any case. Mutations were independent of EBV infection since one or more of the 4 genes were mutated in 3/12 (25.0%) EBV+ PTLD and 4/13 (30.0%) EBV- PTLD. The mutation frequency of each gene in mutated cases ranged from 0.4 to 8.5X10⁻³ bp. Mutations were of somatic origin, as confirmed by analysis of normal DNA from the same patient in selected cases. Mutations were heterozygous and shared features of IgV SHM process, including the predominance of single base pair substitutions ($n=29$) with only one deletion and a preference for transitions ($n=17$) over transversions ($n=13$), with a transition/transversion ratio higher than expected (observed=1.3;

expected=0.5). In c-MYC one mutation was located in the coding exon lead to aminoacid substitution at the transactivation domain of the MYC protein with potential functional consequences. Conclusions: Analogous to DLBCL of the immunocompetent host and HIV-non Hodgkin lymphoma, aberrant SHM maybe also implicated in PTLD pathogenesis. Aberrant SHM displays typical molecular features of IgV SHM, suggesting a derivation from abnormal activity of the physiological IgV SHM process. Aberrant SHM may have relevant functional consequences in PTLD development since it may deregulate gene transcription targeting the regulatory region or may alter the biochemical properties of the protein by introducing aminoacid substitutions

009 STROMA AND INFILTRATING INFLAMMATORY CELLS BUT NOT THE TUMOR CELLS ARE THE SOURCE OF APRIL/TALL-2 EXPRESSION IN HUMAN MALIGNANT LYMPHOPROLIFERATIVE DISORDERS.

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Background: The tumor necrosis factor family member APRIL/TALL-2 has been shown to play an important role as regulator of B-cell homeostasis. In addition up-regulation of APRIL expression has been demonstrated in cell lines as well as primary tumor cells.

Aims & Methods: To learn more about a putative role of APRIL in lymphomagenesis we analyzed in situ APRIL expression by using multi-tissue microarrays including 200 malignant lymphoproliferative lesions and 20 normal tissue biopsies. In addition, APRIL expression was analyzed in a large panel of human B-cell lymphoma and Hodgkins disease cell lines at the mRNA and protein level.

Results: In normal tissue (tonsil, lymph node) as well as in most low-grade B-cell lymphomas occasional APRIL expression was observed in monocyte-like cells around germinal centers. In contrast strong APRIL expression was seen in over 50% of diffuse large B cell lymphoma (DLBCL) and Hodgkins disease (HD). The specificity of the in situ reactivity was confirmed in Western-Blot analysis and blocking experiments. With the use of an antibody reactive with cell-associated APRIL and different antibodies recognizing secreted APRIL, we observed a strong overlap in >95% of the positive cases. However, detailed analysis revealed that cells stained for cell-associated APRIL were confined to the stroma (monocytes, neutrophils, fibroblast-like), while secreted APRIL was found within the extracellular space, concentrated around the tumor cells. Our observation indicates that, first, the source of APRIL in these tumoral lesions are mostly stromal cells, and, second, that secreted APRIL may bind to tumor cells, probably for signaling purposes. APRIL expressing cells are most probably of myeloid origin which is further underlined by in vitro experiments. The absence of APRIL expression in lymphoma cells was further confirmed by RT-PCR analysis, showing very low levels of APRIL expression in a large panel of DLBCL and HD cell lines.

Conclusion: In contrast to previous report suggesting an autocrine loop of APRIL favoring proliferation and survival of lymphoid tumor cells, we found no significant expression of APRIL in tumor cells of a large number of lymphomas analyzed, but rather APRIL expression by tumor-infiltrating inflammatory cells. Ongoing experiments are trying to understand the functional consequence of APRIL expression by stroma cells in high-grade B cell lymphoma and Hodgkins disease.

010 CELL CYCLE REGULATORY GENES EXPRESSION IN DIFFUSE LARGE B-CELL LYMHOMAS

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Background: Diffuse large B-cell lymphoma is an aggressive malignancy of mature B cells with a heterogeneous prognosis. Biological markers have been used in an attempt to improve the discriminative capacity of the International Prognostic Index (IPI). Abnormalities of cell cycle constitute a marker of clonal expansion and may contribute to disease progression; thus the investigation of cell cycle regulatory genes may be of prognostic relevance.

Aim: The aim of the present study was the investigation of gene expression levels of cell cycle regulatory factors in diffuse large B-cell lymphomas and their relation to prognosis.

Methods: The analysis included 26 newly diagnosed cases of diffuse large B- cell lymphomas and 4 cases of non-neoplastic lymphadenopathies used as controls. The IPI was available for all lymphoma cases. RNA from fresh frozen tissue was analyzed by the RNase Protection Assay (RPA) with the following Riboquant Kits (BB Biosciences, Pharmingen): hCC-1 which detected and quantified mRNA for cdk-1, cdk-2, cdk-3, cdk-4, p16, p27 p21, PISSLRE and hCYC-1 for cyclins A,B, C, D1, D2, D3, and A1. The intensity of each band was compared to that of the housekeeping genes GAPDH and L32 using the Image Master Software (Pharmacia).

Results: cdk-1, cdk-2, cdk-3, cdk-4 and p21 were expressed in 6/26, p27 in 5/26, PISSLRE in 14/26 and p16 in 8/26 lymphomas. Cyclin A was expressed in 8/26, cyclin B in 5/26, cyclin C in 9/26, cyclin D1 in 14/26, cyclin D2 in 10/26, cyclin D3 in 11/26 and cyclin A1 in 2/26 lymphomas.

There was not a significant difference in the percentage of positive cases for all cell cycle regulatory genes, between the lymphoma and the control group. Moreover the expression level of each of these genes did not differ significantly between patients and controls and within the lymphoma group between the low and high IPI risk categories.

Conclusion: Cell cycle regulatory genes including cdk's, p21, p16, p27, PISSLRE and cyclins are expressed in some cases of diffuse large cell lymphomas but their expression level was not found to be significantly different from controls or between different risk groups. The analysis of larger number of cases, as well as the simultaneous detection of apoptosis regulating genes, is currently being investigated by our group and may help to clarify the significance of these biological markers in the group of diffuse large B-cell lymphomas.

011 IF IT IS IN THE MARROW, IT IS ALSO IN THE BLOOD.

CONCORDANCE BETWEEN MARROW AND BLOOD EXAMINATIONS IN 826 PAIRED SAMPLES FROM 561 LYMPHOMA PATIENTS

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Background: Bone marrow (BM) examination is a standard procedure in non-Hodgkins lymphoma (NHL) staging and follow-up. However, this procedure is not free of risk and uncomfortable for the patients. **Aims:** We have retrospectively evaluated our consecutive 4-year record of four-color flow cytometry and qualitative PCR evaluation of BM and peripheral blood (PB) samples to investigate concordance between these two parameters in NHL patients.

Methods: A total of 826 BM samples from 561 patients were investigated. PB was also investigated at the time of BM collection. Most frequent diagnoses were follicular NHL (27%), diffuse large B-cell NHL (24%), mantle cell NHL (6%) and marginal NHL (5%).

Results: In 242/826 cases (29%), four-color flow cytometry identified a monoclonal (B-cell NHL) or a phenotypically aberrant (T-cell NHL) cell population. Concordance between BM and PB was 91% (p<0.0001). Most frequent diagnosis of discordant cases was lymphoplasmacytoid-Waldenstrom NHL (41% of discordant cases). Qualitative PCR (sensitivity 10-6) was used to investigate the presence of translocations involving BCL-1 or BCL-2 genes in mantle cell or follicular NHL, respectively. A total of 84/286 samples (29%) were positive. Concordance between BM and PB was 90% (p<0.001). Discordance was found in 11% of mantle cells NHL and 9% of follicular NHL cases.

Summary/Conclusions: Taken together, our data indicate that BM and PB examination by means of four-color flow cytometry and qualitative PCR offers nearly-overlapping results. With the exception of lymphoplasmacytoid-Waldenstrom NHL, PB examination may be considered a sensitive and specific alternative to BM examination in NHL patients.

012 THE 'CUTANEOUS VARIANT' EXHIBITS A FAVORABLE CLINICAL BEHAVIOR WITH RESPECT TO OTHER CLINICAL PRESENTATIONS OF INTRAVASCULAR LYMPHOMA (IVL): THE I.E.L.S.G. EXPERIENCE ON 40 CASES

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Background: Clinical studies on relatively large series of IVL cases are lacking.

Aim: To better delineate clinical forms, histopathological features, natural history, and appropriate therapy of IVL in Western Countries.

Methods: Clinico-pathological data of the largest reported series of HIV-negative patients (pts) with in vivo (n=30) or post-mortem (n=10) diagnosis of IVL were reviewed.

Results: Median age of the 40 pts was 70 yrs (34-90; M:F ratio: 1; ECOG-PS>1 in 25 cases). B-symptoms (52% of cases; fever in 40%), cutaneous lesions (37%) and neurological symptoms (35%) were the most common presenting features. All cases but one were constituted by large B-cells; hemophagocytic syndrome was never observed. Ann Arbor stage was IE in 12 (30%) cases and IVE in 28 (70%). Ten pts (25%) showed a disease limited to the skin after conventional staging (defined as 'cutaneous form' of IVL). Hepatosplenic involvement was observed in 16 cases (40%) and marrow infiltration in 12 (30%), and 4 pts (10%) had nodal disease. Anemia was observed in 25 (62%) cases, thrombocytopenia in 12 (30%), elevated ESR in 16 (40%), and elevated serum LDH in 25/29 (86%) cases.

Twenty-two of the 30 pts diagnosed ante-mortem were treated with chemotherapy (anthracycline-based in 19): response was CR in 10 cases, PR in 3 (ORR=59%); 7 pts experienced PD and two died of toxicity. Seven responders experienced relapse. Three pts treated without anthracycline showed early PD. High-dose chemotherapy supported by ASCT was used as consolidation after conventional chemotherapy in two pts, who are relapse-free at 19 and 47 months, and at relapse in the other two, who died early. Five pts treated with chemotherapy had CNS involvement: four of them (CHOP in 3, CVP in one) died early; the fifth pt was treated with MACOP-B regimen followed by high-dose chemotherapy and ASCT, being relapse-free at 19 months from diagnosis. Eight pts with in-vivo diagnosis did not receive chemotherapy: 4 died early due to IVL or complications, one died without disease progression at 24 months; three young women with 'cutaneous form' are alive and NED at 7-14 years. Overall, 11 pts are alive (9 NED) with a median follow-up of 41 months, and a 3-yr OS of 24%_{95%CI}.

Pts with 'cutaneous form' were all females with normal platelet and leukocyte counts and ECOG-PS<2, exhibiting a better outcome than the others (3-yr OS: 5616% vs. 157%; p=0.0004). In multivariate analysis, ECOG-PS<2, 'cutaneous form' and stage-I disease were independent favorable prognosticators.

Conclusions: Hepatosplenic and bone marrow involvement is an unexpectedly common feature in IVL. The frequent involvement of the brain and skin and the absence of hemophagocytic syndrome are the main differences with respect to Japanese series. Therapeutic outcome is disappointing; early consolidation with high-dose chemotherapy and ASCT may improve survival in young pts with unfavorable features. Drugs with a higher CNS bioavailability should be used in cases with brain involvement. IVL limited to the skin ('cutaneous form') exhibits distinctive clinical properties that deserve to be better characterized on biological grounds.

013 IGVH MUTATIONAL STATUS, GENE USAGE AND IMMUNOPHENOTYPE IN MANTLE CELL LYMPHOMA/LEUKAEMIA.

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BACKGROUND. Mantle cell lymphoma/leukaemia (MCL) has been re-designated recently as an aggressive non-Hodgkins lymphoma. Median

survival is in the region of three years, although gene expression profiling has identified two major subgroups, with median survivals of 0.8 years and 6.7 years, on the basis of proliferation gene expression. We have reported previously on two major immunophenotypic subgroups in MCL, one found in approximately 40% of our patients, with a typical B-CLL immunophenotype, whilst the other showed stronger expression of CD20, CD22, CD79b, FMC7 and CD38, and weaker expression of CD23 and CD43, than typical B-CLL.

AIMS. This study was designed to compare IgVH mutational status, gene usage and immunophenotype in MCL, to determine if associations existed between mutational status and/or gene usage, and immunophenotypic subgroup.

METHODS. Thirteen patients were studied. All had atypical, pleomorphic morphology. t(11;14) was confirmed in all patients by PCR and/or FISH. Mutational status and gene usage were determined using BIOMED-2 protocol and primers. Immunophenotyping was performed by direct two or three colour labelling, and analysis on a Becton Dickinson FACScan. **Results:** Seven patients had unmutated IgVH genes whilst, in the remainder, IgVH genes were mutated. Seven patients had a typical MCL immunophenotype but this was not the same group of patients as that with unmutated IgVH genes. Three patients with unmutated IgVH genes had a B-CLL immunophenotype whilst the remaining four had a typical MCL pattern. Of the six patients with mutated IgVH genes, two had a B-CLL immunophenotype and the remaining four had a MCL pattern. Gene usage showed no clear-cut pattern but VH1-8, VH3-21 and VH3-9 were only found patients with the MCL immunophenotype.

CONCLUSIONS. IgVH mutational status and gene usage showed no clear-cut associations with immunophenotypic sub-groups in this series of MCL patients. Despite earlier expectations based on the concept of the MCL cell as pre-germinal centre, six out of thirteen MCL patients had mutated IgVH genes. These findings confirm reports of other groups. Immunophenotypic sub-type does not appear to be associated with other prognostic markers examined in this study. However, B-CLL immunophenotype should not be used as the sole evidence for excluding a diagnosis of MCL as it can be found in a sub-group of MCL patients. It remains to be determined if gene expression profiling sub-groups will correlate with these immunophenotypic sub-groups.

014 DETECTION OF TCR-GAMMA AND TCR-DELTA GENE REARRANGEMENT BY PCR IN SKIN AND PERIPHERAL BLOOD OF PATIENTS WITH EARLY MYCOSIS FUNGOIDES.

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Background:

At diagnosis a dominant T-cell receptor (TCR) gene rearrangement can be detected in the skin in 50-75% of patients with early MF; an identical T-cell clone is detectable also in the peripheral blood in 15%, according to the literature data regarding TCR-gamma (TCR-g) gene rearrangement analysis and this evenience seems to be correlated with a higher risk of progression. Compared to TCR-gamma, TCR-delta (TCR-d) gene has been very rarely studied by PCR in MF.

Aims: The purposes of this study were to analyse the pattern of TCR-d gene rearrangement in the skin and peripheral blood lymphocytes of 11 early MF patients, and to compare the obtained results with those of TCR-g analysis performed in 14 cases. Other aims were to explore the prognostic significance of a T-cell clonality both in skin and blood specimens at the time of diagnosis.

Methods: PCR products for TCR-g and TCR-d rearrangement were visualised, respectively, by 10% polyacrylamide gel electrophoresis and high resolution electrophoresis on automated DNA sequencers ABI PRISM 310.

Results:

A monoclonal TCR-d rearrangement was detected in all 11 examined cases in the skin; 4 cases showed the same clone also in the peripheral blood. Two molecular patterns seemed to be prevalent, as Vd3-Jd3 was observed in 7 patients and Vd1-Jd1 in 4. These patterns were also associated with a Vd2-Jd1 mono/oligoclonal expression in 2 cases in the peripheral blood.

Out of the 14 cases analysed by TCR-g PCR, including the 11 studied for TCR-d, a dominant clone was observed in 13 in the skin biopsies and in 7 also in the peripheral blood.

TCR-d PCR analysis has allowed to detect monoclonality in 100% of the cases in the skin and an identical clone in the peripheral blood in 37% cases, whereas TCR-g analysis detected monoclonality in 93% of skin samples and the same dominant clone in peripheral blood in 50% of the cases: our detection rates for TCR-g seem to be higher than those reported in the literature and it is probably related to the combination of primers utilised for J1/2 and JP1/2 regions. All the 14 patients analysed by TCR-g PCR were included in a controlled and prospective study exploring the activity of low-dose IFN α + PUVA administered for 14 months. At last follow-up, all 6 patients without monoclonal peripheral blood T lymphocytes, showed an overall response (83% CR and 17% PR), whereas out of the 7 patients with a circulating clone, one showed a CR (14%), 3 a PR (43%), 2 SD (29%) and 1 PD (14%).

Conclusions:

On the basis of our results on TCR-d and TCR- γ analysis, we can conclude that T-cell clones are frequently detected also in the peripheral blood in early MF and that their presence at diagnosis seems to be associated with a worse response to therapy. Finally, the occurrence of a similar VJ-d TCR profile suggests the involvement of constant unique antigens, currently unknown, in the pathogenesis of the disease.

015 ROLE OF CD38 EXPRESSION IN B-CELL NON-HODGKINS LYMPHOMAS IN LEUKEMIC PHASE: CLINICO-BIOLOGICAL CORRELATIONS

A. Pulsoni, A.R. Guarini, M.S. De Propriis, N. Frattarelli, E. Cavalieri, V. Gianfelici, F. Mancini, F. Cucchi, F.R. Mauro, R. Foà
Ematologia Università 'La Sapienza', ROMA, Italy

Background: Few data are currently available concerning the prognostic significance of CD38 expression in non-Hodgkins lymphomas (NHL). Several reports have suggested an association between CD38 expression and worse prognosis in B-cell chronic lymphocytic leukemia (CLL), especially when associated with an unmutated immunoglobulin gene status. The improvement of immunophenotypic techniques allows nowadays to recognize a growing proportion of cases which cannot be defined as typical CLL.

Aims: To evaluate the incidence and prognostic role of CD38 expression in patients with histologically documented B-cell NHL in leukemic phase. Methods/Results: Between January 2001 and December 2002, 78 consecutive, previously untreated patients with NHL in leukemic phase with immunophenotypic features that differed from those of typical CLL (CD5+, slg+ (weak), CD23+, CD79b-, FMC-7-) were studied. In all patients, a specific histologic diagnosis was obtained by lymph node biopsy (23 cases), by bone marrow trephine needle biopsy (12 cases) or both (43 cases). The series included 52 males and 26 females. The median age of the whole population was 58 years, ranging between 36 and 81 years. The clinical and biologic features of the patients enrolled in the study and the response to treatment were analyzed and compared according to CD38 expression. A significantly higher proportion of CD38+ cases was found in the aggressive with respect to the indolent histotypes (diffuse large B-cell and mantle cell NHL vs. small lymphocytic, marginal zone, lymphoplasmacytic, follicular and unspecified small cell lymphoma: 75% vs. 45%; $p < 0.024$). A higher proportion of patients with WBC $> 20,000$, Plts $< 100,000$, increased LDH, more than 5 involved lymph nodes, early need of treatment, was found among CD38+ cases. No relationship between CD38 expression, splenomegaly and presence of bulky disease was found. With regard to cell morphology, a higher proportion of typical small lymphocytes was observed among the CD38 negative population. Furthermore, among those patients for whom a watchful waiting policy was possible, the therapy-free duration was shorter in CD38+ patients. The short follow-up does not allow to conclusively assess the prognostic importance of CD38 expression on survival. CD38 expression was also evaluated on the normal T-cell population to explore the possibility of a protective effect of an activated T-cell response. A lower count of CD38+ T-cells was found in large B-cell NHL.

Conclusions: Our findings suggest that a higher CD38 expression is more frequently observed in the more aggressive histotypes of NHL in leukemic phase and that this is associated with poor prognostic clinical features. Further studies and a more prolonged observation time are needed to fully assess the prognostic role of CD38 expression in patients with leukemic NHL.

LYMPHOMA: TREATMENT WITH MONOCLONAL ANTIBODIES

016 FRACTIONATED RADIOIMMUNOTHERAPY WITH DOTA-CONJUGATED, 90Y-RADIOLABELED, HUMANIZED ANTI-CD22 MONOCLONAL ANTIBODY (EPRATUZUMAB) APPEARS SAFE AND EFFICACIOUS ACROSS NHL PATIENT GROUPS

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⁵University Hospital Dresden, DRESDEN, Germany

⁶Immunomedics, Inc., MORRIS PLAINS, United States of America

Background: A fractionated schedule of delivery may prove safe and efficacious, with tolerable hematologic toxicity allowing a higher total radioimmunotherapy dose to be administered.

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Methods: Eligible pts had $< 25\%$ BM involvement, plts $> 100,000$ cells/mm³, and measurable disease by CT with no single mass > 10 cm. Pts received 1.5 mg/kg/wk epratuzumab with 111In-epratuzumab co-administered the 1st wk. Twenty-six pts with a median of 3 prior treatments have been treated, including 15 pts without prior bone marrow transplant (BMT) at 5-10 mCi/m²/wk (total 90Y dose, 15-22.5 mCi/m²) and 11 pts with prior BMT escalated separately at 2.5-5 mCi/m²/wk (total 90Y dose, 5-10 mCi/m²).

Results: Dose escalation is continuing, with no serious AEs considered treatment-related. One pt in each escalation arm had protocol-defined DLT (> 12 wk platelet recovery); otherwise, therapy was well tolerated with no significant toxicity besides transient hematologic depression (19 pts with Grade 1-2 depression, 7 pts with Grade 3 thrombocytopenia/leukopenia, 1 pt with Grade 4 leukopenia after salvage chemotherapy). 111In imaging demonstrated antibody tumor targeting, and hot/cold PK demonstrated stability of DOTA chelation > 1 wk. In 26 pts with treatment evaluations, 13 pts (53%) had an objective response (OR) by IWG criteria, including pts with and without BMT [5/11 (45%) and 8/15 (53%), respectively], with indolent and aggressive disease [8/11 (73%) and 5/15 (33%), respectively], across histologies [follicular NHL, 9/13 (69%); DLCL, 1/6 (17%); mantle cell, 3/7 (43%)], and in pts failing rituximab (10/19, 53%). Most OR's were complete responses (CR/CRu, 10/13, 77%), and 5/7 pts assessed after CR onset now have responses > 6 mo, including 2 pts continuing > 1 yr. Four pts have now been retreated without additional toxicity.

Conclusion: This study shows the feasibility and safety of a dose-fractionation schedule of a 90Y-labeled anti-CD22 Mab, obtaining therapeutic responses across all pt groups, including durable complete responses, and achieving higher cumulative doses than other RAIT agents given as single doses. These results justify continuing current dose escalation and opening a third escalation arm for patients with $> 25\%$ bone marrow involvement.

017 RITUXIMAB IN PATIENTS WITH HAIRY CELL LEUKEMIA RELAPSING AFTER TREATMENT WITH 2-CHLORODEOXYADENOSINE (2-CDA)

R.Z. Zenhäusern¹, A. Von Rohr², K. Rufibach³, M. Solenthaler⁴, S. Meyer-Monard⁵, A. Gratwohl⁶, U. Hess⁶, T. Cerny⁶, M. Bargetzi⁶, T. Kovacovic⁷, L. Leoncini⁸, A. Tobler⁴

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⁸Oncology, BELLINZONA, Switzerland

Hairy cell leukemia (HCL) is a chronic B-cell leukemia. Patients (pts.) commonly present with pancytopenia, splenomegaly and marrow infiltration. Splenectomy is generally palliative, while treatment with interferon alpha (IF) leads to partial remission (PR) in 30-70% and complete remission (CR), often of short duration, in 5 to 10% of pts.

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Hairy cell leukemia (HCL) is a chronic B-cell leukemia. Patients (pts.) commonly present with pancytopenia, splenomegaly and marrow infiltration. Splenectomy is generally palliative, while treatment with interferon alpha (IF) leads to partial remission (PR) in 30-70% and complete remission (CR), often of short duration, in 5 to 10% of pts.

Use of the purine analogue 2-CDA, considered the treatment of choice, induces CR in up to 85% of pts. and PR in 5 to 25%. Therapy with Rituximab, a chimeric monoclonal antibody against CD20, induces responses in a half of the pts. with relapsed low-grade lymphomas. CD20 is present on human B lymphocytes as well as on lymphocytes of mature B-cell lymphoma and leukemia. We thus investigated the efficacy and safety of rituximab in pts. with HCL relapsing after 2-CDA (SAKK 31/98). Patients and methods: A total of 26 pts. were included. The clinical documentation of a patient who died after the first infusion was never made available. Of the other 25 pts. 24 had a classical HCL, one a prolymphocytic subtype. There were 20 men and 5 women with a median age of 58 years (range 38-83 y.). All pts. were pretreated (2-CDA n=25, splenectomy n=4, IF n= 9, chemotherapy n= 2). Nine had relapsed and 16 had progressive disease after 2-CDA. Median blood counts at study entry: Leucocytes 2.8 G/l (range 0.4-52.8 G/l), platelets 95 G/l (3-330 G/l), haemoglobin 132 g/l (81-163 g/l). 4 courses of rituximab, 375 mg/m² i.v. at weekly intervals were given.

Response (n=26): First follow-up visit: CR 19% (95% CI, 7-39), CR+PR 50% (95% CI, 30-70). Best response over all follow-up visits: CR 27% (95% CI, 12-48), CR+PR 58% (95% CI, 37-77). The median relapse-free survival was 15.9 months (95% CI, 8-19.2 months), the median remission duration 21.2 months.

Toxicities: One patient died one day after the first rituximab infusion due to respiratory and cardiac failure (possibly related to rituximab). Another patient died of gastrointestinal bleeding 12 weeks after the 4th treatment course; this death was considered to be unrelated to Rituximab. 2 pts. had infection grade 3, 7 pts. leucocytopenia grade 3 and 4.

Conclusion: Rituximab has significant efficacy and minimal toxicity in pts. with HCL relapsing after 2-CDA. Special attention should be given before starting therapy with the drug to pts. with underlying cardiac disease.

018 HIGH CLINICAL AND MOLECULAR RESPONSE AFTER A BRIEF CHEMO-IMMUNOTHERAPY FND + RITUXIMAB IN ELDERLY PATIENTS WITH DE NOVO ADVANCED STAGE FOLLICULAR LYMPHOMA (FL)

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Background and aims: Elderly patients with advanced stage FL are usually managed with palliative treatments. The addition of Rituximab to Fludarabine containing regimens may enhance response and its duration and might allow to reduce the amount of chemotherapy. This study aimed at defining a brief chemo-immunotherapy plan specifically devised for elderly patients.

Methods: from March 1999 to March 2003, 80 elderly patients (age >60) with advanced stage FL at diagnosis were enrolled. Treatment consisted in: 4 courses of FND (Fludarabine 25 mg/m² days 1-3, Mitoxantrone 10 mg/m² day 1 and Dexamehasone 20 mg days 1-3) followed by 4 Rituximab infusions at 375 mg/m²/week; patients in partial remission received 2 further FND and 2 Rituximab infusions. PCR molecular monitoring for the presence of IgH/Bcl2 and/or Ig heavy chain gene rearrangement was performed at the beginning of the treatment, after FND, after Rituximab and during follow-up time on bone marrow (BM) samples.

Results: median age was 65 (range 60-78); 42 males and 38 females; 13% had stage II, 12% stage III and 75% stage IV disease; 64% had bone marrow involvement; 42% had bulky disease, 28% had 2 extranodal sites and 27% were at high risk according to IIL score. PCR molecular analysis was performed in 54 patients at diagnosis: 63% were Bcl2 rearranged and 37% were not; a IgH rearrangement was detected in 35% of Bcl2 negative patients. Up to date, 64 patients are evaluable. Clinical response rates at the end of the whole treatment program was: complete remission (CR) 72%, complete remission unconfirmed (CRu) 12%, partial remission (PR) 5%, non response (NR) 10% and toxic death (TD) 1%. Complete responses (CR+CRu) increased from 44% after FND to 84% with the addition of Rituximab. Seventy per cent of responding patients did so with a brief chemo-immunotherapy (4 FND + 4 Rituximab). A thorough molecular analysis is ongoing, to date a molecular marker of disease was detectable in 28 patients. After FND 7/28 patients did not show anymore BM molecular disease, while PCR negativity was achieved in 22/28 patients after Rituximab treatment. PCR negative status was strongly associated with clinical CR: all PCR negative patients were in CR compared to 66% of PCR positive ones (p<0.05). With a median follow-up of 24 months, 2-yr progression free survival was 62% for the whole series of patients. Among the 28 patients who had a

molecular monitoring, progressions were seen more frequently in PCR+ patients: 3/6 progressed compared to only 2/22 PCR- patients. The program was entirely performed in an outpatient setting. The only severe toxicity observed was neutropenia in 22% of FND courses, but only two patients developed bacterial infection and one severe diabetic patient died of neutropenic sepsis during FND chemotherapy. Rituximab toxicity was mild. Conclusions: a brief course of chemo-immunotherapy is effective with low toxicity in elderly patients with advanced stage FL. Clinical and molecular responses are improved by the addition of Rituximab to FND. The duration of clinical response is promising and PCR- patients seem to have a lower progression rate.

019 SUBSEQUENT THERAPY FOR NON-HODGKINS LYMPHOMA IS FEASIBLE AFTER RADIOIMMUNOTHERAPY WITH YTTRIUM-90 IBRITUMOMAB TIUXETAN (ZEVALIN)

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Background: Yttrium-90 ibritumomab tiuxetan (Zevalin®) is a first-in-class, dual action radioimmunotherapy approved by the FDA and the European Commission for the treatment of patients with relapsed or refractory follicular B-cell non-Hodgkins lymphoma (NHL). In clinical trials, Zevalin has achieved consistent overall response rates (ORR) of 73% to 83%, and a complete response/complete response unconfirmed (CR/CRu) rate of 34% to 51%. In patients with low-grade NHL, the ORR and CR/CRu rate was 80% and 34%, respectively.

Aims: This analysis addresses the question of whether prior therapy with Zevalin impacts the feasibility of administering subsequent therapies for NHL.

Methods: Of the 725 patients treated with Zevalin in clinical trials to date, information on the first antilymphoma therapy subsequent to Zevalin was recorded for 254 patients.

Results: Efficacy data are available for 171 patients who received subsequent NHL treatment after Zevalin therapy; of these, 95 patients (56%) responded to future therapy (see Table). In a separate analysis, we compared the safety of subsequent chemotherapy (CT) in patients previously treated with Zevalin (n = 41) versus age-, sex-, and histology-matched historical controls previously treated with CT (n = 40). Subsequent CT was similarly tolerated in both groups, with no significant differences in the incidence of grade 4 cytopenias, neutropenic fever, transfusion requirements, or hospitalization rate.

Summary/Conclusions: Zevalin radioimmunotherapy does not preclude subsequent safe and effective therapeutic options for patients with relapsed or refractory low-grade B-cell NHL, including chemotherapy, bioimmunotherapy, and radiotherapy.

	N	Responders (%)	Nonresponders
All therapies	171	95 (56)	76
Chemotherapy	82	37 (45)	45
Alkylator ± prednisone	5	3 (60)	2
CHOP	6	3 (50)	3
CVO or COP	6	5 (83)	1
Purine analogs	16	6 (100)	11
Other aggressive	49	21 (43)	28
Bioimmunotherapy	41	24 (59)	17
Radioimmunotherapy	46	32 (70)	14
ASBT	2	2 (100)	0
Peripheral stem cells			

Caption 1: Efficacy of first therapy subsequent to Zevalin

020 A PHASE I TRIAL OF TWO SEQUENTIAL DOSES OF ZEVALIN RADIOIMMUNOTHERAPY FOR RELAPSED LOW-GRADE B-CELL NON-HODGKIN'S LYMPHOMA

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Background: Yttrium-90 ibritumomab tiuxetan (Zevalin) is a radioimmunotherapy agent that combines the tumor targeting power of an anti-CD20 monoclonal antibody and the cytotoxicity of yttrium-90 beta radiation. Zevalin radioimmunotherapy is approved in both the United States and Europe for the treatment of relapsed or refractory follicular B-cell non-Hodgkin's lymphoma (NHL). Multiple studies using single doses of Zevalin have demonstrated an 80% overall response rate (ORR), with a 30% complete remission (CR) rate. Currently, there are no data available on the safety and efficacy following a second dose of Zevalin.

Aims: To determine the maximum tolerated dose of Zevalin that can be administered following standard dose Zevalin given 12 to 24 weeks apart for patients with relapsed low-grade CD20+ NHL.

Methods: Patients were required to have relapsed after chemotherapy and have measurable disease, an absolute neutrophil count (ANC) ≥ 1500 , a platelet count (PLT) $\geq 150,000$, and $<25\%$ marrow involvement. All patients were required to have peripheral blood stem cells cryopreserved ($\geq 2 \times 10^6$ CD34+ cells/kg) prior to Zevalin treatment. Patients were treated with the standard Zevalin dose (0.4 mCi/kg; maximum 32 mCi) and assessed for response at 3 months. Responders (CR/PR) were eligible for re-treatment with Zevalin with cohorts receiving 0.2 mCi/kg or 0.3 mCi/kg. No prophylactic growth factors were used during this study. Protocol-defined dose-limiting toxicity (DLT) was grade 4 ANC (<500) or PLT ($<10,000$) for ≥ 14 days or grade 3 ANC (500 to 1000) or PLT (10 to 50K) for ≥ 28 days or requiring stem cell re-infusion.

Results: Eighteen patients were enrolled and received the first dose of Zevalin (0.4 mCi/kg). The ORR was 83% (15/18), with 3 patients progressing prior to the second dose. Fifteen patients received a second dose of Zevalin patients at 0.2 mCi/kg and 6 patients at 0.3 mCi/kg. There was no DLT at the 0.2 mCi dose, but 2/6 patients experienced hematologic DLT at the 0.3 mCi dose; thus, the MTD of 2 sequential doses of Zevalin is 0.4 mCi/kg followed by 0.2 mCi/kg. No patients required the re-infusion of stem cells, as there was no significant nonhematologic toxicity. Of the 15 patients who received the second dose of Zevalin, 4 patients eventually progressed, and the other 11 (61% of the original 18) remained in remission up to 27 months from the first dose without any additional therapy.

Summary/Conclusions: Zevalin re-treatment at a dose of 0.2 mCi/kg for patients responding to an initial standard dose of Zevalin (0.4 mCi/kg) is safe and well tolerated. There was no additional toxicity with the second dose of Zevalin other than reversible myelosuppression.

021 RITUXIMAB PLUS CLADRIBINE IN HEAVILY PRETREATED LOW-GRADE LYMPHOMA

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Background: Cladribine (2-CdA) and rituximab (RIT) are active agents in indolent lymphoid malignancies. The combination of RIT with 2-CdA induces additive pro-apoptotic effect in vitro.

Aims: The aim of the study was to evaluate the feasibility, efficacy and toxicity of the combined regimen consisting of RIT and 2-CdA (RC regimen) in the treatment of patients with advanced B-cell chronic lymphoid malignancies, such as chronic lymphocytic leukemia (CLL) and low-grade non-Hodgkins lymphoma (LG-NHL).

Methods: The study was performed in adult patients with CLL or LG-NHL, with confirmed expression of CD20 antigen on tumor cells, who had refractory or recurrent disease. The treatment consisted of RIT at a dose of 375 mg/m² on day 1 and 2-CdA at a dose of 0.12 mg/kg in 2h infusion given on days 2-6. The courses were repeated at 4 week intervals or longer if severe myelosuppression occurred.

Results: Thirty one patients, 17 with CLL and 14 with LG-NHL were enrolled to the study. Ninety cycles of RC with median of 3 cycles (range 1-5 cycles) per patients were administered. Four patients (12.9%) (95% CI 1.1- 24.6%), 1 with CLL and 3 with LG-NHL, achieved a complete response (CR). Eighteen patients (58.1%) (95% CI 40.775.5%) including 12 with CLL and 5 with LG-NHL, had a partial response (PR). Overall response rate (OR) was 71.0% (95% CI 55.0-86.9%) in the whole group. The median failure free survival (FFS) of responses was 11.0 months. Hypersensitivity to RIT was the major toxicity of RC regimen

and occurred in 10 patients (32.2%) mostly during the first infusion of RIT. Severe neutropenia (grade III) was seen in 3 patients (9.7%) and thrombocytopenia in 2 patients (6.4%). Five (16.1%) episodes of grade III-IV infections were observed. There was no treatment related mortality.

Conclusion: The RC regimen is highly effective in heavily pretreated patients with indolent lymphoid malignancies and its toxicity is relatively low.

This work was supported by the grant No 515-02-003 from the State Committee for Scientific Research, Poland

022 COMBINATION OF THE ANTI-CD20 ANTIBODY RITUXIMAB WITH SEQUENTIAL HIGH-DOSE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CELL SUPPORT IN RELAPSED AND REFRACTORY AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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Background: Combination chemotherapy can cure patients (pts) with Non-Hodgkin's lymphoma (NHL), but those with relapse still have a poor prognosis. High-dose chemotherapy (HDCT) with autologous stem cell support (ASCT) can improve the outcome of these pts as shown in the precursor study with response rates of 46% (32% CR, 14% PR) and FTF/OS of 20%/40% at the final evaluation (Data recently published). Chemosensitivity and achievement of minimal disease status prior to HDCT are important prognostic factors in NHL pts treated with HDCT. Rituximab demonstrated encouraging activity in aggressive NHL and showed low toxicity in the setting of combined immunochemotherapy. Patients and methods: We modified the intensified salvage program by addition of rituximab to the chemotherapy cycles followed by a final myeloablative course with stem cell reinfusion. Eligibility criteria include pts with age 18-65 years and eligible for HDCT with histologically proven CD20+ relapsed NHL. Treatment program consists of two cycles DHAP (dexamethasone, cytarabine, cisplatin) plus rituximab (375mg/m²); pts with PR or CR receive cyclophosphamide (4g/m²) plus rituximab followed by PBSC harvest; methotrexate 8g/m² and vincristine 1.4mg/m² plus rituximab; and etoposide 2g/m² plus rituximab. The final myeloablative course is BEAM plus rituximab followed by ASCT.

Results: 20 pts (median age 57 years, range 22-65) with relapsed (16 pts) and refractory (4 pts) aggressive NHL have been enrolled (stage I/II: 6, stage III/IV: 11, NA: 3). 18 pts had CHOP or CHOP-like regimens as first-line therapy, one pt. was treated with fludarabine + cyclophosphamide + alemtuzumab and one with BEACOPP. The median time to progression was 9 months. This chemoimmunotherapy combination regimen was well tolerated in all pts without side effects exceeding the toxicity expected from chemotherapy alone. 19/20 pts were available for restaging after 2 cycles DHAP with 2 CR, 12 PR, 2 SD and 3 PD, one patient died on myocardial infarction. At the final response evaluation from 9/13 pts were in CR, 3/13 in PR and 1/13 was not available. Treatment was discontinued in one pt after HD'cyclophosphamide due to severe heart failure.

Conclusion: The preliminary results suggest feasibility and safety of this regimen with a overall response rate of 60% (45% complete remission and 15% partial remission); toxicity was tolerable. The combination regimen allows effective mobilization of stem cells and the tolerability of the final myeloablative BEAM was not affected by rapid sequential administration of DHAP and high doses of cyclophosphamide, methotrexate and etoposide, each in combination with rituximab.

023 EARLIER YTTRIUM-90 IBRITUMOMAB TIUXETAN (ZEVALIN) TREATMENT IN PATIENTS WITH PREVIOUSLY-TREATED B-CELL NON-HODGKINS LYMPHOMA: HIGHER RESPONSE RATES AND LONGER DURATIONS OF RESPONSE

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Background: Yttrium-90 ibritumomab tiuxetan (Zevalin®) is a first-in-class, dual action radioimmunotherapy that combines the tumor targeting

power of an anti-CD20 monoclonal antibody and the cytotoxicity of yttrium-90 radiation. Zevalin is approved in both the United States and Europe for the treatment of relapsed or refractory follicular B-cell non-Hodgkin's lymphoma (NHL), including patients with rituximab-refractory NHL.

Aims: To compare the safety and efficacy of Zevalin in patients that received 1 prior therapy (second-line) to patients who received ≥ 2 prior therapies (third-line and subsequent therapy).

Methods: An integrated analysis was used to compare the results from 211 patients from 4 different clinical trials.

Results: A total of 63 patients received Zevalin as second-line therapy, and 148 patients received Zevalin as third-line or later therapy (range: 29 prior therapies). Patient characteristics were similar between the 2 groups; however, the rate of lymphomatous marrow involvement in patients receiving second-line therapy was greater than in those receiving later therapy (57% vs 39%; $P = .023$), and a trend towards bulkier disease in patients receiving later therapy versus second-line therapy. Overall response rates (ORR) in patients receiving second-line therapy were greater than in those receiving later therapy (86% vs 72%; $P = .051$), with a corresponding improvement in complete response/complete response unconfirmed rates (CR/CRu) (49% vs 28%; $P = .004$). Similar improvements were seen in median time-to-progression (TTP) (12.6 vs 7.9 months; $P = .038$) and median duration of response (DR) (13.7 vs 8.2 months; $P = .163$) for patients receiving second-line therapy compared to those receiving later therapy, respectively. In patients achieving a CR/CRu following second-line therapy, TTP was 23.9 months and DR was 22.8 months, while patients receiving later therapy had a TTP and DR of 15.6 and 14.6 months, respectively. In patients with follicular NHL ($n = 169$), ORR in patients receiving second-line therapy ($n = 116$) was greater than in those receiving later therapy (90.6% vs 79.3%; $P = .082$), as were CR/CRu rates (54.7% vs 30.2%; $P = .004$). Moreover, patients with follicular NHL receiving second-line therapy had a longer TTP (14.9 vs 9.2 months; $P = .038$) and DR (14.2 vs 8.2 months; $P = .093$) compared to patients receiving later therapy. Overall, Grade 3/4 adverse events (AEs) were primarily hematologic in nature, with comparable incidence rates for both groups, and nonhematologic AEs were typically Grade 1 or 2 in severity.

Summary/Conclusions: Analysis of these results demonstrated that patients treated with Zevalin as second-line therapy for B-cell NHL had higher response rates and a longer DR compared to those treated with Zevalin after ≥ 2 prior therapies. The benefits of earlier therapy with Zevalin were particularly notable among patients with follicular NHL.

024 DELAYED-ONSET PERIPHERAL BLOOD CYTOPENIA AFTER RITUXIMAB. FREQUENCY AND RISK FACTOR ASSESSMENT IN A CONSECUTIVE SERIES OF 77 TREATMENTS

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Introduction: The occurrence of unexplained peripheral blood cytopenia, particularly neutropenia, has been reported after rituximab (RTX). Its prevalence may be underestimated since it may occur late after treatment.

Aims: We analysed all cases of unexplained cytopenia occurring in a unselected series of patients treated with RTX to evaluate its prevalence and significance.

Patients and Methods: Seventy-seven courses of RTX given to 72 consecutive patients (M/F 32/40) affected by NHL (DLCL 38, secondary DLCL 6, FL 19, Lymphoplasmacytic 3, MCL 3, Burkitt 2, MZL 1) and treated from January 2002 to May 2003 at a single Center were evaluated. RTX was given alone (9 cases) or associated with chemotherapy (68) and autologous stem cell transplantation (18, in vivo purged in 12). Forty-three cases were at presentation, whereas 18 and 16, in first or subsequent relapses, respectively. Median age was 60.5 (range 18-85). Marrow was involved in 20/77 (26%). RTX dosage was 375 mg/sqm and administrations number ranged between 2 and 6 (median 4). All cases of unexplained WHO grade 2-4 cytopenia were recorded, occurring 21-180 days after the last dose of RTX or of chemotherapy/stem cell transplantation. Median number of blood cell counts performed in single patients during the study period was 3 (range 1-21).

Results: Twenty cases of cytopenia (26%) were observed. Neutropenia developed in 18 cases (23%) (WHO grade II: 4, grade III: 6, grade IV: 8), thrombocytopenia in 9 (12%) (grade II: 2, grade III: 3, grade IV: 4),

anemia in 7 (9%) (grade II: 4, grade III: 2, grade IV: 1). Cytopenias were combined in 9 cases (trilinear 5, neutropenia+thrombocytopenia 3, neutropenia+anemia 1) and isolated in 11 cases (neutropenia 9, thrombocytopenia 1, anemia 1). Marrow findings were aspecific except for showing PRCA in one anemic and aplasia in one pancytopenic patient. The median time between the last RTX dose and cytopenia detection was 7 weeks (range 3-20) for neutropenia, 4 weeks (range 3-15) for thrombocytopenia and 5 weeks (range 3-11) for anemia. Treatment was observation +/- corticosteroids +/- supportive therapy, including G-CSF in 9/18 neutropenic patients. Infections occurred in 4/18 (22%) neutropenics (1 bacterial pneumonia, 1 TBC, 1 CMV reactivation, 1 PCP+CMV reactivation), compared to 3/59 (5.2%) among non neutropenics ($p=0.03$) (1 perianal abscess, 1 PCP, 1 aspergillosis). One pancytopenic patient died of infection. Cytopenia eventually resolved in 10 of 15 evaluable patients after a median of 10 weeks (range 1-23+). Development of cytopenia was associated with relapsed NHL (14/34, 41.2% vs 6/43, 13.9%) ($p=0.005$), higher number of RTX doses (RTX courses > 5 : 8/18 [44.4%] vs < 4 : 12/59 [20.3%] $p=0.04$), stem cell transplantation (9/19 [47.3%] vs 13/58, [22.4%]) ($p=0.03$). Bone marrow involvement was not a risk factor for cytopenia (5/20 [25%] vs 15/57 [26.3%]) as were age, sex, and histology. **Conclusions:** We confirm that delay-onset neutropenia, as well as anemia and thrombocytopenia, may develop after RTX treatment + chemotherapy/stem cell transplantation, causing an increased risk of infection. Stem cell transplantation, relapsed lymphoma and > 5 courses of rituximab were risk factors for cytopenia development.

025 COMBINATION OF EPRATUZUMAB PLUS RITUXIMAB IN RELAPSED/REFRACTORY INDOLENT AND AGGRESSIVE NHL: MULTI-CENTER, PHASE-II UPDATE

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Background: The humanised anti-CD22 antibody, epratuzumab has shown single agent anti-lymphoma activity in indolent and aggressive NHL in Phase I/II studies, and a small single-center Phase I/II study of epratuzumab plus rituximab demonstrated very promising efficacy results in patients with refractory NHL.

Aims: A multi-center study to confirm that a combination antibody therapy targeting two receptors on neoplastic B cells with different functions, is a safe and efficacious modality for patients with relapsed/refractory indolent and aggressive NHL.

Methods: Sixty-five pts (57% male; 43% female) with a median age of 61 yrs (range 23-87, 30% > 65) were enrolled, 45 with indolent histology (36 follicular lymphoma) and 20 pts with aggressive histology (13 DLBCL). Three pts had stage II, 20 stage III, and 42 stage IV disease; 45% had bone marrow involvement; 37% an elevated LDH; 35% had > 2 prior treatments with a median time from last therapy of 1.1 yr (range 0.1-12); and 77% of pts were rituximab-naïve. Pts received epratuzumab 360 mg/m² i.v. over 60 min, followed by infusion of 375 mg/m² rituximab, weekly for four consecutive weeks.

Results: Combination antibody therapy was tolerated well; 44 pts (68%) experienced 1 or more adverse events (AEs). The most frequent AEs were rigors (22%), fever (15%), hypotension (14%) and nausea (11%). One infusion reaction (hypotension) required termination of therapy. To date, 20 (57%) of 35 evaluable pts with indolent follicular lymphoma (FL) achieved an objective response (OR) as determined using the IWG response criteria, including 8 (23%) complete /unconfirmed complete responses (CR/CRu) and 12 (34%) partial responses (PR), while 6 (46%) of 13 pts with DLBCL achieved an OR, including 3 (23%) CR/CRu. As of February 2004, 6 months after the last pt completed treatment, the median TTP has not been reached in pts with indolent FL (43% have progressed so far, whereas 69% of pts with DLBCL have progressed, and 36% of the all responding pts have progressed with 6 to 18 months follow up).

Conclusions: This combination monoclonal antibody therapeutic regimen appears feasible, well tolerated, and has promising anti-lymphoma activity that warrants further study in a prospective, randomised clinical trial.

026 ANALYSIS OF LONG-TERM RESPONDERS: DURABLE REMISSIONS WITH YTTRIUM-90 IBRITUMOMAB TIUXETAN (ZEVALIN) RADIOIMMUNOTHERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY B-CELL NON-HODGKINS LYMPHOMA

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Background: Yttrium-90 ibritumomab tiuxetan (Zevalin®) is a first-in-class, dual action radioimmunotherapy (RIT) that combines the tumor targeting power of an anti-CD20 monoclonal antibody and the cytotoxicity of yttrium-90 (90Y) radiation. Zevalin has been approved in the United States and Europe for the treatment of relapsed or refractory follicular B-cell non-Hodgkin lymphoma (NHL), including patients with rituximab-refractory NHL. Zevalin is a RIT comprised of the murine monoclonal antibody ibritumomab covalently bound to a high affinity metal chelator, tiuxetan, providing a stable linkage between the antibody and the pure β -emitting radionuclide, 90Y, for therapy. Between 1996 and 1999, 211 patients with relapsed, refractory, or transformed low-grade B-cell NHL were treated with Zevalin in clinical trials. There is evidence of long-term durable responses with prolonged follow-up of these patients (Witzig et al, ASCO 2003).

Aims: To further characterize patients with long-term response (LTR), we identified those patients with time to progression (TTP) ≥ 12 months.

Methods: By this defined criteria (TTP ≥ 12 months), durable responses were achieved by 78 of the 211 patients (37%). The median patient age was 58 years (range, 24 to 80 years): 44% were >60 years of age, and 55% were male. Most patients (76%) had follicular lymphoma, and 41% had lymphomatous marrow involvement. The median number of prior regimens was 2 (range, 1 to 9 prior regimens): 59% had ≥ 2 prior therapies, 33% had ≥ 3 prior therapies, and 37% had no response to their last prior therapy. Bulky disease (tumor size >5 cm) was present in 30% of these patients, and 83% had stage III/IV disease.

Results: The median follow-up for patients with LTR at the time of this analysis was 45.6 months (range, 12.7 to 75.5+ months). The median duration of response (DR) and TTP for LTR patients were 28.1 months (range, 10.5 to 74.3+ months) and 29.3 months (range, 12.1 to 75.5+ months), respectively. This compares favorably to the DR obtained with the last prior therapy for LTR patients, which was 12 months. The complete response/complete response unconfirmed (CR/CRu) rate for LTR patients was 66%, and the median DR and TTP were 29.3 months (range, 10.8 to 74.3+ months) and 31 months (range, 12.1 to 75.5+ months), respectively. The median DR and TTP in ongoing responders were 48 months (range, 40 to 74.3+ months) and 50.4 months (range, 41 to 75.5+ months), respectively.

Summary/Conclusions: Zevalin produces durable long-term responses (TTP ≥ 12 months) in patients with relapsed or refractory B-cell NHL. The ability to achieve long-term responses with Zevalin radioimmunotherapy is not affected by failure to respond to treatment immediately prior to Zevalin therapy. In 4 clinical trials conducted at 30 centers in the United States, 37% of patients treated with Zevalin achieved durable long-term responses, with some responses lasting longer than 75 months. A large proportion of these patients was >60 years of age and had received ≥ 3 prior therapies.

027 RITUXIMAB, GEMCITABINE AND OXALIPLATIN (R-GEMOX): A PROMISING REGIMEN FOR REFRACTORY/RELAPSED B-CELL LYMPHOMA

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Background: High-dose therapy with autologous stem cell support (HDT) is an established treatment for chemosensitive relapse in aggressive lymphoma. However, not all patients are candidates for HDT because of age, comorbidities or previous HDT. Effective and well tolerated salvage therapies with minimal toxicities are needed.

Methods: We designed the R-GEMOX regimen, with rituximab 375mg/m² d1, gemcitabine 1000 mg/m² d2 and oxaliplatin 100 mg/m² d2 (recycling on d15). Between January 2002 and September 2003, 29 patients with refractory/relapsing B-cell lymphoma not eligible for HDT were enrolled in an open unicenter pilot study whose primary objective was to determine the overall response rate (ORR) after 4 cycles of R-GEMOX. Patients were

planned to receive 8 cycles if a good response (at least PR) was observed after 4 cycles. Median age was 63 years (range: 43-77) and histological subtypes were: diffuse large B-cell lymphoma (n=19), follicular (n=6) and mantle cell (n=4). Prior treatment included anthracyclin (n=28), rituximab (n=14) and HDT (n=7). The median number of prior treatments was 2 (range: 1 to 5) and 8 patients had received at least 3 prior regimens.

Results: 208 cycles were given. The dose administered was 100% of the intended dose for the three drugs in all patients but 5, for whom the dose of oxaliplatin was reduced due to neurotoxicity (n=4) or preexisting renal insufficiency (n=1). The median number of cycles per patient was 8 (range: 3-8). Two patients progressed after 3 cycles. After 4 cycles, observed responses were: 7 CR, 6 CRu, 13 PR and 1 failure resulting in an ORR of 90%. At the end of treatment, among the 26 responder-patients at 4 cycles, 21 patients achieved CR or CRu, 4 remained in PR and 1 progressed. As of February 2004, 27 patients are alive, 21 in continuous complete remission, 1 in CRu, 2 in PR and 3 in progression. NCIC grade 3-4 neutropenia and thrombocytopenia were reported in 47% and 24% of the cycles. Five patients developed a grade 4 infection during one cycle. There was no renal toxicity.

Conclusion: The R-GEMOX regimen shows promising activity with an acceptable toxicity. It is now evaluated in an ongoing multicentric phase II study on diffuse large B-cell lymphoma patients at first relapse.

028 CNOP VS. CNOP-RITUXIMAB VS. RITUXIMAB ALONE AS FIRST LINE THERAPY FOR INDOLENT NON-HODGKIN LYMPHOMA (INHL): PRELIMINARY DISEASE-FREE/OVERALL SURVIVAL ANALYSIS

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A randomized three-arm study was started in October 2001 in order to evaluate the efficacy, safety and disease-free/overall survival for standard (CNOP) versus immunological treatment (Rituximab) versus combined therapy (R-CNOP) for patients with INHL as first-line therapy.

Methods: Until January 15th, 165 patients were randomized to one of the three-arm treatment groups, with a median follow-up of 32 months.

Inclusion criteria: Age <85 yrs, indolent CD20+ NHL, stage IIb to IV, ECOG 0-2, normal liver, renal and cardiac function, negative for CNS infiltration and HIV.

Treatment arms: Arm A: Rituximab 375 mg/SM weekly x 6, Arm B: Cyclophosphamide 750 mg/m²SM, Mitoxantrone (N) 12mg/m²SM, Vincristine 1.2mg/m²SM, on day 1 and Prednisone 100mg PO x 5 days each 21 days x 6, Arm C: CNOP-R x 6. If needed, radiotherapy was allowed. **Results:** Demographic: 47.6% of patients were male. Age: 25-85 years with a mean of 58.7 (SD 14.5). Disease Stage II = 23.8%, III = 27.9%, IV = 45.6%; no differences between groups were found in gender, age or disease stage. Efficacy and safety was reported previously (Rubio-Borja et al. www.hematology.org/meeting/abstracts.cfm). In a cut-off at month 32, the median disease-free survival for patients in arm A was 30 months, for arm B was 25.84 months and for arm C was 29.01 months. The median overall survival for patients in arm A was 27 months, in the arm B was 21.36 and in the arm C was 24.94 months. **Conclusions:** The patients randomized to Rituximab alone showed a longer disease-free survival and a longer overall survival.

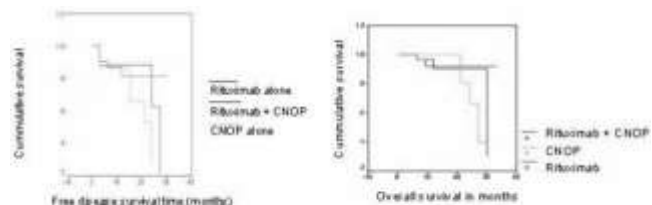


Figure 1: Free disease survival time in the studied groups

Caption 1:

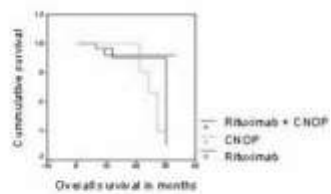


Figure 2: Overall survival in the studied groups

029 DELAYED SEVERE NEUTROPENIA AFTER RITUXIMAB THERAPY IN 12 OUT OF 282 PATIENTS WITH NON HODGKIN'S LYMPHOMA TREATED IN A SINGLE INSTITUTION. A RARE ADVERSE EVENT OF UNKNOWN MECHANISM

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Background: First described by Saikia et al. (Ann Oncol 2002), delayed severe neutropenia after rituximab therapy is a rare adverse event of undetermined mechanism.

Aims: The characteristics of severe neutropenia with delayed onset following administration of rituximab for treatment of B-cell malignancy, in a single institution are here reported.

Methods: Between Oct. 1996 and Feb. 2004, among 282 treated patients, we identified and explored 12 episodes of grade 4 neutropenia.

Results: Rituximab was delivered alone in 3 cases and combined to chemotherapy in 9 other cases. The median delay of occurrence of neutropenia was 5 months (1.5 - 9). At the time of neutropenia, 6 patients were in complete remission and had completed treatment and 6 were still on therapy. All patients had a normal neutrophil count before rituximab treatment. Among the 12 patients (diffuse large B-cell lymphoma: 7, follicular lymphoma: 3, post transplant lymphoproliferative disorder: 1, chronic lymphocytic leukemia: 1), 6 had been treated with consolidative high-dose therapy followed by autologous stem-cell transplantation. In 4 cases, neutropenia was complicated by fever requiring hospitalization. None of the known causes of neutropenia were found. Neutropenia was associated with selective marrow depletion of neutrophil precursors in all episodes. Tests for antineutrophil antibodies were negative and no other immune disorder was identified (no hemolytic anemia or autoimmune thrombocytopenia, normal gammaglobulinemia, no large granular natural killer lymphocytosis). Filgrastim was administered to 3 of the 4 patients with fever and in one patient who had recently developed severe opportunistic infection. The median time of neutrophil recovery was 36 days (range: 2-180).

Conclusion: Mechanism of this rare event already reported (Saikia et al. Ann. Oncol 2002, Voog et al. NEJM 2003, Chaiwatanatorn et al. BJH 2003) remains to be established. Since it seems to be more frequent in patients who have received extensive prior chemotherapy, it is tempting to speculate that cumulative immunosuppression might favour the development of such an event.

MULTIPLE MYELOMA: BIOLOGICAL ASPECTS

030 SOCS1 AND SHP1 HYPERMETHYLATION IN MULTIPLE MYELOMA: IMPLICATIONS FOR EPIGENETIC ACTIVATION OF THE JAK/STAT PATHWAY

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Background: SOCS1 and SHP1 negatively regulate the Jak/STAT signaling pathway.

Aim: Investigate the role of promoter hypermethylation leading to epigenetic inactivation of SOCS1 and SHP1 in myeloma.

Method: The methylation-specific polymerase-chain-reaction, PCR (MSP) was used to define SOCS1 and SHP1 methylation in leukemia and myeloma cell lines and 34 diagnostic myeloma marrow samples. To investigate the biologic significance of SHP1 methylation, the U266 human myeloma line was used as an in vitro model.

Result: For SOCS1, MSP primers located 3' to the translation start site were unreliable and gave positive results in normal controls. However, MSP primers in the 5' promoter region were specific, although no myeloma samples showed methylation. For SHP1, however, 27/34 (79.4%) myeloma samples showed SHP1 hypermethylation. U266 contained completely methylated SHP1. Furthermore, there was constitutive phosphorylation of STAT3. Treatment with 5-azacytidine led to progressive de-methylation of SHP1 on days 2-5, with consequent increasing re-expression of SHP1 as shown by reverse-transcription PCR. Concomitant with increasing SHP1, a parallel down-regulation of phosphorylated STAT3 occurred, so that by day 5, phosphorylated STAT3 was barely detectable. The overall survivals of patients with and without SHP1 methylation were similar.

Conclusion: Methylation of SHP1 leading to epigenetic activation of the Jak/STAT pathway might be an important step in the pathogenesis

of myeloma. Our study also supported the presence of a methylation boundary in genes of normal cells, and underscores its importance on selection of MSP primers.

031 EXPRESSION OF ADHESION MOLECULE CD58 (LFA-3) ON CD138HIGH AND CD138LOW CELLS IN MULTIPLE MYELOMA.

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Background: Expression of CD58 on tumour cells is a condition of lysis of those cells by cytotoxic lymphocytes. A main ligand for CD58 is CD2 present on those lymphocytes.

Aim: The aim of the study was analysis of expression and intensity of expression of CD58 on plasma cells in patients with multiple myeloma. Methods: The study group included 50 patients: 7 at stage I, 9 at II and 34 at III acc. to DS. Monoclonal protein IgG was found in 35, IgA in 13 and only light chain in 2 cases. Immunophenotyping was done on fresh bone marrow samples triple stained with a set of anti- CD138/ CD58/CD38 antibodies using flow cytometry (FACSCalibur Becton Dickinson). Plasma cells were identified on the basis of strong CD38 reactivity, reactivity for CD138 and typical light scatter. The antigen expression was presented as proportion of cells with expression of a given antigen. Antigen expression intensity was determined in form of Relative Fluorescence Intensity indices (RFI) and direct quantitative measurements presented as Antibodies Binding Capacity (ABC). Results: Study patients plasma cells showed expression of CD58; proportion of CD58+ cells ranged from 4 to 89% (mean: 39,527,5%) and three expression patterns could be distinguished. The CD58 expression intensity was homogenous (RFI = 10,2 1,5). A mean CD58 antigen density on plasma cells determined in 12 cases in form of ABC was 2256 630. In 26 patients myeloma plasma cells characterised relatively low CD138 expression intensity (RFI=13,63,2) that was associated with high expression of CD58 (55,623%). In 14 cases there was found high CD138 expression intensity (RFI=21,62,0) on plasma cells. Those cells simultaneously presented with low CD58 expression (9,94,6%). In 10 cases there were distinguished two subsets of myeloma plasma cells differing in CD138 expression intensity i.e. CD138++ cells with high (RFI= 20,72,3) and CD138+ cells with low (RFI= 11,3 2,6) CD138 expression intensity (p<0,0001). Those two cell subsets showed differential CD58 expression: CD138++ cells presented with low CD58 expression (17,8 2,1%) while CD58 expression on CD138+ cells was high (62,0 9,0%). Generally, the study revealed a negative correlation between values of CD138 expression intensity and expression of CD58 (r=-0,89). Analysis of antigen expression intensity confirmed also equivalency of two methods (direct and indirect) used for antigen expression intensity determinations (r=0,87).

Conclusions: The results suggest that myeloma cells with high expression intensity of CD138 show simultaneously low CD58 expression. Deficiency of adhesion molecule (LFA-3) may contribute to difficulties in elimination of these cells by NK cells and cytotoxic T lymphocytes. On the other hand, differential CD138 expression intensity on myeloma cell may modify the adhesion of these cells to bone marrow environment.

032 EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND CD31 IN HUMAN MYELOMA CELLS.

POTENTIAL RELATION TO DISEASE PROGRESSION AND TREATMENT RESPONSE

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Background. Adhesion properties of tumor cells and neoangiogenesis are critically important for metastatic progression of tumor. Expression of adhesion proteins mediating transendothelial migration of cells and production of vascular endothelial growth factor (VEGF) by myeloma cells play an important role in the progression of multiple myeloma (MM). The aim of the current study was to examine the expression of CD31 and VEGF in myeloma cells from patients with different stages, clinical course and morphological type of MM. Methods. Eighty-one samples of aspirated bone marrow (BM) and 73 BM biopsies were obtained from 41 patients with MM. Samples of control BM were obtained from 10 individuals with no hematological disease. Stage of MM was determined according to Durie-Salmon staging criteria. Plasmablastic MM was diagnosed in cases with >10% BM plasmablasts. Expression of CD31 and CD58 adhesion molecules, and CD19, CD38, CD45, CD138 differentiation markers on cells was measured by 4-color flow cytometry of BM cells stained with different combinations of monoclonal antibodies. Myeloma cells were

identified and gated as either CD38brightCD24- or CD38brightCD138+ events with light scatter characteristics of mononuclear cells. Level of analyzed antigen was expressed as percentage of positive myeloma cells and mean fluorescence intensity (MFI) of myeloma cells stained with corresponding antibody. Immunohistochemistry analysis of CD31 and VEGF in myeloma cells was performed on BM biopsies. Results. Flow cytometric analysis showed that myeloma cells expressed lower levels of membrane CD38 (MFI=95.2+14.8) and CD19 (0.9+0.1) than normal plasma cells (218.0+28.5 and 3.6+0.6; $p<0.01$). Myeloma cells from patients with different stages of disease did not differ by the level of CD19, CD31, CD38, CD45, CD58 or CD138. Expression of surface antigens on myeloma cells did not correlate with serum concentration of β 2M, CRP, LDH, creatinine and M-protein. Immunohistochemistry analysis demonstrated that intracellular expression of VEGF was higher in myeloma cells from patients with more advanced stage of disease (stage III) as compared with patients with stage I or II. Flow cytometry analysis showed that expression of CD31 (MFI=11.14.1) and CD138 (MFI=11.73.4) was significantly lower in myeloma cells from patients with plasmablastic myeloma as compared with more mature cells (MFI of CD31=26.14.7; MFI of CD138=39.811.3). Reduced expression of CD31 in myeloma cells from patients with plasmablastic myeloma was confirmed by immunohistochemistry analysis of BM biopsies. Association between antigenic properties of myeloma cells and efficacy of VAD therapy was sought in 14 patients selected for first line therapy with vincristine-adriamycin-dexamethasone (VAD). Flow cytometry analysis of myeloma cells was performed at diagnosis. Effect of therapy was evaluated after 2-4 cycles of VAD therapy by measurement of myeloma disease burden. Myeloma cells from patients which did not respond to VAD therapy had lower level of CD31 (MFI=8.83.6) than the cells from patients responded to VAD therapy (23.52.2; $p<0.03$). Conclusions. Low expression of CD31 on myeloma cells may be associated with low efficacy of VAD therapy in patients with MM. High expression of VEGF in myeloma cells is apparently associated with advanced stage of disease.

033 A ROLE FOR RHAMM IN MITOTIC INTEGRITY AND MYELOMA GENESIS

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BACKGROUND/AIMS: Multiple myeloma (MM) is a B-cell malignancy characterized by chromosomal instability (CIN) and elevated expression of RHAMM. Splice variants of RHAMM (i.e. RHAMM-exon4) are specifically expressed in MM clonal cells. Moreover, elevated expression of RHAMM in newly diagnosed MM patients is significantly correlated with overall survival. Recently we identified RHAMM as a centrosomal protein that maintains mitotic spindle integrity. Centrosome duplication, mitotic progression, and spindle integrity are regulated by auroraA kinase (aurA); a ser/thr kinase that is directly activated by TPX2 and regulates p53. Here, we investigate isoform specific expression of RHAMM in purified MM bone marrow (BM) plasma cells (PC), aggregate populations and single cells, and elucidate the connections between RHAMM overexpression, centrosomal aberrations and mitotic dysregulation.

METHODS: RT-PCR, using fluorescent primers flanking RHAMM exon4 followed by fluorescent capillary electrophoresis (FCE), experiments determined the extent of RHAMM splicing within BM (n=99), and peripheral blood (PB) (n=5), MM patient samples. The resolving power of FCE is 5 orders of magnitude greater than agarose detection. The extent of RHAMM-exon4 expression was correlated to absolute RHAMM expression, by qRT-PCR, and with survival as a measure of clinical relevance. Transient overexpression of RHAMM-GFP determined RHAMM effects on centrosome structure and spindle integrity in 8226 and HeLa. Confocal microscopy and 3-D imaging software detected volumetric centrosome abnormalities induced by RHAMM overexpression. Bone marrow biopsies from 42 MM, 8 MGUS and 4 control patients were similarly examined for numerical, structural and volumetric centrosome abnormalities. Immunoblot and FACS determined RHAMM expression within transfectants, MM PC and normal PC. Colocalization and coprecipitation experiments investigated the association between RHAMM and other spindle pole proteins, including dynein, TPX2 and NuMA. RNA inhibition investigated the effects of RHAMM depletion on spindle structure and integrity.

RESULTS: qRT-PCR demonstrates a near linear relationship between absolute RHAMM expression and an increase in RHAMM-exon4/RHAMMFL ratio in MM BMPC. In the PB, extensive RHAMM-exon4 splicing, as measured by FCE, correlates with relapse. Within the BM, RHAMM-exon4 expression is an adverse prognostic factor ($p<0.01$). RHAMM-GFP overexpression induced volumetric

centrosomal abnormalities and mitotic abnormalities, including mono/multipolar spindles, in 25% of transfectants. Significant centrosomal abnormalities characterize all 42 studied cases of MM in comparison to MGUS and control PCs ($p<0.05$ and $p<0.005$); MM centrosomal abnormalities parallel those identified within RHAMM transfectants. RHAMM colocalizes and coimmunoprecipitates a significant proportion of TPX2 during mitosis. Inhibition of RHAMM function disrupted spindle integrity by inducing fragmentation and multipolar spindles. **CONCLUSIONS:** In MM patients, an increase in absolute RHAMM expression is accompanied by a relative increase in the RHAMM exon4/RHAMMFL ratio; both absolute RHAMM expression and the RHAMM ratio predict patient survival. Taken together these data demonstrate an important role for RHAMM in myelomagenesis. Mechanistically, our data shows a ternary RHAMM-TPX2-dynein complex is essential for mitotic integrity; disruption of this complex through overexpression, inhibition or potentially isoform (-exon4) imbalances, induces spindle fragmentation and chromosomal missegregation. RHAMM may also affect TPX2 regulation of aurA with consequent dysregulation of aurA pathways. Within MM, overexpression of RHAMM likely modifies centrosomal structure and dysregulates mitotic stability inducing CIN and disease progression.

034 ASSOCIATION OF CYTOKINE GENES POLYMORPHISMS WITH MULTIPLE MYELOMA

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Background. Multiple myeloma is a currently incurable B-cell malignancy caused by expansion of a single clone of neoplastic plasma cells that synthesize abnormal amount of monoclonal immunoglobulins. Nowadays, most of recommended diagnostic criteria for the diagnosis of myeloma are well established and widely accepted. But, it is important to determine additional standards to segregate patients with early-stage myeloma into risk groups. There is a greater appreciation of the pathogenic role of the cytokines in the proliferation and survival of myeloma cells. **Aim.** The aim of our study was to test the 308TNF and +252LTA, -174IL-6 genetics polymorphisms for association with myeloma. **Methods.** Genomic DNA was extracted from whole blood by a standard proteinase K digestion and phenol/chloroform method. PCR-RFLP-based genotyping assays were used to determine the frequency of polymorphic variants of the following genes: -308G/A TNF, +252A/G LTA, -174G/C IL-6 in a case-control study comprised 68 adult patients with myeloma and 167 healthy individuals from Bashkortostan Republic. In analyzed group 35 patients had smoldering or indolent myeloma (SM), 33 - multiple myeloma (MM).

Results. We have found that TNF*GG (OR=1,4) and LTA*GG (OR=3,2) genotypes were in association with SM. We determined the most frequent genotype combinations of TNF and LTA genes in the patients groups. For SM more frequent combinations were TNF*GG/LTA*AA, TNF*GG/LTA*GG and for MM - TNF*GG/LTA*AG, TNF*GA/LTA*AG.

The frequency of IL-6*GG (higher risk) genotype was increased in MM patient group (OR=2,4). It is important to note, that IL-6*CC genotype with low constitutive and inducible transcriptional level was found in 28% in the group of SM versus 3% in MM (OR=12,8; $p=0,01$).

Summary. According to results of our work, we could assume, that IL-6 plays an important role in the progression of myeloma. Genotyping of IL-6*CC might be predictable for development of smoldering or indolent myeloma and could be indication for less aggressive treatment. e-mail: elya74@mail.ru

035 MULTIPROBE INTERPHASE CYTOGENETICS DEFINES TWO SUBGROUPS OF MULTIPLE MYELOMA

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BACKGROUND: Genetic instability plays a critical role in the pathogenesis of multiple myeloma (MM). Deletions of chromosome 13 and hypodiploidy detected by metaphase cytogenetics are clearly defined as poor prognostic factors. However, the low mitotic index often combined with low bone marrow (BM) infiltration limits the metaphase analysis to 30% of newly diagnosed MM patients (pts). Using interphase fluorescence in-situ hybridization (FISH) cytogenetic abnormalities are detected in almost all MM pts. **THE AIM** of this study was to analyse and define possible

MM subgroups applying multiprobe interphase cytogenetics. **PATIENTS AND METHODS:** The study included 50 pts with newly diagnosed MM. The group consisted of 35 male and 15 female pts. Mean age was 63 years (range, 44-83 years). Clinical stage of disease according to Salmon and Durie was distributed as follows: I (5 pts), II (7 pts), and III (38 pts). Renal impairment existed in 3 pts. There were 26 pts (52%) with IgG monoclonal (M) protein, 13 pts (26%) with IgA, and 8 pts (16%) with secretion of kappa/lambda light chains only. Non-secretory MM was diagnosed in 1 patient. Elevation of Beta 2 microglobulin (>2.5 mg/l), was detected in 32 pts (64 %). Interphase FISH was applied with 8 specific probes for chromosomes 1q21, 6q21, 8p12, 9q34, 11q23, 13q14.3, 17p13, and 22q11 on CD138-selected BM cells (median purity, 95%). A copy number score (CS) was calculated for each patient by subtracting the number of probes expressing losses from the number of probes indicating gains. **RESULTS:** Signal changes were detected for a median of 3 probes per patient. The most frequent gained signals were found for 9q34 (66%), 11q23 (60%), 1q21 (48%), and 6q21 (22%). Common losses were observed for 13q14.3 (50%), 17p13 (28%), 6q21 (20%), and 8p12 (20%). CS>0 was found in 28/50 pts (56%) and CS<=0 in 22/50 pts (44%). Of note, two peaks (CS=-1 and CS=+2) were detected by plotting patient numbers over CS values, indicating a bimodal distribution. Monosomy of 13q14.3 was present in 19/22 pts (86%) with CS<=0, and 6/28 pts (21%) with CS>0 ($p=0.0001$). Gains of 9q34 and 11q23 were highly associated and more frequent in clones with CS>0 ($p=0.0004$). Gains of 1q21 as well as deletions of 17p13 and 8p12 were distributed evenly between clones with CS>0 vs. CS<=0. No significant difference was found in the distribution of the type of M-protein between groups defined by CS. Also, there was no significant difference regarding mean values of Beta 2 microglobulin, CRP and LDH in these pts groups. **SUMMARY:** Using highly-purified CD138+ BM cells and interphase FISH with 8 probes, at least one aberration (median 3, range 1-8) could be detected in each patient. FISH results delineated two MM subgroups based on CS, exhibiting predominant losses vs. predominant gains. These findings correspond to the metaphase-defined non-hyperdiploid and hyperdiploid MM types, respectively. The prognostic significance will be subject of further evaluation.

036 PRE-TRANSPLANT VEGF LEVELS IN MULTIPLE MYELOMA ARE PREDICTIVE FOR PROGRESSION FREE SURVIVAL.

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Angiogenesis is involved in the development and progression of multiple myeloma (MM). It has already been shown that angiogenesis as assessed with micro-vascular density (MVD) in bone marrow of MM patients plays a significant role on the prognosis of MM. It has also been reported that autologous stem cell transplantation for MM (ASCT) does not affect angiogenesis. Vascular endothelial factor (VEGF) is a potent stimulator of angiogenesis in vivo and levels of VEGF at diagnosis has been shown to have a significant effect on survival of MM patients. Angiogenin is an angiopoietin that has also been found to be increased in MM.

The aim of this study was to evaluate the role of serum VEGF and angiogenin in a group of patients with MM.

We studied 36 patients with MM undergoing ASCT after high dose therapy with melphalan. The median age of patients (11F/25M) was 58 years and the median follow up was 14 months. Four patients were transplanted in CR, 1 in good partial response, 28 in partial response, 1 had progressive disease, and 2 had no response prior to transplant. Ten patients were undergoing a second ASCT and 5 patients had previously been treated with Thalidomide.

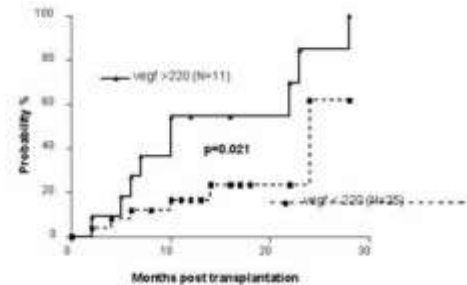
Serum VEGF and angiogenin were measured before and 3 months after transplantation by ELISA (R&D systems)

The mean VEGF concentration pre transplant (206.10 ng/mL) did not differ significantly with the one post transplant (189.08ng/mL). Similarly the levels of angiogenin before (339703.81ng/mL) and after transplant 385318.78ng/mL did not change significantly. The levels of VEGF of the patients that have received thalidomide did not differ significantly with the values of the rest of the patients. The change of VEGF levels did not correlate with the status of the disease at 3 months post transplant.

The mean period of the follow-up was 15.5 months (range from 4 to 29 months). During this period 15/36 patients relapsed. Using 220ng/mL (average normal value) as a cut off point, the patient population was divided into two groups, with high and low pre-transplant VEGF levels. Those with low pre-transplant VEGF levels had a statistically significant

longer median time to progression (24 months) compared to those with high pre-transplant levels (10 months)($p=0.0212$). (Fig.1)

We conclude that although autologous transplantation does not seem to decrease the levels of the circulating VEGF and angiogenin, higher VEGF levels before transplantation can predict for earlier progression of the disease. The fact that VEGF is produced by myeloma cells and may be involved in a paracrine loop to promote tumour growth might be a possible explanation for these findings.



Caption 1:

037 HIGH INCIDENCE OF MONOCLONAL B-CELL DISEASE (MCBD) IN SIBLINGS (SIBS) OF PATIENTS (PTS) WITH MULTIPLE MYELOMA (MM)

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Background: Intensive first line treatment with autologous (auto) stem cell transplantation (SCT) has been demonstrated to be beneficial for MM pts. In pts with more advanced disease and/or adverse prognostic factors, the therapeutic approach of an auto-, followed by an allogeneic (allo)-SCT is currently pursued within trials. At our institution, MM pts opting for allo-SCT were HLA-typed, and preferably a sibs-, or matched unrelated donor (MUD)-search was performed. In those sibs being screened as allo-donors, we unexpectedly observed familial clustering of MCB. Familial MM was first described in 1956. Despite previous reports, its occurrence has been suggested as an infrequent event, and it has not been excluded that its detection in sibs is coincidence.

Aims: To evaluate the incidence of familial MCB, all MM pts being scheduled for allo-SCT and their sibs were analysed.

Methods: Between 1/1992 and 12/2003, 35 allo-SCT for MM were performed at our institution. With the accidental observation of familial MCB in one MM family in 1999, a prospective analysis was performed in all MM pts and sibs being scheduled for allo-SCT. The analysis of MM pts and sibs included routine clinical screening, imaging studies, HLA typing, cytogenetics and a detailed family history.

Results: Five MM pts opting for allo-SCT were observed to show familial MCB. Of a total of 27 sibs and 20 being analysed (all being asymptomatic), seven were found to have MCB, with MGUS in five, and MM and CLL in one sibling each. The analysis of these families showed that 1.) the gender of pts and their affected sibs was the same in 4/5 families, 2.) heavy and light chain paraproteins were predominantly IgG (4/5 in pts and 3/7 in sibs) and kappa (4/5 in pts and 5/7 in sibs), 3.) the age at diagnosis in pts and sibs was comparable (median 57 vs. 61 years), 4.) cytogenetics available in 3/5 pts were normal, 5.) HLA-identical sibs showed the subgroup A9 in 3/5 families and 6.) with seven sibs showing MCB, and a total of 12 pts and sibs being affected, the frequency of MCB was 25.9% (7/27) and 37.5% (12/32), respectively. With the detection of MCB in our families and only one pt undergoing allo-SCT, the incidence in allo-SCT was 2.9%.

Conclusions: With this report we underline the hypothesis, that there are distinct characteristics in familial MCB with marked similarities between MM pts and their affected sibs. Our data demonstrate that thorough screening of sibs before allo-SCT is strongly advisable. With the MCB incidence among sibs of around 25%, an autosomal-recessive inheritance may be postulated. Although additional analyses of possible genetic and molecular changes of familial MCB are needed to determine its genetic background and path of inheritance, our data already suggest the heritable etiology for this malignancy. The further analysis of familial MCB will allow to more clearly define the pathogenesis of this disease.

038 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND ITS SOLUBLE RECEPTORS VEGFR-1 AND VEGFR-2 IN THE SERUM OF PATIENTS WITH MULTIPLE MYELOMA

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Background. Vascular endothelial growth factor (VEGF) is the main proangiogenic cytokine in haematological malignancies including multiple myeloma (MM). Their two receptors VEGFR-1 and VEGFR-2 were investigated in ex-vivo conditions, on myeloma cell lines, however there is no clinical data correlating VEGF and its receptors in MM.

Aims. The aim of the study was to investigate the serum concentration of vascular endothelial growth factor (VEGF) and its two receptors (VEGFR1 and VEGFR2) in multiple myeloma (MM) patients and healthy subjects. The interactions between VEGF and its receptors as well as correlation with some clinical and laboratory parameters associated with the disease activity were studied.

Methods. The study group consisted of 79 patients (48 F and 31 M, mean age 64 years) with multiple myeloma and 15 healthy controls (8 K and 7M, mean age 68 years). All clinical and laboratory parameters of patients were estimated on the day of blood sampling for cytokine assay. The serum samples obtained were assayed for the concentration of VEGF, VEGFR1 and VEGFR2 by means of commercially available enzyme linked (ELISA) assay kits Quantikine, RD Systems Inc.(USA). In 16 patients we also measured plasma concentration of VEGF and its receptors to compare it with respective serum levels. Statistical evaluation was performed according to the procedures of Statistica for Windows package.

Results. The VEGF serum concentration was significantly increased in MM patients compared with healthy persons (mean 559 and 427 pg/mL) ($p=0.01$). The level of VEGF correlated positively with advanced stage of disease estimated according to the Salmon and Durie classification (I vs III stage $p=0.02$). Higher values of VEGF were stated in patients with anaemia, hypercalcaemia and correlated with the elevated concentrations of β_2 -microglobulin. The level of VEGFR1 was also significantly higher in MM patients than in controls (mean 79 and 58 pg/mL) ($p=0.02$) and correlated with III stage of disease and renal failure. The concentration of VEGF correlated positively with VEGFR1 levels ($p=0.01$). The level of VEGFR2 was no significantly higher in healthy persons than in MM patients (15089 and 14822 pg/mL, respectively) ($p>0.05$). We failed to find any correlation of VEGFR2 with analysed parameters as well as VEGF and VEGFR1. We stated that plasma values of VEGF, VEGFR1 and VEGFR2 are significantly lower than respective serum values. **Summary.** In conclusion we can state that VEGF and VEGFR1 concentrations are higher in MM patients than in healthy controls in comparable age and are correlated with disease advance.

039 SERUM LEVELS OF MIP-1-alpha- ARE USEFUL FOR STUDY OF BONE DISEASE IN MULTIPLE MYELOMA (MM) AND DIFFERENTIATION FROM MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE (MGUS) AND BENIGN OSTEOPOROSIS (BO)

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Background Macrophage Inflammatory Protein 1 alpha (MIP-1 α) is a chemokine that has shown a relevant role as Osteoclast activating factor in MM patients. MIP-1 α is produced by plasma cells (PC) and is responsible for osteoclastsrecruitment, proliferation and differentiation. Whether or not these effects are directly produced or through a receptor activator of NF- κ B ligand (RANKL) pathway remains to be elucidated.

Aims: To investigate if serum levels of MIP-1 α correlate with the extent of bone involvement in MM, and contribute to differentiate MM with early bone disease from Monoclonal Gammopathies of Unknown Significance (MGUS) or Benign Osteoporosis (BO). In addition we have explored the potential correlations of MIP-1 α with other cytokines involved in the pathogenesis of MM. **Materials and Methods:** Overall 159 patients were included in the study: 72 newly diagnosed MM patients (median age- 67.5; M/F- 1.25), 37 MGUS patients (median age- 68.5; M/F- 0.6) and 18 cases with BO (median age- 64; M/F 0.06). In addition, 22 healthy individuals (control group) (median

age- 62; M/F 0.17)were studied. Serum levels of MIP-1 α were measured by ELISA (Quantikine-RD System®). We have also analyzed other serum cytokines (Osteoprotegerin (OPG), soluble RANKL (sRANKL), IL-6, IL-1 β , TNF- α , sIL-6R, HGF and VEGF), bone resorption markers (CTX (β -crosslaps), Pyridinoline total (PyrT), Deoxypyridinoline total (DpyrT)) and bone formation markers (Osteocalcine (OC), bone alkaline phosphatase (bAP)). **Statistical methods:** Non-parametric test (Mann-Whitney U, Kruskal-Wallis and Spearman correlation); survival curves of Kaplan-Meier were compared by long-rank test.

Results: Serum levels of MIP-1 α (Table I) were significantly higher in MM patients compared with MGUS patients, BO patients or controls ($p<0.05$ vs. MGUS; $p<0.01$ vs. BO; $p<0.05$ vs. HI). When we divided MM patients by the radiological degree lytic of bone disease (group A- MM without lesions or just osteoporosis; group B- MM with lytic lesions and/or pathological fractures), we observed that patients with extensive bone lesions have significantly higher serum concentrations of MIP-1 α ($p<0.01$ vs. Group A MM and BO; $p<0.05$ vs.HI), but there werent differences between the levels of MIP-1 α observed in group A MM and those present in MGUS patients, BO patients or controls. We also assessed the possible correlations between MIP-1 α and other serum cytokines, and bone remodeling markers. A significant correlation with bone resorption markers as well as IL-6, IL-1 β , sIL-6R, HGF was observed. By contrast, there was no correlation with OPG or S-RANK. MM patients with serum levels of MIP-1 α greater than 55 pg/ml showed significantly lower survival ($p<0.05$). **Conclusions:** Serum levels of MIP-1 α correlates with the extent of MM bone disease as well as bone resorption markers and it is a useful parameter for discrimination between active MM and MGUS or BO but not for MM without lytic lesions. Supported by grant Spanish FISS Thematic Network G03/136

	MIP-1 alpha Median (Min-Max) (n)
MM	29.5 (4.5-85) (72)
>MM with lytic	33.5 (4.5-85) (45)
>MM without lytic	24.5 (3.5-85) (27)
MGUS	24.1 (5.6-85) (37)
BO	18.5 (4.5-85) (18)
HI	20.1 (5-85) (22)

Caption 1:

040 T CELL SUPPORT OSTEOCLASTOGENESIS IN AN IN VITRO MODEL DERIVED FROM HUMAN MULTIPLE MYELOMA BONE DISEASE

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Multiple myeloma (MM) is a hematological malignancy in which immunoglobulin producing plasmacells undergo clonal expansion within the bone marrow microenvironment characterized by cell-cell interactions involving myeloma, hematopoietic and stromal cells. These interactions are critical not only for the growth and survival of the tumor cells, but also for the development of MM characteristic lytic bone disease, which is mediated by the increased number and activity of osteoclasts (OCs). The cellular and molecular mechanisms responsible for OC activation are not well understood; however, an important role of the two secreted molecules receptor activator of nuclear factor (NF)- κ B (RANKL) and osteoprotegerin (OPG) in this process is emerging from the recent studies. RANKL, a member of the TNF family expressed by osteoblasts and activated T cells, induces OC differentiation and bone resorption by binding to its signaling receptor RANK, on the surface of OC precursors (OCPs) and mature OCs. OPG, a soluble decoy receptor member of the TNF receptor family, can bind to RANKL and prevent its interaction with RANK, thus inhibiting OC bone resorption. In the present study we show that OCs, derived from cultures of unfractionated peripheral blood mononuclear cells (PBMCs) of patients with MM bone disease spontaneously developed and displayed a longer survival in a T cell-dependent way. In T cell-depleted MM PBMC cultures, the addition of macrophage colony stimulating factor (M-CSF) and RANKL was necessary to induce the formation of OCs, not

showing however a longer survival. Furthermore, we detected differences in the expression of osteoclastogenesis regulatory cytokines in fresh T cells purified from PBMCs and BM of MM patients with or without lytic bone disease as well as from the controls consisting of subjects affected by non-neoplastic disease without any skeletal involvement. In patients with MM bone disease, T cells overexpressed RANKL at both mRNA and protein levels whereas TNF α was detected only at mRNA level. In fresh T cells from MM patients without lytic bone lesions a very low expression of both RANKL and TNF α was demonstrated; none of these cytokines was found in T cells from the controls. No difference was detected in M-CSF expression among MM patients with or without osteolysis and the controls. OPG resulted overexpressed in the fresh T cells from MM patients with osteolysis whereas it was detected at low levels in those without osteolysis; neither mRNA nor proteins were found in the controls. Our data highlight the T cell regulation of OC formation and survival through a disruption of RANKL/OPG axis, which is responsible for osteolysis observed in most of MM patients.

041 PRENYLATION INHIBITORS ALTER MULTIPLE MYELOMA CELL BIOLOGY

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Recent studies have demonstrated efficacy of prenylation inhibitors such as farnesyl transferase inhibitors (FTIs), geranylgeranyl transferase inhibitors (GGTIs) and the HMG-CoA reductase inhibitor lovastatin, in multiple myeloma (MM). Aims: In order to investigate the biological effects of prenylation inhibitors, multiple myeloma cell lines and primary cells from a plasma cell leukemia (PCL) patient were assayed for cell proliferation/viability, prenylation status of RAS-family proteins, MEK-1/2 activation, and chemotaxis following treatment with FTIs, GGTIs and lovastatin. Methods: MM/PCL cell growth was measured using an MTS proliferation assay. Synergy was evaluated by the method of Chou and Talalay. Apoptotic DNA strand breaks were quantified using the TUNEL method. Protein prenylation was monitored by Western blotting. MEK-1/2 activation was measured by flow cytometry after double staining with propidium iodide and antibodies specific for activated, diphospho-MEK-1/2. MM cell chemotaxis was evaluated in transwell migration assays using modified Boyden chambers. Results: Co-treatment of MM cell line NCI-H929 with FTI L-744,832 and GGTI-2147 resulted in synergistic inhibition of proliferation (IC₅₀ combination 0.28 μ M vs IC₅₀FTI 2.04 μ M and IC₅₀GGTI 0.94 μ M). Combination of L-744,832 with lovastatin also induced synergistic growth inhibition of NCI-H929 (IC₅₀ combination 0.15 μ M vs IC₅₀FTI 1.78 μ M and IC₅₀lovastatin 0.85 μ M). Similar results were obtained in other MM cell lines (RPMI-8226, LP-1, OPM-2, L-363, U266) as well as in primary PCL cells. Co-treatment of OPM-2 with 20 μ M FTI FPT-3 and 5 μ M lovastatin resulted in increased apoptosis as compared with either compound alone (90% vs. 10% and 4%, respectively). While FTIs were observed to block H-RAS prenylation, more complete inhibition of N- and K-RAS prenylation was observed in cells treated with a combination of both FTI L-744,832 and GGTI-2147 versus either compound alone. Although 20 μ M lovastatin potentially blocked N- and K-RAS processing, 10 μ M of the drug was found to be at least as effective when combined with 10 μ M L-744,832. Additionally, lovastatin blocked the prenylation of RAS-related proteins Rheb, RhoA and RhoB. Rho protein processing was rescued by co-incubation with mevalonate or GGPP, but not FPP. Prenylation inhibitors elicited different effects on cell cycle-dependent levels of activated MEK-1/2. FPP-based FTI FPT-3 caused a decrease of PP-MEK-1/2 in both G0/G1 and G2M cell cycle populations, while CAAX-based FTI-277 and L-744,832 resulted in higher levels of activated MEK-1/2 in G2M. GGTI-2147 caused an increase of PP-MEK-1/2+ cells in the G0/G1 cell cycle phase and lovastatin caused a decrease in cells containing activated MEK-1/2 in G0/G1. Transwell migration assays demonstrated that lovastatin and FTIs (FPT-3, L-744,832) each inhibited myeloma cell migration and this inhibition was potentiated upon co-treatment of OPM-2 cells with lovastatin and FTIs. In conclusion, our results demonstrate that prenylation inhibitors alter several aspects of MM cell biology including proliferation, intracellular signaling and chemotaxis. Furthermore, our results suggest that combining lovastatin with FTIs may be a more successful therapeutic strategy in multiple myeloma versus FTI mono-therapy.

042 THE ACTIVATION OF THE PI3K-AKT PATHWAY IS NOT SUFFICIENT TO PROVIDE LONG TERM SURVIVAL OF HUMAN MALIGNANT PLASMA CELLS

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Background:

Interleukin-6 (IL-6) is well known as growth and survival factor in multiple myeloma. Recently, Insulin growth factor-1 (IGF-1) was identified to be also important for plasma cell growth. However, the mechanism by which IGF-1 induces myeloma cell growth as well as the interaction of both cytokines remains to be elucidated.

Aims:

The aim was to clarify the long-term effects of IL-6 and IGF-1 on the survival of human malignant plasma cells.

Methods:

We tested six human malignant plasma cells (INA-6, JK-6E, JK-6L, L363, RPMI8226, U266) for their response to IL-6 and IGF-1 in a proliferation assay ([³H]-thymidine uptake). In order to better define the effects of IL-6 and IGF-1, propidium iodide-triton X staining was used to measure cell cycle and apoptosis. Western blot analysis was performed with phosphospecific antibodies to find out whether the Ras-MAPK or the JAK-STAT pathway is activated by these cytokines. The activation of the PI3K-AKT pathway was measured by a phosphospecific ELISA.

Results:

Growth of JK-6E, raised in our laboratory, was stimulated by IL-6 (2.5 ng/ml) as well as IGF-1 (100 ng/ml) under serum free conditions. JK-6 E cells showed optimal proliferation upon IL-6 and IGF-1 co-stimulation. Interestingly, the cytokines had very similar effects on JK-6 E cells: the rate of sub-cellular apoptotic bodies in the sub G1 phase was significantly reduced after 48 h stimulation while the rate of G2/S phase cells was increased. Despite these similarities, great differences in long term effects induced by these cytokine were seen. The JK-6 cells supplemented with IGF-1 stopped proliferation at 6 days, showed a slowly rising rate of apoptosis and finally died around day 18 while IL-6 was able to maintain cell growth even at very late time points. A western blot showed a constitutive activation of the Ras dependent MAPK, caused by an activating K-Ras mutation, and the pathway was not affected upon stimulation with either cytokine. However, IL-6 and IGF-1 activated the PI3K-AKT and the JAK-STAT pathway differentially. IGF-1 activated AKT as measured by phosphospecific ELISA, while IL-6 had no effect. In contrast, IL-6 showed a significantly increased rate of phosphorylated STAT3, which is not a downstream target of IGF-1.

Summary/conclusion:

Thus, both the activation of the JAK-STAT and the PI3K-Akt pathway promote plasma cell growth and co-stimulation provides an additive effect. However, the PI3K-AKT pathway is not sufficient to provide long term survival of malignant plasma cells. Continuous activation of the JAK-STAT pathway is required for long term growth and survival.

043 RELATIONSHIP BETWEEN POLYMORPHISMS OF METHYLEN TETRAHYDROFOLATE REDUCTASE (MTHFR) AND SUSCEPTIBILITY TO MULTIPLE MYELOMA

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Background: MTHFR reduces methylenetetrahydrofolate to methyltetrahydrofolate which is the main methyl donor for homocysteine remethylation to methionine and for DNA methylation. Two MTHFR polymorphism have been recently described 677C to T and 1298 A to C. These polymorphism are associated with reduced activity of the enzyme promoting a genomic hypomethylation which would result in a reduced risk of carcinogenesis. Moreover the reduced function leads to the increase of 5,10 methylenetetrahydrofolate which increases the amount of thymidilate available for the DNA synthesis. A relationship between these polymorphisms and the susceptibility to colorectal cancer and acute leukemia has been recently reported demonstrating that individuals with variant allele (677T and 1298C) have lower susceptibility to these cancers than individuals with the wild type variants (677C and 1298A). Aims: In the present study we examined the prevalence of both MTHFR polymorphism among 91 multiple myeloma (MM) patients and 73 healthy controls.

Methods:Patients characteristics were: M/F 53/38, median age 63y (range 34-87). Of these patients 8 were in IA stage, 37 in IIA, 2 in IIB, 41 in IIIA and 3 in IIIB stage. Thirty-three patients received melphalan with prednisone or dexamethasone, 20 chemotherapy, 25 chemotherapy followed by autologous/allogeneic peripheral blood stem cell transplantation, 1 patient radiotherapy alone and 12 were untreated. Eighty-five patients are alive, while six patients died after a median time of 42 months (range 12-48). The median follow-up was 24 months (range 2-120). Forty-six treated patients showed response to treatment, while 31 had progression of disease, 2 patients were still not evaluable. The control population consisted of 73 individuals (M/F 40/33) without cancer history with a median age of 65y (36-89).

Results: Among the 91 patients the frequency of 677 wild type (CC) was 33% compared to 37% in controls. The heterozygous mutants genotype (CT) was detected in 42.8% of MM patients vs 43.8% in controls, while the homozygous mutants (TT) had a frequency of 24.3% vs 19.2% in patients vs controls. The frequency of 1298 wild type (AA) was 47.2% in patients group compared to 46.5% in controls. The heterozygous mutants genotype (AC) was detected in 43.9% of MM patients vs 35.6% in controls, while the homozygous mutants (CC) had a frequency of 8.7% vs 17.8% in patients vs controls. Statistical analysis did not disclose any significant association between MTHFR polymorphism and Myeloma although a trend toward a reduction of incidence of MM was observed in the group carrying MTHFR 1298CC.

Conclusions: In the present study we report an association between the susceptibility to MM and polymorphisms in the gene encoding the enzyme MTHFR. In particular we found that individuals with 1298CC mutation were less likely to contract multiple myeloma although there was not statistical significance and the small sample size imposes some cautions. Further studies are necessary to confirm the influence of the MTHFR and the role of folate in myeloma pathogenesis.

044 SERUM FREE LIGHT CHAINS ARE A SENSITIVE MARKER OF SEROLOGICAL REMISSION IN MULTIPLE MYELOMA PATIENTS

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Multiple myeloma is a lymphoproliferative disorder usually characterised by the presence of monoclonal immunoglobulin in serum and/or urine. Levels of these paraproteins are used to assess the response to treatment. The current definition of a complete response to treatment includes the absence of monoclonal proteins in serum and urine as measured by immunofixation (IFE). Serum free light chain (FLC) measurements are a sensitive method for the detection of light chain multiple myeloma (LCMM) and non-secretory myeloma and may prove useful in the monitoring of intact immunoglobulin multiple myeloma (IIMM) patients. Given the potentially greater sensitivity of serum FLC assays, we undertook a study to determine whether the serum free kappa/lambda ratio is a better measure of complete response than IFE in multiple myeloma. A total of 96 patients from the UK MRC VI and VII Myeloma trials were selected for this study: 61 with IIMM and 35 with LCMM. All were in complete serological remission i.e. LCMM patients were IFE negative in serum and urine and IIMM were IFE negative in serum. FLC concentrations in serum were measured on the Behring Nephelometer II (Freelite, The Binding Site Ltd). 54/96 patients (11/35 LCMM and 43/61 IIMM) had normal serum FLC ratios, concordant with the assessment of complete response by IFE. However, the remaining 42/96 (24/35 LCMM and 18/61 IIMM patients) had abnormal serum FLC ratios suggesting the presence of residual disease. In a preliminary analysis of available overall survival data for 52 patients, we found that IFE negative patients with abnormal FLC ratios had shorter overall survival times compared to IFE negative patients with normal FLC ratios (mean overall survival of 1040 versus 1309 days). A Mann-Whitney U test showed that the differences reached significance ($p < 0.03$). This study indicates that serum FLC assays identify residual disease in some IFE negative multiple myeloma patients. Analysis of the overall survival in these groups indicates that abnormal FLC ratios are predictive of reduced overall survival. The increased sensitivity to detect residual disease in LCMM may be due to the use of serum FLC rather than urine FLC measurement. Measuring FLC in urine is not reliable because proximal tubules can reabsorb and catabolise 10-30 g of protein/day preventing FLC entering into the urine. In IIMM, the reasons for the increased sensitivity of serum FLC assays to detect residual disease are not fully understood. In summary, serum free light chains are a more sensitive marker of serological remission in multiple myeloma patients.

045 GENE EXPRESSION PROFILING OF PLASMA CELL DYSCRASIA REVEALS MULTIPLE MYELOMA MOLECULAR HETEROGENEITY

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Introduction: Multiple Myeloma (MM) is a fatal malignancy of bone marrow neoplastic plasma cells characterized by a high degree of genomic instability involving both ploidy and structural rearrangements. The disease has also a highly varied clinical course, ranging from the presumed pre-malignant condition named monoclonal gammopathy of undetermined significance (MGUS), to truly overt and symptomatic MM and extra-medullary myeloma (plasma cell leukemia-PCL). The genetic heterogeneity of MM has been limiting our knowledge on molecular mechanisms of pathogenesis and disease progression, prognostic risk groups and new targeted therapies. Although recent advances have contributed towards establishing a molecular classification of the disease, many issues remain to be clarified such as (i) the molecular events leading to the transition from the MGUS to MM, and (ii) the contribution of the genes deregulated by 14q32 IGH translocations to the biological and clinical variability of MM. **Aims:** To correlate the gene expression profiles of patients affected by different forms of plasma cell dyscrasias with clinical-pathological parameters and distinct types of chromosomal lesions. **Patients and methods:** Highly purified bone marrow plasma cells isolated by CD138 immunomagnetic positive selection from 3 normal donors, 8 MGUS, 39 newly diagnosed MM and 6 PCL patients were analyzed by means of Affymetrix® GeneChip® HG-U133A oligonucleotide microarrays, representative for more than 18.000 transcripts. All the patients included in the study have been characterised for the more frequent genetic lesions at 14q32 IGH locus. **Results:** Hierarchical clustering analysis of the 56 patients showed that MGUS could be differentiated from most of the MM and PCL, although MM samples were not identifiable as a clear distinct entity. The analysis identified a relevant number of genes differentially expressed between MGUS and PCL patients, mostly cell cycle and immunoglobulin-related transcripts. Notably, only a relatively limited number of genes could distinguish MM from either MGUS or PCL plasma cells, the most of them representative of the same functional categories identified by the previous analysis. MM patients displayed a heterogeneous pattern of gene expression profiles, whose clustering was driven by the different types of associated-IGH translocations, while no correlation was found either with clinical or prognostic parameters. **Conclusions:** By means of microarray analysis MM represents a highly heterogeneous entity and only slight differences can be evidenced at the gene expression level in relation with MGUS. Our results may provide insights into the understanding of the biological mechanisms involved in the MM pathogenesis.

046 INCREASED RISK FOR MULTIPLE MYELOMA IN INDIVIDUALS WITH THE HOMOZYGOUS 66GG POLYMORPHISM OF THE METHIONINE SYNTHASE REDUCTASE (MTRR) GENE

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Background: Folate and methionine metabolism play essential roles in DNA synthesis and DNA methylation. A relationship between functional polymorphisms in the folate and methionine metabolising enzyme genes, such as methylenetetrahydrofolate reductase gene (MTHFR), methionine synthase (MS), methionine synthase reductase (MTRR), and thymidylate synthase (TS) and the development of malignancies has been described in the last decade. However, their role in multiple myeloma (MM) susceptibility remains not fully clarified. **Aims:** In this study, we verify whether the MTHFR C677T and A1298C, MS A2756G, MTRR A66G, and a double (2R2R) or triple (3R3R) 28bp tandem repeat in the promoter region of TS gene polymorphisms altered the risk for MM. **Methods:** The diagnosis of 127 MM was established according to conventional criteria. The control group consisted of 300 consecutive newborns from the same university hospital in order to provide a representative group of the general population that seeks medical assistance in this region. Genomic DNA from bone marrow of MM patients and peripheral blood of controls were analysed using the polymerase chain reaction (PCR) followed by restriction digestion with HinfI, MbolI, HaeIII, and NdeI

for MTHFR, MS, and MTRR polymorphisms, respectively. The TS polymorphisms were analysed by Variation Number Tandem Repeat PCR. Statistical significance of the differences between groups was calculated by chi-square or Fischer exact test. Crude odds ratios (ORs) were calculated and were given within 95% confidence intervals (CI). All analyses were performed using the statistical package Epi Info. Results: Similar frequencies of the MTHFR 677TT genotype (11.0% vs 7.4%) and MTHFR 1298CC genotypes (8.7% vs 6.0%) were found in patients and controls. There were no differences in the frequencies of the MS 2756GG genotype in MM patients and controls (7.9% vs 3.4%, $P=0.07$). Similar frequencies of the TS genotypes were also observed in MM patients and controls (18.2% vs 17.0% for 2R2R, 48.4% vs 47.7% for 2R3R, 33.1% vs 35.3% for 3R3R). In contrast, the frequency of the MTRR 66GG genotype was higher in patients than in controls (25.2% vs 15.0%, $P=0.02$). Individuals with the MTRR 66GG genotype had an estimated 1.91 (95% CI=1.11-3.28) fold increased risk for the disease. Conclusion: Our observation of a significant increased risk of MM associated with the MTRR 66GG genotype presents preliminary evidence that this inherited characteristic of the methionine metabolising pathway could be an important determinant of this disease. Supported by FAPESP

047 OPG AND sRANKL IN SERUM OF UNTREATED MULTIPLE MYELOMA (MM) PATIENTS

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Background: The main system regulating bone turnover is constituted by the receptor activator of NF- κ B (RANK) on osteoclasts surface, that induces their differentiation and activation, its ligand RANKL and osteoprotegerin (OPG), a soluble decoy receptor, that blocks RANK/RANKL ligation thus inhibiting osteoclasts differentiation and function. RANKL may be cleaved into a soluble form (sRANKL). The role of this system in MM is under investigation. Aims: To determine serum OPG and sRANKL levels in untreated myeloma patients and to correlate them with parameters of disease activity and with prognosis. Patients and Methods: We studied 58 untreated MM patients, 3 patients with solitary plasmacytoma (SP), 12 subjects presenting monoclonal gammopathy of undetermined significance (MGUS) and 15 age-matched healthy individuals (HI). 54% MM patients were males and the median age was 69y (42-80), 2/3 of SP patients were females (median age 65y) and 80% of individuals with MGUS were females (median age 62y). MM patients stage was as follows: 14 stage I, 23 stage II, 21 stage III according to Durie-Salmon staging system and 18 stage I, 16 stage II, 24 stage III according to the new International Scoring System (ISS). Paraprotein subtype was IgG in 37 patients, IgA in 13, BJ in 6, IgD in 1 and non-secretory in 1. Median follow-up was 20 months. Serum OPG and sRANKL measurements were performed by ELISA (RD, Quantikine for OPG and Biomedica for sRANKL). In 16 MM samples OPG was also determined with another ELISA KIT from BioVendor. Results: In HI median OPG was 1700pg/ml (500-8000) and median sRANKL 5.9 pg/ml (2.6- 160). In MM patients, pretreatment median serum OPG was 2950 pg/ml (820-25000), 2200pg/ml (2100-2600) in SP and 2275 pg/ml (1500-6200) in MGUS. Serum OPG was higher in MM patients than in HI ($p=0.02$). Median serum sRANKL was 3.8pg/ml (1.6-200) in MM patients, 3.6pg/ml (3.6-6) in SP patients and 3.6 pg/ml (3.2-5.6) in MGUS; differences are not statistically significant. Serum OPG levels were correlated to beta2-microglobulin (Spearman's rho 0.24, $p=0.05$), weakly to sRANKL (Spearman's rho 0.2, $p=0.13$) and inversely to hemoglobin (Spearman's rho 0.25, $p=0.07$). There was no correlation of OPG values obtained by the two different ELISA KITs in 16 patients (Spearman's rho 0.13, $p=0.62$). Serum sRANKL correlated negatively with beta2-microglobulin (Spearman's rho 0.25, $p=0.06$). No correlations were found with disease stage or bone status. The sRANKL/OPG ratio inversely correlated with beta2-microglobulin, CRP, serum creatinine (Spearman's rho 0.32, $p=0.01$; -0.24, $p=0.09$; -0.25, $p=0.07$ respectively). The 6-year overall survival was 54% (37 patients; 4 deaths) vs 33% (16 patients; 5 deaths) in patients with normal vs elevated serum OPG/sRANKL ratio ($p=0.01$). Conclusions: Interpreting serum OPG (and possibly sRANKL) measurements is difficult because different assays using different antibodies may detect different forms of OPG and because most assays measure both free and sRANKL-bound OPG; correlations found between OPG and sRANKL values may be due to this. Nevertheless in this study, increased serum OPG correlated with adverse prognostic parameters (beta2-microglobulin) and elevated serum OPG/sRANKL ratio with inferior survival.

048 SERUM SOLUBLE SYNDECAN-1 (S-SYND-1), INSULIN LIKE GROWTH FACTOR (IGF-1) AND MACROPHAGE INFLAMMATORY PROTEIN-1 ALPHA (MIP-1A) IN UNTREATED MULTIPLE MYELOMA PATIENTS. RELATIONSHIP WITH PROGNOSIS

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Background: Multiple myeloma (MM) is partly regulated by cytokines released upon interaction between plasma cells and the bone marrow microenvironment. Among them IL-6 and its receptors were proved to constitute plasma cell growth factors. New cytokines, such as syndecan-1 and its soluble form s-synd-1, IGF-1 and MIP-1a, are now proposed to play an important role in MM disease biology, by mechanisms that are still under investigation. Furthermore their serum levels may predict prognosis. Aims: To determine serum levels of s-synd-1, IGF-1 and MIP-1a in untreated myeloma patients and to correlate them with parameters of disease activity and prognosis. Patients and Methods: Frozen sera from 70 untreated MM patients, 3 patients with solitary plasmacytoma (SP), 12 subjects with monoclonal gammopathy of undetermined significance (MGUS) and 15 age-matched healthy individuals (HI) were studied. MM patients included 34 women and 36 men (median age 69y). 16 MM patients had Durie-Salmon stage I, 30 stage II and 24 stage III while 21 were in stage I according to the new International Scoring System (ISS), 22 in stage II and 27 in stage III. Patients received conventional treatment when required and were subsequently followed for a median time of 20 months from diagnosis. Serum s-synd-1, IGF-1 and MIP-1a levels were determined by commercially available ELISA kits (Diacclone Research for s-synd-1, Quantikine, RD for IGF-1 and MIP-1). Results: In HI median s-synd-1 was 20 ng/ml (9-64), median IGF-1 96 ng/ml (46- 210) and median MIP-1a was 24.5 pg/ml (15-60). In MM patients, median pretreatment serum s-synd-1 was 100 ng/ml (9.2-3500). They were 80 ng/ml (34-200) in SP and 40 ng/ml (12-220) in MGUS. Median serum IGF-1 was 86 ng/ml (13-260) in MM, 150 ng/ml (98-220) in SP patients and 86 ng/ml (50-190) in MGUS and median serum MIP-1a was 23 pg/ml (14-100) in MM, 32 pg/ml (19-38) in SP patients and 25 pg/ml (17-47) in MGUS. Differences between HI, subjects with MGUS and MM patients as well as between patients of different D-S stage were significant for syndecan-1 and MIP-1a ($p<0.01$) but not for serum IGF-1 levels. Differences between patients of different ISS stage were significant only for MIP-1a ($p=0.03$). Serum s-synd-1 and MIP-1a correlated with beta2-microglobulin (Spearman's rho 0.35, $p=0.003$ and 0.45, $p=0.0002$ respectively) and inversely with hemoglobin (Spearman's rho -0.35, $p=0.004$ and 0.41, $p=0.001$ respectively). Furthermore s-synd-1 correlated with the degree of depression of IgA serum levels in IgG MM and MIP-1a with CRP, serum creatinine and sRANKL/OPG ratio. Overall survival was decreased in patients with elevated serum s-synd-1 and MIP-1a (6-year overall survival 75% vs 21%, $p=0.006$ and 94% vs 19%, $p=0.01$ respectively). Conclusions: Elevated serum s-synd-1 and MIP-1 were correlated to established parameters of poor prognosis (stage, beta2-microglobulin and anaemia) and had an adverse impact on survival. In our experience serum IGF-1 levels were not related to parameters of disease activity or prognosis.

ALL: BIOLOGY.

049 REAL-TIME QUANTITATIVE PCR DETECTION OF WT1 GENE TRANSCRIPTS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background and Aims: Monitoring of minimal residual disease (MRD) during treatment has become a golden standard in the modern management of children with acute lymphoblastic leukemia (ALL). However, different approach method of MRD has their limitation in the assessable number. The Wilms tumor gene (WT1) is overexpressed in a variety of hematologic malignancies including ALL. To evaluate the usefulness of WT1 transcript levels in bone marrow samples as a marker for MRD detection in children with ALL, we analyzed WT1 expression level in bone marrow samples of children with ALL using quantitative real time PCR (RQ-PCR) assays with Taqman technology. **Methods:** Total RNA was extracted from bone marrow mononuclear cells of patients and normal controls. Duplicated amounts of 100ng cDNA were assayed for WT1 transcript expression using RQ-PCR with simultaneous amplification of beta-actin as an internal amplification control. WT1 expression level was prospectively measured at diagnosis and the end of remission induction. Results were described as level per 100,000 beta-actin levels when the WT1 expression level in K562 cell line was defined as 1.0. **Results:** From Dec.12 2000 to Jan. 1, 2004, 56 newly diagnosed ALL children were enrolled with a median observation period of 15.8 months (range, 2.2-38.5 mo). Forty-nine samples (87.5%) at diagnosis were found to be over-expression for WT1 transcript and 7 (12.5%) were within normal levels. Ten normal BM samples had minimal amount of WT1 transcript expression (median 49.4; range, 0-163.3). By contrast, higher levels of WT1 transcript expression were found in BM samples of children with ALL at diagnosis (median 2654.1; range, 7.8-325,217.1). No correlation was found between initial expression levels and gender, initial white count, lineage and risk groups. WT1 expression in the BM samples was monitored at the end of remission induction in 49 out of the 56 patients (87.5%). Of the 49 studied at the end of remission induction, 8 had not achieved morphologic remission. The remaining 41 patients were in morphologic remission but 10 (23.8%) had detectable MRD by RQ-PCR of WT1 assay. Four patients experienced a clinical relapse course and all of them displayed a significant increase in WT1 expression levels at the end of their remission induction ($p=0.022$). **Conclusions:** Our findings suggest that the WT1 level quantified by RQ-PCR is a useful and applicable marker for MRD detection in the majority of children with ALL. Our preliminary data demonstrate that there is detectable WT1 transcript in the normal subjects and the level of WT1 expression at the end of remission induction in children with ALL might be related to disease relapse.

050 ANALYSIS OF BONE MARROW LYMPHOPOIESIS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA DURING AND AFTER THE CHEMOTHERAPY

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It is known for a long time that chemotherapy inhibits bone marrow (BM) lymphopoiesis. In spite of this fact, the effect of chemotherapy on different stages of lymphocyte differentiation remains poorly studied. In patients with acute lymphoblastic leukemia (ALL), study of lymphopoiesis during and after chemotherapy might provide new data regarding the recovery of normal hematopoiesis. Study of early lymphoid progenitors during chemotherapy might be used for optimization of schedule for autologous BM transplantation. Significant progress had been recently achieved in antigenic phenotyping of human B cells on different stages of maturation. It rendered flow cytometry as an effective tool for analysis of BM lymphopoiesis in various diseases. The aim of this study was to characterize normal lymphopoiesis in patients with ALL in different phases of chemotherapy. We used multiparametric flow cytometry to measure early B-, pro-B-, pre-B, immature B and mature B cells in hematologically normal childhood and adult BM and to establish reference ranges for normal BM lymphopoiesis. Different fractions of B cells were then determined in BM samples of 16 pediatric patients with ALL. The patients were treated in accordance with BFM-99 guidance and had no evidence for relapse and minimal residual disease during follow-up period. The

samples of BM were collected in the end of 1st phase of induction therapy, prior to re-induction, during or after re-induction, twice during maintenance, after the end of treatment, and during recovery. Amounts of CD19+CD34+CD45dim pro-B cells, CD10+CD19+CD20-CD45dim pre-B cells and CD10+CD19+CD20+CD45+ immature B cells were dramatically reduced after the 1st phase of induction and remained reduced until the end of therapy. Amount of CD10-CD19+CD20+CD45++ mature B cells was not changed after the 1st phase of induction, and declined significantly after the end of induction therapy. Chemotherapy had variable and inconsistent effect on the amount of CD19-CD79a+TdT+CD45dim early B-cell progenitors. Transient and moderate reduction of early B-cell progenitors was found in all patients in the beginning of maintenance. Flow cytometric analysis of multidrug resistance (MDR) in B cells by rhodamine 123 expel assay showed that CD10+CD19+ immature B cells had lower MDR-activity than CD10-CD19+ mature B cells. Analysis of lymphoid cells from patients with regenerating BM revealed increased amount of CD19-CD24+CD45dim B cells. These cells expressed VpreB (CD179a) proteins hallmarked the maturation of early B-cell progenitors into pre-B cells. Half of CD19-CD24+CD45dim cells were TdT+. Few CD19-CD24+CD45dim B cells might be also found in BM of patients during chemotherapy, and most of them were VpreB-. Our data indicate that chemotherapy damages mainly on pro-B and more mature B cells, while early B-cell progenitors are partially resistant to chemotherapy and may contribute to the recovery of lymphopoiesis after the end of therapy. Difference in MDR-activity between immature and mature B cells might somewhat explain the different effect of chemotherapy on these cells. Analysis of CD24 on B-cell progenitors shows that early B cells seem to express CD24 during their maturation into pro-B cells prior to the expression of CD19 pan-B cell marker.

051 SYNERGISTIC CYTOTOXIC EFFECT OF RAPAMYCIN IN COMBINATION WITH PURINE NUCLEOSIDE ANALOGUES ON PRE-B ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Background: Rapamycin (RAPA), is an inhibitor of mTOR kinase, exerting an immunosuppressive effect by arresting G1 phase of the cell cycle. An anti-tumor efficacy of RAPA is currently tested in patient with malignant lymphomas. Recently, RAPA activity against pre-B acute lymphoblastic leukemia (pre-B ALL) was also reported. **Aims:** The study was aimed to investigate the effects of RAPA in combination with drugs targeting cells in G1 phase, purine nucleoside analogues (PAN) , on pre-B ALL cells.

Methods: This in vitro study was performed on pre-B ALL cell line, NALM-6. The cell cultures were treated for 24h with different concentrations of RAPA (0.05-100ng/ml) alone and in combination with two PAN: 0.1-10mg/ml 2-chlorodeoxyadenosine (2-CdA) or 0.1-1.0mM fludarabine (FA). In some experiments cells were initially incubated with RAPA for 12h and then, FA or 2-CdA were added to the cultures for the next 12h. Untreated cultures, or treated for 24h with RAPA, FA or 2-CdA alone, were maintained as respective controls. Each experiment was repeated at least three times. The pro-apoptotic effect was assessed by both Annexin-V and TUNEL assays; the percentage of positive cells were defined as an apoptotic index (AI). The effect on cell cycle was analyzed using histograms of DNA content in propidium iodide/RNase stained cells. Additionally, intracellular expression of cyclin B, D3 and E was evaluated. All fluorescence measurements were performed by flow cytometry.

Results: We found two different, strictly dose-dependent modes of RAPA activity against NALM-6 cell. In low concentrations (0.05-10ng/ml) RAPA exclusively inhibited proliferation, arresting NALM-6 cells in G1 phase of cell cycle. Interestingly, the highest rate of G1 phase cells, approximately 85%, was found to induce by RAPA in concentration 0.25ng/ml. An increasing evidence of apoptotic cell death was observed in higher doses, starting from 5.0ng/ml (median AI-10%) up to 100ng/ml (median AI-49%). When NALM-6 cells were treated with the combination of RAPA+PAN, the highest, synergistic pro-apoptotic effect was found at 24h for 0.5ng/ml RAPA and 0.5nM FA (median AI: 1.5% with RAPA and 3.5% with FA vs 11.0% with RAPA+FA; $p<0.001$). Interestingly, when NALM-6 cells were pretreated for 12h with 0.5ng/ml RAPA, and then 0.5nM FA was added only for the next 12h, this effect was even higher (median AI-14.5%; $p<0.0001$). From the other hand, the combination of RAPA+2-CdA induced some additive cytotoxic effect, statistically significant only for 1ng/ml RAPA and 0.5nM 2-CdA (median AI:

2.5% with RAPA and 5.5% with 2-CdA alone vs 8.5% with RAPA+FA; $p < 0.05$).

Conclusions: RAPA exerts dual effect on pre-B ALL cell line in vitro: from arresting cell cycle in G1 phase with low concentrations to subsequently increased pro-apoptotic effect with high doses. In low, G1 phase-affecting concentration RAPA sensitizes those cells to pro-apoptotic activity of PAN, mainly FA. Pretreatment with RAPA even amplify cytotoxicity of FA. These data provide strong rationale for future applying RAPA in combination chemotherapy of lymphoproliferative diseases. This work was supported by the grant No 507-11-248 from the State Committee for Scientific Research, Poland

052 VARIATIONS IN THE RECOMBINATION ACTIVATING GENES (RAG1/2) IN RELATION TO SUSCEPTIBILITY TO CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: The recombination activating genes (RAG-1, RAG-2) are required for the targeted recombination of the T-cell receptor (TCR) and immunoglobulin (Ig) V(D)J genes during lymphoid ontogeny. The RAG1/2 proteins introduce DNA double strand breaks (DSB) at recombination signal sequences (RSS) flanking the V(D)J coding segments prior to TCR and Ig recombination. About 10-15% of childhood acute lymphoblastic leukaemias (ALL) arise in T cell progenitors. In a proportion of cases, oncoprotein activation giving rise to T-ALL arises as a result of illegitimate RAG-mediated TCR recombination.

Aims: The RAG1/2 genes are known to be polymorphic, and also to be mutated in Omenn syndrome and B-cell negative SCID. We set out to investigate whether coding sequence variation in the RAG1/2 genes is associated with susceptibility to childhood T-ALL. Methods: Constitutional genomic DNA from remission blood samples obtained from children with pro-B, B cell precursor (BCP) and T-ALL and normal new born controls were screened for RAG1-1/2 sequence variants using SSCP, and variants were identified by direct DNA sequencing.

Results: In all, we found 7 RAG1 and 3 RAG2 sequence variants during the screening of 108 childhood leukaemias and 190 new born controls. None of these included known RAG1/2 pathogenic mutations. We detected a possible T lineage bias in the frequency of the R249H bi-allelic polymorphism in T-ALL (n=22) as compared to BCP (n=54) and pro-B ALL (n=18) and new born controls (n=190). The frequency of RAG1 histidine 249 homozygotes was lower in T-ALL (32%) than in BCP ALL (52%) or pro-B ALL (67%), whilst the frequency of RAG1 arginine homozygotes was higher in T-ALL (18%) than in BCP ALL (6%) or pro-B ALL (0%). Similar results were obtained in a larger combined series of T-ALL (n=73), when compared to pro-B ALL (n=28), BCP ALL (144) and newborn controls (n=541). Apart from certain other rare variants, we found no significant excess or deficit of other RAG1/2 polymorphisms in childhood leukaemia.

Summary: Our results suggest that RAG1 R249H may be associated with a T lineage bias in the risk of developing childhood ALL. Although we have yet to analyse this in relation to chromosome 7 or 14 rearrangements in T-ALL, we suggest that illegitimate TCR rearrangement leading to oncoprotein activation may be influenced by the RAG1 genotype, but probably not by the RAG2 genotype

053 MEK INHIBITOR IN PRIMARY ADULT ACUTE LYMPHOBLASTIC LEUKEMIA CELLS: OPPOSITE REGULATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE-1/2 (ERK) AND P21CIP-1/WAF1

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Extracellular Signal-Regulated Kinase-1/2 (ERK) are serine/threonine kinases involved in the Mitogen Activated Protein Kinase (MAPK) pathway, that appear to convert a variety of extracellular signals, such as growth factors and cytokines, to cell growth, differentiation and proliferation. However, few data are available explaining the role

of ERK1/2 in leukemogenesis. Moreover, prolonged MAPK activation may increase p21CIP-1/WAF1 expression, resulting in cell cycle arrest and cytoprotective effects against chemotherapeutic agents. The aim of our study was to evaluate the role of phospho-ERK (p-ERK) and p21 in primary blasts from adult acute lymphoblastic leukemia (ALL) enrolled in the GIMEMA protocols. Primary samples from 110 untreated ALL patients and human leukemic cell lines were evaluated for p-ERK expression by flow cytometry using a p-ERK1/2-specific MoAb (clone E10). Phospho-protein expression was analyzed using the Kolmogorov-Smirnov statistic test (D-value). Human K562 cells express p-ERK (D-value=0.47), as well as normal PMA-activated PBL (D-value=0.37), whereas resting PBL and normal CD34+ cells exhibited minimal levels of p-ERK (D-value=0.04 and 0, respectively). Expression of p-ERK (D-value >0.10) was found in 38/110 adult ALL cases (34.5%), ranging between 0 and 0.72. These results were confirmed by Western blot analysis. Expression of p-ERK did not correlate with age, WBC number, and leukemia phenotype. Flow cytometry analysis of selected ALL samples for p21 expression using the WAF1 MoAb showed positivity in 53% of cases (44/83) and its expression was significantly associated with higher WBC values (>20 x 10⁹/L, $p=0.04$) and a higher percentage of cells in G0 phase ($p=0.03$). In vitro exposure to the MEK inhibitor PD98059 (1.5 mM) induced a significant downregulation of p-ERK expression from a D-value of 0.090.11 (T=0) to 0.02 0.06 (after 24h of PD98059 incubation; $p=0.0157$) in 7/8 samples examined. In contrast, in the same samples, the expression of p21 increased after incubation with PD98059 (D-value 0.070.10 and 0.30 0.28, respectively; $P=0.05$). In summary, our study shows that both p-ERK and p21 are expressed in a significant proportion of adult ALL samples: p-ERK expression appears to be associated with a higher leukemic mass, p21 expression is similarly associated with a higher leukemic mass but, at variance from p-ERK, it is also associated with kinetic quiescence. Clinical follow-up will clarify impact of both parameters on patient prognosis and chemoresistance. These data suggest an opposite regulation of p-ERK and p21 expression in adult ALL, which may reflect their different role in leukemic cell proliferation and chemoresistance.

054 ZAP-70 IS EXPRESSED IN NORMAL B-CELLS OF IMMATURE PHENOTYPE AND IN SOME B ACUTE LYMPHOBLASTIC LEUKEMIAS

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BACKGROUND: ZAP-70 is a protein tyrosine kinase of the Syk/ZAP-70 family that plays a critical role in the signal transduction of the T-cell receptor. In human lymphocytes, ZAP-70 gene is found in T and NK derived cells, and in IgVH unmutated B-chronic lymphocytic leukemia cells. More recently, ZAP-70 expression has been shown to be required for B-cell development in mice, its absence blocking the B cell development at the pro-B cell stage. Consistent with this, ZAP-70 expression was found in pro-B, pre-B and CD19+ splenic B cells from mice. To date, the expression of ZAP-70 gene in human immature B-cell stages has not been ascertained.

AIMS: To analyze ZAP-70 expression in: 1) normal human B cells at different stages of B cell maturation, including pro/pre B cells, and 2) tumor cells from acute lymphoblastic leukemias (ALL) with pro/pre B phenotype. METHODS: ZAP-70 was analyzed in lymphocytes from peripheral blood or bone marrow of 18 patients with B-ALL, normal bone marrow (2 cases), one tonsil, and in NALM-6 (pre B ALL), JVM-2 (mantle cell lymphoma) and Jurkat (T cell leukemia) cell lines. ZAP-70 expression was assessed by flow cytometry (FC) and/or by Quantitative Real Time RT-PCR (QRT-PCR) using Jurkat as a positive control. In addition, normal tonsil was also analyzed by immunohistochemistry (IHQ).

RESULTS: In normal bone marrow, ZAP-70 expression was found only in T and in immature B (CD19+ / CD10+ / CD20 -) cells. Moreover, T cells -but no CD19+ cells- from normal tonsil expressed ZAP-70, as assessed by QRT-PCR and IHQ. By flow cytometry, 9/12 B-ALL cases exhibited high expression of ZAP-70 (mean, 79%, range 60-99%), whereas in 3 cases ZAP-70 was barely detectable (mean, 10%). By QRT-PCR, 9/13 B-ALLs showed ZAP-70 expression levels similar to that found in cell lines not expressing ZAP-70 (JVM-2) and normal mature B-cells, whereas in the remaining cases ZAP-70 was 3-4 times higher than in normal mature B-cells. In the 7 samples analyzed by both methods, an absolute concordance in ZAP-70 expression was observed. Taken together, we found a high expression of ZAP-70 in 10 out of 19 (53%) B-ALLs. No relationship was observed between the B-ALL maturation status, as assessed by FC, and the level of ZAP-70 expression.

CONCLUSIONS: Among normal B cell subsets, ZAP-70 expression was restricted to B-cells with immature phenotype (pro/pre B cells), as previously described in mice. Moreover, ZAP-70 was expressed in 53% of B-ALLs, probably reflecting the B-cell origin of these tumor cells.

055 HISTONE DEACETYLASE INHIBITORS ARE ABLE TO CHANGE LEUKAEMIA-SPECIFIC PHENOTYPE OF TEL/AML1-POSITIVE LEUKAEMIC CELLS

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The cryptic translocation t(12;21), resulting in TEL/AML1 chimaeric gene, is the single most frequent non-random genetic aberration in childhood ALLs (~25%). Both partner genes are important for the development and maintenance of normal haematopoiesis. The TEL part of the fusion protein contains domains interacting with the mSin3, N-CoR and HDAC-3 corepressors. A part of the AML1 gene involved in the fusion carries a DNA binding domain that controls the interaction with the cis-regulatory elements of the target genes. TEL/AML1 is supposed to negatively regulate the expression of the genes that, under normal conditions, are transactivated by AML1. TEL/AML1 chimaeric protein seems to affect gene responsible for the differentiation during haematopoiesis by chromatin remodelling via association with histone deacetylases (HDAC).

We use HDAC inhibitors (HDACi) to overcome the presumptive differentiation block mediated by HDAC recruited with TEL/AML1. Trichostatin A (TSA) and valproic acid (VPA), common antiepileptic drug with newly attributed HDACi activity, were used as HDACi. We tested different concentration of TSA (120nM, 240nM and 600nM) and VPA (0.5mM, 1.0mM and 2.0mM; concentration close to the VPA plasmatic level used in the treatment of epilepsy). We determined the cell cycle and state of differentiation using flow cytometry 24 and 48 hours after HDACi administration.

We compared TEL/AML1[+] (REH) and TEL/AML1[-] (Nalm-6) leukaemic cell lines to the non-leukaemic lymphoid cell line (NC-NC). Under HDACi treatment, leukaemic cells were arrested in proliferation and entered apoptosis. In REH cell line, the apoptosis rate was 11% using 1mM of VPA, and 30% using 240nM of TSA in contrast to 3.7% in control. Similarly, Nalm-6 apoptosis rate reached 16% with VPA, and 39% with TSA compared to 0.6% in control. In contrast, NC-NC cell line showed only negligible shift in cell cycle. The Nalm-6 and REH cell lines reduced their proliferation activity by 36% and 19%, respectively. Moreover, live TEL/AML1[+] cells showed dose-dependent maturation drift from CD10+/CD20- to CD10+/CD20+ after both TSA and VPA in contrast to TEL/AML1[-] cells.

Our results support the hypothesis that HDACi is generally able to affect maturation stage and viability of leukaemic cells. We suppose that the differentiation effect of HDACi on TEL/AML1[+] cells is directly related to the function of TEL/AML1 protein. The advantage of Valproate lies in its proven low toxicity as known from the epilepsy treatment.

Supported by grants: 7433 (IGA MZ), 301/D0189 (GACR), 111300001 and 111300005 (MSMT)

056 SHP-2 AND RAS FUNCTION IN INFANT ALL

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Background: We recently provided evidence that PTPN11 is recurrently mutated in childhood B-cell precursor acute lymphoblastic leukemia (ALL). PTPN11 encodes SHP-2, a SH2-containing protein tyrosine phosphatase that positively controls cell proliferation and survival of hematopoietic cells by acting as positive modulator in RAS signaling. Consistently, we showed that PTPN11, NRAS and KRAS2 mutations were largely mutually exclusive, and their combined prevalence accounted for approximately one-third of pediatric cases aged 1-18 years with common ALL. Significantly, while PTPN11 mutations appeared to be preferentially associated with the common immunophenotype, and with cases negative for the most frequent translocations, mutations in NRAS and/or KRAS2 were uniformly distributed among the B-cell precursor ALL subtypes. We also showed that PTPN11 mutations occurred in 4/12 pediatric cases with acute monocytic leukemia (FAB-M5); three of them showed rearrangement of the MLL gene. By contrast, RAS mutations

occurred in children with different AML subtypes. No NRAS mutation was identified among the four cases with mutated PTPN11.

Aim: we have analyzed 37 pediatric ALL patients aged <1 year (infant), in order to explore whether alterations of the RAS pathway genes may identify specific subgroup within this heterogeneous patient population.

Results: MLL gene rearrangements were fully characterized in 28/37 cases (75.7%). Six patients did not show rearrangement using the 11q23 FISH 'splitted signal' probe (21%); 15/28 (53.6%) were t(4;11) positive, 3 were t(11;19) positive, 2 were t(9;11) and 2 cases had 11q23 rearranged with an unknown MLL partner gene. None of the 32 cases carried a PTPN11 somatic mutation. Only one case was positive for the G35C mutation of the KRAS2 gene (1 out of 25). Five cases out of 28 (17.9%) carried NRAS mutations: G35A, n=3; G34C, n=1; G38A, n=1. Interestingly, in two cases DHPLC analysis showed the presence of the RAS mutation only in a fraction of the blast cells population. Point mutations of the FLT3 gene were detected in 3/35 case at diagnosis, by PCR followed by restriction enzyme digestion and sequencing. Of note, none of those cases were positive for RAS pathway alterations. Eleven out of 14 cases with MLL rearrangements showed high levels of the FLT3 gene expression (DeltaCt=CtFLT3-CtABL>3, by RQ-PCR). More precisely, out of the 20 cases successfully analyzed for FLT3 expression, 7 had DeltaCt<3.

Conclusions: 1. PTPN11 gene mutations do not occur in infant ALL as compared to older children; 2. By contrast, the overall incidence of RAS genes mutations are comparable; 3. The alterations of RAS and FLT3 pathways occurred in different infant ALL subgroups. Whether these genetic differences could influence the heterogeneous clinical behavior of infant ALL, is still unknown.

057 EXPRESSION OF THE PRE-T CELL RECEPTOR ALPHA CHAIN IN ACUTE LYMPHOBLASTIC LEUKEMIA CELL LINES AND ITS POTENTIAL ROLE AS A THERAPEUTIC TARGET

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Background: The early stages of T cell development ensures a selection of thymocytes with functionally rearranged T-cell receptor (TCR) * loci which is mainly controlled by the pre-TCR consisting of disulfide-linked TCR* / pre-TCRCR consisting of disulfide-linked TCR* / pre-TCRCR consisting of disulfide-linked TCR* / pre-TCRCR consisting of disulfide-linked TCR* / pre-TCRCR consisting of disulfide-linked TCR* / pre-TCRt this developmental checkpoint might play a crucial role in the emergence of T lymphoblastic leukemias.

Aims: In the present study we aimed to quantify the gene expression level of pTα and its splicing variant in leukemic cell lines of different developmental stages. Based on additional protein studies pTα expressing cell lines were selected for further functional analyses testing its relevance as potential therapeutic target. **Methods:** Gene and protein expression patterns of pTα and its splicing variant were studied by RT-PCR, real-time RT-PCR, westernblot analysis and immunoprecipitation in T lymphoblastic cell lines (n=16) in comparison to non-T-lymphoblastic leukemias and healthy controls (n=18). Functional assays were performed by using a PTK inhibitor selectively and strongly interfering with downstream signaling events of pTα. A MTT assay was applied for the determination of the IC50 values. Cell cycle analyses and apoptosis staining assays were performed for the characterization of the underlying inhibitory mechanism.

Results: T-lymphoblastic cell lines (2.36 x 10⁴ copies; mean value) expressed high levels of pTα in comparison to non-T-lymphoblastic leukemias (1.01 x 10⁴ copies; mean value). The highest gene expression levels were found in immature T-ALL cell lines (prethymic and thymic stages; 3.17 x 10⁴ copies; mean value), whereas mature T-ALL (4.76 x 10⁴ copies; mean value) and even B-ALL (3.0 x 10⁴ copies; mean value) cell lines only expressed small amounts of pTα. In contrast, myeloid leukemias show a lack of pTα expression. Surprisingly, alternatively spliced pTα was also detectable in healthy controls. The protein amount of pTα in the tested cell lines did not show a correlation to the quantified gene expression level. The tested PTK inhibitor shows a remarkable effect on cell proliferation in 7 out of 12 pTα protein expressing cell lines. Preliminary results indicate that a block at the G0/ G1 phase of the cell cycle is mainly responsible for the growth inhibition of leukemic cells.

Summary/ Conclusions: The early TCR functions as critical regulator of the normal T cell development and cell survival. The high gene and protein expression levels of pTα in T-ALL cell lines emphasize its potential role in dysregulated proliferation of lymphoblastic leukemias. Furthermore, the blocking of p56lck tyrosine kinase, which delivers signals from molecules of the pre-TCR, may inhibit the leukemic cell growth. These functional data support the leukemogenic potency of the early TCR and suggest new molecular targets for therapeutic intervention.

058 UPREGULATION OF ASPARAGINE SYNTHETASE FAILS TO AVERT CELL CYCLE ARREST INDUCED BY L-ASPARAGINASE IN TEL/AML1-POSITIVE LEUKAEMIC CELLS

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L-Asparaginase (Asnase) is a standard component in the chemotherapy of childhood acute lymphoblastic leukaemia (ALL). Leukaemic cells carrying TEL/AML1 fusion gene were shown to be more sensitive to treatment with Asnase compared to other subtypes of ALL. We hypothesised that this effect of Asnase is based on the diminished activity of Asparagine Synthetase (AsnS) in leukaemic cells. We simulated in vitro the treatmentlike administration of Asnase. We analysed the expression of AsnS by quantitative RT-PCR and cell cycle changes by flow cytometry. AsnS expression was also analysed in newly diagnosed ALL patients. Significantly higher expression of AsnS was found in leukaemic cells from children with TEL/AML1[+] ALL (n=20) in comparison with the group of age-matched children with B-cell precursor ALL bearing no known fusion gene (n=25; p=0.0043). Interestingly, non of TEL/AML1[+] patients with high AsnS level relapsed whereas 10/15 pts with AsnS below median relapsed (p=0.00028). We demonstrated in vitro prolonged growth suppression of TEL/AML1[+] cells compared to TEL/AML1[-] leukaemic cells after repeated treatment with Asnase simulating the treatment protocol. No difference in upregulation of AsnS expression was observed when TEL/AML1[+] and TEL/AML1[-] leukaemic cells were compared to non-leukaemic lymphoid cells. Cell cycle analysis revealed that after treatment with Asnase, TEL/AML1[+] cells were accumulated in G1/G0 phase: 81-98% with a single dose and 91-98% with a repeated dose compared to TEL/AML1[-] cells (51-60% and 47-57%, resp.). We conclude that sensitivity to Asnase is not directly related to the ability to upregulate AsnS expression. We hypothesise that TEL/AML1[+] cells are unable to progress into S phase of cell cycle under nutrition stress caused by Asnase. Quantitative determination of AsnS levels in diagnostic samples might distinguish between TEL/AML1[+] leukaemias with excellent prognosis and those in higher risk of subsequent relapse. Grant support: #7433 from the Ministry of Health and #111300003 from MSMT.

059 INCIDENCE AND SIGNIFICANCE OF 12P13/ETV6 DELETION IN 269 ADULT ACUTE LYMPHOBLASTIC LEUKEMIA ENROLLED IN THE ALL 0496 AND 2000 GIMEMA TRIAL

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Background: Chromosome 12p deletions occur at a 3-5% incidence in adults with acute lymphoblastic leukemia (ALL) as assessed by cytogenetic analysis. Usually, the 12p13 band where the ETV6 gene is located is involved. The clinico-biological significance of this deletion is unknown. **Aims:** To better define the incidence and significance of 12p/ETV6 deletion in adult ALL.

Methods: 269 patients enrolled in the ALL 0496 and 2000 GIMEMA trials were studied by interphase FISH using a 12p13/ETV6 probe and an AML1 probe as control. The cases were submitted to cytogenetic analysis and molecular investigation for the detection of BCR/ABL fusion, MLL rearrangement, PBX/E2A fusion and TEL/AML1 fusion. Samples showing a 12p/ETV6 deletion were studied with a probe that recognizes

DUSP16, a candidate tumor suppressor gene at 12p13 coding for a MAPK phosphatase.

Results: 12 out of 269 cases (4.5%) carried a 12p/ETV6 deletion in 40-90% of interphase nuclei (median 67%). Concomitant DUSP16 deletion occurred in 7/12 cases in 26-70% of the cells (median 54%). Using the MRC hierarchical molecular-cytogenetic classification, every patient was assigned to a single specific cytogenetic category as follows: 74 patients had the t(9;22)-BCR/ABL fusion (3 with 12p/ETV6 deletion); 25 had the t(4;11)-MLL rearrangement, or 6q- or t(1;19) (no case with 12p/ETV6-); 9 had a 12p/ETV6 deletion; 53 had a pseudodiploid, hypodiploid or hyperdiploid karyotype; 80 had a normal karyotype or no mitosis and no detectable molecular defect. Hematologic features in the 9 patients assigned to the 12p-/ETV6 deletion group were as follows: 7/9 patients displayed a B-immunophenotype (pre-B in 6 cases, pro-B in 1 case), whereas 2 cases were classified as T-lineage ALL; 7/8 cases were CD34 positive; CD33 with or without CD13 was found in 5/7 cases tested; the age of the patients ranged between 16 and 60 years (median 42), the WBC count at presentation ranged between 3,850 and 63,000 (median 15,900); 8/9 patients attained CR after induction treatment; however 5 relapsed after 2-13 months (median 3 months); 3 patients are alive in 1st CR at 3, 24 and 36 months; 2 are alive in 2nd CR; 1 is alive with resistant disease after allogeneic BMT, 3 patients died after 1-20 months.

Conclusions: Based on the above results, the following conclusions can be reached: a) the 12p/ETV6 deletion is the fourth most frequent anomaly occurring in adult ALL after t(9;22), 11q23 rearrangements and 6q-; b) the DUSP16 gene is involved in the deletion in a fraction of cases; c) with the exception of t(9;22), the 12p deletion is not found in association with other classical anomalies (11q23 rearrangements, 6q-, t(1;19)); d) it is usually associated with a pre-B immunophenotype. Though the number of cases is relatively small, the outcome appeared severe, even when patients with t(9;22) were excluded from the analysis.

060 MYELOID ANTIGEN EXPRESSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: IMPACT ON PROGNOSIS

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Background: The prognostic value of myeloid antigen (MyAg) expression in adult acute lymphoblastic leukemia (ALL) is still controversial; while early adult ALL studies had suggested an inferior outcome for MyAg positive ALL patients, other published series with protocols based on high dose chemotherapies failed to confirm a prognostic correlation. **Aims:** We reviewed the co-expression of the myeloid antigens CD13 and/or CD33 in a large number of adult patients with de novo ALL, in order to analyze the impact on prognosis. **Methods:** Between October 1996 and July 2000, 377 patients registered and uniformly treated in the GIMEMA LAL0496 protocol were studied. All patients were homogeneously diagnosed through a central handling of the samples at presentation and were investigated for morphology, immunophenotype, cytogenetics, molecular biology and expression of multidrug resistance (MDR); CD13 and/or CD33 surface markers were considered positive when 20% or more of the blasts expressed the antigen(s). **Results:** A MyAg (CD13 and/or CD33) aberrant co-expression was documented in 35% of the 377 adult ALL cases analyzed. Overall, there were 153 females and 224 males, with a median age of 30.1 years (range: 14.0-59.8), a median hemoglobin value (Hb) of 9.2 gr/dl (range: 3.4-16.8), a median white blood cell (WBC) value of 16.1 x 10⁹/l (range: 0.5-848.0) and a median platelet (Plts) value of 51.0 x 10⁹/l (range: 1.0-407.0); the Kruskal-Wallis test showed no distinction between MyAg positive cases and MyAg negative cases in relation to age and Hb, while a significant difference was found for WBC (p=0.03) and Plts (p=0.004). 114 (38%) of the 301 patients classified as B-lineage and 18 (24%) of the 76 T-lineage ALL patients were MyAg positive (p=0.02). No diversity was observed in the expression of MyAg between normal and abnormal cytogenetics subgroups and high risk (BCR-ABL+, ALL1-AF4+, E2A-PBX1+) and low risk B-ALL. Furthermore, an equivalent expression of MyAg was recorded among MDR positive patients and MDR negative patients (MDR was evaluated as expression). In our ALL series, we failed to observe any effect of the aberrant MyAg expression in the achievement of complete remission (76.5% vs 81.2%; p=ns), as well as in the prediction of relapse. In addition, no differences were found between MyAg positive and MyAg negative cases in terms of disease-free survival (33.8% vs 32.1%; p=ns) and overall survival (33.2% vs 31.8%; p=ns) at 5 years. **Conclusions:** The small numbers of patients in adult series and the differences in the treatment protocols have led

to divergent results in various studies; we have hereby examined the clinical impact of an aberrant MyAg expression in a large cohort of adult patients uniformly treated with the GIMEMA LAL0496 protocol. Our data suggest that the MyAg positive expression is not associated with adverse presenting clinical and biological features. In addition, we observed that response to treatment was comparable in MyAg positive and MyAg negative cases in terms of both complete remission rate and overall survival; thus, when analyzed in a large number of adult ALL cases, the expression of myeloid antigens seems to lack prognostic significance.

CLL: ZAP-70 AND OTHER MARKERS MUTATIONAL STATUS

061 PH CHROMOSOME-POSITIVE CML PATIENT WITH SUPPURATIVE PERIODONTITIS DURING TREATMENT WITH IMATINIB (STI571)

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Background: The treatment of Philadelphia positive chronic myeloid leukaemia (CML) has undergone a dramatic change with the introduction of imatinib. Manifestations of extramedullary hematopoiesis are rare in CML (< 10%) and identify patients with CML in accelerated and blastic-phase. There are only a few reported cases in which the extramedullary manifestations preceded the blastic phase of bone marrow or peripheral blood: the median time from diagnosis of extramedullary disease (ED) to blast crisis is 4 months and the median survival from development of ED is 5 months; thus, it is well known that ED represents a negative prognostic factor in the natural history of CML.

Aim and Results: We report the case of a 36-year-old male patient, affected by newly diagnosed Philadelphia positive low Sokal risk CML in chronic phase, treated with imatinib, who presents a possibly related adverse event of suppurative periodontitis. On May 2003 the patient received for the first time imatinib at conventional dose of 400 mg/day. Soon after the patient presented occasional muscle cramps in the legs and myalgias with normal creatinina phosphokinase levels; there were no other important adverse effects attributable to imatinib. Bone marrow aspirate, performed after three months of treatment, showed a complete haematologic remission, while cytogenetic monitoring showed a partial cytogenetic response. After approximately five months of treatment with imatinib, on physical examination the patient presented hypertrophy of the gums, toothache, alveolaris pyorrhoea and fever superior to 38°C for one day; there were no other clinically significant features; liver and renal function tests were normal; the haemoglobin level, absolute neutrophil count and platelet count were normal. The imatinib drug was temporarily stopped due to this event for four weeks. Morphological examination of peripheral blood did not show haematopoietic progenitors. Bone marrow aspirate confirmed a complete haematologic response. Gingival biopsy, performed to rule out possible ED, showed a diffuse involvement of neutrophil cells; there were no blasts, no promyelocytes and no basophil cells. Cytogenetic analysis performed both on bone marrow aspirate and gingival lesions did not show the additional chromosomal abnormalities. These istological findings reflected local irritation and focal infection of the gums. A diagnosis of 'suppurative periodontitis' was made and the patient started the antibiotic therapy with ceftriaxone. In our case the histological and cytogenetic features and the disappearance of clinical symptoms after the antibiotic therapy confirmed the absence of an extramedullary blastic infiltrate.

Conclusions: The 'suppurative periodontitis' in our patient was probably an intercurrent bacterial infection possibly related to imatinib. Bacterial infections, during treatment with imatinib, were usually related to neutropenia; in our experience the patient did not develop neutropenia. The appearance of a bacterial infection in our patient, thus, might suggest the presence and selection of dysplastic neutrophil cells unable to carry on their killing activity and phagocytosis. Chromosomal abnormalities associated with Myelodysplastic Syndromes were reported in Ph-negative cells of imatinib-responder patients.



Caption 1:

062 CD38 EXPRESSION AND NF-KB ACTIVITY IN B-CLL: EFFECT OF TGF-BETA MODULATION

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Background. B-chronic lymphocytic leukaemia (B-CLL) is an haematological malignancy characterised by the accumulation of mature-like monoclonal B lymphocytes and that can follow either an indolent or a progressive course. CD38 expression is a negative prognostic factor for B-CLL, but it is also tightly regulated during B-cell ontogeny, suggesting that its not merely a marker in B cells. In particular, CD38 plays a role in cellular proliferation and shows an anti-apoptotic activity in mature B cells. Lack of apoptosis and constitutive high level of NF-kB are key mechanisms of accumulation of malignant B mature-like cells. Furthermore, several cytokines play a role in the leukaemic growth or in prolonging cell survival of B-CLL cells in vitro, as well as in regulating NF-kB activity. In particular, TGF-beta showed a pro-apoptotic role in reducing the high constitutive level of NF-kB.

Aims. To investigate, in 26 patients with B-CLL (mean age+SD 71+10 years, range 52-86, 10 female and 16 male), the expression of CD38 mRNA and NF-kB activity and their modulation by TGF-beta; to evaluate the relationship between CD38 mRNA expression and clinical parameters (stage, therapy) or standard markers of disease progression (lymphocytic count, beta2 microglobulin).

Methods. The percentage of CD38 expressing cells was estimated in peripheral blood by standard cytofluorimetric assay. PMBC from B-CLL patients were cultured in the absence and in the presence of PMA and TGF-beta. RNAs were extracted from PMBC and reverse-transcribed to make cDNA, amplified for CD38 and GAPDH and PCR products were quantified with the PCR-ELISA Kit. The results of mRNA CD38 expression were normalised for GAPDH. To evaluate NF-kB activity, nuclear extracts were obtained from B-CLL patients PBMC and EMSA assay.

Results. We found that PMA stimulation significantly reduced CD38 mRNA expression compared with resting cultures ($p<0.005$). On the contrary, NF-kB activity was constitutively high in unstimulated PBMC cultures and no further inducible by PMA stimulation. Addition of TGF-beta significantly reduced NF-kB activity ($p<0.04$), whereas it has no effect on CD38 mRNA expression. However, considering patients with CD38 expression higher than median value, TGF-beta was able to significantly reduce CD38 levels ($p<0.05$, TGF-beta stimulated versus unstimulated cultures), whereas it had no effect in patients with CD38 expression lower than median value. Finally, beta2 microglobulin levels were significantly higher in B-CLL patients with CD38 mRNA higher than median value (3.82 ± 3.2 versus 2.23 ± 0.55 mg/L, mean+SD, $p<0.05$), whereas CD38 mRNA levels showed no correlation with stage, therapy, and lymphocytic count.

Conclusions. Lack of correlation between CD38 expression and clinical parameters is in line with the recently questioned negative prognostic value of CD38 expression. The ability of TGF-beta to down regulate

CD38 mRNA in B-CLL patients with high expression of this molecule is in favour of a signalling molecule role of CD38, rather than merely a marker. Finally, we confirm that TGF-beta is an important negative regulator that can induce apoptosis in several cell systems, as observed for NF-kB. div_emat@policlinico.mi.it

063 VASCULAR ENDOTHELIAL GROWTH FACTOR IN PATIENTS WITH B- CHRONIC LYMPHOCYTIC LEUKEMIA. ASSOCIATION WITH THE STAGE AND OTHER PROGNOSTIC MARKERS OF THE DISEASE

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Background. The vascular endothelial growth factor (VEGF) promotes the angiogenesis and is related with the growth and the dissemination of certain neoplasias. The prognostic importance of the concentrations of VEGF in the serum of patients with B-chronic lymphocytic leukemia (B-CLL) has not been studied sufficiently.

Aim. The study of the concentrations of VEGF in the serum of patients with B-CLL, their role in the growth and development of the disease and their correlation with the stage and other prognostic markers of the disease. **Material - Methods.** We studied 28 patients with B-CLL at diagnosis (RAI stage: 12/0, 7/I, 2/II, 2/III and 5/IV). We measured: a) serum concentrations of VEGF using ELISA b) the percentage of CD38 + B lymphocytes using flow cytometry c) the b2 microglobulin and LDH using classic methodology and d) the histologic type of the infiltration of the disease in the bone marrow. Moreover we studied the immunoglobulin heavy chain variable region genes (IGHV) somatic mutation status in clonal rearranged IGHV genes after RT-PCR amplification, direct sequencing, sequence alignment to the BLAST/IMGT databases and analysis of the distribution of mutations.

Results: 1) the mean value of serum concentrations of VEGF was found $X = 368.9 \text{ pg/ml}$ in patients of stages 0, I, II (group A) and $X = 789.2 \text{ pg/ml}$ in patients of stages III and IV (group B), $P < 0.01$ 2) the mean value of percentage of CD38 + B lymphocytes were found $X = 5\%$ in group A and $X = 56\%$ in group B, $P < 0.001$ 3) the mean value of b2 microglobulin was found $X = 2.1 \text{ mg/l}$ and $X = 6.4 \text{ mg/l}$, while the mean value of the LDH $X = 155.1 \text{ IU/l}$ and $X = 246.1 \text{ IU/l}$ in A, B groups respectively, $P < 0.01$ and $P < 0.001$ 4) nodal bone marrow infiltration presented in 16/21 (76%) and in 2/7 (29%) patients of groups A and B respectively 5) in group A, we found 17 (81%) patients with mutated status of IgHV genes (VH homology to germline $< 98\%$) and 4 (19%) with unmutated status. In group B we found 2 (29%) mutated and 5 (71%) unmutated cases.

Conclusions: 1) patients with B-CLL in advanced stage (III, IV), presented high serum VEGF concentrations. 2) the high serum VEGF concentrations were correlated with bad prognostic predictors, as unmutated IgHV genes, increased percentage of CD38+ B-lymphocytes, diffuse bone marrow infiltration and increased concentrations of b2 microglobulin and LDH 3) the VEGF should play important biological role in the growth and the dissemination of the disease.

064 IDENTIFICATION OF NOVEL MARKER GENES FOR B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background

Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in the Western world. It is a malignant disorder of slowly proliferating CD5+ B-lymphocytes. The epidemiology of CLL suggests that genetic factors influence disease susceptibility. Whereas some patients have an indolent course and die after many years from unrelated causes, others progress rapidly and succumb within a few years from this currently incurable leukaemia. Prognostic markers poorly predict the outcome for individual patients in early stages. Furthermore, the variable clinical course points to that the disorder is heterogeneous as to which oncogenic program is operative.

Aims

In the present study the aim is to identify susceptibility and marker genes for CLL using DNA microarray technology, and to identify

different gene profiles, that differentiates 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 complete blood cell counts, conventional cytogenetic studies, immunofenotyping by flow cytometry, and B-cell isolation. CD19+ B-lymphocytes were isolated by density gradient centrifugation and immunomagnetic cell separation. The purity of isolated cells was determined by flow cytometry. Then RNA was isolated using the Chomczynski method, followed by RNeasy clean up. Briefly, RNA was transcribed into cDNA and biotin-labelled cRNA was synthesized. Hybridization to DNA microarrays (Human Genome U133A; Affymetrix, Santa Clara, CA) and detection of hybridized target cRNA was performed according to the Affymetrix Gene Chip Expression Analysis manual. This gene chip covers more than 20 000 human genes. Genes were clustered and selected using Genespring 5.0.

Results

The diagnosis of the included patients was confirmed by immunophenotyping. Follow up time since diagnosis was at least 18 months (mean 80, range 18-154 months). Chromosome analysis showed trisomy 12 in one indolent and one aggressive patient where the indolent patient also had deletions of chromosome 17. In addition, one patient in the aggressive group showed a translocation involving 11q. 830 of 22 283 genes analysed by DNA microarray were differently expressed between normal B-cells and CLL cells. BCL-2 was upregulated in the CLL patients compared to the controls. Furthermore, the immunoglobulin gene expression was downregulated in the CLL cells. The gene encoding the autocrine motility factor receptor (GP78) was overexpressed in indolent CLL but not detected in aggressive CLL or normal B-cells. This protein is a cell surface receptor suggested to be involved in metastasis in several cancer forms. The expression of GP78 will be analysed by flow cytometry in a new set of CLL patients and compared to prognostic markers such as IgVH mutational status and expression of CD38 and ZAP-70.

Summary/conclusions

CLL is currently incurable and more information about its tumour cell biology and susceptibility genes is highly warranted. Also the identification of markers distinguishing the indolent and aggressive forms of CLL could help to predict the outcome for the patients and help in planning the treatment strategy. Using DNA microarray GP78 was suggested to be overexpressed in indolent CLL.

065 DETECTION OF ZAP-70 BY FLOW CYTOMETRY USING THE HIGHLY FLUORESCENT ALEXA FLUOR DYE IN CELLS FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Early biological prognostic markers for CLL including staging, immunological markers, and chromosomal and molecular abnormalities have limited ability to predict disease outcome. Lately, immunoglobulin mutational status (IgVH) and the expression of the tyrosine kinase ZAP-70 appear to offer a more robust alternative to the other parameters. We studied ZAP-70 expression in 120 patients with CLL (80M/40F) using a highly improved fluorescent antibody conjugate that results in minimal quenching upon conjugation and consequent brighter photostable staining, easier instrument compensation and greater accuracy in detection of proteins using flow cytometry. A human ZAP-70 antibody (clone 2F3-2) was conjugated to the Alexa Fluor 488 dye using a zenon mouse IgG labelling kit. The FACS data from this conjugate was initially compared to a standard FITC/FACS technique and to western blots on protein lysates from highly purified CLL cells ($< 1.0\%$ CD2+ve cells) using a B-cell isolation kit. Results from the Alexa/FACS analysis was expressed as a ratio of B-cell mean cell fluorescent (MCF) to T-cell MCF with a ZAP-70+ve cut-off ratio of ≥ 0.75 . Concordance was demonstrated between both FACS methods and western blot data with greater concordance using the Alexa/FACS protocol. ZAP-70 protein expression was positive in 31 (25.8%) patients with a median MCF ratio of 0.89 (range 0.76-1.46) while the remaining 89 (74.2%) were ZAP-ve with a median MCF ratio of 0.57 (range 0.19-0.72). IgVH status was analysed in 66 (55%) of the samples. In 7 (10%) patients, no clonal IgVH rearrangement was detected and all 7 were negative for ZAP-70 expression. In the remaining 59 samples, 37 (62.7%) carried mutated IgVH ($> 2\%$) and were

ZAP-70-ve while 12 (20.3%) had unmutated IgVH and were ZAP-70+ve. Discordant ZAP-70 and IgVH data were detected in 10 (17%) patients. Three patients with mutated IgVH were ZAP-70+ve and 7 unmutated IgVH patients were ZAP-70-ve. In this cohort of patients, ZAP-70 expression and IgVH status correlated with WBC and lymphocyte counts. There was also a correlation between ZAP-70-ve/IgVH mutated patients and Binet stage. The associated tyrosine kinase, Syk-72, expressed in normal B-cells was also studied in 71 highly purified protein lysates by western blotting. In contrast to earlier reports, in our cohort of CLL samples 10% (7/71) did not express Syk-72 protein, including 3 that were also negative for ZAP-70 expression (Syk-72-ve/ZAP-70-ve). This study confirms that there is good correlation between ZAP-70 expression and the IgVH mutational status in CLL. The concordance of the two parameters was 84% (37/44) for ZAP-70-ve/IgVH mutated status and 80% (12/15) for ZAP+ve/unmutated IgVH.

066 A NOVEL FLOW CYTOMETRY ANALYSIS METHOD FOR THE QUANTITATION OF ZAP-70 EXPRESSION IN B-CLL CELLS

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Background: In recent years IgVH mutational status and molecular cytogenetics have improved the ability to predict the prognosis of CLL patients. These tests are highly sophisticated and too complex for routine use. ZAP-70, a newly described marker when highly expressed in B-CLL cells was shown to correlate with poorer prognosis and shorter survival. A flow cytometry assay, recently described by Crespo et al (1) for ZAP-70, appears to be the simplest approach for routine clinical stratification in B-CLL. This analysis is highly informative, and demonstrated a clear correlation between the expression of ZAP-70 in CLL cells and poorer clinical outcome. However, there are some technical aspects that could be improved to enable standardization of this assay for routine flow cytometry. Currently ZAP-70 expression in B-CLL cells is not quantitative but assessed relative to its expression in the T and NK cells (CD3+CD56+). This can be problematic because ZAP-70 levels in T cells varies in both normal blood and in CLL, probably due to upregulation following activation. An additional problem is that all results are relative to the subjectively delineated T cell gate. Small changes in gating can dramatically alter the results in some samples.

Aims: We attempted to establish a method for the analysis of ZAP-70 in B cells which would be uncoupled from T cells as well as less subjective, quantitative and have the potential to be standardized in any laboratory. **Methods:** We stained normal and patients' samples by the method described by Crespo et al (1). However we set a consistent ZAP-70 quadrant marker at 95% positive in the T/NK cell population for each sample. ZAP 70 levels in B cells from CLL patients (CD19+CD5+) and healthy volunteers (CD19+) were determined using a standard curve generated by an absolute fluorescent standard of FITC high levels beads with range of 50,000 to 2,000,000 molecules of equivalent soluble fluorochrome (MESF) units per microsphere. Quantitation of expression levels were generated using Quick Cal V2.2 via www.bangslab.com. (Bangs laboratories)

Results: Using this analysis system the mean expression levels of ZAP 70 were calculated in healthy B cells (n=10) to be 11,3611,993 MESF units while in CLL patients (n=27) the mean value was >139,000 MESF units. To determine the reliability of this new method and its clinical relevance we compared our results to data generated using the analysis method of Crespo et al (1). We found a significant correlation between the two methods (r= 0.7850). Using ROC curve analysis with maximum sensitivity and specificity our minimum positive value was found to be 50,000 MESF. This analysis is highly specific as it is quantitative, and not subjective.

Conclusions: We believe that this type of assay will provide a more reproducible and reliable analysis of ZAP-70 expression in B-CLL, which could easily be standardized. This methodology provides a quantitative assay, which, is not subjective, and is also independent of the possible T cell activation in the sample.

1. Crespo et al., N. Engl. J. Med., (348); 1764-1775, 2003

067 INTEGRITY OF THE DNA SOMATIC HYPERMUTATION (SHM) MACHINERY IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS

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Background: Mutational analyses of the IgVH genes have demonstrated that around 50% of CLL cases undergo SHM. Although the molecular

mechanisms producing SHM are not completely understood, it has recently been proved that the AID enzyme and several error prone DNA polymerases are essential for both SHM and class switch recombination processes. In this context, a defect in the SHM machinery has been proposed to explain the unmutated CLL phenotype. **Aim:** The aim of this study was to analyze the expression of AID and error-prone DNA polymerases (Iota, Eta, Zeta, Kappa, and Mu) in a series of IgVH mutated and unmutated CLLs. B-CLL cells from 26 patients were negatively purified by magnetic separation of CD3 positive cells, yielding a purity of > 99% CD19 positive cells.

Methods: IgVH mutational analysis was performed by direct sequencing, cases with < 98% of identity with the germline being considered as mutated. Expression of AID and DNA polymerases was analyzed by using a quantitative Real-Time RT-PCR. The centroblast-like Ramos cell line, a constitutively hypermutating Burkitt cell line, was used as positive control. In addition, cDNA from an AID knock-out cell line was used as a negative control for AID expression. Results were expressed as relative expression with respect to the Ramos cell line.

Results: There was a trend for a higher AID expression in the IgVH unmutated group (cutoff: 0.1%; 29% vs. 0%, respectively; p=0.06). However, AID expression exhibited a high variability from case to case, with samples in both IgVH mutated (21%) and unmutated (14%) groups showing no expression. DNA polymerases were expressed in all the cases, with no differences between the IgVH mutated and unmutated groups. In CLL cells, Iota and Zeta polymerases were expressed at similar levels than in the Ramos cells, whereas Kappa and Mu polymerases were higher (mean 5.7 and 3.2 time-fold, respectively) than in the Ramos cells. Therefore, AID expression was variable from case to case, whereas DNA polymerases related to SHM processes were expressed in all the cases with no relationship with the IgVH mutational status.

Summary: In conclusion, this study demonstrates the integrity of the DNA SHM machinery in both IgVH mutated and unmutated CLL cases.

068 CCR7 RECEPTOR EXPRESSION IS GREATER IN ZAP70 POSITIVE CLL-B CELLS AND IS ASSOCIATED WITH GREATER RESPONSIVENESS TO CCL19 AND 21.

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Background: The chemokine receptor CCR7 has a prominent role in mediating the trafficking of lymphocytes and the surface expression of CCR7 and CXCR5, another chemokine receptor, determine their exact intra-nodal location (1). CCR7 and its ligands, CCL19 and CCL21, are predominantly involved in localising T cells and dendritic cells to the T zone of the node. Recent studies show that antigen-induced B cells also increase their CCR7 expression allowing them to locate to the boundary between the B and T zones. The B-CLL B cell phenotype resembles an activated B cell that has been exposed to antigen. This study examines the expression of CCR7 and the effect of CCR7 ligands (CCL19 and 21) on chemotaxis of CLL B cells (ZAP-70 +ve and -ve) compared to normal B cells.

Methods: Expression of CCR7 and ZAP-70 were determined by flow cytometry. Polymerisation of the actin cytoskeleton is an early indicator of chemotaxis. Changes in actin polymerisation were measured by flow cytometry to determine CCL19 and 21-mediated effects on chemotaxis. **Results:** B-CLL B cells exhibited a significantly higher level of both surface and intracellular CCR7 expression (MFI) than healthy B cells (p<0.001; 6.5 fold increase). ZAP-70+ve B CLL B cells had significantly higher levels of surface CCR7 than ZAP-70-ve cells (p=0.02; 1.4 fold increase). B-CLL B cells response to CCL19 and CCL21 was significantly greater than seen in controls, in keeping with the expression data. ZAP-70+ve patients were shown to be more responsive to both CCL19 and CCL21 when compared with ZAP-70-ve patients.

Summary: B-CLL B cells express high levels of CCR7, which is in keeping with these cells being activated in response to antigen. Patients who were ZAP-70+ve had significantly higher levels than ZAP-70-ve patients, which appeared to be sufficient to enable the ZAP-70+ve cells to be more responsive to CCL19 and CCL21, the ligands of CCR7. These results concur with those of a previous study, which showed that CCR7 expression is increased in patients with progressive disease (2). Therefore ZAP-70+ve B-CLL B cells may be more able to migrate to a favourable microenvironment in which to receive signals for both survival and proliferation, for example, contact with CXCR4, produced by stromal cells within the lymph nodes.

Refs: 1. Reif K, Ekland E H, Ohl L, et al. Nature 2002; 416: 94-99

2. Till K J, Lin K, Zuzel M, Cawley J C. Blood 2002; 99: 2977-2984

069 VH GENE USAGE AND CHARACTERISTICS DIFFERS IN GERMLINE AND MUTATED B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA TO REVEAL TWO DISEASE GROUPS

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Background: Given the prognostic relevance that the identification of mutated and germline subgroups of chronic lymphocytic leukemia (CLL) has recently acquired we set out to analyze in depth individual VH gene usage rearrangements in patients with mutated and germline CLL.

Methods: Using sequence analysis of FR1/JH polymerase chain reaction products, the VH immunoglobulin gene configuration was analysed in 212 rearranged IgH alleles from 205 CLL patients. Having previously identified a spatial relationship between VH gene usage and JH proximity in patients with acute lymphocytic leukemia (ALL), we performed linear and Poisson regression analysis on patients with germline and mutated CLL against VH rearrangements from normal peripheral blood (n=74 clones). The length of the CDR3 region was also examined as an indicator of cell maturity.

Results: Sequence analysis showed that 135 patients (64%) had mutated sequences (>2% DNA base pair changes) while 77 (36%) had germline sequences. Overall the V, D and J gene usage in the mutated and germline groups were similar when compared to the control group with a few exceptions. VH3-48 and VH3-21 were greatly overused in CLL when compared to controls (p=0.0004 and p=0.0124 respectively). VH3-23, VH4-34 and JH4 (p= 0.0237, p=0.0102 and p=0.0011, respectively) were over used in the mutated group, while VH1-69 and DH3-3 were greatly overused in the germline group (p=0.024 and p<0.0001 respectively). The germline CLL group showed JH proximal over-usage similar to that reported in ALL patients, while the mutated CLL group showed a pattern comparable to that of the control group. The CDR3 region was statistically longer in the control group compared to the CLL patients (p<0.0001). Among CLL patients, the CDR3 region was longer in the germline than in the mutated CLL group (p=0.0029).

Interpretation and Conclusions: This study highlights differences in the VDJ profile in mutated versus germline CLL. The presence of longer CDR3 region and over-usage of JH proximal genes in the germline group suggests the 'CLL cell' may derive from a more immature pool unable to follow or complete the normal pathway of B-cell differentiation. In addition, higher mean white blood cell count and predominance of disease stage B or C were observed in this group. By contrast, shorter CDR3 region and a heterogeneous VH gene usage profile, lower mean white blood cell count and predominance of disease stage A was observed in the mutated CLL group.

070 BIOMED-2 STANDARDISED PRIMERS AND PROTOCOL FOR ROUTINE ANALYSIS OF IGVH MUTATIONAL STATUS OBLIATE THE NEED FOR SURROGATE MARKERS IN B-CLL

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BACKGROUND: The biological diversity of B- chronic lymphocytic leukaemia (CLL) has become evident during the past few years. Traditional methods to predict outcome have failed to identify those patients with early clinical stage at diagnosis, but whose clinical course will be progressive. Recent studies have identified IgVH mutational status as highly predictive of clinical outcomes. Current methods for the detection of IgVH mutational status, which identifies two subgroups of patients with significantly different outcomes, are laborious, expensive and do not lend themselves to a routine diagnostic setting. With the introduction of BIOMED-2 protocol and primers, a rapid method is now available.

AIMS: To compare the BIOMED-2 protocol and primers with an RT-PCR method and investigate reported associations between unmutated IgVH genes, and recurring chromosomal abnormalities linked to poor outcomes.

METHODS: The BIOMED-2 protocol and primers were used to examine DNA from 100 CLL patients. Conventional methods using RNA, and FISH analysis for recurring chromosomal abnormalities, were carried out on 20 and 60 of these patients, respectively.

RESULTS: There was complete concordance between the BIOMED-2 protocol and the RT-PCR method, in both mutational status and gene usage. Unmutated IgVH genes showed association with 17p and 11q23

deletions, and trisomy 12, linked with poor and intermediate outcomes, respectively. Abnormalities at 14q32 breakpoint were also observed in several patients, predominately with unmutated IgVH genes.

CONCLUSIONS: No surrogate marker for IgVH mutational status has so far shown 100% concordance, there are technical complexities with the methods used and no gene usage information, which has been shown recently to be associated with prognosis in some populations, is available with this approach. This study demonstrates that IgVH mutational status can be determined rapidly by the BIOMED-2 protocol and can be adopted as a clinical test in reference centres, for all patients presenting with CLL, obviating the need for detection of surrogate markers. Associations between unmutated IgVH genes, and chromosomal abnormalities associated with poorer outcomes, were confirmed.

071 ZAP-70 OVEREXPRESSION IN T-CELLS OF B-CLL PATIENTS CORRELATES WITH INCREASED LEVELS IN B-CLL CELLS

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Background: In recent years it has become evident that there are two major subtypes of B-cell chronic lymphocytic leukemia (B-CLL), mainly based on the mutational status of the IgVH gene. There is general agreement that B-CLL patients in the unmutated group are more likely to have aggressive disease and a shorter survival than those who display mutated genes. Recently ZAP-70, a protein tyrosine kinase, which plays a critical role in T cell antigen receptor signaling, was shown to serve as a reliable surrogate marker for the mutational status of the IgVH gene in B-CLL patients. ZAP-70 is overexpressed in the unmutated group and this high expression correlates with progressive disease, and poor survival. The expression of ZAP-70 in B-CLL cells raises a number of questions regarding its possible role in the pathogenesis of the disease. In B-CLL it is known that there is impaired regulation and altered T-cell function, which may affect the eventual lifespan of the leukemic B-cells. In the light of this, we decided to determine ZAP-70 expression in circulating T lymphocytes from patients with B-CLL.

Aims: To evaluate the ZAP-70 expression in the B-CLL T lymphocytes compared to normal controls and to examine whether there was any correlation between the degree of T-cells ZAP-70 expression and the levels detected in the leukemic B-CLL cells.

Patients and Methods: Using a novel flow cytometry analysis (details in another submitted abstract from our group) we measured absolute ZAP-70 levels in the circulating T lymphocytes and leukemic B cells from 25 B-CLL patients with a typical immunophenotype (male 13, female 12, age range 30-83 years). ZAP-70 expression was also measured in T lymphocytes from 7 normal controls.

Results: ZAP-70 was overexpressed in the peripheral T lymphocytes of B-CLL patients compared to T-cells from normal controls (p 0.001). Furthermore, when ZAP-70 expression in T lymphocytes from B-CLL patients were determined according to low and high ZAP-70 expression in the leukemic B-cells (using a cut-off of 17.5% as positive for B-CLL cells), the T lymphocytes in the high ZAP-70 B-CLL subgroup expressed significantly more ZAP-70 than the T lymphocytes in the low ZAP-70 B-CLL subgroup (p=0.003). In addition the degree of T lymphocytes expression of ZAP-70 in B-CLL also had a positive linear correlation with the levels of ZAP-70 evident in the B-CLL cells (R²=0.769). In this respect, our preliminary in vitro results also showed that exposure of the lymphocytes to phytohemagglutinin (PHA) stimulation increased ZAP-70 levels both in the T-cells and the leukemic B-CLL cells, suggesting that interaction of B-CLL cells and activated T cells may influence ZAP-70 expression in the leukemic B-CLL population.

Conclusions: Zap-70 is overexpressed in B-CLL T lymphocytes and the degree of ZAP-70 expression in the B-CLL cells, may be related to its overexpression in the T cells.

072 ZAP-70 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA: FLOW CYTOMETRY, IMMUNOCYTOCHEMISTRY AND WESTERN BLOT ANALYSIS, AND CORRELATION WITH THE IGVH MUTATIONAL STATUS

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Background: Chronic lymphocytic leukemia (CLL) is an heterogeneous disease. Many patients have an indolent clinical course that never

requires treatment, while others have an aggressive outcome. Nowadays, biologically-based prognostic markers are necessary at the time of diagnosis to design a targeted therapeutic algorithm. The presence or absence of somatic mutations in the variable region of the Ig heavy chain gene (IgVH) allows to distinguish between good or poor prognosis. Recently, gene profile studies have suggested that the expression of the ZAP-70 gene correlates with an unmutated IgVH status. Furthermore, different authors have demonstrated that patients in whom the leukemic cells express ZAP-70 have a significantly shorter median survival.

Aims: To compare different methods for the detection of the ZAP-70 antigen in CLL cells and to evaluate the correlation between the levels of expression of the ZAP-70 molecule and the IgVH mutational status.

Methods: Thirty-nine CLL patients have been extensively evaluated at the time of diagnosis, prior to treatment, from January 2003 at the Hematology Institute of 'La Sapienza' University, Rome, for morphology, immunophenotype, cytogenetics and molecular genetics. ZAP-70 expression has been investigated by flow cytometry, immunocytochemistry and Western blot analysis on peripheral blood leukemic cells using the human monoclonal antibody clone 2F3.2 (Upstate Biotechnology). The IgVH mutational status of CLL cells has been evaluated after amplification and purification of cDNA with the 3100 Avant Genetic Analyzer (Applied Biosystems).

Results: Twenty-five of the 39 patients studied presented more than 2% IgVH mutations; 25/39 cases showed less than 10% ZAP-70 positive leukemic cells by immunocytochemical determination. In 23 of them, ZAP-70 negativity corresponded to an IgVH mutated status; in 13 patients the presence of ZAP-70 corresponded to an IgVH unmutated status; in 3 patients a discrepancy was found. ZAP-70 expression was confirmed by PCR analysis. Two patients had mutated IgVH (3.3% and 3%, respectively), but expressed the ZAP-70 molecule (81% and 32%, respectively) on the neoplastic cells; both patients expressed other negative prognostic factors: a 11q- cytogenetic alteration and a CD38 antigen expression. One patient showed an unmutated IgVH status, but no expression of ZAP-70 by the leukemic cells nor any other negative prognostic factor. Western blot analysis showed an incorrect interpretation of the result when the ZAP-70 protein was measured on the total mononuclear cell population compared to CD19+ purified cells. The cytometric method showed good results when a conjugated antibody was utilized, but was less reliable when 10-20% of the leukemic cells expressed the ZAP-70 antigen.

Conclusions: We documented an overall good correlation between ZAP-70 expression and Ig mutational status in CLL cells. The immunocytochemical method has proven in our hands more reliable than the other two methods (Western blot and flow cytometry), without requiring any further purification of the leukemic cell population. A clinical diagnostic laboratory can utilize this tool as a predictor marker of the IgVH mutational status for the prognostic stratification of CLL patients.

073 ZAP-70 EXPRESSION IN B CHRONIC LYMPHOCYTIC LEUKAEMIA (B-CLL): MULTIPARAMETER FLOW CYTOMETRY OR IMMUNOCYTOCHEMISTRY?

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Background: Mutational status of IgVH region is one of the most powerful prognostic factors reported in B-CLL. However, this technique is time-consuming and remains accessible in few laboratories. In a recent report (Crespo, NEJM2003;348:1764-75), expression of ZAP-70 kinase, normally expressed in circulating T/NK-cells but not B-cells, was shown to be associated to unmutated status and was proposed as a surrogate for the molecular technique.

Aim: To compare flow cytometry (FCM) and immunocytochemistry (ICC) to ascertain ZAP-70 expression in B-CLL.

Patients (patients): 73 B-CLL patients, including 68 patients at diagnosis and before therapy. M/F=2.65/1; age=37-91y (mean=69y). Staging according to Binet was A, B and C in 55%, 37% and 8% respectively. Immunophenotype was CD5+CD23+ in all patients with Matutes score of 5, 4 and 3 in 56%, 30% and 14% patients, respectively.

Methods: FCM was performed in all cases, on peripheral blood mononucleated cells according to the technique described by Crespo, using indirect labelling with an anti-ZAP-70 antibody (Upstate Biotechnology) and co-labelling of CD5, CD19, CD3 and CD56. B-CLL cells were considered as positive if fluorescent signals were at least that of CD3+/CD56+ cells from the relevant sample, serving as an internal positive control. ICC was performed on blood smears from 53 cases, using the same anti-ZAP-70 antibody and Envision peroxidase revelation

(Autostainer, Dako). B-cells were considered as positive if brown colour was near that of the few T/NK-cells from the same smear.

Results: FCM allowed to assess ZAP-70 expression in 69/73 patients (95%): four failures due to absence of T/NK-cells within the relevant samples. According to the patients, positive B-cells ranged from 0 to 84% (mean=18%, median=9%). Using cut-off of 20% positive cells proposed by Crespo, 24/69 patients (35%) expressed ZAP-70. However, this threshold fell within the cloud of B-cells, leading to ambiguous results in 11/69 instances. Using more stringent criteria, we assessed ZAP-70 expression (>30% positive B-cells) or its absence (<15%) in 15/69 and 43/69 cases, respectively. With ICC, ZAP-70 expression status was ascertained in 52/53 cases (one case with absence of labelling). Positivity was observed in 0 to 96% lymphocytes according to the patients (mean=27%, median=12%). Using 20% positive cells as cut-off, 35/52 patients (62%) expressed ZAP-70. Using more stringent thresholds, ambiguous results (>15% and <30% positives cells) were observed in 5/52 cases. Although overall correlation was good between both methods, some patients were considered as positive using FCM but negative using ICC, and the opposite was also observed. If threshold was 20% positive cells or <15% and >30%, discrepancies were observed in 22% and 9% of cases, respectively.

Conclusion: both FCM and ICC methods can be used for routine assessment of ZAP-70 expression. However, it must be kept in mind that some cases cannot be determined by FCM, due to absence of internal positive T/NK-cells control, and some other cases fell within a range of ambiguous results, leading to ascertain clear-cut expression (or not) in 79% cases. It must also be stressed that at least another 10% cases demonstrated discordance of expression after comparison between both techniques.

CLL: THERAPY

074 FLUDARABINE AS INDUCTION THERAPY FOR CHRONIC LYMPHOCYTIC LEUKAEMIA: A SYSTEMIC REVIEW AND META-ANALYSIS.

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BACKGROUND

Chronic Lymphocytic Leukaemia (CLL) is one of the chronic lymphoproliferative disorders. The historical backbone of chemotherapy for CLL has been the use of alkylating agents such as chlorambucil or cyclophosphamide. More recently, there has been extreme interest in the use of purine (nucleoside) analogues as primary or secondary treatment of CLL, for which the most extensively studied agent is fludarabine and it has shown good results.

AIM

We performed a systemic review and meta-analysis on studies comparing fludarabine with several alkylator- based combination regimens in the primary treatment of CLL to assess if there is indeed a superiority of fludarabine over conventional therapy.

METHOD

Candidate studies for this meta-analysis included all clinical trials comparing the efficacy of fludarabine versus alkylating agents in the treatment of CLL published through June 2003, and identified using MEDLINE and OVID databases. Abstracts from conference proceedings were also hand-searched, checking under 'CLL' and 'Fludarabine' in the index. A study was eligible for inclusion only if it was a randomised controlled trial. The primary end-points were complete and partial response (CR and PR) rate at 6 months, overall survival and tolerance to therapy.

RESULT

Five randomised controlled trials involving approximately 1300 patients with CLL were identified as comparable to be included in the meta-analysis. Complete response rate at 6 months was significantly better for fludarabine alone than with alkylator-based chemotherapy with relative ratio (RR) at 1.87 (95% CI between 1.10 to 3.19, p=0.02). The overall response rate (CR and PR at 6 months) also showed a trend in favour of fludarabine, but did not reach statistical significance (RR=1.22, 95% CI between 0.88 to 1.69, p=0.24). However the overall survival was similar for fludarabine and alkylator-based therapy. Treatment

associated toxicities such as infection, was significantly higher compared to alkylator-based regime (RR=1.58, 95% CI = 1.10 to 2.27, p=0.01), but no significant difference was seen for incidence of neutopenia, thrombocytopenia and anaemia.

CONCLUSION

The superiority of fludarabine over conventional therapy as primary induction therapy for patients with CLL has been shown in several studies, but no study has yet reported a pooled estimate of the treatment effect. This meta-analysis supports the findings that fludarabine as an induction agent for patients with CLL yields a better clinical response with acceptable toxicity when compared with alkylator-based combination therapy, but without a survival benefit by 5-6 years follow-up.

075 ALEMTUZUMAB (CAMPATH, MABCAMPATH) CONSOLIDATION AFTER FLUDARABINE PHOSPHATE AND CYCLOPHOSPHAMIDE SECOND-LINE CHEMOTHERAPY FOR PROGRESSIVE CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Fludarabine phosphate (Fludara, F) and cyclophosphamide in combination (FC) are highly effective in treating B-CLL, but prolonging duration of relapse-free survival is a challenge. Alemtuzumab (Campath) is effective therapy in CLL. Aims: This study is to determine efficacy and safety of alemtuzumab consolidation after FC chemotherapy in patients with progressive B-CLL, who received one prior line of chemotherapy, alkylating agents or F, and who gave informed consent. Methods: Treatment consists of 4 courses of FC (F: 25mg/m², Days 1-3; C: 250mg/m², Days 1-3) every 4 weeks. Alemtuzumab (Schering AG) is administered iv 2 months after chemotherapy. The target alemtuzumab dose (30mg over 2 hours, 3 x weekly) is given to maximum response, with a planned duration of 4-8 weeks. Patients are initially given a low dose (3mg), increased step-wise to 30mg. To prevent infusion-related events, premedication was given. All patients receive cotrimoxazole, itroconazole and acyclovir anti-viral prophylaxis, and are monitored for CMV reactivation. Ciprofloxacin is given in the event of neutropenia (neutrophils <1x10⁹/L). In severe infection or CMV reactivation, alemtuzumab is withheld until the problem resolves. Response is evaluated according to 1996 NCI-WG criteria. Flow cytometry and ZAP-70 expression are performed on bone marrow at start of treatment. Molecular analysis is performed using PCR, to monitor VDJ Ig rearrangement, IgVH mutational status and MRD. Clinical and molecular responses are examined after the first 4 courses of FC chemotherapy; after every 4 wks of alemtuzumab therapy; and during the follow-up period. Results: the trial is ongoing. Up to now, 10 patients with advanced disease have been enrolled. The mean age was 60 (range 50-65) years; 8 patients were in Binet stage B, 1 in Binet stage C and 1 in progressive stage A. Prior treatment comprised alkylating agents (n = 6) and F (n = 4). To date, IgVH somatic mutation analysis has been performed in 9 patients: 6 had an unmutated (high-risk) status and 3 had a mutated status. To date, all patients completed the FC chemotherapy: 1 patient achieved CR, 7 achieved PR and 2 had stable disease. Up to now 4 patients received alemtuzumab consolidation with improved responses: 3 patients demonstrated a CR, 1 NV. To date 4 patients are still in treatment with alemtuzumab. After FC, MRD was still evident in all patients except one; MRD after alemtuzumab therapy has been assessed on bone marrow in 2 patients; both are MRD-negative after consolidation. CMV reactivation was documented 6/8 patients and received oral gancyclovir, one patient was symptomatic with slight fever and was treated iv. Alemtuzumab was withheld until after clearance of CMV antigenemia. Infusion-related symptoms were mild, and no other major toxicities or infections were reported. Conclusions: Sequential use of FC and alemtuzumab appears effective in patients with progressive CLL. As a lower dose of alemtuzumab may reduce the risk of CMV reactivation, while maintaining efficacy, a 15mg x 3-week dose of alemtuzumab will be investigated.

076 CANNABINOIDS AS POTENTIAL ANTILEUKEMIC AGENTS

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BACKGROUND: Cannabinoids comprise the psychoactive components of cannabis and their synthetic derivatives. They have been reported to exhibit multiple biological activities, some of which may have therapeutic value. Two cannabinoid receptors which differ in their tissue expression have been identified: type I (CB1), which is expressed mainly in the central nervous system and type II (CB2), which is expressed largely on peripheral tissues. These compounds display a variety of biological activities in cells, including the ability to regulate processes of cell life and death.

AIMS: In our current study we tested the effect of δ 9THC, a natural cannabinoid; HU-210, a synthetic cannabinoid; and the two endocannabinoids, 2-arachidonyl-glycerol (2-AG) and anandamide (AEA), on CCRF-CEM T-lymphoblastic and HL-60 promyelocytic cell lines as well as on primary CLL and AML cells in vitro.

METHODS: Heparinized peripheral blood was obtained from CLL and AML patients. Mononuclear cells isolated by ficoll-density-gradient centrifugation as well as CCRF-CEM and HL-60 cell lines were cultured in RPMI medium containing 10% serum unless otherwise indicated. Cell viability was measured by XTT reduction, and apoptosis was measured by double staining with acridine-orange and ethidium-bromide.

RESULTS: CCRF-CEM and HL-60 cell lines were affected by HU-210 at concentrations of 10 μ M and higher. The drug induced an apoptotic mode of cell death. Treatment of CLL cells with cannabinoids resulted in a concentration dependent decrease in cell viability, caused mainly by HU-210. The average LC50 value was 12.81.59 μ M (n=7) with LC50 ranges between 6.5 μ M and >20 μ M, and median of 14. The natural cannabinoid δ 9THC was active only at serum concentration of 5%, for which the LC50 value was about 14 μ M. AEA was inactive in regular medium, and caused complete cell death only in serum-free medium. 2-AG did not exert any activity under either condition. Drug interaction between cannabinoids and fludarabine in CLL cells, or between cannabinoids and ARA-C in AML cells was found to be additive. We also found that cannabinoids induced an apoptotic mode of cell death in the primary cells.

CONCLUSION: The natural cannabinoid δ 9THC and the synthetic compound HU-210 were more effective in killing leukemia cells by apoptosis induction than were the endocannabinoids. The results suggest that these compounds may provide an effective anticancer agent for treating different types of leukemia.

077 IMMUNOTHERAPY OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA WITH AUTOLOGOUS IRRADIATED LEUKAEMIC CELLS AND BCG

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BACKGROUND: Chronic B-cell lymphocytic leukemia (B-CLL) is a heterogeneous disorder, it generally follows an indolent clinical course, with most of patients do not requiring any therapeutic intervention for some time from initial diagnosis. Since currently available therapies do not cure patients with B-CLL, and they may be associated with significant toxicities, the appropriate time to initiate therapy becomes an important decision, especially in patients with low-risk disease. Immunotherapy may be an advantageous therapeutic option for these group of patients, because in early stages of the disease the tumour burden is relatively small, and slow growth of tumour allows time for generation of an immune response against leukemic cells. AIMS: Our attempt was directed on use of irradiated peripheral blood autologous leukaemic B-CLL cells, as a vaccine in patients with B-CLL. We hypothesized, that apoptotic leukemia cells, specially in presence of BCG vaccine, will induce skin dendritic cells maturation and than their activation following migration to regional lymph nodes. In lymph nodes the dendritic cells may present the processed leukemia antigens to T cells and activate them to CTLs directed against B-CLL cells of the patient. These may break the tolerance of active T cells as well. PATIENTS AND METHODS: From June 2002 till February 2004 10 untreated B-CLL patients in stage 0 or 1 acc. Rai were included into the study. First two patients finished vaccination schedule in January 2003. After 10 months observation period in November 2003 next eight patients started therapy which has being continued till now. Patients were vaccinated with autologous irradiated leukaemic cells. Vaccines were administered once a week during first month of therapy, and then every

two weeks until 12 doses. In the first two injections the irradiated cells were mixed with viable fresh frozen BCG suspension. The mixture of cells was injected intradermally in close proximity to axillary or inguinal lymph nodes. The phenotype of peripheral blood leukocytes and lymphocytes in a whole blood of patients was assayed by cytometry before every vaccination and every 3 months after the end of therapy. RESULTS: In one of the patients vaccinated from June 2002 to January 2003 the number of leukocytes and lymphocytes remained on the similar level and in the other one decreased during 18 months observation period. In the patients treated from November 2003 stable disease is observed so far. Most remarkable phenotypic change during vaccination was rise in CD3+CD8+ population of T lymphocytes. CD4/CD8 ratio decreased and populations of monocytes and NK cells normalized after vaccination. No treatment-related adverse events were observed. Most common side effects were local ulceration at site of BCG injection healing within 2-3 months and flu-like signs for about one day after first vaccinations with irradiated cells. CONCLUSIONS An attempt was made for the first time in CLL patients to use directly of apoptotic B-CLL cells. After 18 months from start of vaccination the patients are in a stable phase of the disease. Further patients are vaccinated and observed now.

078 INTERIM SAFETY SUMMARY OF ALEMTUZUMAB (CAMPATH, MABCAMPATH) VS CHLORAMBUCIL AS FRONT-LINE THERAPY FOR PATIENTS WITH PROGRESSIVE B-CELL CHRONIC LYMPHOCTIC LEUKEMIA

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Background: MABCAMPATH has shown promising results as single agent or in combinations in the treatment of relapsed or refractory B-CLL, leading to molecular remissions and potentially prolonged survival in some patients. Aims: This study seeks to evaluate the efficacy and safety of MABCAMPATH used in front-line treatment of progressive B-CLL. The primary study endpoint is progression free survival; secondary endpoints include safety, overall survival and response rate.

Methods: CAM307, an open-label Phase III, international, randomized trial is accruing therapy-naïve, Rai stage I-IV B-CLL patients with progressive disease requiring treatment. Exclusion criteria include autoimmune thrombocytopenia, prior bone marrow transplant, active infection, positive CMV PCR assay at baseline, secondary malignancy, or central nervous system involvement. Patients are randomized 1:1 into two treatment arms: MABCAMPATH 30 mg IV 3 times a week for a maximum of 12 weeks or chlorambucil 40 mg/m² PO once every 28 days, up to a maximum of 12 cycles. MABCAMPATH patients receive antibiotics and antivirals until CD4+ counts recover to >200 cells/L post-treatment.

Results: Currently, patient disposition data is available for 152 patients (103 males, 49 females) with a median age of 59 years. Two-thirds of the patients have Rai stage I/II and one-third have Rai stage III/IV disease. Patients treated with MABCAMPATH had 276 adverse events (AE), while patients on the chlorambucil arm had 218 AE (data available for a total of 71 patients). The mean grade for all adverse events was 1.5 in the MABCAMPATH, and 1.6 in the chlorambucil arm. Most AEs in the MABCAMPATH arm were infusion-related (41% of all AEs), and in the chlorambucil arm most AEs were related to the GI system (30% of all AEs). More serious adverse events (SAE; data available for 213 patients) were noted in the MABCAMPATH arm (59 and 42, MABCAMPATH and chlorambucil, respectively). CMV infection reactivation occurred in 32 patients (MABCAMPATH 29; chlorambucil 3). Seven MABCAMPATH patients had CMV infections with fever as the only symptom, and were treated with IV ganciclovir or foscarnet. In all 32 cases the CMV infection reactivation resolved, no CMV-related deaths were observed and the majority of patients were able to complete treatment per protocol.

Conclusions: Preliminary safety summary suggests that therapy nave CLL patients treated with MABCAMPATH and chlorambucil have comparable safety profiles. Active screening allows for early diagnosis and effective CMV prophylaxis and CMV infection treatment.

079 PENTOSTATIN AND RITUXIMAB COMBINATION FOR TREATMENT OF RELAPSED/REFRACTORY HAIRY CELL LEUKAEMIA - A REPORT OF 3 CASES

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Background:

Overall response rates of 25-64% with complete responses (CR) of 10%-55%, have been reported with the use of standard dose, single agent rituximab in patients with relapsed/refractory hairy cell leukaemia (HCL). The recognised synergistic effect and safety observed when rituximab is combined with chemotherapy in B-cell lymphoproliferative disorders provides the rationale for investigating the combination of pentostatin and rituximab in the treatment of HCL.

Aims:

To report the efficacy and safety of pentostatin in combination with rituximab in 3 patients with relapsed/refractory HCL.

Methods:

Three patients with relapsed/refractory HCL were treated with rituximab (375mg/m²) for 4 doses. In 2 patients this was given in conjunction with the first 4 doses of pentostatin treatment (4mg/m²) on a 2 weekly basis. In 1 patient, rituximab was given weekly, in tandem with pentostatin. CR was defined as complete disappearance of all evidence of disease with normalisation of blood counts and partial response (PR) as > 50% improvement in these criteria, but short of CR.

Case1: A 47 year-old man diagnosed with HCL in 1981 was treated with splenectomy and CVP resulting in 16 years remission. First relapse in 1998 was treated with 9 doses of pentostatin with CR of 2 years. Second relapse in 2000, proved resistant to 2 courses of cladribine, single agent rituximab and interferon-alpha, and was associated with the presence of larger cells with blastic appearance. He progressed in May 2003. Pentostatin (12 doses) in combination with rituximab (4 doses) achieved PR lasting 9 months to date.

Case2: A 26 year-old man diagnosed with HCL in 1997 was treated with splenectomy and cladribine resulting in a 3-year remission. First relapse in 2000 was treated with cladribine with only minor improvement. A second course of cladribine resulted in a PR (5% residual BM infiltration) lasting 2 years. Pentostatin (5 doses), in combination with rituximab (5 doses) was given for his second relapse in 2003. Larger, blastic cells were noted. The patient achieved CR (including immunohistochemistry) and will receive 3 further combined doses.

Case 3: A 34 year-old man was diagnosed with HCL in 2001. After initial therapy with G-CSF, erythropoietin and interferon- alpha to improve blood counts, he received 12 doses of pentostatin achieving a PR (15% residual BM infiltration). 4 doses of weekly rituximab were given to clear residual BM disease. CR was achieved, lasting 9 months to date.

Results:

All patients responded to combination therapy with CR in 2 patients and PR in 1. In the patient achieving PR, this was the first response achieved since his disease became refractory. No toxicity or infective complications were observed. Marked, transient peripheral blood and bone marrow eosinophilia was seen in 1 patient.

Conclusion:

Use of rituximab in combination with pentostatin in advanced/refractory HCL, or following purine analogue treatment in order to augment response, has not been previously reported. Our series suggest good efficacy and safety of this combination in these contexts, with superior quality of responses.

080 LOW DOSE ORAL FLUDARABINE PLUS CYCLOPHOSPHAMIDE IN ELDERLY PATIENTS WITH CHRONIC LYMPHOPROLIFERATIVE DISORDERS

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Background. Fludarabine (Flu) is highly effective in B-cell chronic lymphocytic leukemia (B-CLL) and is now used as first line treatment. A strong synergistic effect of Flu and Cyclophosphamide (CY) has been demonstrated in the treatment of chronic lymphoproliferative disorders (CLD), although a high incidence of severe neutropenia and infectious complications, particularly in elderly patients, have been reported.

Aims. Based on previous clinical experiences in elderly CLD patients treated with a combination of low dose i.v. Flu and CY in whom we obtained good response rates and negligible toxicity, we tested efficacy

083 ALEMTUZUMAB AS FIRST-LINE THERAPY IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL): LONG-TERM FOLLOW-UP OF CLINICAL EFFECTS, INFECTIOUS COMPLICATIONS, AND RISK OF RICHTER TRANSFORMATION (RT)
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Background: Alemtuzumab is a humanized anti-CD52 monoclonal antibody that has shown promising results when used in advanced B-CLL and as first-line therapy in previously untreated, but symptomatic B-CLL patients (Lundin et al. Blood 2002). As alemtuzumab may induce long-lasting immunosuppression in such patients (Lundin et al. Leukemia 2004), it is of major importance to study the incidence of infectious complications and Richter transformation (RT).

Aims: To study the incidence of infectious complications and RT in patients with B-CLL treated with alemtuzumab.

Methods: Forty-one patients with B-CLL receiving alemtuzumab as first-line therapy in a phase II trial (Lundin et al. Blood 2002) were subject to long-term follow-up with regard to time to treatment failure (TTF), infectious complications, and incidence of RT. The incidence of RT was compared with data obtained from 310 historical B-CLL control patients identified retrospectively from the Regional Cancer Registry in Stockholm (1990-1997). All suspected cases of RT were reexamined by a hematopathologist.

Results: The median TTF in patients responding (PR and CR) to alemtuzumab therapy was 35+ months (range 572+ months). No severe late infectious complications were observed, with the exception of one symptomatic EBV reactivation 21 months after completion of alemtuzumab therapy. The incidence of RT was 12% (5/41) in the alemtuzumab-treated patients compared to 16% in 76 historical control patients who matched the inclusion criteria of the alemtuzumab study (no significant difference). The incidence of RT in the total control population (n = 310) was 5%. Results will be updated at the meeting.

Summary/Conclusions: Alemtuzumab is a highly active agent when used as first-line therapy in B-CLL and induces long-lasting remissions and few late occurring infectious complications. The incidence of RT appears to be similar in the study group compared to matched historical controls, but a longer follow-up of the alemtuzumab-treated patients is required.

084 CYTOTOXIC T CELL CLONES AGAINST B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS CAN BE GENERATED FROM HLA-MATCHED SIBLING DONORS: IMPLICATIONS FOR ADOPTIVE IMMUNOTHERAPY

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Allogeneic stem cell transplantation (SCT) is increasingly considered for the treatment of patients with advanced CLL. The rationale for allogeneic SCT relies on adoptive transfer of donor-derived alloreactive T cells that may eradicate refractory or recurrent CLL. Complete sustained remissions in CLL patients have been observed after allogeneic SCT suggesting a susceptibility of CLL cells to a graft-versus-leukemia (GVL) effect. However, CLL may escape T cell-mediated immune reactivity since these cells lack expression of costimulatory molecules. Recently, we have demonstrated that CLL cells can be modified into antigen presenting cells by CD40 triggering in the presence of IL-4 (CLL-APC). To examine whether CLL-reactive cytotoxic T lymphocytes (CTL) from HLA-matched sibling donors can be generated using these malignant CLL-APC as stimulator cells, three sibling combinations were tested. Patient 1 and 2 had received an allogeneic SCT as treatment for their CLL. In contrast to patient 1, patient 2 had a durable clinical response concomitant with graft-versus-host disease (GVHD). The third patient was candidate for allogeneic SCT, was positive for the minor histocompatibility antigen (mHag) HA-1, and had a related HA-1 negative donor. T cells from the donors were stimulated with irradiated primary CLL or CLL-APC. After three cycles of (re)stimulation responding CTL lines were tested in a cytotoxicity assay at various effector/target (E/T) ratios. CTL clones were generated by plating cytotoxic CTL lines one cell/well using a FACS-Vantage flow cytometer. In general, all CTL clones were derived from CTL lines, generated against CLL-APC as stimulators. In the first patient/donor combination, which did not respond to DLI, no cytotoxicity against CLL-specific targets was observed. Sorting of viable

CD4+ or CD8+ T cells resulted in 32 CD4+ and 5 CD8+ proliferating clones, but none of these clones was cytotoxic to primary CLL. In the second patient/donor combination, which responded to DLI, cytotoxicity of the CTL lines against primary CLL (12.2%) and CLL-APC (13.6%) was obtained. Following single cell sorting 3 out of 6 proliferating CD8+ CTL clones killed primary CLL (median 14.4%, range 8-22%), and 4 of 14 CD4+ clones recognized primary CLL (median 15.3%, range 12.2-16.6%) in a 4-hour chromium release assay at an E/T ratio 10:1. Finally, in the HA-1 incompatible patient/donor combination, more than 200 proliferating clones could be tested. 4 CD8+ cytotoxic CTL clones effectively lysed primary CLL (26%), CLL-APC (47%) and EBV-LCL from the patient (38%) and not EBV-LCL from the donor. These results are compatible with mHag-specific CTL clones. One CD8+ clone was tetramer-HA-1 positive, the specificity of the other mHag-specific clones remains to be determined. Blocking studies of these clones using anti-HLA class I antibodies confirmed class I restricted recognition of the target cells. In conclusion, using CLL-APC as stimulators, CLL-reactive and mHag-specific CTL clones from HLA-matched sibling donors can be generated. Probably due to the low precursor frequency of CLL-reactive T cells in the donor PBMC, only a limited number of CTL clones could be obtained and further enrichment for these CLL-reactive T cells is essential to allow introduction in clinical practice.

AML THERAPY

085 TREATMENT OF NEWLY DIAGNOSED PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA USING INTRAVENOUS ARSENIC TRIOXIDE

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Background: There is limited data on the use of arsenic trioxide (As₂O₃) as primary therapy for newly diagnosed patients with acute promyelocytic leukemia (APML). We describe our experience with the use of intravenous arsenic trioxide in these patients.

Methods: Between Oct 1998 and December 2003, 51 patients with newly diagnosed APML were treated with intravenous arsenic trioxide. As₂O₃ was administered daily over 4 hours at a dose of 0.15mg/kg/day. Therapy consisted of induction therapy where As₂O₃ was administered till hematological remission was achieved (or a maximum of 60 days) followed by consolidation therapy where As₂O₃ was administered for 28 days, one month after completing induction therapy and then maintenance therapy which consisted of cycles each lasting for 10 days monthly for a period of 6 months. Patients were followed up for molecular remission using RT-PCR from peripheral blood.

Results: There were 11 children (< 15 years) and 40 adults with a median age of 27 years (range: 6 - 60). This included 27 males and 24 females. All patients had the hypergranular type of APML. The mean WBC count at diagnosis was 9.4 x 10⁹/L (range: 0.5 to 96.5). The major isoform identified was bcr 1 in 69% followed by bcr 3 in 27%. 40/51 patients (80.4%) achieved hematological remission in a median duration of 43 days (range: 26 - 60). Eleven patients expired during induction therapy due to intracranial hemorrhage. All patients who achieved hematological remission also achieved molecular remission in a median duration of 80 days (range: 36 - 123). There was no major toxicity seen during treatment except for hyperleukocytosis in 37 patients (72%), hyperpigmentation in 8 patients, paresthesia in 5 patients, asymptomatic reversible prolongation of SGPT in 6 patients, prolonged neutropenia in 1 patient with fibrosis in the bone marrow and ECG abnormalities in 1 patient. Hyperleukocytosis was controlled in a majority with hydroxyurea while anthracyclines were used in 3 patients. There was no major toxicity that required discontinuation of arsenic except for the patient with marrow fibrosis. Four patients relapsed at a median duration of 15 months (3 in the marrow, one with an isolated CNS relapse); of these three patients have achieved a second remission while one patient expired due to an intracranial bleed. At a median follow up of 30 months (range: 3 - 74), 40 patients (78.4%) remain in hematological and molecular remission.

Conclusion: Arsenic trioxide achieves hematological and molecular remission in a majority of patients with newly diagnosed APML with no major toxicity. Long term follow-up is required to completely define the role of arsenic trioxide in the treatment of acute promyelocytic leukemia.

086 SECONDARY ACUTE MYELOID LEUKEMIA: RESULTS OF CONVENTIONAL TREATMENTS. EXPERIENCE OF GIMEMA TRIALS

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BACKGROUND: Acute myeloid leukemia (AML) in patients who received chemo- and/or radiotherapy (sAML) for a previous malignancy (PM) is a well-documented event, representing approximately 5-10% of all AML. A major cause for this event is considered the leukemogenic activity of several drugs (i.e. alkylating agents or epipodophyllotoxins) utilized in the treatment of primary cancer; radiation therapy is also considered to be potentially mutagenic. The clinical course of sAML is considered progressive and relatively resistant to conventional therapies used for AML arising de novo, only few studies demonstrated good results with a standard induction therapy only in those sAML with favorable cytogenetic pattern.

AIMS: To evaluate the outcome of patients with a previous malignancy treated with chemo and/or radiotherapy and enrolled in conventional trials.

METHODS: In a multicenter setting, a prospective non-concurrent analysis was performed on 2513 new AML patients, aged 12-78 years, consecutively enrolled in EORTC-GIMEMA trials (AML 8A and 8B, AML 10, AML 13) between 1987 and 2001. Thirty-eight patients (16 males and 22 females) with sAML were identified and compared with a group of 114 de novo AML matched according to age; F.A.B. morphology criteria; WBC count at AML diagnosis; enrolment trial; time of diagnosis of AML. Induction treatment response; diseases free survival, duration and overall survival was evaluated in the two groups.

RESULTS: Comparing the CR rate between 38 sAML and 114 de novo AML, selected according the previous reported criteria, no difference was observed [25/38 (66%) vs 66/114 (58%), Pearson χ^2 0.7393, $p = 0.390$] as well as no differences were observed comparing the DFS and the OS between the two groups.

SUMMARY/CONCLUSIONS: The results of this study suggest that sAML patients, cured from a previous malignancy and characterized by a good performance status that favor a recruitment in conventional trials, present the same possibility of cure than de novo AML.

087 RISK-ADAPTED THERAPY OF ADULT ACUTE MYELOID LEUKAEMIA (AML) INCLUDING MULTIPLE HD-ARA-C CYCLES WITH LOW-DOSE STEM CELL SUPPORT: EXCELLENT RESULTS FROM A SINGLE CENTRE

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In adult AML, effective postremission consolidation is obtained with HD-ARA-C and either autologous or allogeneic stem cell transplantation (SCT). HD-ARA-C is markedly myelotoxic, causing some remission deaths and other nonlethal toxicity that may preclude the administration of multiple courses. Since 2000, we are evaluating in a multicentre setting a risk-oriented strategy that specifically considers 'for selected patients- the use of multiple, stem cell-supported HD-ARA-C courses. The results from a single centre are presented to underline the potential benefits from this treatment when correctly applied in a wider cooperative context.

After double induction (ICE-IC or ICE-sequential HAI if primary resistance is documented), ARA-C 1 g/m²/bd on dd 1-4 is given followed by G-CSF with the intention to collect CD34+ blood stem cells (1-2x10⁶/kg per bag). As final treatment step, patients with high risk cytogenetics or with intermediate risk cytogenetics plus any other adverse feature (WBC >50.000, MDS/secondary, late CR, FLT-3 mutation, hepatosplenomegaly, FAB M0,6,7), i.e. the high-risk (HR) group, are offered allogeneic SCT; in other cases, i.e. the standard-risk (SR) group, three HD-ARA-C-based cycles (2 g/m²/bd on dd 1-5, plus idarubicin) are planned, followed by the reinfusion of one stem cell bag on day 6 of each course and G-CSF; HR patients unable to undergo SCT are transferred to HD-ARA-C, and

those with an insufficient CD34+ cell yield are given instead one-two courses with intermediate-dose ARA-C.

Sixty-five unselected patients (males 33) aged 17-65 years (median 50) with either de novo or secondary/MDS-related (17%) non-M3 AML (cytogenetics: favourable 8, intermediate 10, normal 30, unfavourable 11, unknown 6) are thus far evaluable: 48 HR and 17 SR. CR rate was 83% (100% in SR, 75% in HR). So far, SCT was performed in 13 HR cases, while 60 HD-ARA-C courses were administered to 26 other patients, with a median intercycle time of 37 days (only 15% >45 days), a median duration of neutropenia <0.5 and thrombocytopenia <20 of 9 and 6 days, respectively, and no toxic death. By treatment intention, projected overall survival at 3 years is 47% (81% in SR and 37% in HR), and DFS rate at 3 years is 54% (74% in SR and 46% in HR).

In summary, low-dose autologous CD34+ cell rescue (plus G-CSF) may sensibly limit HD-ARAC-related toxicity in unselected adults with AML, up to an age of 65 years, allowing an effective increase in dose intensity. Survival and DFS rates at 3 years in SR category appear outstanding, and DFS is appreciable in HR group too. These results from a single centre highlight the importance of a correct prognostic assignment and treatment application in a larger multicentre trial.

088 CYTARABINE BASED REGIMENS IN ACUTE MYELOID LEUKEMIA: - ASSESSMENT AND COMPARABILITY OF TREATMENT INTENSITY - USE OF THE CONCENTRATION COEFFICIENT WEIGHTED AREA UNDER THE CURVE (N-AUC)

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Background: Current approaches to improve the results of antileukemic treatment against AML usually involve 'treatment intensification'. However rational treatment intensification requires knowledge about the parameter that is to be intensified. I.e. there must exist a clearly established relation between that particular parameter and treatment effect (e.g. CR rate). A correlation of such parameters such as total drug dose, dose intensity or area under the curve (AUC) (esp. of cytarabine) and treatment effect has so far never been systematically been demonstrated in AML. Esp. AUC is often considered to best characterize an applied regimen, however it does not take into account the relative relevance of concentration versus exposure time for antileukemic efficacy. This shortcoming may be overcome by weighing the AUC by the so called concentration coefficient N which can be derived from the paradigm 'CexpNT = constant' for equitoxic effects (termed N-AUC).

Aims: 1) To establish the concentration coefficient N for cytarabine in AML. 2) To extrapolate from published pharmacokinetic analyses of cytarabine in AML the average AUC in each arm of a large set of randomized studies. 3) To demonstrate from these studies the existence of a linear relationship between response (CR rate) and cytarabine dose, dose intensity, AUC, N-AUC.

Methods: 1) N was derived from in-vitro experiments that involved measuring equitoxic effects (LC-50%) at varying exposure times in 54 AML samples. 2) Pharmacokinetic parameters of diverse cytarabine regimens (doses ranging from 0,02 to 6,0g/m²/d; exposure times ranging from 0,5 to 24h per day) were extracted from the literature. 3) Randomized cytarabine based studies since 1990 were identified in the literature, in which total dose, dose intensity and CR rate could be identified.

Results: 1) (Equi-)Antileukemic effects of cytarabine were well described by the paradigm 'CexpNT = constant', which therefore made the derivation of N possible: 0,45 - N.B. that a concentration coefficient < 1 implies a higher relevance of exposure time for increased effects as compared to concentration. 2) Published cytarabine AUCs are highly linear to dose and are not affected by the infusion time, which therefore made plausible the assignment of average AUCs to the various treatment arms of other AML studies (n = 50) in which only doses and infusion times were stated. 3) No relationship could be found between CR rate and cytarabine dose (r=0,1211, p=0,4122), dose intensity (r=0,1388, p=0,3366) or AUC (r=0,1068, p=0,4955). However there was a strong linear correlation between CR rate and N-AUC (r=0,5901, p<0,0001).

Conclusions: 1) We established the concentration coefficient N of cytarabine in AML, which is a useful parameter to formally quantify the relative relevance for the clinical effect of exposure time versus concentration. 2) N-AUC of cytarabine is highly correlated with the clinical effect, which suggests that it might be a better parameter for 'treatment intensification' than dose, dose intensity or AUC. 3) The concept of N-AUC allows the realistic comparison of pharmacokinetically widely diverse applications of cytarabine and immediately suggests rational ways of treatment intensification.

089 RELATIVE SURVIVAL RATES IN AML PATIENTS IN SWEDEN 1973 - 2000 . WHAT IMPACT HAVE NEW TREATMENT STRATEGIES HAD ON SURVIVAL?

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Background

Approximately 400 new patients with AML are diagnosed annually in Sweden. According to the Swedish Cancer Registry there has been no change in incidence over the years. Multiagent chemotherapy and development of allogenic and autologous stem cell transplants have improved response and survival rates during the last decades. The aim of this study was to investigate what subgroups of patients who have had most benefit from new therapies and if there were any differences in survival between geographical regions. In the present study we used central Swedish registries to elucidate relative survival rates (RSR) in a population-based cohort of approximately 6000 AML patients.

Methods

In order to identify AML cases we used the Swedish Cancer Registry which contains information on sex, date of birth, diagnosis, date of diagnosis, region and in which health care institution the diagnosis was made. The individually unique national registration number allow complete long term follow up of survival by record linkages to the nationwide Register of Causes of Death. Relative survival was computed as the ratio of the observed survival among the cohort to the expected survival among individuals of the same age, sex and calendar year of observation.

Results

5809 AML patients diagnosed between January 1, 1973 and December 31, 2000 were identified. The cases were divided into six age groups; 0-18, 19-40, 41-60, 61-70, 71-80, and 80+. For the whole study period, the 5-year RSR for the age groups were 46%, 31%, 21%, 11%, 5.2%, and 1.4% and the 10-year RSR were 43%, 28%, 16%, 7.7%, 3.4%, and 3.3%, respectively. The study period was arbitrarily divided into 7-year intervals. There was a major improvement in RSR over the years for the younger patients but not for the elderly (table 1).

A comparison between regions with different therapeutical traditions was made. The regions of Stockholm, Uppsala and rebro has cooperated with treatment protocols since 1971 in the Leukemia Group of Middle Sweden (LGMS) and was therefore considered as one group and the rest of the country was considered as one group. Patients resident in other counties than those belonging to LGMS had a 9% higher excess mortality. The difference in RSR between the regions was large in the first seven-year period and after that the differences diminished (table 2).

Conclusion

Improvement in survival of AML patients was mainly confined to young patients (<60 years). Differences in RSR in the elderly (70+) patients over the study period were very small. There were differences in RSR between the regions with better rates in the LGMS during the first period (1973-1979). This might, at least in part, be explained by the cooperation that started in 1971 with more intense treatment protocols.

Table 1

5-year RSR	1973-1979	1980-1989	1990-1999	2000-2002
0-18	17%	21%	22%	46%
19-40	14%	17%	18%	31%
41-60	4.7%	13%	22%	21%
61-70	3.8%	5.8%	12%	11%
71-80	1.1%	3.2%	4.7%	5.4%
80+	0.8%	0	0	1.0%

Table 2

5-year RSR	1973-1979	1980-1989	1990-1999	2000-2002
LGMS	17%	13%	14%	20%
other	14%	8.7%	17%	22%

Caption 1: table 1& 2

090 THE TREATMENT COST AND SURVIVAL OF PATIENTS WITH ACUTE LEUKEMIA –TAIWAN EXPERIENCE

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The aim of this study was to analyse the treatment outcome and total treatment cost of 106 cases with acute myeloid leukemia (AML, No.=94) and acute lymphoblastic leukemia (ALL, No.=12), who were treated in Taipei-Veterans General Hospital since the implementation of National Health Insurance Program between January 1995 and January 2002. The median age of our patients were 45.5+18.5 years old, and 30 patients were old than 60 years old. The CR rate is 63.1% (70/111). Twenty patients have refractory disease (18%, 20/111) and 21 patients had treatment related mortality during first 30 days (18.9%, 21/111). After achieving CR, 54 young patients received further intensive consolidation therapy with HiDAC chemotherapy with or without autologous or allogeneic PBST, 14 patients received conventional consolidation chemotherapy and 37 patients received only supportive care. After a median follow-up of 14 months (range: 1 ' 101 months), 25 patients (23.5%) remained alive and the estimated 5 years overall survival rate is 23%. Patients treated with intensive consolidation have significantly higher 5 years survival rate than those with conventional/no chemotherapy (40% and 2.5%, respectively; p<0.0001). The estimated 5 years overall survival rate in the patients treated with HiDAC, either alone or followed by subsequent auto-PBST, is higher than those treated with allo-PBST (50% vs. 21.3%), but without statistical significance (p=0.1164). Subsequent auto-PBST following HiDAC therapy showed no survival benefit in the 5 years overall survival as compared with those received only HiDAC therapy (p=0.8267). Further analysis showed that young age (<60 years) and good karyotype (t(8;21), t(15;17) and inv 16) had significant higher 5 year disease free survival. The average duration of total hospital care and costs were 115 days USD 41,690+31,022 per AML patient , and 130 days & USD 51,397+40,674 per ALL patient. The average costs during different treatment phases were as following: remission induction US\$15,084 conventional consolidation, \$6,724; high dose arabinoside chemotherapy (HiDAC) \$16,667; allo- peripheral blood stem cells transplantation (PBST) \$29,208; auto-PBST \$10,037; supportive care during follow-up period \$3,013; and terminal care \$15,725. The total average cost in patients who survived for < 100 days, 100-365 days, 365-730 days and > 730 days were US\$13,249, 54,275, 60,095, and 50,732, respectively. Patients received post-remission intensive consolidation spent 57.3% more in the total cost (p<0.001), 47.3% more in the hospital day (p<0.001), but was also associated with significantly more survival days (median: 1209 days vs. 124 days; p< 0.001). We conclude that total cost of treating AML patients is very expensive in Taiwan, but is cost-effective in prolong patients' survival.

091 THE COMBINATION OF 2-CdA, CYTARABINE, MITOXANTRONE AND G-CSF (CLAG-M) AS INDUCTION THERAPY IN RESISTANT AND RELAPSED ACUTE MYELOID LEUKEMIA

PHASE II STUDY OF POLISH ADULT LEUKEMIA GROUP (PALG)
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Background: Primary resistant and relapsed AML have poor prognosis. Addition of purine analogues in induction treatment seems to be an attractive option in this group of patients. Aim of the study: To assess the efficacy and toxicity of the CLAG-M regimen 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 or idarubicine, 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 less than 6 months in duration 3) second or subsequent relapse 4) recurrence after stem cell transplantation.

Results: 26 patients in a median age of 44,5 years (range,20-58), from 5 centers were registered. 23(82%) patients were refractory and 5 (18%) patients had first relapse after CR1>6 months in duration. After CLAG-M regimen CR was achieved in 16 (61%) of the whole group of patients, 8 (31%) were refractory and 2 (8%) died early. 11 (52%) of refractory patients and all of 5 patients with first relapse of AML after CR1>6 months achieved CR. In the group of refractory AML, 3 patients relapsed

after autoSCT and all of them achieved CR after CLAG-M treatment. Hematological toxicity was the most prominent toxicity of this regimen. At the time of analysis, 17 (62%) of the 26 patients were alive, the median OS was 24 weeks for the whole group of patients and 31 weeks for patients in CR. The OS of one year for the whole group of 26 patients and the group of 16 patients in CR was 52% and 90%, respectively. Median DFS was 20 weeks. DFS of one year was 33%. Prognostic value of following factors was analyzed for CR probability: age, WHO performance status 0-1 vs 2, karyotype at diagnosis according to SWOG classification, number of preceding salvage regimens. None of these factors influenced CR probability significantly.

Conclusions: The CR rate of 52% after CLAG-M salvage regimen in primary resistant and early relapsed AML is better than CR rate achieved with many other salvage regimens. 100% CR in 5 patients with AML relapsed after CR1>6months needs further evaluation. Despite advanced stage of AML and prominent hematological toxicity of CLAG-M the treatment-related mortality is relatively low.

092 EFFICACY OF GEMTUZUMAB OZOGAMICIN (MYLOTARG) ON ATRA- AND/OR ARSENIC-RESISTANT ACUTE PROMYELOCYTIC LEUKEMIA (APL) CELLS

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(Background) Acute promyelocytic leukemia (APL) cells express a considerable amount of CD33, which is a target of Mylotarg. Its laboratory and clinical effectiveness on APL cells has been reported by some authors including our group. On the other hand, all-trans retinoic acid (ATRA) and arsenic trioxide (As) have specific effects in the treatment of patients with APL. However, their resistance is relatively easily induced even in initially sensitive cells. Therefore, it is important to understand the efficacy of Mylotarg on ATRA- or As-resistant cells. (Materials and Methods) Cells used in this study were an APL cell line in which MDR related P-gp was undetectable (NB4), ATRA-resistant NB4 (NB4/RA), NB4/RA transfected with MDR-1 cDNA (NB4/RA/MDR), arsenic-resistant NB4 (NB4/As), all drug resistant NB4 (NB4/RA/As/MDR) and blast cells from 4 patients with clinically ATRA-resistant APL and from 2 patients with clinically As-resistant APL. After 12- and 24-hour incubations with 5, 10, and 100 ng/ml of Mylotarg, its efficacy was analyzed by 3H-thymidine uptake, the dye exclusion test with propidium iodide staining, cell cycle distribution and morphological examination. (Results) Mylotarg suppressed the growth of NB4, NB4/RA and NB4/As cells in a dose-dependent manner. Analysis of cell cycle distribution revealed that, after a 24-hour incubation with Mylotarg, the number of cells in a hypodiploid portion was increased in NB4, NB4/RA and NB4/As cells and less in NB4/RA/MDR and NB4/RA/As/MDR cells. Identical results were obtained using samples from patients with APL. (Discussion) These results indicate that Mylotarg is effective in ATRA- or As-resistant APL cells that do not express MDR. It has been reported that APL cells in relapsed cases hardly express MDR and Mylotarg may be effective in the treatment of these cases. Our results also indicate that the resistance mechanism for Mylotarg is independent of the ATRA- or As-resistance mechanism in APL.

093 TREATMENT OF NEW CASES OF ACUTE PROMYELOCYTIC LEUKAEMIA BY ARSENIC TRIOXIDE

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Purpose: Arsenic Trioxide approved for treatment of relapsed or refractory APL to ATRA. We studied the effects of Arsenic Trioxide as first line treatment of new cases of APL and their follow up.

Material and methods: we studied 63 new cases of APL diagnosed by morphologic criteria and confirmed by cytogenetic, RT-PCR for PML/RARA and/or FISH. Our patients were 28 males and 35 females with median age 27+/- 11.98. Patients treated by infusion of 0.15mg/kg/d of Arsenic Trioxide to complete remission by morphologic criteria or till day +60. In patients who complete remission achieved, after 28 days rest, again we began Arsenic Trioxide 0.15mg/kg/d for 28 days as consolidation.

Results: complete remission were achieved in 57 patients(90.5%) and 6 early mortality. Median time to complete remission was 30+/-6.6 days. Most common cause of mortality was APL maturation syndrome (4 cases) Most common toxicities during induction phase were, APL maturation syndrome (14.7%), serositis(11.4%) and hepatotoxicity (18%). 88.5% of patients are alive with a median follow up of 12+/-10.02 months. 11 relapses observed in our patients and complete remission achieved with retreatment by Arsenic trioxide in 8 of them. Mean survival time of patients by Kaplan-Meier method was 33.91 months(CI95% 30.98-37). Most common cause of death were APL maturation syndrome in 3 patients and relapse in 3 cases.

Conclusion: Arsenic Trioxide is acceptable as first line treatment of APL and its result is comparable to ATRA with chemotherapy.

094 ACUTE LEUKEMIA - CLINICAL REGISTER (ALERT) IN THE CZECH REPUBLIC 1996-2004

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Background: The highly effective and safe treatment of acute leukemias (AL), perhaps with the exception of PML, is still searched for and the treatment results, despite the very high costs, are still not fully satisfied.

Aim: The aim of the Acute Leukemia ' Clinical Register project (ALERT) is to give general information about diagnostic and treatment results in AL patients in the Czech Republic, clinical analysis of different prognostic factors, including cytogenetics and molecular genetics, clinical analysis of different treatment modalities and to enable the start of cooperative clinical trials in this country. Project ALERT initiated the Czech hematologists in 1996.

Methods: ALERT is clinical registry for parametrically standardized observations of diagnostic, prognostic and clinical data of AL patients. Project is based on progressive software tools including structured databases ' communication and analytical tools, and provides information service available on-line for all engaged centres. Project has professionally guarantee by the Czech Hematology Association, and all 6 centres of intensive hematological are involved.

Results: Since 1996 full-scaled clinical data from 1412 patients with AL have been collected and coded in ALERT database. It contains data of 1169 AML patients (83% of all AL) with the median of age 58 years (10-90% limit 27-74 years), and 344 ALL patients (17% of all AL) with the median of age 45 years (10-90% limit 20-69 years). The cytogenetic data of 76,2% of intensive treated AML patients are available and were analysed. Total 72% of AML patients (89% of patients in the group of younger than 56 years and 59% in the group of older ones) were treated with intensive induction, the remaining with palliative treatment. CR was achieved in 72,1% intensively treated AML patients younger than 56 years, and in 47,3% of older patients. As the main independent prognostic factors for CR and OS were identified age, PML subtype and poor cytogenetic karyotype. Performed analyses documented significant prognostic impact not only for risk factors at the time of diagnosis, but also for applied consolidation therapy. Significant longest OS in comparison with other treatment modalities was achieved in patients treated by hematopoietic stem cell transplantation. No differences in OS according to the type of transplantation were identified yet. No statistic significant differences were found for CR rate, DFS and OS between centres involved, even if they used different treatment strategies. More detailed data will be given.

Summary/conclusion: Project ALERT provides very important epidemiological data, it allows us to do profound analysis of prognostic factors and treatment results on large series of AL patients in the Czech Republic, to analyse different treatment modalities, costs benefit and evidence based medicine evaluation. Introduction and using of this approach is also the starting point for closer cooperation on treatment projects between hematological centres in the Czech Republic. This project is also important for future accreditation of intensive hematological care and transplant centres in the Czech Republic. Project ALERT is supported by grant of Ministry of Health Czech Republic NR/8080-3

095 SAFETY PROFILE OF ZARNESTRA (TIPIFARNIB) IN PATIENTS WITH RELAPSED AND REFRACTORY ACUTE MYELOGENOUS LEUKEMIA (AML)

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Background: Tipifarnib is a selective, orally bioavailable farnesyltransferase inhibitor. It disrupts signal transduction pathways associated with oncogenic cellular transformation and growth. Central neurotoxicity (ataxia and confusion) had been identified as the dose-limiting toxicity of tipifarnib monotherapy when continuously or administered at high doses. (Blood. 2001;97:3361-3369). Aims: To further characterize the safety profile of tipifarnib, we performed an open label, international phase 2 study in 252 adults with refractory or relapsed AML. Methods: All patients were heavily pre-treated. Tipifarnib (600 mg p.o. bid x 21 days q 4 weeks) was administered in the outpatient setting until disease progression or appearance of unacceptable toxicity. Results: The major toxicity of tipifarnib was myelosuppression. Consistent with pre-treated AML, grade 3/4 thrombocytopenia, neutropenia, or anemia was documented at the time of study entry in 67%, 63%, and 12% of patients, respectively. In this context, occurrences of grade 3/4 thrombocytopenia, neutropenia and anemia were documented in 95%, 86% and 67% of patients. Approximately 50% of patients with normal values at baseline reported Grade 3/4 values during the study. The contribution of tipifarnib was judged by the investigators to be responsible for any grade of thrombocytopenia, neutropenia, or anemia in 32%, 21%, and 20% of subjects, respectively. Drug-related non-hematologic toxicity was noticeably less common. Fatigue (62%) was the most prominent adverse event. Nausea, diarrhea, and vomiting were usually mild (<5% grade 3/4) and seldom considered related to tipifarnib. In contrast, skin rash was often considered related to tipifarnib (27/48 patients). Mucositis was uncommon (8.7%) and rarely severe (2%). Hypokalemia was the most common biochemical abnormality (44%). The majority of these patients were receiving concomitant furosemide and/or amphotericin B. The safety profile was similar across age groups except for drug-related grade 3/4 fatigue (26.3%), diarrhea (15.8%), and sepsis (10.5%), which were more common in patients older than 75. Serious adverse events were reported by 218 patients (87%), but were considered related to tipifarnib in only 98 (39%). These pertained mostly to neutropenia and its attending infectious complications. 73 patients (29%) discontinued treatment due to adverse events. Few (7%) died due to toxicity induced by tipifarnib. Overall, less than 10% of study time was spent in hospital. Disease complications were the most common reasons for hospitalization. Only 58 patients (23%) were hospitalized for toxicity related to tipifarnib. Summary/Conclusions: There were no clinically significant differences between the refractory and relapsed cohorts. Correlation with pharmacokinetic and pharmacogenetic endpoints is ongoing. Evaluation of this dosing regimen is underway in newly diagnosed elderly patients.

096 DRUG EXPOSURE IN A PHASE 2 STUDY OF PATIENTS WITH RELAPSED AND REFRACTORY ACUTE MYELOGENOUS LEUKEMIA (AML) TREATED WITH ZARNESTRA (TIPIFARNIB)

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Background: Tipifarnib is a selective, orally bioavailable farnesyltransferase inhibitor. It disrupts signal transduction pathways associated with oncogenic cellular transformation and growth. A phase 1 study had established a recommended dose of 600 mg po BID x 21

days q 4 weeks. (Blood. 2001;97:3361-3369). Aims: We performed an open label, international phase 2 study in 252 adults with refractory or relapsed AML. Methods: All refractory patients were refractory to first or second line regimens. Among the relapsed cohort, 69 patients (51%) were in first relapse and 16 (12%) in second relapse. Overall, 167 patients (66%) had become biologically refractory to chemotherapy at some time during the natural history of their disease. Tipifarnib was administered until disease progression or appearance of unacceptable toxicity. Treatment was withheld for grade 3 or 4 non-hematologic toxicity (grade 2 neurological toxicity) and grade 4 granulocytopenia or thrombocytopenia attributed to tipifarnib. Dose reductions were implemented, to 300 mg p.o. bid after the first occurrence of toxicity, and 200 mg p.o. bid after a second occurrence. Dose escalation to 900 mg p.o. bid was permitted for patients with evidence of disease control but who had not entered into complete remission after 3 cycles of treatment. Treatment compliance was assessed at each cycle based on a review of patient diaries by the participating investigators. Results: All patients received tipifarnib (all treated population, n = 252). Median duration of treatment was 25 days (range:1-296 days). The per protocol population consisted of 169 patients who received at least 1 cycle of therapy with tipifarnib. In the latter, median duration of treatment was 42 days (range: 15-296), with an average dose intensity of 852 mg/day (95% of planned dose). 137 patients (81% of per protocol population) received more than 1 cycle of therapy. 19% received 4 cycles or more. Both duration and intensity were studied in the <65, 65-74, and 75 age groups. Duration of treatment was shorter for older subjects but median dose intensity was similar. 74 patients (29%) required dose reductions or treatment delays, which represents 54% of patients who received more than 1 cycle of tipifarnib. The incidence of treatment delays increased with duration of therapy. Dose escalation to 900 mg was implemented in 18 patients (7%) and was well tolerated in two thirds. Summary/Conclusions: There was no clinically significant difference in drug exposure between the refractory and relapsed cohorts. Correlation with pharmacokinetic and pharmacodynamic endpoints is ongoing. Evaluation of the same dosing regimen is underway in newly diagnosed elderly patients.

097 EFFICACY OF LOW-DOSE ALL-TRANS RETINOIC ACID IN THE TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA

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All-trans retinoic acid (ATRA) is an effective agent in the achievement of complete remissions and long term survival of patients with acute promyelocytic leukemia (APL) and the standard doses are 45 mg/m²/day. In a few studies low-dose ATRA (15-25 mg/m²/day) therapy was found to be as effective as standard doses in achieving complete remissions, but its influence on survival was not evaluated. In this study, the efficacy of low-dose ATRA therapy in remission induction of APL was investigated retrospectively. For induction of complete remissions, 15 cases diagnosed as de novo APL, between April 1994 and September 2002, received continuous oral ATRA therapy at the dose of 25 mg/m²/day. Of these, eight patients received low-dose ATRA alone until remission was induced, meanwhile 7 patients were also given Ara-C plus anthracycline (7+3) on day 4 to 22 of ATRA therapy. There were 9 females and 6 males. The median age was 40 years (range, 15 to 62 years). At diagnosis the leukocyte count of all patients was <10000/ μ L with a median of 1300 (range, 514 to 6100)/ μ L. Fourteen patients (93.3 %) achieved complete hematological remission after 30 to 104 days (median, 41 days) of low-dose ATRA treatment, while an increase in the ATRA dosage to the standard dose of 45 mg/m²/day was needed in the remaining one patient to induce complete remission. For consolidation therapy after induction of remissions, patients who did not receive any chemotherapy at remission induction were treated with Ara-C+daunorubicin (1 course 7+3, 2 courses 5+1-2) and the rest were given 2 courses Ara-C/ idarubicin (in one patient daunorubicin) 5+2. Three patients (20 %) developed retinoic acid syndrome (RAS), two were treated, but one (6.6 %) died at the 75th day of therapy, while still on remission due to complications of RAS. At a median follow-up of 67.5 months (range, 36.2 to 106.1 months), three of the 15 patients relapsed after 9, 11 and 18 months. Another patient died during follow-up because of a traffic accident. Thus, ten patients are still alive in complete remission. The median overall survival (OS) and disease-free survival (DFS) have not been reached yet. The 8-year Kaplan-Meier estimates OS and DFS were 67%. Our results suggest that low-dose ATRA therapy is as effective as standard dose therapy in treating APL patients, with a leukocyte count of <10000/ μ L, which could not be included the high-risk group.

098 ATTITUDE TOWARDS REMISSION INDUCTION (RI) FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND SURVIVAL. ANALYSIS FROM THE SWEDISH ADULT ACUTE LEUKEMIA REGISTRY

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Background: Combination chemotherapy is routinely given to young patients with acute leukemia, but to a lesser extent to older people, according to clinical status and the physicians choice. In Sweden, both clinicians and pathology departments are since 1958 compelled to report all patients/diagnostic samples with malignancy to the Swedish National Cancer Registry. The Swedish Adult Acute Leukemia Registry started in 1997, and contains 1993 patients diagnosed 1997-2001, i.e., 98% of all cases with acute leukemia in the Cancer Registry. Data in the Leukemia Registry include use of diagnostic procedures (cytochemistry, flow cytometry, cytogenetics), and reports on the individual patients eligibility for remission induction, according to their local physician; such data were available in 98% of the cases. The proportion of elderly patients judged by their physician to be eligible for RI was known to be different between the six Swedish health care regions (HCR), each with a population of 0.9-1.9 million.

Methods: The regional differences were confirmed, and the consequences as regards overall survival were prospectively evaluated in the current analysis, with survival updated to May 2003 through the Swedish Population Registry. Overall survival from diagnosis of patients with AML excluding APL (n=1610) were analysed according to age group and HCR. In the reports, intention of induction was documented for 95% of the patients below 60 years (n=390), for 85% of those 60-69 years (n=337), for 54% of those 70-79 years (n=507), and for 16% of those 80 years or older (n=376). In patients aged 70-79 years the RI rate ranged from 36% in one HCR to 76% in another.

Results: Survival rates for patients aged 70-79 years at 18 months (range 8-31% in the six HCRs) with no censored cases were associated with remission intention (Chi2=16.3, p<0.0001). There were also correlations between RI rates and both 2-year (range 5-22.5%) and 3-year (range 2-19%) projected overall survival (r2 =0.91 and 0.79, respectively, both p<0.05 using non-parametric analysis), according to HCR. The median survival of patients in their 70s was 4 months. In a landmark analysis on patients surviving 90 days, the survival was better in HCRs with a high intention for RI (p=0.0001). Similar data were achieved with landmark analyses at 48 and 60 days. There was no excess early mortality among treated patients in HCRs with a high RI rate. Differences were not explained by demographics, proportion secondary leukemias, or patient selection. The survival of the overall Swedish population aged 70-79 years during the study period was not different between the HCRs.

Conclusion: Long-term survival of AML patients between 70 and 79 years is better in health care regions where more elderly patients are intended for remission induction.

099 EFFICACY AND TOXICITY OF FLAI VS ICE FOR INDUCTION TREATMENT OF NEWLY DIAGNOSED AML PATIENTS, YOUNGER THAN 60 YEARS

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Acute myeloid leukaemias (AMLs) are a heterogeneous family of hemopoietic malignancies that share a high frequency and a high degree of Pgp-mediated multidrug resistance (MDR), one of the major causes of treatment failure. Induction treatment (FLAI), including 5 days administration of Idarubicin (IDA 10mg/sqm/day), in combination with

high-dose Arabinosyl Cytosine (HDAC- 2g/sqm/day) and Fludarabine (FLUDA- 25mg/sqm/day) was adopted as induction treatment of newly diagnosed patients with AML, except acute promyelocytic leukemia (APL). In our previous experience (Russo et al. Leukemia Lymphoma, 2001), FLAI regimen showed to be highly effective (CR rate 72%), also in AML MDR-pos patients, with a low extra-hematological toxicity. In this prospective randomized trial, FLAI was compared with ICE (IDA 10mg/sqm/day x 3 days + AC p.c 100mg/sqm/day x 10 days + VP16 100mg/sqm/day x 5 days) for induction of remission. Post-induction treatment program included: HDAC (3g/sqm/12h x 6 days), MEC (Mitoxantrone 12 mg/sqm/day x 4 days, Etoposide 100 mg/sqm/day x 4 days, Cytarabine 1 gr/sqm/day x 4 days) and allogeneic or autologous BMT. Over a period of 2 years, 122 patients were randomly assigned to FLAI (67) or ICE (55). The clinical and hematological characteristics of the two patient population were not different. In the FLAI group, the complete remission (CR) rate was 72% after the first course and 76% after HDAC in the ICE group, the CR rate was 50% and 63%, respectively (P <0.02). Interestingly, the CR rate of MDR-pos. and MDR-neg. patients treated with FLAI was similar (68% vs 65%), while the CR rate of MDR-pos. and MDR-neg. patients treated with ICE showed a significant trend in favour of MDR negative patients (20% vs 54%). The median time to recovery of neutrophils > 1.0 x 10⁹/L and platelets > 50 x 10⁹/L was significantly better in FLAI arm than in ICE arm. In both groups, it was seen an approximately equal rate of FUO, Gram negative/Gram positive bacteremias and systemic fungal infections. Infections and haemorrhages caused death during induction (DDI) in 3% of patients treated with FLAI and in 9% of patients treated with ICE. Non-hematological toxicity of FLAI was mild and significantly lower than ICE. In particular, in the FLAI arm, only 3/67 patients developed a grade 3 or 4 gastro-intestinal toxicity, whereas in ICE arm 16/55 patients experienced this toxicity (p=0.0001). Other grade 3 or 4 toxicity (ipertransaminasemia, cutaneous toxicity, renal or cardiac failure) were seen in 1/67 patients in FLAI arm versus 7/55 patients in ICE arm (p=0.02). In conclusion, these preliminary results strongly suggest that FLAI, as single induction course, is an highly effective regimen with a limited non-haematologic toxicity. Furthermore, FLAI seems to be more effective than ICE to overcome Pgp-mediated multidrug resistance (MDR).

Aknowledgements: supported in part by Cofin 2002 (prot. MM06268917_004, Prof. Domenico Russo), CNR, AIRC, AIL, FIRB 2001, Ateneo 40% (Prof. Baccarani), Fondazione del Monte di Bologna e Ravenna.

100 ATTENUATED FLAIG REGIMEN IN ELDERLY PATIENTS WITH HIGH RISK ACUTE MYELOID LEUKEMIA OR MYELODYSPLASIA

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Seventy consecutive elderly patients with acute myeloid leukaemia (AML=41), high risk myelodysplasia (MDS=13) or secondary AML (sAML=16) were treated with an attenuated FLAIG protocol. The median age was 67 years (range 60-76). The male/female ratio was 1.18. Successful karyotype was obtained in 56 patients (80%). A normal karyotype was found in 33 patients. In 5 patients no mitoses were obtained and in 9 cytogenetic was not available. An complex karyotype was detect in 16. The induction therapy was Fludarabine 30 mg/sqm (25 mg/sqm in patients>70 years) and Cytarabine 1 g/sqm for 5 days (4 days in patients> 70 yrs) plus Idarubicine 10 mg/sqm in 24 hours continuous perfusion on days 1-3-5. Glycosilated G-CSF was administered from day 7 to granulocyte recovery. 44 patients (62.9%) obtained a complete remission (CR) (AML 27=65.8%, MDS 8= 61.5%, sAML 9= 56.2% - p= n.s.) 9 patients died during the induction phase (2 early death and 7 death during aplasia) and 17 were resistant. Patients in CR were treated with a first outpatient consolidation course with Fludarabine 30 mg/sqm (25 mg/sqm in patients>70 yrs, Cytarabine 1 g/sqm for 2 days and Idarubicine 10 mg/sqm on day 2. A second consolidation was given with Cytarabine 100 mg/sqm/day s.c., Thioguanine 50 mg/sqm for 5 days and oral Idarubicine 15 mg/sqm on day 1. Maintenance treatment with Cytarabine 100 mg/sqm once a week and Thioguanine 50 mg/sqm/day for 5 days a week was planned for one year or until relapse. Two patients with an HLA identical sibling underwent non-myeloablative allogeneic stem cell transplantation. The median survival (OS) was 11 months with a median follow-up of 11 months (range 2-51) for censored patients and with an actuarial probability of survival at 4 years of 6%. The median disease-free survival (DFS) was 10 months. Age appeared to be the most important determinant of survival (median OS 14 months for patients < 67 years and 8 months for patients

> 67 years; $p=0.02$). No difference in OS and DFS was observed between patients with AML, MDS and sAML and between different cytogenetic risk groups. This FLAIG regimen provided an high incidence of CR with reduced toxicity, induction mortality and time of hospitalisation. However the remission duration was short, particularly in the more advanced age group. Better consolidation and maintenance strategies should be devised to improve the outcome without affecting the quality of life.

101 FLUDARABINE, ARABYNOSIL CYTOSINE AND IDARUBICIN (FLAI), A COMBINATION OF NON MDR-RELATED DRUGS FOR TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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One of the most important causes of treatment failure in AML is the non specific multidrug resistance (MDR). This is mainly due to the overexpression of 170-Kd trans-membrane glycoprotein (p170) which enhances the efflux of several drugs from the cells. The availability of agents which are not at all or minimally involved in the MDR efflux mechanism have recently allowed to test new therapeutic approaches in AML. We report here on the results of 138 newly diagnosed AML patients (FAB M3 excluded) treated with a combination of Fludarabine, Arabinosyl Cytosine and Idarubicin (FLAI) as first-line induction therapy. Patients responding to FLAI were scheduled for consolidation with a second course of FLAI or high dose cytosine arabinoside (HDAC), and autologous stem cell transplantation (ASCT) or allogeneic BMT, when feasible. A complete remission after a single course of FLAI was achieved in 99/138 (72%) patients. The median time of granulocyte recovery (ANC > 1.000/mL) was 24 days (range 15-55) and a platelet count > 50.000/mL was reached after a median of 22 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 6/138 patients developed a grade 3 or 4 gastro-intestinal toxicity. Before therapy, the p170 expression in bone marrow blasts was evaluated in 95 patients (69%) by an indirect immunofluorescence method with the anti-p170 monoclonal antibody MRK-16. The results were expressed as the mean fluorescence index (MFI). At present, no significant difference neither in the CR rate nor in the overall survival (OS) and disease free survival (DFS) was observed between the patients who were overexpressing the p170 glycoprotein (MFI > 6) and the patients who had lower p170 levels (MFI < 6). These results suggest that the FLAI regimen is a good first-line induction therapy for patients with newly diagnosed AML. Our results suggest that FLAI regimen can be useful to overcome primary MDR-Pgp mediated.

Acknowledgements: supported in part by Cofin 2002 (prot. MM06268917_004, Prof. Domenico Russo), CNR, AIRC, AIL, FIRB 2001, Ateneo 40% (Prof. Baccarani), Fondazione del Monte di Bologna e Ravenna.

102 THE ROLE OF ADENOSINE AND ADENOSINE KINASE IN ACUTE MYELOID LEUKEMIAS WITH METHYLTHIOADENOSINE PHOSPHORYLASE (MTAP) INACTIVATION

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Background: The metabolic enzyme methylthioadenosine phosphorylase (MTAP), which is involved in purine metabolism, is codeleted with tumor suppressor gene p16INK4a in approximately 30% of all human malignant tumors due to their close localization on chromosome 9p21. The availability of the adenylosuccinat-synthase inhibitor L-alanosine initiated several studies with this antineoplastic drug in MTAP-negative tumors with the goal to completely inhibit AMP synthesis in these tumors. Nevertheless, several tumors could be identified which are MTAP-deficient but do not respond to L-alanosine putting emphasis on the existence of additional pathways of AMP synthesis.

Aim: The aim of this study was to evaluate the importance of other AMP-synthesis pathways and to prove that these salvage pathways are of particular importance in MTAP-negative acute myeloid leukemia cells and that they might influence the effective treatment of this malignancy with L-alanosine.

Methods: We examined the presence of the MTAP gene as well as of the MTAP protein in 19 different human acute myeloid leukemia (AML) cell lines and primary myeloid blasts from 23 patients with AML. MTAP deficiency at the protein level was confirmed by western blot analysis using a monoclonal mouse-anti-human MTAP-antibody. The effect of L-alanosine, the adenosine kinase inhibitors HC-7 or iodotubercidine on cell proliferation was measured by cell counting using trypanum blue staining and/or intracellular ATP determination using a commercially available kit (Amersham). Adenosine kinase activity was determined using a recently described kinase assay based on [3H]-adenosine. L-alanosine was kindly provided by the National Cancer Institute (NCI) and the adenosine kinase inhibitor HC-7 by Dr. H. Cottam, UCSD, La Jolla, USA.

Results: Using PCR and fluorescent in situ hybridization (FISH) techniques, we found a homozygous MTAP-deletion in 7/19 AML cell lines and a hemizygous in one AML line. Although nearly 25% of the primary AML samples showed no MTAP protein, only one primary AML sample had a homozygous deletion on chromosome 9p21 including the MTAP locus. Adenosine kinase activity was higher in MTAP-negative tumors (3.68 pmoles/mg protein/min) compared to MTAP cell lines (1.67 pmoles/mg protein/min). The sensitivity of MTAP negative AML cell lines to L-alanosine treatment was significantly higher than the sensitivity of MTAP-positive tumors. The addition of the adenosine kinase inhibitors HC-7 or iodotubercidine had an additive effect to the L-alanosine induced inhibition of cell proliferation, underlining the importance of the adenosine kinase pathway in AMP/ATP synthesis. Interestingly, the addition of adenosine to the cell culture medium completely neutralized the L-alanosine effect, indicating that the increased uptake of extracellular adenosine and the intracellular modification by adenosine kinase is a potent salvage pathway of AMP/ATP synthesis in MTAP-deficient cells.

Conclusion: Our results indicate that ATP synthesis via adenosine kinase is an important salvage pathway in MTAP negative tumors. Therefore the combination of L-alanosine and an adenosine kinase inhibitor or an adenosine uptake inhibitor such as dipyrindamole could be a promising new therapeutic regimen for MTAP negative tumors/leukemias. Lowering the intracellular ATP levels might also sensitize the cells, so that L-alanosine and adenosine kinase inhibitors could be useful combinational drugs.

103 5-YEAR SURVIVAL OF ACUTE MYELOID LEUKEMIA IN ITALY: RESULTS FROM THE EUROCORE-3 STUDY COMPARED TO GIMEMA MULTICENTRIC PROSPECTIVE TRIALS

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Background: recently, the Eurocare working group reported results of a 5-year survival analyses on patients with tumor utilizing data from different European registries, and recorded between 1990 and 1994. This study showed some differences in survival for hematological malignancies in Italy versus other countries, with a lower improvement rate than the one reported in other diseases; in particular, poorer results were obtained in acute leukemias, with less than 30% relative survival versus about 35% in other European countries. For instance, the 5-year age-standardized relative survival (%) in AML was 10.1 in Italy versus 14.2 and 17.0 in England and France respectively for males, and 13.8 in Italy versus 16.1 both in England and France respectively for females.

Aims: in order to verify whether these findings were limited to a comparison of data from tumor registries or were also evident for data from clinical trials in acute leukemias, we analyzed a cohort of AML patients treated in GIMEMA trials in the same lapse of time (1990-1994), with a minimum follow up of 5 years from diagnosis.

Methods: a total of 9 GIMEMA trials have been analyzed, all recruiting prospectively patients with acute myeloid leukaemia referring to the main national hematologic hospitals in Italy between 1990 and 1994, with a total of 1,723 evaluable cases. To compare results with the tumor registries Eurocare data, the actuarial overall survival at 1 and 5 years, and survival stratified by age category (15-44, 45-54, 55-64, 65-74) and by sex (male/female) have been analyzed with the same modalities utilized in the Eurocare study.

Results: the survival at 1 and 5 years for all AML (including APL) treated in the GIMEMA protocols was 62% (95% C.I.: 59.5 - 64.2) and 40% (95% C.I. 37.2 - 42.0) respectively, versus 12% at 5 years reported by the Eurocare study. Excluding APL, the 1 and 5 year survival probability are 57% (C.I. 95%: 53.8 - 59.3) and 33% (C.I. 95%: 30.5 - 35.8) respectively. These data are clearly superior to those reported by the

Italian tumor registries for acute myeloid leukemias, and are completely superimposable with data from the main international groups in Europe and the USA for multicentric cooperative trials.

Summary: the different outcome reported by the Eurocare study for survival in AML in Italy versus the other EU countries does not seem to be confirmed by a comparison between results of clinical trials carried out in Italy versus those reported in Europe. Thus, the information from the registries, at least as concerns AML, seems not to be adequately informative of the actual results of the outcome for this disease in Italy. To clarify this issue, a prospective study in cooperation with registries is needed in Italy, to thoroughly understand the difference in terms of patients registered and in terms of accuracy of the diagnostic information reported.

104 CYTOREDUCTION RATE AFTER HYDROXYUREA PRE-TREATMENT IS AN INDEPENDENT PROGNOSTIC FACTOR FOR COMPLETE REMISSION IN ADULT AML

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1. Background. During the last years, a central handling of BM and PB samples at diagnosis has become more and more relevant to allow an accurate evaluation of biological characteristics of the disease with prognostic relevance and to investigate and identify new genetic alterations which could influence treatment and outcome.

2. Aims. To evaluate the feasibility of collection and centralization - on a national basis - of PB and BM samples at diagnosis in all adults newly diagnosed with AML before starting the induction treatment.

3. Methods. Since 1999, a 5-day hydroxyurea (HU) treatment at the dosage of 2 g/sqm/day has been administered before starting the induction to adult (15-60 yrs) AML patients treated according to GIMEMA protocols as to allow an adequate interval time for the collection and centralization of PB and BM samples. At the end of the HU pre-treatment all patients started the same 3-drug induction cycle with DNR (50 mg/sqm d 1, 3, 5), cytarabine (100 mg/sqm d1-10), etoposide (100 mg/sqm d1-5) followed by an intensive consolidation treatment with cytarabine (500 mg/sqm/q12 hrs d1-6) and daunorubicine on d 4-6. Following consolidation, eligible pts with a HLA compatible sibling were allografted, and the remaining patients were autografted with autologous peripheral stem cells collected during recovery from consolidation.

4. Results. Out of 366 patients who started the HU pre-treatment, 353 (96%) were evaluable for response to pre-treatment. CR after the first induction cycle was obtained in 233 (66%) cases. The probability to achieve CR after the first induction cycle was associated with WBC count at diagnosis, age, and percentage of WBC reduction after HU pre-treatment. In particular, patients with a rate of WBC reduction at the end of pre-treatment >30% ('responders') have a higher probability of CR versus 'non-responders' (OR=2.3, 95%CI: 1.4-3.8, p=0.001) after adjustment for WBC value at diagnosis and for age. On the contrary, in the post-remission phase no difference in outcome has been detected, with similar DFS, non-relapse mortality and relapse risk in both groups.

5. Summary GIMEMA administered a 5-day HU pre-treatment to AML patients to allow collection and centralization of biological samples on a national basis, and to standardize a debulking pre-treatment in all new patients. This analysis shows that: (a) a standard HU pre-treatment is feasible, with a CR rate comparable to what obtained with same treatment protocols in this category of patients, thus formally demonstrating that a delay in starting the induction to complete all the extended diagnostic evaluations needed today is feasible and does not affect the treatment outcome; (b) response to HU pre-treatment predicts the probability of obtaining CR independently of WBC count and age, thus contributing

to identify risk categories avoiding difficult and expensive evaluation of other prognostic factors.

105 TREATMENT OF OLDER AML PATIENTS WITH LOW-DOSE 5-AZA-2'-DEOXYCYTIDINE (DECITABINE)

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Background: Epigenetic mechanisms of gene inactivation, such as cytosine methylation, provide powerful potential targets for treatment approaches aiming at altering the transcriptional program of malignant cells. Treatment of AML is often limited by patient (pt) age, performance status and comorbidity. Pioneering studies by Pinto and colleagues demonstrated clinical activity of non-intensive doses of the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (Decitabine, DAC) in elderly AML pts. This approach was also successful in preleukemic MDS, resulting in a response rate of 49% in pts with 21-30% blasts (RAEB-t or AML according to FAB or WHO, respectively; Wijermans et al., Blood 100:96a).

Aim: To develop a treatment schedule of low-dose DAC for older AML patients.

Results: Four pts screened for studies of DAC in high-risk MDS had already progressed to AML at time of planned treatment start. Due to their age and/or comorbidity (median age 75 years, range, 62-79) they were not eligible for, or did not consent to, intensive chemotherapy. The median performance status was 2 (range, 1-3). Comorbidities included coronary heart disease, carotid artery stenosis, cardiac insufficiency. 2/4 pts had received previous low-dose treatment. Pts received a median of two low-dose DAC courses (range, 1-8), in two pts resulting in an antileukemic effect (complete clearance of peripheral blood blasts and >50 % reduction of bone marrow blasts); in two pts in both a hematologic CR and karyotypic normalization after 2 and 5 DAC courses, respectively (duration 8 and 15 months from 1. response). Median survival from start DAC was 8 months (range, 5-21). Based on these very encouraging clinical results of low-dose DAC in bona fide AML patients, a phase II protocol was developed that allowed AML pts >60 years and ineligible for induction to be treated with low-dose decitabine (135 mg/m2 total dose per course) given over 72 hours. One complete treatment consists of 4 DAC courses. Based on in vitro data of reactivation of the retinoic acid pathway in non-M3 AML cells by pre-treatment with DAC, the addition of ATRA (45 mg/m2 given orally over 28 days) is part of the 2nd course. In pts with >20 000 WBC/ l at initial diagnosis, a short course of hydroxyurea is allowed. So far 9 AML pts (median age 70 years, range 63-75) have been treated on the protocol, receiving a total of 23 courses. So far, no unexpected toxicities have been observed with low-dose DAC given alone or in combination with ATRA. Hematologic results will be presented.

Conclusions: Preliminary results imply feasibility and activity of repeated courses of low-dose intravenous DAC also in AML. The phase II study initiated to determine toxicity and efficacy of this approach in AML pts >60 years which are not eligible for induction treatment is ongoing.

106 ALL-TRANS-RETINOIC ACID (ATRA) AND VALPROIC ACID (VPA) COMBINATION INDUCE TERMINAL DIFFERENTIATION IN AML BLASTS

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Aberrant recruitment of the histone deacetylase (HDAC) complexes is a usual event in different subtypes of acute myeloid leukaemia (AML). These complexes are able to block the retinoic acid (RA) signalling pathway preventing differentiation of haematopoietic precursors of myeloid series. Treatment with HDAC inhibitors (HDACi) and ATRA has been demonstrated to induce in vitro differentiation in AML blast cells. VPA, extensively used in the therapy of epilepsy, has been recently recognised as a novel HDACi agent. To study the response of blasts cells to VPA in combination with ATRA we cultured bone marrow samples from 31 primary tumours, comprising 22 adult non-promyelocytic AML (3 AML-M1, 4 M2, 5 AML-M4, 3 AML-M5, 5 AML-others) and 8 adult MDS samples. The clinical characteristics of the series were as follows: 14M/8F; median age 64 yr (33-81), WBC 9.8 x109/L and blast count median

MUD transplants, and 9% for auto-transplants. Relapse-free survival at 48 months was 52%, 51%, and 9%, respectively. The only three survivors in the auto-transplant arm all had CBF-leukemias.

Conclusion: HR patients as defined in our trial seem not to benefit from autografting even after intensive induction and early consolidation. In these patients, allografting from either related or unrelated donors should be considered in first CR.

108 CHARACTERIZATION OF GENOMIC DELETION OF 5' ABL AND 3' BCR IN DER (9) CHROMOSOME IN CHRONIC MYELOID LEUKEMIA AND RESPONSE TO IMATINIB MESYLATE

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Conclusion: The data shows that genomic deletions involving 5 ABL sequences, ABL along with adjacent sequences (9q34@ter) occur in substantial number of Ph+ CML patients (30%). Large 9q deletions (9q@ter) were visualized by presence of single bcr-abl fusion with loss of both residual abl and bcr sequences on derivative 9. The results also indicate that large 9q deletions are more common than mere 5 abl deletion. The detection of deletion of abl/9q simultaneously along with bcr-abl fusion at diagnosis indicates that these deletions occur early at the time of Ph translocation. There was no significant difference in short-term response to imatinib with or without deletion of abl/abl-bcr. Only a longer follow-up may reveal any possible biologic significance of this abnormality while receiving a targeted therapy like imatinib.

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Myeloid and plasmacytoid dendritic cells (MDC, PDC) are key players in the initiation of immune responses. PDCs are also crucial effectors in innate immunity and might play a key role in antitumor immunity. Chronic myeloid leukemia (CML) is a malignant clonal disorder of primitive haematopoietic progenitors characterized by a balanced translocation between chromosomes 9 and 22, leading to the fusion of a portion of the ABL gene with the BCR gene. The resulting BCR/ABL gene has been shown to play a critical role in the pathogenesis of CML. The BCR/ABL gene product demonstrates constitutive activation of tyrosine kinase activity that appears critical for BCR/ABL-induced transformation. We have previously shown a reduction of DC subsets among CML patients in chronic phase at diagnosis. These data suggested that restoration of DC functions may be a goal for CML immunotherapy. Interestingly, imatinib mesylate (Gleevec[®], STI571) has been demonstrated to be a

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Results: 174 patients were classified as HR, of whom 102 (59%) achieved CR (as compared to 90% of the SR patients), and 24 (13%) of the HR patients died during induction. After 48 months, overall survival was significantly worse for HR patients (25%) than for SR patients (52%). In the HR group, there was no difference in survival between those patients classified as HR because of bad response to IVA-I (n = 49), unfavourable karyotype (n = 65) or both characteristics (n = 60). Regarding the different regimens for late consolidation, the 'as treated' analysis of the HR patients revealed that overall and relapse-free survival was significantly better after an allogeneic HLA-matched sibling (n=21) or MUD (n=20) transplantation than after autoPBSCT (n=20). At 48 months, the actuarial overall survival was 60% for HLA-matched sibling transplants, 65% for

potent inhibitor of ABL tyrosine kinases. Several preclinical and clinical studies demonstrated high levels of imatinib activity against BCR/ABL-containing cells with remarkable results in the treatment of CML. Despite the exciting success of this targeted cancer therapy, it is still needed to determine whether imatinib can also influence the immune functions in CML patients, allowing for immune-mediated durable responses as it has been already shown after allogeneic stem cell transplantation. In this study, we show that imatinib, in the presence of Flt3-Ligand, could induce CD34+ progenitors from CML to give rise to typical BDCA-2+ type I interferon-producing PDCs. The impact of imatinib on PDC generation was related to upregulation of Flt3 on leukemic CD34+ progenitors. Moreover, CML patients who were in complete cytogenetic or molecular response after imatinib treatment restored their blood PDCs both quantitatively and functionally comparable to healthy donors, in contrast to patients not responding to imatinib, further confirming that imatinib can restore PDC function in vivo. These findings provide some evidence that imatinib treatment is capable to restore crucial DC-related immune functions in CML and might help restoration of full immunocompetence, resulting in long term disease control.

110 DOSE-ADJUSTMENT OF IMATINIB BASED ON PLASMA LEVEL MEASUREMENT IN A PATIENT WITH CML

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Background:

Many trials have tested the activity of imatinib in patients with chronic, accelerated, and blastic-phase chronic myeloid leukaemia (CML) at dosages of 400, 600 and 800 mg/day (d). Despite interindividual variations of clinical response, measurement of imatinib plasma level is generally not recommended.

PATIENT

Caucasian male, aged 26 years, was diagnosed as having CML in chronic phase at low risk according to Hasford Score. He was placed on hydroxyurea, and after haematological remission, was treated with interferon-alpha at 8 mio IE/d. Interferon-alpha was stopped because of haematological side effects. Imatinib was commenced at 400 mg/d and after 9 months cytogenetic analysis showed Philadelphia (Ph) chromosome positivity in approximately 70% of metaphases and no decrease of BCR-ABL transcripts was noted by quantitative polymerase chain reaction (PCR). Because of a body weight of 110kg (BMI 32.5 kg/m²) plasma level measurement of imatinib was performed, which revealed half of expected plasma level. The imatinib dose was therefore increased to 600 mg/d, administered in two doses. After 3 and 4 months repeated cytogenetic analysis showed marked reduction of Ph+ metaphases to 7% in standard method and to 1% by FISH analysis as well as a 2 log reduction of BCR-ABL transcripts.

METHOD

Imatinib concentration measurement was performed with a validated high-performance liquid chromatography (HPLC) -UV method developed for the pharmacokinetic study of imatinib. This method enables the concentration determination up to 10000 ng/ml, with a limit of quantification of 50 ng/ml. Concentrations obtained in this patient were visually compared to concentration-time curves determined in cancer patients at steady-state.

CONCLUSION

Recently published data have reported a wide range of peak plasma concentrations in patients which may possibly modulate cellular influx changes as compared to extracellular concentration. We describe here a case where dose-adjustment of imatinib based on plasma level measurement in a patient with CML resulted in improved cytogenetic response. Additional pharmacokinetic-pharmacodynamic studies on imatinib are nevertheless warranted to systematically recommend dose-adjustments based on plasma levels in patients lacking adequate responses.

111 ON THE EXPECTED SURVIVAL ADVANTAGE OF IMATINIB OVER IFN IN CHRONIC PHASE CML PATIENTS

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Background: The promising safety profile and the high response rates demonstrated by imatinib make it difficult to perform a standard phase

III trial in order to compare survival with IFN-treated patients, since most of them cross over to imatinib (1). Nevertheless, patients and physicians would appreciate to be able to assess the expected survival advantage with imatinib. Aims: Whereas IFN treatment is obviously given up in patients with high risk according to the New CML score (2), the notion was to estimate the rise in survival probabilities for intermediate-risk and low-risk patients treated with imatinib by considering its higher major cytogenetic response (MCR) rates. Methods: Our data base comprised 680 IFN-treated intermediate-risk or low-risk patients with complete data on cytogenetic response and survival. Within 21 months of treatment, 83% of all first MCR had been achieved and the survival of 583 patients was still under observation. The 269 patients of the low-risk group (46%) consisted of 88 patients (33% of 269) with MCR within 21 months and 181 patients without. The 10-year survival probabilities of MCR vs. no MCR were 0.74 vs. 0.20. Within the 314 intermediate-risk patients, the MCR rate was 24% (n=75) while 239 patients remained without MCR during the first 21 months. The respective 10-year survival probabilities equalled 0.60 and 0.12. To estimate survival probabilities under imatinib treatment, two assumptions were met: (i) Within each of the four groups determined by the specific combination of New CML score risk (low, intermediate) and MCR (yes, no), the survival probabilities under imatinib and IFN treatment were expected to be the same. (ii) For both risk groups, among imatinib-treated patients a 21 months MCR rate of 85% was presumed. For the estimation of survival probabilities under imatinib treatment, in accordance with (i) and (ii), a sample of low-risk patients was constituted by randomly drawing 85% of its patients and their survival data out of the 88 patients with MCR and 15% out of the 181 patients without MCR. In the same way, a patient sample representing imatinib treatment in the intermediate-risk group was gained. Results: The estimated 10-year survival probabilities for imatinib-treated low-risk and intermediate-risk patients were 0.66 and 0.53, respectively. Compared to the 10-year survival probabilities of the 269 low-risk and 314 intermediate-risk patients actually treated with IFN, 0.37 and 0.22, increases of around 0.30 were prognosticated for both samples, a larger improvement than seen with the more conservative approach where all risk groups were estimated together (3). Conclusions: So far, in the long run, it is not known for sure that MCR under imatinib induces the same survival advantage as observed with IFN. However, there is strong evidence that imatinib with its high response rates will lead to an increase in survival probabilities in comparison to IFN. Our prognosis suggests that this increase will be decisive, both in the low-risk as well as in the intermediate-risk group. (1) O'Brien et al., NEJM 2003; 348:994-1004 (2) Hasford et al., JNCI 1998; 90:850-858 (3) Hasford et al., Blood 2003; 102:905a

112 TARGETING OF MCL-1 WITH ANTISENSE OLIGONUCLEOTIDES COUNTERACTS GROWTH AND VIABILITY OF CML CELLS: ROLE OF MCL-1 AS A NOVEL BCR/ABL-DEPENDENT TARGET IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disease in which leukemic cells exhibit the reciprocal translocation t(9;22) and the BCR/ABL oncogene. Recently, anti-apoptotic members of the Bcl-2 family such as Bcl-XL and Bcl-2 have been implicated in the pathogenesis of CML. However, so far, little is known about the expression and function of Mcl-1 in CML. Aims: In the current study, we have investigated expression of Mcl-1 and underlying signal transduction pathways in CML cells and evaluated the effects of targeting of Mcl-1 on growth and viability of these cells. Methods: Expression of Mcl-1 was detected by Northern and Western blot analysis in primary CML cells as well as in Ba/F3 cells inducibly expressing the BCR/ABL-oncogene. Underlying signaling pathways were characterized employing various pharmacologic inhibitors. To downregulate expression of Mcl-1 we used Mcl-1 antisense oligonucleotides (ASO). Results: Independent of the phase of disease, primary CML cells were found to express Mcl-1 in a constitutive manner and the BCR/ABL-inhibitor ST1571 downregulated expression of Mcl-1 in these cells. Correspondingly, BCR/ABL was found

to enhance Mcl-1 promoter activity and expression of Mcl-1 mRNA in Ba/F3 cells. BCR/ABL-dependent expression of Mcl-1 in Ba/F3 cells was counteracted by the MEK-inhibitor PD98059, but not by the PI3-kinase inhibitor LY294002. Identical results were obtained for constitutive expression of Mcl-1 in primary CML cells and the CML-derived cell lines K562 and KU812. Downregulation of Mcl-1 by ASO led to a substantial decrease in cell viability in both STI571-sensitive and STI571-resistant K562 cells. Moreover, the Mcl-1 ASO were found to sensitize K562 cells against the growth-inhibitory effects of STI571. Summary: Together, our data identify Mcl-1 as a novel BCR/ABL-dependent survival factor in CML. Whether Mcl-1 can indeed serve as a novel molecular target in CML remains to be determined.

113 ALPHA-1-ACID GLYCOPROTEIN SHOWS INCREASED BINDING TO IMATINIB AT NEW HIGHER DOSES RECENTLY PROPOSED

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Background: Alpha-1-acid glycoprotein (AGP) is an acute phase protein whose plasma concentration is increased under conditions of stress and disease. AGP is known to bind drugs in the plasma and decrease their efficacy by reducing the free, active, concentration of drug. The glycosylation pattern of AGP is altered in disease, with changes in the branching of its oligosaccharide chains and the degree of sialylation and fucosylation. We have previously shown (Blood 2002;99:713) that AGP did not affect imatinib mesylate efficacy in chronic myeloid leukaemia (CML) at the standard dose of 400mg/day, however with recent reports (Blood 2003;102:abstract 94) of increased efficacy of imatinib at between 600 and 800mg/day it was important to determine AGP-imatinib interactions at these increased doses, which would result in increased plasma concentration of imatinib.

Aims: To determine the degree of binding between AGP and imatinib at the increased plasma concentration resulting from increased drug dosage. To compare the microheterogeneity of AGP from CML patients with healthy individuals.

Methods: The degree of AGP-imatinib binding was determined spectrophotometrically. This technique is based on the intrinsic fluorescence at 300-400nm of tryptophan residues present in the protein backbone. The fluorescence spectrum of AGP was compared to that of AGP-drug solution, and the percentage decrease (quenching) in fluorescence at 335nm calculated. A high percentage decrease in fluorescence correlates with a high level of binding between drug and AGP. AGP was isolated from the plasma of CML patients (n=10) using low pressure chromatography. The monosaccharide content of AGP was analysed using high pH anion exchange chromatography.

Results: At an imatinib concentration of 5uM (peak plasma concentration for 400mg/day imatinib = 4.6uM), the percentage quenching obtained for the interaction with AGP was 3.91 +/- 1.03%. In comparison, at an imatinib concentration of 10uM (peak plasma concentration for 800mg/day imatinib approx. 9.0uM, assuming linear kinetics), the percentage quenching was 11.60 +/- 4.68%. Although there was increased interaction with AGP at the higher imatinib concentration, there was no significant difference (p>0.05) in the degree of interaction between AGP and imatinib at 5uM or 10uM (400mg/day vs 800mg/day). Of the CML patients who exhibited no cytogenetic response to imatinib, 2 of 3 had fucose levels greater than the normal control samples. 4 of 7 CML patients with a complete cytogenetic response (CCyR) to imatinib had fucose levels within the normal range. One CCyR patient had fucose levels raised above normal, and two patients with CCyR had fucose levels below normal.

Summary: There was higher binding observed between AGP and imatinib at plasma concentrations resulting from dosing at 800mg/day compared to 400mg/day, however the difference was not statistically significant. Levels of fucose present on AGP could be used as a prognostic factor for response to imatinib, with increased fucose levels suggesting a lack of cytogenetic response.

114 CLINICAL SAFETY OF IMATINIB: A PROSPECTIVE STUDY OF POSSIBLE CLINICAL EFFECTS OF INHIBITION OF OTHER TYROSINE KINASES IN PATIENTS TREATED FOR CHRONIC-PHASE CML

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Background: Imatinibs high efficacy and good tolerability have resulted in it becoming a standard therapy for patients with chronic-phase CML. However, imatinibs rapid clinical development inevitably meant less time was available to study long-term side effects. Imatinib targets bcr-abl therapeutically, but also inhibits c-abl, arg, c-kit, pdgfra and pdgfrb experimentally. In 'knock-out' mice lacking these kinase genes throughout development, loss of the kinases functions causes defects in the respiratory, renal, reproductive and immunologic systems. However, it is not clear if inhibition of these other kinases is achieved or has deleterious effects in adult patients on imatinib. Aims: We aimed to look for effects of inhibition of these other kinases, as a sub-study of a clinical trial of escalating doses of imatinib in 103 patients with untreated early chronic-phase CML. Methods: 45 patients consented to participation in aspects of this sub-study, with ages ranging from 37-75 (median 51), and receiving average daily imatinib doses from 342-600mg (median 559mg). We evaluated respiratory function, urinary protein excretion, male and female hormonal status, sperm counts, serum immunoglobulin levels, blood B-, T- and K- cell numbers, since each of these parameters has been reported to be affected by knock-out of one of imatinibs target kinases in mice. Patients had samples taken prior to starting imatinib and after 6 months on therapy. (We plan to report subsequently on samples taken after 12 and 24 months.) Results: With regard to respiratory function, in 13-19 patients studied median vital capacity fell from 4.7 to 4.5L (p=0.035), and median DLCO rose from 27 to 28 (p=0.009), but median FEV1/VC, and median PEFR did not change significantly. Median urinary excretion of protein fell (in 16 patients) from 180 to 120mg/day (p=0.043). With regard to hormone levels, in 11 male patients median serum oestradiol rose from 88 to 120pmol/L (p=0.043), but median testosterone, FSH and LH levels stayed unchanged. Too few semen analyses and female hormone levels were measured for meaningful analysis at this stage. With regard to immunologic parameters, in 20 patients studied there were falls in median IgG from 10.45 to 9.95g/L (p=0.0058), and in median IgM from 1.31 to 0.68g/L (p<0.0001), but not in median IgA. In 15 patients, no significant changes in blood counts of CD3, CD4, CD8, CD19 or CD56 cells were found, but there was a trend to lower CD4 counts (710 to 540 cells/uL; p=0.066). Clinically, no serious infections were noted in the cohorts of patients studied for these parameters. Summary/conclusion: In CML patients on imatinib therapy for six months, there were moderate reductions in median serum IgM levels, and in urinary protein excretion, and a moderate increase in oestradiol levels in male patients. No deleterious clinical consequences of these changes were noted.

115 SEMI-SYNTHETIC HOMOHARRINGTONINE FOR PATIENTS WITH CML IN ACCELERATED PHASE RESISTANT TO IMATINIB

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Background: Semi-synthetic homoharringtonine (HHT) is a chemically modified preparation derived from the Cephalotaxus evergreen tree which has anti-neoplastic properties.

Aims: We aimed to explore the value of HHT in the management of accelerated phase (AP) CML.

Methods: Nine patients with Ph-positive CML who had progressed to AP defined by WHO criteria while receiving imatinib mesylate were entered into a phase I/II study. Their median age was 65.5 years (range 54-72); there were 4 males and 5 females. Five patients received other drugs before changing to imatinib while still in chronic phase and 4 patients received imatinib only after diagnosis of AP. All 9 patients were judged to have progressive disease at time of starting HHT. Patients were monitored for mutations in the Abl kinase domain of their leukaemia cells. Each course the planned dose of HHT was 2.5 mg/kg administered daily by subcutaneous injection for seven consecutive days. Patients

were taught to administer the drug themselves and could then continue treatment without daily supervision. Courses were repeated at intervals of 28 days if blood counts permitted.

Results: With a median follow-up of 12 months (range 4-14) the mean total number of courses was 9 (range 3 to 11). The cumulative incidence of achieving second chronic phase at 12 months was 80% and the cumulative incidence of complete haematological response was 67%. There were no cytogenetic responses. One patient progressed to blastic transformation and died but 8 patients survive. HHT was well tolerated with minimal non-haematological toxicity. Grade IV neutropenia was seen in three patients and did not recur after dose reduction. Grade IV thrombocytopenia occurred in 2 patients after the first and subsequent courses despite dose reduction and required platelet support; in these 2 patients a median of 8 (4-12) pools of platelets was required per course. Erythropoietin (10,000 units x 3 per a week subcutaneously) was administered to 4 patients to maintain the haemoglobin level. In one patient a kinase domain mutation detected at start of treatment was no longer identified detectable after 6 months on HHT. The other 8 patients are currently being studied for changes in mutation status.

Conclusions: Semisynthetic HHT may be a valuable agent in the management of CML patients who have become resistant to imatinib.

116 AN INTERNATIONAL STUDY TO STANDARDIZE THE DETECTION OF BCR-ABL TRANSCRIPTS FROM STABILIZED PERIPHERAL BLOOD PREPARATIONS BY QUANTITATIVE RT-PCR
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Quantitative determination of residual disease levels after treatment for patients with BCR-ABL positive leukemias may be achieved by various methods. However, there is a need to standardize methodologies and interpretation of results to reduce interlaboratory variation. The amount of BCR-ABL transcripts has been shown to be a reliable surrogate parameter for progression free survival after treatment with interferon alpha or imatinib and after allogeneic stem cell transplantation. The major aim of quantitative RT-PCR analyses is to identify patients who need alternative treatment strategies. Standardized molecular response data are required for new treatment protocols and to compare different treatment strategies. Hence, a multicenter study was performed to detect BCR-ABL transcripts from peripheral blood (PB) preparations utilizing RNA stabilization and extraction (PAXgene Blood RNA Kit, Preanalytix, Hombrechtikon, Switzerland) and real time RT-PCR technology (Lightcycler, LC, Roche Diagnostics, Mannheim, Germany, and TaqMan, TM, ABI Prism, Foster City, CA). Objectives were the definition of a clinically relevant detection limit for low tumor burden (optimal sample volume), the applicability and comparison of the two control genes glucose-6-phosphate dehydrogenase (G6PD) and total ABL, and comparison of the LC and TM RT-PCR methodologies to develop a standardized PCR strategy for the molecular monitoring of residual disease in CML patients after therapeutic intervention. A total of 186 PB samples (2.5, 5 and 10 ml) derived from healthy donors and spiked with serial dilutions (1:20 to 1:2x10⁶) of b2a2 (n=11x6), b3a2 (n=6x6), and e1a2 (n=6x6) BCR-ABL+ cells from leukemic patients and negative controls (n=8x6) were stabilized using the PAXgene system, blinded, frozen and distributed to six laboratories. RNA extraction and cDNA synthesis were performed according to a common protocol, but PCR conditions for quantification of BCR-ABL, ABL and G6PD were optimized for the LC (three labs) and TM (three labs) platforms. A calibrator RNA was used as reference point for both target and standard genes. The calculation of the final results was performed using the relative quantification software (Roche). BCR-ABL transcripts were detected in 99/138 positive samples and 0/48 negative controls. The coefficients of variation for samples tested positive were 0.74 and 0.76 for ratios BCR-ABL/ABL and BCR-ABL/G6PD, respectively. There was no significant difference of the results achieved with LC and TM technologies. Up to a dilution of 1:1000, 27/30 2.5 ml samples were tested positive. For higher

dilutions, a volume of 5 or 10 ml PB was required to improve sensitivity. We conclude that (i) standardization of quantitative PCR is feasible independent of the PCR machine used. (ii) Sensitivity of the test depends on the volume of PB used. To detect a three-log reduction of the tumor load, 2.5 ml PB are sufficient in most cases. Higher sensitivities can be achieved using 10 ml PB. (iii) Total ABL should be used as control gene.

117 ANALYSIS OF IMATINIB THERAPY IN 300 PATIENTS WITH BCR-ABL POSITIVE LEUKEMIAS: EVALUATION OF RESPONSE AND RESISTANCE

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Imatinib has shown to be very effective in multicenter studies for patients (pts.) with Philadelphia (Ph) and/or BCR-ABL positive chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL). Open questions are durability of remission, treatment of resistant disease, and long term molecular response rates. Here we report on the experience with 300 pts. (171 m, 129 f, median age 60 years, range 16-82) with CML in chronic phase (CP), resistant or intolerant to interferon alpha, n=139; accelerated phase (AP), n=80; myeloid blast crisis (BC), n=76, lymphoid BC, or BCR-ABL+ ALL, n=5, treated with imatinib within six phase II and expanded access protocols. Initial dosage of imatinib was either 400 mg (n=153) or 600 mg/day (n=147). Median observation time was 33.8 months in CP, 29.9 in AP, 5.8 in myeloid and 4.8 in lymphoid BC. Hematologic response (HR) was reached in 97% of pts. in CP, 91% in AP, 51% in myeloid BC, and 60% in BCR-ABL+ ALL. Major cytogenetic response (Ph+ <35%) was observed in 68% of pts. in CP, 41% in AP, and 24% in myeloid BC. Overall survival at 24 months was 92% in CP, 69% in AP with a median of 43.7 months, and 17% in myeloid BC with a median of 5.9 months. Estimated rate of complete HR was 81% at 24 months in CP, median duration of HR was 38 and 6 months in AP and BC, respectively. Resistance to imatinib was observed in 41% of pts. in CP, 65% in AP, and 92% in BC, whereas hematologic relapse occurred after a median of 17.3 months, 11.9, and 6.3, respectively. In case of resistance imatinib dose was increased in 47% of pts. in CP, 23% in AP, and 19% in BC, therapy was supplemented by additional drugs in 35% in CP, 33% in AP, and 45% in BC to overcome resistance. Nevertheless, progression of disease arose in 39% in CP and AP, respectively and in 81% in BC. Clonal evolution (CE) was observed in 41% of pts. in CP, 41% in AP, and 69% in BC at the time of resistance or relapse. Among the patients relapsing, mutations of the tyrosine kinase domain were detected in 50% in CP, 57% in AP and in 29% BC. In conclusion, the efficacy of imatinib in all phases of the disease was confirmed. However, long-term follow-up data revealed an increasing rate of resistance. Mutations at the ATP binding site occurred in case of relapse rather than primary resistance, and they were more characteristic for the CP. In AP and BC, BCR-ABL independent mechanisms like CE are more common. These findings implicate that resistance cannot be attributed to a single mechanism and depends in part on the natural course of the disease. The response to strategies to overcome resistance, such as dose increase or combination therapies is only temporary, therefore regular molecular and cytogenetic monitoring is suggested. Allogeneic SCT should be considered for resistant pts. in CP or after reinduction of a second CP in advanced disease.

118 THE INFLUENCE OF ARSENIC TRIOXIDE ON THE CLONOGENIC CAPACITY OF BONE MARROW IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC PHASE CML.

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Background: Arsenic trioxide (As₂O₃) is an active agent against acute promyelocytic leukemia. It is being tested as a therapeutic agent for a variety of malignancies. In vitro studies with clinically achievable concentrations of As₂O₃ showed induction of apoptosis and differentiation in Ph+ K562 and HL60/Bcr-abl cells. The aim of our study was to investigate in vitro the effects of increasing concentration of As₂O₃ on clonogenic capacity of haematopoietic and mesenchymal progenitor cells in patient, with newly diagnosed CML, shortly treated with hydroxyurea.

Materials and methods: Bone marrow samples were obtained from 11 patients with diagnosed Ph+ CML in chronic phase before treatment. Mononuclear cells (MNC) were cultured without As2O3 and with increasing concentration of As2O3: 0,1; 0,2; 0,5mmol/l. MNC were plated in standardized methylcellulose medium (MethoCult 4434, StemCell Technologies) and MesenCult (StemCell Technologies). Colony formation of haematopoietic progenitors (CFU-GM and BFU-E) and mesenchymal progenitor cells (CFU-F) based on morphology of the colonies were assessed on day 14 of cultures. CFU-GM, BFU-E and CFU-F expressed as the percentage of decrease versus control and the mean and standard deviation (SD) of colony inhibition for each concentration of As2O3 were calculated across all samples.

Results: All As2O3 concentrations employed cause reduction of CFU-GM and BFU-E formation after 14 days of incubation to 3% and 0,8% of control values respectively. Formation of CFU-F was completely inhibited with each As2O3 concentration.

Conclusions: Our data clearly demonstrate that in vitro conditions exposure to As2O3 even in low concentration is able to induce growth inhibition of haematopoietic progenitor cells in patients with CP CML. Unexpectedly, in contrast to findings presented by some authors As2O3 inhibits also formation of mesenchymal progenitor cells in this group of patients. A combination of direct toxicity against leukemic cells with antiangiogenic activity may be the optimum characteristic activity of a successful antileukemic agent. We plan future experiments to establish the effects of combination of As2O3 with other antiangiogenic factors such thalidomide on the inhibition of pathological Ph+ clonogenic cells in patients with CML.

119 IMATINIB TREATMENT NORMALIZES ABERRANT NEUTROPHIL LEUKOTRIENE C4 SYNTHASE EXPRESSION IN PH+ CML PATIENTS POTENTIAL INDICATOR OF P210 BCR-ABL SIGNALING

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Imatinib mesylate induces clinical, hematological and cytogenetic responses by specifically inhibiting the abnormal tyrosine kinase p210 BCR-ABL in Philadelphia-positive (Ph+) chronic myeloid leukemia (CML) patients. We have earlier shown that leukotriene C4 (LTC4) can stimulate growth of normal human myeloid progenitors and that LTC4 synthase (LTC4S) is markedly and consistently over expressed in CML myeloid cells (Stenke Blood 1993, 81, 352; Sjlander Blood 2000, 95, 1456). We now report on the expression of LTC4S during imatinib treatment in CD16(+) peripheral blood neutrophils from 7 interferon-failing Ph+ CML patients in chronic phase. The patients displayed a significantly increased neutrophil LTC4S activity before treatment, with a mean (SEM) conversion of LTA4 to LTC4 of 16.0 ± 1.5 pmol LTC4 /106 cells (corresponding value in normal neutrophils < 2 pmol). In addition, LTC4S expression was clearly confirmed by Western blot and RT-PCR analyses. After 3 months of imatinib therapy (400mg q.d.), all patients had achieved hematological responses, but none displayed any complete cytogenetic response, with four patients maintaining >50% Ph+ cells, as determined by conventional cytogenetics. In contrast, already after one month of treatment the neutrophil expression of LTC4S decreased in all seven patients. Thus, the mean enzymatic activity was reduced to <4 pmol LTC4 /106 cells, immunoblot was negative in 5 cases, RT-PCR was negative in 2 cases. A similar, but not as pronounced reduction of LTC4S expression was noted as early as after 7 and 14 days of imatinib treatment in individual patients. Theoretically, patients with minor or major cytogenetic responses may release Ph-negative neutrophils into the blood. Using fluorescence in-situ hybridization (FISH) combined with morphologic cell examinations we could however confirm presence of Ph+ also in neutrophil populations with low or non-detectable LTC4S expression during imatinib therapy. We conclude, that initiation of imatinib treatment rapidly reduced or totally normalized the aberrantly increased neutrophil LTC4S expression in Ph+ CML patients. This improvement appeared to be in parallel to the hematological responses but not dependent on the cytogenetic outcome. Our data suggest, that aberrant LTC4S expression may be a down-stream step in the p210 BCR-ABL-induced signaling leading to the pathophysiological events related to Ph+ CML. If so, assessment of LTC4S expression may thus provide a unique tool to monitor the immediate, biological and clinical consequences of inhibition of p210 BCR-ABL, information not obtainable with conventional cytogenetics or FISH.

120 CHRONIC MYELOID LEUKEMIA WITH AN UNCOMMON E8-INT-A2 BCR/ABL FUSION TRANSCRIPT (P200) AND ATYPICAL HEMATOLOGICAL FEATURES: A CASE REPORT.

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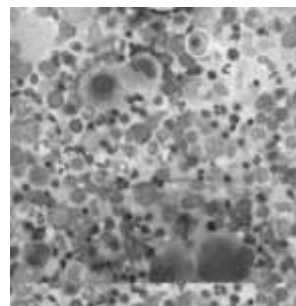
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Background. The hallmark of chronic myeloid leukemia (CML) is the chromosomal translocation t(9;22)(q34; q11.2). In most cases, the resulting fusion genes give rise to different length products corresponding to chimeric BCR-ABL proteins of 210 or, more rarely, 190 or 230 kDa, respectively. We report the case of a patient diagnosed as having CML, showing atypical hematological features and a very uncommon BCR-ABL rearrangement.

Patient. A 45-year old woman, was referred to our attention because of a normochromic anemia, thrombocytosis and a mild basophilia, discovered through a routine blood count. On admission, Hb was 11.7g/dl, Hct 33.1%, white blood cells 4,650/mL (neutrophils 1,870/mL, lymphocytes 2,440/mL, monocytes 190/mL, eosinophils 69/mL and basophils 280/mL) and platelets 531,000/mL.

Methods, Results and Conclusions. A comprehensive laboratory work-up showed no further abnormalities. Physical and imaging examinations revealed no pathological findings; no spleen or liver enlargements were found. The peripheral blood smears confirmed the thrombocytosis and the mild increase in the number of basophils; some hypogranulated and banded neutrophils were also noted. The BM was markedly hypercellular mainly because of a granulocytic and megakaryocytic hyperplasia (see BM picture). Most megakaryocytes were monolobulated. Myelodysplastic features, such as hyposegmented and hypogranulated granulocytes, were also found. The G-banded karyotype revealed a typical t(9;22) chromosomal translocation in 5 of the 25 metaphases analyzed. Reverse transcription-polymerase chain reaction (RT-PCR) analysis, using primers derived from bcr exon e1 and abl a3, showed an atypical amplification band. Sequencing analysis of the PCR product revealed that the breakpoint on the BCR gene fell within exon 8. Moreover, the portion of exon e8 of the BCR gene was not directly joined to ABL exon a2 due to an insertion of 15bp derived from ABL intron b1, giving rise to an in-frame e8-int-a2 BCR-ABL fusion transcript. This latter was translated into a p200 BCR-ABL protein. Further molecular analyses excluded the activation of the genes for the platelet-derived growth factor receptor beta (PDGFRB), the FIP1L1-PDGFR (alpha) and the FGFR1 fusion tyrosine kinases, which are currently under study because of their association with a good response to imatinib. In summary, although rare cases of CML expressing the p200 protein have been reported (Martinelli et al., Haematologica, 2000), our patient, although formally diagnosed as having a CML, showed very atypical hematological features, such as the lack of leukocytosis and of splenomegaly, mimicking a combined myeloproliferative/myelodysplastic disorder. In the rare cases so far reported, a good response to alpha-interferon therapy has been observed, indirectly suggesting that the clinical significance of this rare finding in the patient's outcome may be similar to p210 BCR-ABL. Moreover, to the best of our knowledge this is the first case observed in the imatinib era. To date, the patient is well and continues to receive imatinib at a daily dose of 400 mg. She will be closely followed and the levels of transcript regularly monitored. The clinical outcome and the response to imatinib will be presented at the meeting.



Caption 1: Megakaryocytic hyperplasia

121 CYTOGENETIC AND MOLECULAR MONITORING OF PH+ CHRONIC MYELOID LEUKEMIA (CML) CHILDREN AND YOUNG ADULTS TREATED WITH IMATINIB MESYLATE (STI571) THERAPY AT A SINGLE INSTITUTION

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Background. STI571 has demonstrated high hematologic and cytogenetic response rates in adults and children with CML. **Aim.** Since the degree of tumor load reduction is an important prognostic factor, we monitored CML patients (pts) aged less than 20 years (yrs) treated with STI571 (Glivec).

Methods. Eight chronic phase (CP) pts (5 M and 3 F; median age at diagnosis: 117/12 yrs; 5 previously treated with IFN), recruited between March 2001 and September 2003 (median follow-up: 11 months), were treated with Glivec, kindly supplied by Novartis, at a dosage of 340mg/sqm/day in 6 pts aged <15yrs and at 500 mg/day in the older pts. Cytogenetic analysis was performed on bone marrow (BM) before and during Glivec therapy, at planned intervals; fluorescence-in-situ-hybridization (FISH) and nested real time polymerase chain reaction (RT-PCR) were provided at complete cytogenetic response (CCyR); reverse quantitative PCR (RQ-PCR) was performed on peripheral blood (PB) every month (mo) and on BM every 3 mo. The quantitative results were obtained by normalizing the BCR-ABL copy number with respect to the number of ABL copies used as housekeeping gene and expressed as fusion-gene copy number/104 ABL copies. All experiments were carried out in triplicate with appropriate negative controls.

Results. Overall, cytogenetic analysis and nested RT-PCR were performed on 40 and 36 BM samples, respectively, while 92 PB samples from 8 pts and 14 BM samples from 7 pts were tested by RQ-PCR. The 7 evaluable pts obtained a CCyR after a median of 3 mo. At CCyR, all BM samples were positive for the BCR-ABL transcript (nested RT-PCR), that disappeared in 4/7 pts after a median of 6 months from CCyR. At CCyR, the BCR-ABL copies (RQ-PCR) ranged from 0 to 31 (median 8.5) on PB of 6/7 pts and from 9.5 to 36 on 3 tested BM. At disappearance of the BM BCR-ABL transcript (nested RT-PCR), BCR-ABL copies decreased both on PB samples (range: 0-22, median 3.45) and on 2 tested BM (3.8 and 4, respectively). One of the 8 pt lost the CCyR (20% Ph+ metaphases) after 6 mo, but obtained a 2nd CCyR (confirmed by FISH) 6 mo later continuing STI. A BCR-ABL transcript reappeared in the BM in 1 of the 5 pts, 3 months later (nested RT-PCR). At present, all pts are in CCyR and 4 are negative for the BCR-ABL transcript (nested RT-PCR). The BCR-ABL copy number decreased both on PB and BM samples (RQ-PCR) in 7 patients, progressively in 3 and fluctuating in 4. BCR-ABL transcript levels were not different between PB and BM: the median copy level was 10 on PB and 9.6 on BM.

Conclusions. In conclusion, (i) Glivec induced persistent CCyR in children and young adults with CML; (ii) in pts in CCR and with BCR-ABL undetectable by RT-PCR, RQ-PCR detected BCR-ABL positive cells both from PB and BM; (iii) the RQ-PCR analysis carried out in PB samples can be considered a useful method for monitoring the degree of tumor load reduction in CML pts treated with Glivec.

STEM CELL TRANSPLANTATION EXPERIMENTAL

122 AN FC-GAMMA-RIIA-POLYMORPHIC SITE AS TARGET FOR ACUTE GRAFT-VERSUS-HOST DISEASE IN PARTIALLY T-CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION

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Graft-versus-host disease (GVHD) is a major complication of allogeneic stem cell transplantation (SCT) for haematologic diseases. An antigen mismatch due to a polymorphic difference between the patient and its donor may play a role in the mechanisms of GVHD. Polymorphisms are known for FcgammaRIIa and this molecule is present on endothelial cells, dendritic cells and Langerhans cells. Donor cells reacting against the patient as GVHD can also react against the malignancy of the patient, known as the graft-versus-leukemia (GVL) effect. As the FcgammaRIIa molecule is also present on acute myeloid leukemia (AML) cells, a mismatch due to this polymorphism could be a target both for GVHD and GVL. We retrospectively studied 73 patients with AML and analyzed the differences in GVHD and relapse rate between patients with or without a pro-GVHD/GVL FcgammaRIIa allotype mismatch. We observed a difference in FcgammaRIIa receptor with a pro-GVHD/GVL mismatch in 18 patients (25%). Univariate and multivariate analyses showed the pro-GVHD mismatch as the only significant risk factor for the presence of acute GVHD ($p=0.023$ and $p=0.026$, respectively). No significant difference for the pro-GVHD mismatch in chronic GVHD was found ($p=0.6$). The relapse rate was not different for patients with or without the pro-GVL mismatch ($p=1.0$), though for standard-risk AML the relapse rate was 9% in patients with the pro-GVL mismatch group and 28% in patients without the pro-GVL mismatch group ($p=0.32$). We conclude that the polymorphism of the FcgammaRIIa receptor represents a candidate target for acute GVHD.

123 A NEW TOOL TO OBJECTIFY DEVELOPMENT OF FIBROSIS IN CUTANEOUS GRAFT-VERSUS-HOST DISEASE

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Allogeneic stem cell transplantation is the treatment of choice for many haematological malignancies. One of the major complications that limit the success of allogeneic stem cell transplantation is graft-versus-host disease (GVHD). The major target organ in GVHD is the skin. Cutaneous GVHD can eventually lead to fibrosis of the skin. Fibronectin mediates a variety of cellular interactions with the extracellular matrix. The molecular and functional diversity of fibronectin arises from alternative splicing of pre-mRNA. In normal circumstances endothelial cells and fibroblasts synthesize fibronectin without the ED-A domain. In tissue repair and in pathologic circumstances such as fibrosis the ED-A domain is expressed. We hypothesize that detection of ED-A FN can be used as an objective tool to quantify skin fibrosis in GVHD. In frozen skin biopsies the expression of ED-A FN was measured at the protein level by immunohistochemistry and at the mRNA level by quantitative reverse transcriptase PCR (qRT-PCR). Immunohistochemistry was done by indirect immunoperoxidase staining with mouse monoclonal ED-A antibody. For qRT-PCR the differences in mRNA expression of fibronectin with or without the splice variant ED-A were measured. In normal control skin ($n=10$) immunohistochemistry showed slight deposits of ED-A FN just under the basal layer. The expression of ED-A FN significantly increased in acute cutaneous GVHD ($n=10$) ($p<0.05$) and was strongly deposited in chronic cutaneous GVHD ($n=10$) ($p<0.001$). Quantitative RT-PCR also showed increased expression of ED-A FN in acute and chronic cutaneous GVHD compared to normal control skin ($p=0.07$ and $p=0.039$, respectively). The expression of ED-A FN is increased in the skin of patients with cutaneous GVHD measured both with immunohistochemistry and qRT-PCR. ED-A FN can be used as a marker of fibrosis in the skin of patients with cutaneous GVHD.

124 CYTOMEGALOVIRUS-SPECIFIC IMMUNE RECOVERY FOLLOWING ALLOGENEIC HLA-IDENTICAL SIBLING TRANSPLANTATION WITH ANTI-THYMOCYTE GLOBULIN-BASED REDUCED INTENSITY PREPARATIVE REGIMEN

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Although antiviral prophylaxis has led to significant reduction in early CMV disease, recovery of CMV-specific cytotoxic effectors is still necessary for long term control of CMV reactivation. Reduced intensity conditioning regimens (RIC) could show that durable donor cell engraftment can be achieved after allogeneic stem cell transplantation (allo-SCT). However, it has also been shown that RIC might be associated with a high rate of early CMV positive antigenemia. New biologic tools such as the ELISPOT assay or tetrameric HLA-peptide complexes have dramatically improved our understanding of specific cytotoxic T-cell responses. Here we describe the results of 54 patients (39 hematological malignancies and 15 non-hematological malignancies) who received an anti-thymocyte globulin-, fludarabine- and busulfan-based RIC for allo-SCT with the aim to define elements of protective immunity to CMV. The cumulative incidence of CMV positive antigenemia was 37% (95%CI, 24%-50%), occurring at a median of 43 days (range, 7-104) after allo-SCT. Conditioning regimen (ATG dose) and graft characteristics (stem cell source and CD3+ cell dose) influenced CMV-specific immune recovery. A significant correlation ($P=0.00002$) was found between CMV-specific T cells detected by IFN- γ ELISPOT assay and pp65-specific CD8+ T cells frequency quantified by tetramers. CMV-specific CD8+ T cells presented a phenotype of effector cells being mainly CCR7-, CD45RA-, CD27- and CD28-. These CMV-specific CD8+ effectors were functional with major expression of cytotoxicity markers 2B4 and intracellular perforin. In multivariate analysis, bone marrow (BM) as graft source was the only variable associated with an increased risk of CMV positive antigenemia ($P=0.0001$) in line with the ELISPOT assay showing higher frequency of functional CMV-specific effectors within peripheral blood stem cell grafts as compared to BM, suggesting that protection against CMV positive antigenemia might be conferred by specific T lymphocytes transferred with the graft, at least in the early period after allo-SCT. Overall, these results suggest that individual patient rapid assessment of CMV-specific immune recovery early after allo-SCT, might prevent in some patients unnecessary routine ganciclovir prophylaxis impairing CMV-specific T cell recovery, but can help to better identify those patients at high risk of CMV reactivation who may benefit from preemptive therapy or adoptive transfer of CMV-specific T cells.

125 DIFFERENTIATION OF HUMAN BONE MARROW CELLS INTO CARDIOMYOCYTE-LIKE CELLS: COMPARISON OF DIFFERENT INDUCTION PROTOCOLS

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Background: Recent stem cell studies have opened new perspectives for regenerating damaged tissues in patients. Autologous bone marrow cells (BMCs) can contribute to the repair of a damaged heart. Aims: The aim of this study was to investigate whether mononuclear populations of bone marrow cells can differentiate into cardiomyocyte-like cells in vitro. Therefore, different methods for cardiogenic differentiation of BMCs were evaluated. Methods: Adult human or sheep BMCs were isolated and cultured in different media with different supplements. Starting concentrations of the BMCs varied between 2.5-5.0 x 10⁶ cells/ml in a culture flask with a size of 75 cm. Once cultured, the BMCs were also treated with different induction agents : 5-azacytidine, 5-aza-2-deoxycytidine, DMSO and vitamin C. When the cultured cells became confluent, they were trypsinized and replated (ratio 1:3). After a month, the cells were collected and analyzed by light and immunofluorescence microscopy, flowcytometry and transmission electron microscopy. Results: The BMCs adhere to the bottom of the culture flask and became spindle-shaped. After treatment with different induction agents, the cultured cells formed myotubules observed by light microscopy. By flowcytometry, the cultured cells stained positive for the markers : CD44 and CD90, by immunofluorescence microscopy for the markers : nestin, smooth muscle actin and sporadic desmin. They were consistently negative for Troponin-I. A constant growth pattern could be observed

among cultures. However, outliers with low yield remain possible. Conclusion: BMCs cultured in X-vivo 15 medium supplemented with 2% noninactivated autologous serum grow clearly into spindle-shaped cells. Treated BMCs with either 5-azacytidine or 5-aza-2-deoxycytidine or DMSO or vitamin C differentiate into cardiomyocyte-like cells. Further investigations need to be carried out to determine why these cells do not beat spontaneously. These results may contribute to the development of ex vivo expansion protocols for human applications e.g. transplantation of cardiomyocytes in myocardial scar tissue.

126 DIFFERENTIAL ROLE OF CD97 IN IL-8-INDUCED AND G-CSF-INDUCED STEM CELL MOBILIZATION

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The CXC chemokine interleukin-8 (IL-8) induces rapid (15-20 minutes), neutrophil dependent, mobilization of hematopoietic stem and progenitor cells (HPC) in both mice and monkeys. CD97 is a member of the EGF-TM7 family of class II seven-span transmembrane receptors and is broadly expressed on hematopoietic cells, including neutrophils and is involved in neutrophil migration. Here, we studied a possible role of CD97 in IL-8-induced stem cell mobilization. BALB/c mice were treated with a single i.p. injection of 500 micrograms neutralizing anti-CD97 mAb (clone 1B2) or control IgG. After 24 hours, mice were mobilized with a single i.p. injection of IL-8 (30 micrograms) or saline. No mobilization of HPC was observed in mice receiving anti-CD97 mAb followed by IL-8 (35.2 26.8 CFU-GM per ml blood; n=7), whereas animals treated with control IgG followed by IL-8 exhibited a mean 11-fold increase in HPC (383.4 246.4 CFU-GM per ml blood; n=4). In contrast to IL-8-induced mobilization, G-CSF-induced mobilization was not inhibited after anti-CD97 administration compared to control Ig treated mice (597.4 247.7 CFU-GM per ml blood; n=6 versus 605.9 218.9 CFU-GM per ml blood; n=4 respectively). Anti-CD97 mAb alone did not induce HPC mobilization in vivo, nor did it inhibit GM-CSF-induced colony formation in vitro. A mean of 95.5% of bone marrow mononuclear cells expressed CD97 (n=4). Cell sorting experiments showed that the frequency of colony-forming cells in the CD97Neg fraction was 34-fold increased (purity 85%; 7474 6025 CFU-GM per 10⁶ isolated cells; n=7) compared to the CD97Hi fraction (purity 97%; 220 182 CFU-GM per 10⁶ isolated cells; n=16) and 22-fold increased in comparison with the CD97lo fraction (purity 98%; 341 430 CFU-GM per 10⁶ isolated cells; n=16), indicating that CD97 is not expressed on colony-forming cells. In summary, our results show that 1) CD97 is involved in IL-8-induced stem cell mobilization, 2) the prevention of mobilization by anti-CD97 mAb is not mediated by a direct effect on HPC. We suggest that neutrophils are involved as intermediate cells in this process.

127 TRANSPLANTATION WITH EX-VIVO THROMBOPOIETIN-EXPANDED CD34POS CORD BLOOD CELLS ACCELERATES PLATELET RECONSTITUTION IN NOD/SCID MICE

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Background: Transplantation with umbilical cord blood (UCB)-derived stem cells is characterized by delayed hematopoietic reconstitution in the peripheral blood. The low number of hematopoietic stem cells in CB and/or different maturation kinetics may be factors that cause this delay. Ex-vivo expansion of hematopoietic stem cells may be a strategy to circumvent this problem.

Aims: The aim of this study was to evaluate the effect of ex-vivo thrombopoietin (TPO)-expanded and differentiated CD34+ UCB-derived cells on platelet reconstitution and long term engraftment upon transplantation in NOD/SCID mice.

Methods: CD34+ cells were selected from UCB, split into equal parts and cryopreserved. After thawing, 2x10E5 CD34+ UCB cells were cultured in 50 ng/ml TPO for 10 days and subsequently all cultured cells were transplanted into sub-lethally (3.5 Gy)-irradiated NOD/SCID mice. 2x10E5 non-expanded CD34+ cells from the same cord blood unit were thawed and served as controls. The appearance of human platelets was evaluated weekly for a period of 6 weeks after transplantation, using a sensitive assay specific for human platelets, that reliably detects 1

platelet/l. Engraftment and repopulating capacity was evaluated at 6 weeks after transplantation by enumerating colony forming cells (CFU-GEMM) in the bone marrow (BM).

Results: Platelet recovery started earlier in mice transplanted with expanded CD34+ CB cells compared to recipients of control CD34+ UCB cells. A platelet count of $0.05 \times 10^9/L$ was observed in the peripheral blood already at day 5 in mice transplanted with expanded CD34+ UCB cells, while the control group reached this platelet count at day 10. The number of platelets was similar for both groups after day 15. At 6 weeks after transplantation, the platelet count reached a concentration of $1.15 \times 10^9/L$ in mice transplanted with expanded CD34+ UCB cells, and $0.58 \times 10^9/L$ for control CD34+ UCB cells, suggesting a slight, but not statistically significant, benefit in the expanded group. At day 7 after transplantation, higher numbers (8.24.7 colonies/ 10^4 WBCs) of human progenitor cells were present in the BM of mice transplanted with the expanded CD34+ CB cells compared to the non-expanded control CD34+ UCB cells (1.60.6 colonies/ 10^4 WBCs), while at 6 weeks after transplantation equal numbers of human progenitor cells were found (17.56 colonies/ 10^4 WBCs for the expanded CD34+ cells vs. 21.612 colonies/ 10^4 WBCs for the control CD34+ cells). In addition, at 6 weeks the distribution of human hematopoietic cell lineages in the BM was similar in both groups.

Summary / Conclusion: transplantation of TPO-expanded CD34+ UCB cells enhances the speed of platelet recovery after transplantation in comparison with transplantation of an equal number of unexpanded CD34+ cells derived from the same cord blood unit. This is associated with a higher number of progenitor cells in the BM in recipients of expanded cells at day 7. It appears that the long-term multilineage engraftment capacity of the cells is not compromised by TPO.

128 MONITORING OF CHIMERISM AFTER ALLOGENIC PERIPHERAL BLOOD STEM CELL OR BONE MARROW TRANSPLANTATION USING MULTIPLEX PCR AMPLIFICATION OF SHORT TANDOM REPEAT MARKER

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Background: Chimerism analysis after allogeneic bone marrow transplantation (alloBMT) allows detection of early marrow engraftment, disease relapse, and graft rejection. Several approaches have been published for the detection of chimerism. FISH with X- and Y-chromosome specific probes and PCR-based amplification of a single variable number of tandem repeat (VNTR) or short tandem repeat (STR) markers are frequently performed methods. 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 (PBL) in recipients of alloBMT with the use of STR microsatellite markers for monitoring the efficacy of BMT and donor leukocyte infusions (DLIs).

Methodes: We have investigated 100 alloBMT suffering from different types of leukemia (n=68) or non-malignant hematologic disorders (n=32) 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 (VWA, CSF1PO, TPOX, ARA, F13A1, ADA, D4S2366, TH01, D16S539, D7S820, D13S317, and FES/FPS) were used in Multiplex PCR with a highly discriminative capacity between individuals.

Result: The multiplex PCR showed a sensitivity of 1-2% when analyzed on polyacrylamide gel. Of the 100 patients, 74 patients (74 %) exhibited full donor chimerism, 18 Patients (18%) had mixed chimerism and 8 patients (8 %) had lower chimerism and reject the transplant. Some patients with full donor chimerism converted to mix chimerism before clinical relapse, however, with periodic and frequent monitoring of patients we could detect the patients clinical outcome and their need for DLI or second alloBMT.

Conclusion: 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.

129 IN VITRO COSTIMULATION SIGNIFICANTLY IMPROVES IN VIVO POTENTIAL OF CULTURED HUMAN T LYMPHOCYTES IN HU-PBL-RAG2-/-GAMMAC-/-MICE

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Background

T cell manipulation offers the opportunity to control donor T cells in allogeneic stem cell transplantation. However, initial results of clinical trials showed reduced Graft-versus-Host Disease (GVHD)-inducing capacity of manipulated T cells and low numbers of cells surviving long-term, raising questions towards the maintenance of a Graft-versus-Leukemia effect. [Tiberghien Blood 2001, Markteld Blood 2003]

Aims

We hypothesized that the reduced GVHD effect and survival of manipulated T cells were caused by long-term culture and lack of costimulation, resulting in anergy of the cells. We therefore investigated the effect of culture and different in vitro stimulation conditions on the potential of human T cells and distribution of naive, effector and memory phenotypes in vivo. For this, we used the hu-PBL-RAG2-/- γ c-/- model, which we described recently as a highly efficient intravenous model for human T cell engraftment and development of xenogeneic (X)-GVHD. [van Rijn Blood 2003].

Methods

T cells of 4 human donors were cultured for 7 or 16 days and 3 stimulation conditions were compared: 1) anti-CD3 2) anti-CD3/28 (XCyte™ Dynabeads®) and 3) 4-1BBL/anti-CD3/28 [Maus Nature Biotech 2002]. 81 RAG2-/- γ c-/- mice received macrophage depletion and 350 cGy irradiation followed by 15, 30, 60 or 90.106 cultured T cells. Control mice received pretreatment and 15.106 fresh T cells of the same donor. Engraftment levels and survival of the cells long-term were represented by the area under the curves of the % human cells measured from day 0-45 in the peripheral blood (PB) of surviving mice, and analyzed by a 2-way ANOVA with 3 factors, followed by a Bonferroni post hoc test.

Results

All control mice showed high levels of human T cells in PB associated with lethal acute X-GVHD. Strikingly, after 16 days of culture with anti-CD3, there was no engraftment of 15.10^6 human T cells at all. After 7 days of culture with any stimulation condition, 15.10^6 T cells induced lethal X-GVHD in 9/36 mice. 100% Lethal X-GVHD occurred at about 6 times the dose for anti-CD3 stimulation and about 3 times for anti-CD3/28 costimulation. In 49 surviving mice (dose 15, 30 or 60.10⁶), costimulation with anti-CD3/28 or 4-1BBL/anti-CD3/28 also significantly improved engraftment levels and long-term in vivo survival of human T cells compared to anti-CD3 alone (p<0.01). Of these 49 mice 20 developed chronic X-GVHD (anti-CD3:6/20, anti-CD3/28:11/20, 4-1BBL/anti-CD3/28:3/9), characterized by weight loss and skin abnormalities (hair loss, inflammation) and a highly variable number of human T cells in PB (0.2-75%), indicating maintenance of X-GVHD-inducing capacity by the long-term surviving cells. Long-term surviving CD4 and CD8 T cells were mainly of an effector (CD45RA+CD27+CCR7-) or memory-effector (CD45RA-CD27+CCR7-) phenotype, suggesting an antigen-driven response.

Conclusions

These results show for the first time in a xenograft model the quantified reduction in GVHD-inducing capacity of human T cells following stimulation and culture. Based on these results we recommend short-term culture (<7 days) and anti-CD3/28 costimulation for preservation of human T cell function during manipulation. The hu-PBL-RAG2-/- γ c-/- model will be very valuable for evaluation of in vivo potential of human T cells following in vitro manipulation.

130 ADMINISTRATION OF CYCLOPHOSPHAMIDE SHORTLY BEFORE TOTAL BODY IRRADIATION AND TRANSPLANTATION DECREASES ENGRAFTMENT OF BOTH ADULT BONE MARROW AND FETAL LIVER HEMATOPOIETIC STEM CELLS

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Background: The combination of myelosuppressive and myeloablative agents is the most common strategy used as a conditioning regimen for experimental and clinical hematopoietic stem cell (HSC) transplantation.

Engraftment of HSCs requires that the host hematopoietic tissue accepts and supports grafted HSCs and that these HSCs have the capacity to occupy a portion of marrow sites in the host. However, the mechanisms regulating short- and long-term engraftment of HSCs are largely unknown.

Aim and Method: Our lab has previously demonstrated that cyclophosphamide (CY, 135mg/kg) treatment induces both a unique pattern of hematopoietic regeneration in mice and an overexpression of some cytokine genes (SCF and SDF-1) in the bone marrow (BM) (Sefc, L., et al, 2003). To further investigate the effectiveness of a conditioning regimen using CY in combination with irradiation on engraftment of transplanted HSCs, we used a syngenic murine model of competitive repopulation to evaluate importance of timing between CY administration and total body irradiation (TBI, 5Gy). Adult BM or fetal liver cells (E14.5) were transplanted to CY and TBI treated recipients (Ly5.1 donor cells to Ly5.2 recipients).

Result: CY given two days prior to TBI (CY-2) significantly decreased short- and long-term engraftment of donor HSCs compared to controls (only TBI). On the other hand, CY given five (CY-5) or seven (CY-7) days before irradiation and transplantation significantly increased both short- and long-term engraftment of donor cells. CY given twice, five days and two days prior to TBI (CY-5-2), significantly reduced the engraftment compared to CY-5 alone.

Summary: Prolongation of the interval between CY and TBI enhanced the engraftment of donor HSCs and, on the other hand, administration of CY shortly before TBI and transplantation suppressed it. Our previous results demonstrated that hematopoietic progenitors, detected by the spleen colony assay (CFU-Sday8), intensively proliferate one to three days after CY while they are quiescent five to seven days after CY. Therefore, we assumed that the active proliferation of host HSCs two days after CY provides them with an advantage to compete with transplanted non-proliferating HSCs from adult BM. However, the present experiments that used actively proliferating HSCs from the fetal liver did not support this assumption. The effect of timing of CY administration on engraftment of donor cells is thus independent on the proliferation status of transplanted HSCs.

Reference: Sefc L, Psenak O, Sykora V, Sulc K, Necas E. Response of hematopoiesis to cyclophosphamide follows highly specific patterns in bone marrow and spleen. *J Hematother Stem Cell Res.* 2003; 12:47-61
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131 RHODAMINE123 DYE-EXCLUDING CELL SUSPENSIONS DERIVED FROM CULTURES OF HUMAN CD34+ CORD BLOOD CELLS CONTAIN EARLY HEMATOPOIETIC PROGENITOR CELLS ABLE TO RECONSTITUTE HEMATOPOIESIS IN VIVO

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Background: A better understanding of human immature hematopoietic progenitors (HPC) physiology in vitro, leading to an eventual increase of their number in culture without losing their ability to self-renew would be largely welcomed. To do so, key culture parameters influencing HPC characteristics have to be identified, and specific bioassays aimed at the detection of early HPC are required. Currently, such assays include the family of long-term culture initiating cell in vitro assays (LTC-IC, CAFC, LHTC-IC), and the SCID-mouse xenograft model as a surrogate to human repopulating assay. All these procedures are time-consuming and their results prone to wide variations. More practical assays would be needed to evaluate the presence of primitive HPC in culture. Ex vivo, HPC able to reconstitute lethally irradiated hosts are associated with cells excluding lipocationic dye such as the rhodamine123 (Rho) via the multi-drug resistance gene product (MDR). By contrast to the above methodologies, dye exclusion analysis is very rapid and allows the direct identification, thus also the sorting of early HPC. However, it is not known if this association remains after culture. **Aims:** We evaluated if cultures derived from CD34+ cord blood (CB) cells contained dye-excluding (Rho-low) cells that remained enriched in early HPC. **Methods:** CD34+ cells were purified from human CB using immunomagnetic beads and were cultured for 4-6 days with growth factors. The frequency of Rho-low cells in culture was established by flow cytometry using the MDR-blocker agent verapamil. Cells were also sorted according to their dye-excluding properties and the resulting fractions evaluated for their content in various HPC, including those able to reconstitute SCID mice. **Results:** After short-term culture with FLT3-ligand, thrombopoietin and stem cell factor, Rho-low cell frequency had decreased compared to ex

vivo values, and such a decrease correlated with that of long term-culture initiating cell (LTC-IC) ($R=0.997$, $p<0.04$), whereas late HPC (CFU) frequency remained unaltered. Analyses performed after culture on ex vivo sorted CD34+CD38- and CD34+CD38+ cells showed that Rho-low cells were more numerous in the cultures derived from CD34+CD38-cells. Moreover, Rho-low cells sorted after 6 days of culture contained 2.8-fold more secondary CFU and 1.8-fold more LTC-IC compared to Rho-high cells. When cells sorted after culture were injected into sublethally irradiated SCID mice, human cells recovered 6 weeks later represented 1.1 0.7% and 0.4 0.4% of the bone marrow of animals injected with Rho-low and Rho-high cells, respectively (mean SD, $n=5$, $p<0.056$), and 4/5 animals injected with Rho-low cells contained >1% of human cells, whereas none of those injected with Rho-high cells reached such an engraftment level. **Conclusion:** these data demonstrate that HPC actively excluding Rho after culture exhibit an identical hematopoietic potential than that of ex vivo cells, and suggest that the dye exclusion assay could be used as a relevant alternative to identify immature HPC after culture.

132 MOLECULAR MONITORING OF MINIMAL RESIDUAL DISEASE AFTER REDUCED INTENSITY CONDITIONING AND ALLOGENEIC TRANSPLANTATION IN RELAPSED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND LOW-GRADE LYMPHOMAS

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Background: Graft-versus-leukemia effect (GVL) after allogeneic stem cell transplantation (allo-SCT) has been already described in chronic lymphocytic leukemia (CLL) and indolent lymphomas. Clinical responses can be achieved after cyclosporin withdrawal, donor lymphocyte infusions (DLI), and with the onset of graft-versus-host-disease (GVHD).

Aims: We have evaluated if molecular remissions could be achieved after reduced intensity conditioning (RIC) and allo-SCT.

Methods: Thirty-nine patients with relapsed disease (21 CLL/SLL, 16 FCL, 1 MALT, 1 lymphoplasmacytic lymphoma) were enrolled in a prospective phase II study. Median age was 55 years (range: 32-69). The median number of previous chemotherapy regimens was 3 (range 1-5) and 10 of 39 patients (26%) had already failed a previous autograft. Before transplant, 38% of patients had a chemorefractory disease and 59% were in partial or complete remission (CR=8; PR=15); the remaining patients were minimal responders. The conditioning regimen consisted of thiotepa 10 mg/kg, fludarabine 60 mg/ms and cyclophosphamide 60 mg/kg. GVHD prophylaxis consisted of short course of methotrexate and cyclosporin from day -5 to day +180 (tapering from day +120). Minimal residual disease (MRD) was monitored by nested PCR for IgH or Bcl-2 genes. In 4 PCR-positive patients (2 CLL and 2 FCL), a TaqMan based quantitative monitoring was also employed. For the two patients affected by CLL we designed a patient-specific IgH TaqMan system, whereas for the two FCLs we took advantage of a previously described TaqMan primer and probe set for Bcl-2 rearrangement.

Results: All patients engrafted; 22 of 39 were in CR at day +30 after transplant. Eighteen of 22 patients attaining CR were monitored for MRD. At a median follow up of 495 days (range 60-1067), 10 of 18 patients were alive in clinical and molecular remission; one patient died of transplant-related-mortality (GVHD and infection) while he was still in molecular remission. Seven patients never attained PCR negativity and 3 of them relapsed within a median time of 270 days from allo-SCT; one of them achieved the molecular remission after chemotherapy and DLI, without developing GVHD. Six of 11 patients who became PCR-negative between day +90 and +180 developed acute and/or chronic GVHD, suggesting a GVL effect. Two of the 4 patients monitored by quantitative PCR achieved the PCR negativity at day +30 and +90 after allo-SCT, while the other 2 patients were persistently MRD positive; in the latter patients the TaqMan PCR could detect an increase of tumour genomes in the marrow prior to the clinical relapse.

Summary: Qualitative and quantitative PCR monitoring can be used to monitor patients at high risk of disease recurrences. The role of cyclosporin withdrawal and DLI for molecular disease is currently under investigation.

133 KINETICS OF CHIMERISM IN DIFFERENT PREPARATIVE CONDITIONING REGIMENS AND GVHD

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Background: we report clinical outcome and results of chimerism analysis in 53 patients (32 male, age: 30-60, mean: 47 yrs) with different malignancies (6 HD, 16 NHL, 11 MM, 3 CML, 4 AML 1 CLL, 6 Kidney and 6 Breast cancer) that received different non myeloablative conditioning, followed by allogeneic SCT: 18 Flu/Mel 16 Flu/TBI 19 Flu/Cy. In all cases unmanipulated PBSC from fully matched HLA-identical siblings were used; median number of infused CD34+ cells was $3.30 \times 10^6/\text{kg}$ (range 0.90-10.61). GVHD prophylaxis consisted of CSP/MTX.

Methods: chimerism analysis was performed using multiplex PCR amplification of shorth tandem repeat markers and fluorescence detection. Fifteen STR loci and the Amelogenin locus are co-amplified in a single reaction. Donor engraftment was evaluated on unfractionated peripheral blood (PB), bone marrow samples and on immuno-magnetically selected PB CD3+ cells. Median follow-up was 225 days (34-912).

Results: the rates of complete donor chimerism (CDC=>95% donor cells) at day +15,+30, +90, +180, +270, +360 and beyond +360 were 23%, 36%, 60%, 86%, 93%, 93% and 93% respectively. Kinetics of engrafting donor cells was different in the three conditioning regimens. The rate of CDC at days +30 were 73%, 20%, 0%, respectively; at days +90 were 92%, 57%, 0%. Acute GVHD occurred in 5/18 (28%) patients (pts) undergoing Flu/Mel, three of them were grade > II (2 died), while 7/18 (39%) developed chronic GVHD, none of them died of it. In two (12%) pts treated with Flu/TBI protocol, grade III-IV acute GVHD was observed (1 died), chronic GVHD occurred in 7/16 (44%) pts and one of them died of it. Finally in Flu/Cy protocol acute GVHD was absent, while 4/19 (21%) pts developed chronic GVHD. Thirty-two patients (60%) are alive to date.

Conclusion: Our study shows that Flu/Mel protocol has unincreased transplant related mortality, mainly due to higher incidence of acute GVHD, while chronic GVHD occurred in all three protocols. We cannot evidenciate acute GVHD in patients that underwent Flu/Cy conditioning regimen because at day +90 none of them has achieved complete donor chimerism.

134 ISOLATION AND CHARACTERIZATION OF LYMPHOCYTES DERIVED FROM INTESTINAL BIOPSIES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION AND IN GRAFT-VERSUS-HOST DISEASE

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Background: The gastrointestinal (GI) tract is one major target in graft-versus-host disease (GvHD) but little is known about a) the infiltration of the GI tract with donor-derived lymphocytes after allogeneic stem cell transplantation (SCT) and about b) lymphocyte-subpopulations in GI-epithelia in GvHD.

Aims: To characterize lymphocyte-subpopulations in the lamina propria and in epithelium we developed a method to isolate GI-lymphocytes directly from GI-biopsies. In addition, paraffin-embedded sections were studied to evaluate the localization of lymphocytes.

Methods: Biopsies and peripheral blood were taken from 6 patients day +34, +36, +45, +60, +415 and day +486 after allogeneic stem cell transplantation. All patients had given informed consent. Biopsies derived from duodenum or colon sigmoideum were preincubated for 18 hours (4C) in collagenase-containing medium (RPMI, 10% FKDS) and then incubated for 4 hours (37C) in a humid chamber. GI-lymphocytes were then separated from epithelia by Ficoll-gradient centrifugation. Cells were stained for CD3, CD4, CD8, CD2, CD56, CD45RO, and the activation marker CD25 (IL-2 receptor) and analysed by flow-cytometry.

Results: Lymphocytes could have been isolated in relatively large numbers with a vitality of >90% which allowed a characterization by flow-cytometry. Preliminary results of six patients who underwent allogeneic stem cell transplantation showed significant differences of lymphocyte-subsets in GI-tract. In two patients who developed histological GI-GvHD grade I to II the lymphocyte population derived from the GI-tract was dominated by CD3+/CD8+/CD45RO+ T-lymphocytes (73 and 85%), while CD3+/CD4+ lymphocytes were quantitatively reduced (3 and 19%). In 4 patients without GI-GvHD CD4+/CD8+-ratio was approximately 1:1. The IL-2 receptor (CD25), which is the target of the monoclonal antibody basiliximab, was expressed on 6% to 18% of the GI-lymphocytes with GvHD (1% to 6% in patients without GI-GvHD). One patient with GI-GvHD had 6% CD3+/CD25+ GI-lymphocytes on the onset of GvHD. After receiving treatment with the anti-CD25 monoclonal antibody Basiliximab,

CD25 expression on GI lymphocytes was no more detectable even though 30% of peripheral blood lymphocytes expressed CD25.

Conclusion: The analysis of lymphocyte-subpopulations isolated from the GI tract opens a wider understanding concerning the repopulation of the GI tract after allogeneic stem cell- or bone marrow transplantation and of the mechanisms leading to GI-GvHD.

135 COMPARISON OF LYMPHOID SUBSETS IN THE LATE POSTTRANSPLANT PERIOD BETWEEN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANT AND THEIR RESPECTIVE DONORS USING LYMPHOGRAM®

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Lymphocytes play an essential role in the allogeneic stem cell transplant (allo-SCT) setting. Flow cytometry is the standard tool to evaluate lymphoid subsets, and a simple method has been recently validated for this purpose (Lymphogram®, Cytognos, Spain). It has yet to be ascertained if, in the long term period after an allo-SCT transplant, lymphoid subsets in the patients are similar to that of their respective donors. Therefore, we have evaluated lymphoid subsets using Lymphogram® in 40 patients undergoing HLA-matched sibling Allo-SCT comparing it to that of their respective 40 donors. In 11 cases, patients were under treatment for active chronic GVHD. Samples were simultaneously obtained from both patient and donor. In all cases time from engraftment had exceeded one year and complete chimerism was also confirmed. Peripheral blood samples were stained with this single monoclonal antibody (McAb) combination: CD8/CD19-FITC//CD3/CD56-PE//CD4-PeCy5//CD45-APC. Absolute numbers were calculated using FlowCount® microspheres. Regarding B-cells, both their proportion as well as their absolute number was significantly increased in the patient group (21.3 10% vs 13.1 4% [p<0.001] and 453325 cells/L vs 249110 cells/L [p<0.001], respectively). Total T cell number was similar in both groups but they displayed different numbers and proportions of CD4+ and CD8+ T cells: We found 714384 CD4+ cells/L in the patient group and 909377 cells in the donors (p:0.02), whereas CD8 counts were 701429 cells/L vs 445220 cells/L, respectively (p=0.004). NK cell counts were similar in both groups. When the presence of chronic GVHD under treatment was evaluated, B cell proportion was significantly decreased and CD8+ T cells increased in this subgroup of patients. In summary, our results show that in the long term period after Allo-SCT lymphoid subsets are different in patients compared to that of their respective donors. These differences may be influenced by the presence of chronic GVHD.

136 CYTOTOXIC ACTIVITIES OF HUMAN HLA IDENTICAL DONOR DERIVED CYTOKINE INDUCED CELLS (CIK) AGAINST ACUTE MYELOID BUT NOT LYMPHOID LEUKEMIA BLASTS

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Background: Cytokine induced killer (CIK) cells have been described as strong lytic effectors against different types of solid tumors. Although CIK are CD3+CD8+CD56+ cells, they induce tumor lysis through a T cell receptor (TCR) independent recognition, through NKG2D receptor. Recently it has been reported that CIK cells are able to induce graft versus leukemia (GvL) but not graft versus host disease (GvHD).

Aims of the study: to develop a reproducible clinical grade method to ex-vivo expand CIK cells from peripheral blood of HLA identical hematopoietic stem cell donors, and to characterize their lytic activity against acute myeloid and lymphoid blasts.

Methods: Peripheral blood (30 ml) from HLA identical familiar (N=9) or unrelated donors (N=7) were collected at time of bone marrow donation or in case of G-CSF mobilized donors, at time of their clinical examination. After Ficoll separation, peripheral blood mononuclear cells (PBMC) were cultured in vitro in 10 ml of RPMI 1640, in the presence of 10% clinical grade FCS, 2mM Glutamine, Penicillin/Streptomycin and 1000U/ml IFN-gamma (Gammakine, Domp Biotec SpA). The day after 50 ng/ml of anti-CD3 (OKT-3, Orthoclone Muro mon Ab-CD3, Janssen-Cilag Spa) and 500U/ml of IL-2 (Proleukin, CHIRON) were added to the culture and cells were maintained at a concentration of 0.5×10^6 cells/ml in the presence of only IL-2. The cytotoxicity of CIK cells was determined by Calcein AM release assay.

Results: In our culture condition the proportion of CD3+CD56+ cytotoxic cells, increased from 1-3% of total mononucleated cells of normal donors

at day 0 up to 80% (median 55%) at day +21 of in-vitro culture. Moreover a median 10 times fold increase in the absolute cell count was observed, with a median 25 fold increase of CD3+ cells, while CD3+CD56+CD8+ subset showed a median 350 fold increase. Starting from 30 ml peripheral blood a median number of 400 X 10⁶ CD3+CD56+ effector cells can be obtained over 21 days of in vitro culture. Cell sorting experiments using CD56 magnetic microbeads showed that CD3+CD56+ had a strong cytotoxic potential against the standard NK target K 562 cell line (at 4 hours the median cytotoxicity was 45% at a E:T ratio of 10:1). Donor derived CIK cells were tested against HLA identical patient derived blasts (5 AML, 8 ALL). Cytotoxicity was documented only against myeloid targets, independently from their FAB classification (at 4 hours median cytotoxicity was 20% with a E:T ratio of 10:1). These experiments were performed under GMP conditions so to provide a protocol for further scaling up. Conclusion: HLA identical donor derived CIK cells can be always expanded ex-vivo in a reproducible, clinical grade and effective way, showing a high cytotoxic potential against myeloid but not lymphoid leukemia blasts. These pre clinical data allow the design of new adoptive immunotherapy protocols for the treatment of acute myeloid leukemia patients relapsing after HLA identical allogeneic stem cell transplantation.

STEM CELL TRANSPLANTATION AUTOLOGOUS

137 AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN 102 PATIENTS WITH AGGRESSIVE OR FOLLICULAR NON-HODGKIN'S LYMPHOMA BETWEEN 8/84 AND 9/03 - A SINGLE CENTER REPORT

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ASCT is an established therapeutic option in the treatment of non-Hodgkin's lymphoma. From 8/1984 to 9/2003 ASCT was performed in 102 patients (47 f, 55 m, age 15'65, median: 44,5 years) with either bone marrow (25), peripheral stem cells (73) or both (4). 29 patients were diagnosed as follicular lymphoma (FCL), 59 as aggressive B-NHL, 6 as aggressive T-NHL and 8 as mantle cell lymphoma (MCL). Status at transplantation for FCL was CR 1 (stage III/IV): 8pts., CR 2: 8 pts. or sensitive disease (PR, CR>2 or responding relapse): 13 patients and for aggressive lymphoma including MCL CR 1: 25 pts., CR 2: 16 pts., sensitive disease: 29 pts. or refractory disease: 3 patients. TRM for all patients was 3,9 % i.e. in 3/42 sensitive disease due to bacterial and/or fungal infections and in 1/33 CR 1 due to viral hepatitis. So far, late complications were rare except one patient with cancer of the stomach (d+782), but no secondary hematologic disorder was seen. Relapse rate for FCL was 38 % within the median observation period of 40 months (1'113 mo), occurring in patients transplanted in CR 1: 12,5 %, in CR 2: 37,5 % and in sensitive disease: 53 %. Overall disease free survival was 46 % without reaching a plateau curve yet (CR 1: 75 %, CR 2: 36 %, sensitive disease: 31 %). Overall survival was 52 % (CR 1: 100 %, CR 2: 57 %, sensitive disease: 26 %). Patients with aggressive lymphoma relapsed in 37 % within a median observation time of 31 months (1'229 mo), occurring in patients transplanted in CR 1: 24 %, in CR 2: 37,5 % and in sensitive disease: 41 %. All 3 patients with chemotherapy refractory disease showed progression shortly after ASCT and died within 7 months. Overall disease free survival post transplant was 56 % with a plateau after the last relapse at 56 months (CR 1: 72 %, CR 2: 60 %, sensitive disease: 48 %). Overall survival was 66 % (CR 1: 77 %, CR 2: 92 %, sensitive disease: 49 %). Excluding patients with refractory disease, ASCT for non-Hodgkin's lymphoma was a manageable and a beneficial treatment that led in more than half of the patients to a long lasting disease free survival.

138 RELATION BETWEEN HARVEST SUCCESS AND OUTCOME AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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The prognostic influence of pre-transplant characteristics on response and survival was studied in 104 consecutive patients with multiple myeloma receiving uniform pre-transplant treatment consisting of VAD regimen, stem cell mobilisation, harvesting, and conditioning with melphalan 200 mg/m². At the time of peripheral blood stem cell transplantation (PBSCT), 11% of patients were in complete remission (CR), and 63% in partial remission (PR).

We evaluated the influence of age, sex, pre-transplant response, number of harvested CD34+ cells, number of infused CD34+ cells, splitting and saving part of the harvest for succeeding transplants on overall (OS) and progression-free survival (PFS) times. Following PBSCT, 31% of the patients were in CR and 57% in PR. Median OS and PFS from transplantation were 67 and 36 months, respectively. Transplant-related mortality was 0%. The number of harvested CD34+ cells was the only variable that was prognostic for OS in univariate and multivariate analyses. None of the variables was prognostic for PFS, although pre-transplant response was nearly significant. The procedure of splitting and saving part of the harvest thus reducing the number of cells in the graft had no significant influence on outcome measured as OS or PFS.

139 MYELOMA IS AN INDEPENDANT RISK FACTOR FOR THE DEVELOPMENT OF CYTOMEGALOVIRUS FOLLOWING STEM CELL TRANSPLANTATION.

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Polymerase chain reaction (PCR) quantification and pre-emptive ganciclovir is now the management of choice for CMV reactivation post stem cell transplantation (SCT). Due to the increasing age, more intensive pre-treatment of patients undergoing SCT, expanding disease indications and the advent of novel SCT protocols there has been alterations in both the incidence and clinical pattern of cytomegalovirus (CMV). There is limited data defining the significance of PCR quantification of CMV copy number, pre-emptive therapy and its relationship to end organ disease, particularly since the advent of non myeloablative/ reduced intensity SCT. We analysed 577 consecutive patients undergoing SCT over a 5 year period (405 autologous: 172 allogeneic). autograft patient were monitored for 21 days post SCT or if symptomatic: allogeneic patients were monitored until cessation of immunosuppression and/or quiescence of active GVHD. Patients who became PCR positive on 2 consecutive occasions or had high copy numbers (>log 3) on the first occasion were commenced on ganciclovir 5mg/kg twice daily. Therapy was stopped after 2 negative PCR results, in absence of tissue disease. CMV reactivation was as high as 72% amongst 'at risk' allogeneic SCT patients (those in whom donor and/or recipient were seropositive). Risk factors confirmed the most significant factor in CMV reactivation after allogeneic SCT was donor/recipient serostatus (p<0.001), followed by underlying disease. Myeloma patients were at significantly increased risk compared to non-myeloma patients (p=0.03 by multivariate analysis). In total there were 24 (14%) patients undergoing allogeneic transplant for myeloma and of these patients 15 patients had a seropositive donor or recipient. 15 of these 16 (94%) patients 'at risk' reactivated requiring treatment, whereas 24 of the 69 (35%) of the 'at risk' non-myeloma patients reactivated CMV. Other risk factors significant by univariate analysis included increasing age, peripheral blood stem cell rather than marrow as a source, and reduced intensity conditioning. CMV reactivation occurred in only 6/405 (1.5%) of patients undergoing autologous transplant. 4 out of 6 of these patients were undergoing transplantation for myeloma, and included 2 patients who required treatment. This pre-emptive strategy has led to a safe approach of the treatment of CMV reactivation with low rates of CMV disease and mortality (1.7%). In the 7 patients with end-organ disease 4 patients were transiently or persistently CMV PCR negative in peripheral blood. There was a tendency over time to decreased frequency of CMV pneumonitis, and later emergence of CMV disease in more unusual sites (e.g. colitis, retinitis). This study confirms that PCR monitoring and pre-emptive therapy remains an efficacious strategy following newer modalities of SCT. We however have observed significantly increased reactivation rates within the myeloma patient cohort. we conclude this is likely to be multifactorial, and relates to factors such as immunoparesis, increasingly intense pre-treatment for myeloma, increased age and the utilisation of reduced intensity conditioning SCT.

140 OPTIMIZATION OF CD34+ CELL COLLECTION FOR AUTOLOGOUS TRANSPLANTATION USING EVOLUTION OF PERIPHERAL BLOOD CELL COUNTS AFTER MOBILIZATION CHEMOTHERAPY

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Background: Intensive chemotherapy with peripheral blood progenitor cells (PBPC) rescue is an important therapeutic procedure in hematological malignancies. Several parameters may affect the harvesting of PBPC after mobilization chemotherapy.

Aim: To establish the parameters that best predict the day to start harvesting of PBPC in order to collect most CD34+ cells with few aphereses.

Methods: We analysed data of patients with hematological malignancies from our Institution, who underwent mobilization of PBPC for autologous transplantation. Mobilization was done by cyclophosphamide (4 or 7g/m²) and G-CSF or GM-CSF (from day 1 after chemotherapy and continued until the last leukapheresis), aiming to collect >5x10⁶ CD34+/Kg. We investigated the influence of age, sex, diagnosis, number of previous chemotherapy lines (nr CHT), peripheral blood counts at day D0 of mobilization, day of neutrophils <1.0x10⁹/l and day of nadir (including the interval between these two days - delta) on harvesting. Multivariate linear correlation models were built in order to predict the day of best harvesting respecting principles of parcimony. The quality of the models was evaluated using the Kolmogorof Smirnov test. The stability of the models was evaluated by bootstrap resampling.

Results: 134 patients were analysed: 36 Hodgkin's lymphoma (HL), 65 B-large cell lymphoma (NHL) and 33 multiple myeloma (MM). Median age: 28, 40 and 48 years respectively. Day of apheresis presented a correlation with number of previous chemotherapy lines, hemoglobin value of day 0, day of granulocytes <1.0x10⁹/l and day of nadir and dosis of mobilization therapy. However, for each disease group a different model could be established.

HL: day of apheresis = delta x 1.5 + 9.8.

NHL: day of apheresis = -log gran D0 x 0.95 + nr CHT x 1.5 + delta x 0.70 + 17.0

MM: day of apheresis = delta x 1.4 + 9.1

Conclusion: each disease has its own pattern of mobilization dynamics, possibly related to the amount of previous chemotherapy or the status of the bone marrow microenvironment. The most important parameter, common to all patients, was the velocity of decline of the neutrophil count after mobilization chemotherapy.

141 AUTOLOGOUS STEM CELL RESCUE WITH CRYOPRESERVED PERIPHERAL BLOOD PROGENITORS (PBSC) USING 5% VERSUS 10% DIMETHYLSULOXIDE (DMSO) IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: High-dose chemotherapy followed by autologous stem cell rescue usually requires cryopreservation of hematopoietic progenitors. For decades conventional 10% DMSO has been used as cryoprotective agent. The grade of infusion related toxicities, as well as, cell viability after thawing depends on the amount of DMSO in the PBSC grafts. The aim of the study was to evaluate the reduction to 5% versus 10% DMSO and compare severity of clinical toxicities in autologous recipients.

Material and methods: Progenitor cells were mobilized with chemotherapy + G-CSF, or single G-CSF in 34 patients with hematological malignancies (6 AML; 9 HD; 10 NHL; 7 MM; 1 ALL) and collected using Baxter CS 3000 aphaeresis equipment. PBSC grafts from consecutive patients were cryopreserved in solutions containing 5% DMSO (20 patients) and 10% DMSO (14 autologous recipients), by controlled rate freezing procedure (Nicole plus PC Espace 330) and stored at -196 C. Cell viability was assessed by fluorescence microscopy using acridine orange dye exclusion. All patients were monitored for DMSO infusion related toxicity. Before graft infusion during the autologous setting all patients were administered mannitol, hydrocortisone, clorphenamine and ondansetron.

Results: A total of 71 PBSC collection procedures were performed in our group of patients. Median period from storage of cryopreserved PBSC grafts until thawing was 35 days (32-38). Total number of infused CD34+ cells was between 2.2-16x10⁶/kg and median number of mononuclear cells was 3.85x10⁸/kg (1.71-6.0). The amount of infused DMSO solution ranged between 210-650ml (median 430ml) with DMSO concentration ranging 23ml- 50ml (median 34ml) in a group preserved with 10%DMSO and 13-23ml (median 19ml) in 5% DMSO cryopreserved grafts. Viability of cryopreserved PBSC after thawing revealed higher percent of viable cells with 5%DMSO versus conventional 10% DMSO. In a group of patients that received PBSC grafts preserved with 10% DMSO, 6 (42%) revealed signs of infusion related toxicity, nausea, vomiting, abdominal cramps, hypotension in 2 pts and 1 patient had hypertension and tachycardia. Other side effects were fever with chills (2); chest tightness (3), transitory cystitis (4). Grade of clinical toxicities was significantly

lower in a group of patients that received PBSC solutions with 5% DMSO and nausea was the most frequent side effect during infusion (3 patients). **Conclusion:** In our experience infusion of cryopreserved autologous PBSC with 5%DMSO revealed less toxicity, higher cell viability, which opens potential possibilities for further in vivo engraftment and ex vivo manipulations.

142 IMMUNOABLATIVE THERAPY WITH AUTOLOGOUS STEM CELL SUPPORT IN THE TREATMENT OF 27 PATIENTS WITH POOR RISK MULTIPLE SCLEROSIS

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The immunoablative therapy with hematopoietic stem cell support represents an encouraging experimental method for patients with intractable form of multiple sclerosis (MS). Thirty one patient with poor risk MS was included to the phase I/II clinical trial involving the high dose chemotherapy with autologous peripheral blood progenitor cell (PBPC) rescue. Twenty seven patients underwent high dose conditioning BEAM with at least 2 months of follow up. T cell depletion in vitro was performed in 18 grafts. Nine patients with not purged graft received in vivo ATG 4mg/kg i.v. D+1, D+2 after transplantation. In 3 patients PBPC mobilization failed. One patient refused transplantation after improvement in disability following mobilization. No mortality has been observed, the treatment was feasible. Median follow-up is 30 months (3 - 71). Median EDSS (Expanded Disability Status Scale) of grafted patients at the time of inclusion were 6.5 (5.0-8.5). After initial improvement (36 %) one patient out of 27 (4 %) remains improved significantly (by at least 1.0 point on EDSS), 3 patients (11 %) remain improved not significantly (by 0.5 point). Eleven patients (41 %) did not change their EDSS. Seven patients (26 %) gained their disability significantly (by 1.0 point or more on EDSS) despite the treatment, one of them died 31 months after the transplantation from disease progression. Five other patients (8 %) worsened not significantly (by 0.5 point) on their EDSS. Patients who stabilized their disability or improved represent 56 %. Twenty patients (74 %) did not worsened on their EDSS during the follow up. Among 4 mobilized but not transplanted patients 1 improved by 1.5 point on EDSS, one stabilized, 2 patients worsened by 1.0 and 0.5 respectively. Changes between the EDSS before and after the transplantation in the group were significant (p = 0.015) if clinically not significant changes to both sides were included. If clinically significant changes only were included (1.0 point or more on EDSS), statistics did not reached significance (p = 0.07). The development of disability between the group that was grafted with in vitro purged graft and the group with ATG i.v. was also not significant (p = 0.75). Probability of progression in patients with initial EDSS 6.0 and less was 31 (10 74) %, probability of progression in patients with initial EDSS > 6.0 was 91(79 99) %. Brain MRI finding stabilized in 16 and improved in 3 patients from 23 patients with follow up > 6 months. Twelve months after the transplantation the number of CD4+ and CD4+CD45RA+ cells as well as IRI were significantly decreased. The number of CD4+CD45RA+ cells was lower in patients treated with T cell depleted graft. We consider our results to be promising for such intractable type of disease. Next patients will be included to the new international randomized clinical trial (ASTIMS).

143 ARA-C BASED CHEMOTHERAPY AND G-CSF INDUCE HIGH RATE OF PROGENITOR CELL MOBILIZATION IN ACUTE LEUKAEMIA PATIENTS

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Background. Peripheral Blood Progenitor Cells (PBPC) are widely employed in autotransplant procedures. Autologous rescue with limited amount of 1-2x10⁶/kg CD34+ PBPC allows to support consecutive high dose (HD) consolidation courses, therefore obtaining a high dose-intensity with low treatment-related toxicity. However, many aspects affect the extent of PBPC mobilization; disease subtype and choice of chemotherapy regimen are recognized as principal factors influencing an

effective cell collection. In a recent GITMO survey, a significant fraction of patients with acute leukaemia (AL) were unable to mobilize PBPC with standard alkylating-agents chemotherapy.

Aims. An exploratory trial is being conducted by NILG (Northern Italy Leukaemia Group) to evaluate the role of Ara-C based regimens in the mobilization of PBPC in AL.

Methods. The mobilizing treatment was planned as part of early postremission consolidation: Ara-C 8 g/m² (total dose) + G-CSF 10 mcg/kg in acute myeloid leukaemia (AML); HD-MTX 1.5 g/ m² + Ara-C 8 g/m² (total dose) + G-CSF 10 mcg/kg in acute lymphoblastic leukaemia (ALL). According to the total amount of collected CD34+ cells, patients failing to collect >2x10⁶/kg were identified as poor mobilizers (PM).

Results. So far, 144 AML patients (median age 51, range 21-65), and 112 ALL patients (median age 31, range 15-66) are evaluable for mobilization analysis. Overall, 111 out of 144 (77%) AML patients collected a median 6.25 (range 2-52) CD34+ cells x10⁶/kg; 6 out of 144 were PM according to the definition given. Groups of good mobilizers (GM) and non-mobilizers + PM did not differ significantly with regard to age, sex, FAB class, cytogenetic risk class, and presentation WBC count; moreover, incidence of FLT-3 ITD mutations (18/63 28% in GM, 1/16 6% in NM+PM) and MDS/secondary AML (5% vs 21%) were significantly different (p<0.05). Overall, 99 out of 112 (88%) ALL patients collected a median 13.65 (range 2-74) CD34+ cells x10⁶/kg; 2 out of 112 were PM according to the definition. Groups of good mobilizers (GM) and non-mobilizers + PM did not differ significantly with regard to age, sex, FAB class, immunophenotype, cytogenetics, WBC count.

Conclusions. The schemes adopted with Ara-C based chemotherapy and G-CSF allowed high rates of PBPC mobilization in AL patients. The availability of sufficient amounts of PBPC collected in postremission consolidation phase, give access to consecutive HD consolidation courses in the majority of AL patients.

144 HIGH RISK PATIENTS WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMA SHOULD BE TRANSPLANTED IN FIRST REMISSION (SINGLE CENTER EXPERIENCE)

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The Non-Hodgkin's lymphoma are heterogeneous group of diseases with diversity in their clinical, pathological, biological and genetic characteristics, with diversity in laboratory findings, response to therapy and the final outcome of the disease. To make the right and optimal therapeutic approach we need to stratify those patients in subgroups of patients with 'high' and 'low' risk, which was achieved with this study. High-dose therapy with autologous hematopoietic stem-cell support has been proposed as a potentially curative treatment for patients with recurrent aggressive NHL that remains sensitive to further standard chemotherapy, with proved superiority over standard salvage therapy for treatment of sensitive relapsed patients, as well as optimal therapeutic approach for high risk patient with aggressive NHL in first remission. This study comprises 148 patients with histopathology diagnosis of aggressive Non-Hodgkin's lymphoma treated at the Department of Hematology in the period 1989-2000, which gave us the observation period of 15 to 159 months. There were 88 male patients, median age 53 years and 60 female, median age 52 years. Most of the patients were in the advanced clinical stage at the disease on the initial presentation of the disease, 24% of the patients in the third stage and 43% in the fourth stage. Immunophenotyping suggested that 80% of the cases are B-cell lymphoma. The patients were treated with anthracycline included regimens, most of them being CHOP regimen. After initial chemotherapy complete remission was achieved in 60%, partial response in 4% and there was no response in 32% with early deaths in 4%. At the end of the study 32% of the patients were alive and well, 32% were deceased and the remainder had unknown status. According to the univariate analysis the following parameters had significantly influence the overall survival in the patients with aggressive Non-Hodgkin's lymphoma: initial anemia, initial LDH, the stage of the disease, ECOG score, bone marrow infiltration, number of sites of extranodal infiltration, lymphoma subgroup according to various classification systems, morphology of the lymphoma cell, immunophenotype profile, percent of Ki-67 positively, bcl-2 positively, time to complete remission. The multiply progression analysis produce mathematical model through which we can anticipate the expected survival in each patients individually based on the statistically most influential prognostic markers, those achieving stratification of the patients in risk groups. In our study 32% of the patients with 'high' risk are

alive and 'low risk patients have 70% 5-years overall survival. With the use of this prognostic model for aggressive Non-Hodgkin's lymphoma we achieved risk based stratification of the patients even in the fourth stage of the disease with statistical significance. This prognostic model for aggressive Non-Hodgkin's lymphoma enables the clinical hematologist to create the optimal individual therapeutic approach for each patient with aggressive Non-Hodgkin's lymphoma. The patients with 'high' risk are group of patients where beside the standard chemotherapy, use of aggressive chemotherapy and hematopoietic stem cell transplantation as well as the new therapeutic approaches would improve therapeutic results and overall survival.

145 HIGH INCIDENCE OF NEUTROPENIA IN PATIENTS TREATED WITH RITUXIMAB AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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The occurrence of neutropenia has rarely been reported in patients exposed to rituximab (RTX), although this complication seems more frequent in the set-ting of autologous stem cell transplantation (ASCT). We report a high incidence of neutropenia in patients treated with RTX prior to and following ASCT. Fourteen patients with confirmed histologic diagnosis of follicular (n=9) or mantle-cell (n=5) lymphoma were treated with a protocol integrating chemotherapy, RTX and ASCT. After a phase of debulking with VACOP-B, the patients received RTX, vincristine and cyclophosphamide and under-went mobilization and harvest of CD34+ cells after high dose cytarabine and RTX. The final phase of the protocol consisted of ASCT, using BEAM as the conditioning regimen, followed by two courses of RTX (375mg/m²). The median number of CD34+ cells infused was 17.15x10⁶/kg (range 6.21-32). Engraftment was rapid in all patients. The median time to a neutrophil >0.5x10⁹/l was 9 days (range 8-18). Ten out of 14 patients (71%) received RTX at a median of 52 days (range 34-63) post-transplant while the median neutrophil count was 2.35x10⁹/l (range 1.2-4.2). A second dose of RTX was administered two weeks later. Seven out of 10 patients treated with RTX early post-transplant experienced severe neutropenia at a median of 38 days after the second administration. The median neutrophil nadir was 0.38x10⁹/l (range 0.1-0.8) while the median duration of the neutropenic phase was 12 days. Two patients experienced multiple episodes of neutropenia. All episodes either resolved spontaneously or responded to a few days of treatment with granulocyte colony-stimulating factor. An infectious etiology of the neutropenia was excluded in all cases. An evaluation of bone marrow in the neutropenic period showed hypocellular marrow with normal trilineage hematopoiesis consistent with the recent transplant. Cytogenetic analysis demonstrated a normal karyotype in all cases. At median follow-up of 19 months (range 9-45) from ASCT, 14/14 patients are alive in continue complete remission. In conclusion our experience suggests that early administration of RTX during the post-transplant period may favor the onset of severe neutropenia.

146 THREE YEARS' FOLLOW-UP IN A RANDOMIZED, DOUBLE-BLIND COMPARATIVE STUDY OF PARENTERAL NUTRITIONAL SUPPORT WITH OR WITHOUT GLUTAMINE IN AUTOLOGOUS STEM CELLS TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES

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Background: We have previously shown that during the hospital stay no clinically useful benefit of prophylactic parenteral nutrition (PN) and glutamine supplementation in our autologous transplant patients (pts) with hematologic malignancies could be found.

Aims: Here, we present the long-term outcome data of this group after three years follow-up, in an attempt to find any influence on the overall survival rate and/or relapse rate.

Methods: Since August 2000, 41 adult pts (13 with multiple myeloma, 13 with non-Hodgkins lymphomas, 8 with Hodgkins disease and 7 with leukemias) were randomized to receive either prophylactic PN starting with the cytoreductive regimen (prophylactic P group, n=19), or PN given if oral intake became inadequate (control C group, n=22). In each group, they were further randomized to receive standard PN or

isocaloric, isonitrogenous PN with L-Ala-L-Gln (0,5 g Gln/kg). The groups were compared during follow-up with median 34 months (range 28-42). Significance was tested using Fisher exact test. For survival analysis, Kaplan-Meier method was used.

Results: In the follow-up, of the whole group 11 pts died (overall survival rate 73 %), and 17 relapses were seen (relaps rate 41 %). Of the 17 relapsing pts, median disease-free survival was 15 months (range 4-32). The outcome was slightly but not significantly more favourable in P group than in C group: 7 pts died in C group (overall survival rate 68 %), and 4 pts in P group (79 %), 10 pts relapsed in C group (relaps rate 45 %), and 7 pts in P group (37 %). Surprisingly, in glutamine-supplemented group the overall survival rate (64 %) was worse than in standard formula group (84 %), and also more relapses were found (11 vs. 6, i.e. 50 % vs. 32 %). Again, these differences were not significant but certainly do not favour the glutamine supplementation.

Summary/conclusion: In our three years follow-up, we found no clinically useful benefit of prophylactic PN in autologous transplant pts with hematologic malignancies. Also, glutamine supplementation was not helpful, and was even connected with apparently worse long-term outcome. Recently used techniques of supportive care probably make these strategies unnecessary, and even potentially harmful.

The study was supported by grant IGA, Czech Ministry of Health, No. NB7000-3.

147 PERIPHERAL BLOOD STEM CELL (PBSC) : COMPARISON OF TWO METHODS OF COUNTING; CD34 BY FLOW CYTOMETRY (IF) AND HEMATOPOIETIC PROGENITOR CELL (HPC) COUNT ON SYSMEX XE-2100

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During the organization of collection of PBSC, a crucial problem is to determine the best time to start the harvest. Circulating hematopoietic stem cells (CHSC) evaluation is based on counting of CD34+ cells by IF. This method is expensive, complex, time consuming and induces organization difficulties as regard to bed occupancy and staff timetable. With the increasing acceptance of stem cell transplantation as a standard therapy worldwide, there has been an accompanying increase in demand for technologies that can detect and count progenitor cells. An automatic counter, the Sysmex XE 2100 is equipped with a unique Immature Myeloid Information (IMI) channel, a specially designed unit for the detection of immature myeloid cells which are resistant to lysis reagent and HPC are counted on the basis of direct current and radiofrequency in the IMI-HPC area. The HPC parameter of the IMI channel is used for screening the presence of haematopoietic progenitor (stem) cells in peripheral blood and cord blood samples. Blood samples were tested for CD34+ cells using Coulter flow cytometer, HPC count was determined on Sysmex XE-2100 analyzer in the IMI channel.

The goal of this study was to determine the correlation between the two methods of counting : HPC and CD34 by IF, on peripheral blood sample and harvested cells. We analysed in HPC and CD34 for 51 CHSC samples and 18 PBSC samples. Our results showed a significant correlation between the quantification of the CD34 by IF and HPC on CHSC (Rho Spearman = 0.763, $p < 0.001$); The analysis of these results enabled us to propose a minimal threshold from 20 HPC/mm³, value of lower part of which, a numeration of the CD34 is required. Concerning the counting of the HPC and CD34 on the PBSC collection, there is too a significant correlation (Rho Spearman = 0.488, $p < 0.04$), but the small sampling size does not allow us definite conclusion.

The interest of the HPC count is that it is time and cost saving, and thus can be used as a first time method before harvesting stem cells.

148 PERIPHERAL BLOOD PROGENITOR CELLS (PBPCS) ARE EFFECTIVELY MOBILISED BY PEGFILGRASTIM (NEULASTA)

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Background: Daily injection of filgrastim (r-metHuG-CSF, Neupogen (R)), repeated over several days, alone or with chemotherapy, mobilises adequate numbers of PBPCs for successful transplantation and engraftment. There is evidence that a single injection of pegfilgrastim, a novel, long-acting, pegylated form of filgrastim, provides at least as effective mobilisation. In mice receiving single dose pegfilgrastim,

GM-CFCs peaked earlier and at threefold higher levels than with daily filgrastim. CD34+ cells and GM-CFCs peaked at day 45 in normal human volunteers receiving single dose (30, 60, 100 or 300 mcg/kg) pegfilgrastim, with broad dose dependence (Molineux et al. Exp Hematol 1999;27:1724-1734). Mobilisation of CD34+ cells was evaluated in 13 patients with non-small-cell lung cancer (NSCLC) receiving 5 mcg/kg/day filgrastim for 5 days or single dose pegfilgrastim (30, 100 or 300 mcg/kg) pre and post-chemotherapy. Median peak cell counts were 15, 12, 61 and 43 cells/mcL pre-chemotherapy, and 57, 5, 68 and 28 cells/mcL post-chemotherapy (for filgrastim and pegfilgrastim 30, 100 and 300 mcg/kg groups, respectively). It was concluded that pegfilgrastim mobilises CD34+ cells in a similar way to daily filgrastim (Johnston et al. J Clin Oncol 2000;18:2522-2528).

Aims: To investigate the effects of pegfilgrastim on peripheral blood and bone marrow in a randomised, open-label study in 6 patients with NSCLC. Methods: Group 1 (n = 3) received 10 mcg/kg/day filgrastim pre-chemotherapy for 5 days or until ANC 7.5 x 10⁹/L, and 5 mcg/kg/day filgrastim post-chemotherapy until ANC 10 x 10⁹/L. Groups 2 (n = 1) and 3 (n = 2) received single doses of pegfilgrastim (60 or 100 mcg/kg) pre-and post-chemotherapy. Results: Pre-chemotherapy, counts of neutrophils, CD34+ cells, and GM-CFC and BFU-E colonies peaked between days 47 for all groups. There was a trend of increased neutrophils and CD34+ cells in those patients treated with pegfilgrastim. Treatment with pegfilgrastim resulted in GM-CFC colony number which was 2.5 to 6 fold higher and BFU-E colony number which was 1.7 to 3.5 fold higher compared to those treated with filgrastim. Post-chemotherapy, CD34+ counts increased between days 811 for all groups. GM-CFC counts peaked between days 1114, and BFU-E counts peaked between days 914 for all groups. Conclusion: Single dose pegfilgrastim had a similar effect on PBPC mobilisation post-chemotherapy as multiple doses of daily filgrastim but with the potential for enhanced convenience. Single dose pegfilgrastim may permit effective mobilisation of PBPC without chemotherapy priming and should be explored further. Phase 2 studies of pegfilgrastim for PBPC mobilisation with and without chemotherapy are ongoing.

149 FEASIBILITY OF INTERLEUKIN-2 ADMINISTRATION FOLLOWING AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS IN 1ST COMPLETE REMISSION

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BACKGROUND Interleukin-2 (IL2) is a cytokine with antitumor activity. When administered after autologous stem cell transplantation, it appears to reproduce the graft versus leukaemia effect of allogeneic transplant and possibly prolong disease-free survival (DFS).

AIMS To investigate the feasibility and tolerability of IL2 in AML patients in first complete remission (CR) after peripheral blood stem cell transplantation (PBSCT).

PATIENTS AND TREATMENT PLAN Since 1999 at Hematology Department of Rome, 42 AML patients in 1st CR received PBSCT after BU-CY conditioning regimen. All patients received high-dose hydroxyurea followed by cytarabine, daunorubicin and etoposide as induction treatment, and daunorubicin and high dose cytarabine as consolidation. In 27 out of 42 patients, a post- autograft immunotherapy with IL2 was planned. An absolute neutrophil count higher than 1,000/mL, a stable platelet count greater than 50,000/mL and no evidence of active infections were required to start the treatment. IL2 was administered subcutaneously on 5 consecutive days, on a monthly basis, for 1 year or until relapse. The dosage of IL2 was 4x10⁶ IU on day 1, followed by 8x10⁶ IU on days 2 through 5. All patients received during IL2 therapy prophylactic trimethoprim/sulfamethoxazole and paracetamol. RESULTS 13/27 patients were treated with IL2 after PBSCT. Two patients are too early. One patient refused treatment and 13 were not eligible: 2 patients because of psychological disorders, 3 patients because of documentation of active infection after transplant, 2 patients who relapsed without PLTS recovery and 4 patients who showed a delayed platelet recovery at a median of 149 days (range 124-209) from transplant. Among the 13 evaluable patients, IL2 therapy was started after a median of 107 days from autograft (range 35-180). No patient had high grade toxicity requiring treatment discontinuation. All patients showed fever (38-39°C) with arthralgia (5/13 patients), 4-6 hours after IL2 administration. The majority of patients showed gastrointestinal toxicity (grade 1-2) in the form of nausea and vomiting (10/13), diarrhoea (4/13) and transient transaminase increase (2/13). Skin toxicity (grade

1-2) was observed as desquamation (5/13), rash and pruritus which required systemic measures (2/13) and injection site reactions (4/13). With regard to hematological toxicity (grade 2-3), transient neutropenia (2/13) and thrombocytopenia (4/13) were observed, requiring a 50% dose reduction in 2 patients. One patient showed irritability, during drug administration, not requiring dose modification. One patient showed herpes simplex infection requiring oral treatment. In all cases, toxicity completely recovered within 48 hours from IL2 discontinuation. Six patients completed the treatment and are in CCR. Three patients are still on therapy. Three patients showed disease relapse after 2 (CNS relapse), 4 and 11 months of IL2 treatment, respectively.

CONCLUSION Our study shows that the IL2 therapy was often delayed after PBST because of a delayed stable platelet reconstitution. Based on our experience, it appears that low-dose IL2 after PBST is a feasible approach devoid of serious toxicity. A randomized trial is currently undergoing in the context of EORTC/GIMEMA AML12 protocol to document whether or not IL2 is capable to enhancing the likelihood of DFS after PBST.

ANTICOAGULATION AND THROMBOPHILIA

150 FXIII VAL34LEU POLYMORPHISM IS ASSOCIATED WITH THROMBIN GENERATION IN HEALTHY SUBJECTS

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Background: The role of haemostatic gene polymorphisms in arterial and venous thrombosis has not been completely clarified yet, with the exception of FV Leiden and the prothrombin G20210A mutation in venous thromboembolism. Although the haemostatic phenotypes associated with some of these genetic variations have been partially identified, their effects on thrombin generation remain unknown.

Aims: The aim of our study was to further investigate whether these polymorphisms could be involved in the appearance of a prethrombotic state, by measuring the levels of coagulation activation markers in a group of healthy subjects, comparing carriers and non-carriers of the different polymorphisms.

Methods: Coagulation activation markers, prothrombin fragment 1+2 (F1+2), thrombin-antithrombin complexes (TAT) and D-dimer (DD), were measured in a group of 70 healthy subjects, non-carriers of FV Leiden or the G20210A prothrombin mutation, without previous history of thrombosis. Levels of coagulation activation markers were compared between carriers and non-carriers of the FXIII Val34Leu, FVII Arg353Gln and H5-H7, and PAI-1 4G/5G polymorphisms. Since coagulation activation markers did not follow normal distribution, the non-parametrical Mann-Whitney and Kruskal-Wallis tests were used depending on the number of groups compared (two or more than two).

Results: The allelic frequencies observed were similar to those previously reported in other South-European populations. Heterozygous carriers of the FXIII Leu34 allele had lower F1+2 levels than non-carriers, almost reaching statistical significance ($p = 0.06$). When heterozygous and homozygous FXIII Leu34 subjects were combined, F1+2 levels were significantly lower than in Val/Val controls (median 1.1 nmol/L, percentiles 25-75 range: 0.9-1.5 for Val/Val controls versus median 0.9 nmol/L, percentiles 25-75 range: 0.8-1.1 for the polymorphism carriers group; $p = 0.03$) (Fig. 1).

On the other hand, differences in TAT concentrations (median 2.3 g/L, percentiles 25-75 range: 1.2-3.6 for Val/Val subjects versus median 2.0 g/L, percentiles 25-75 range: 1.3-3.4 for FXIII Leu34 carriers) did not reach statistical significance ($p = 0.37$). We found no significant association between FXIII Val34Leu polymorphism and plasma DD levels in healthy subjects. Levels of F1+2, TAT and DD were similar in carriers and non-carriers of the FVII Gln353 or H7 alleles. We did not find any significant differences between homozygous carriers of the PAI-1 4G and 5G alleles either.

Conclusions: Our results show that healthy carriers of FXIII Leu34 variant have a lower thrombin generation rate than non-carriers, suggesting an involvement of FXIII Val34Leu polymorphism in the development of a hypercoagulable state.

This work was partially supported by grants FIS 99/0045-01 and PIUNA-98.

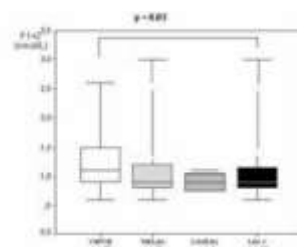


Fig. 1: F1+2 concentrations in heterozygous carriers of the FXIII Val34 allele (white plot), heterozygous subjects (dotted plot), heterozygous Leu34 subjects (grey plot) and combined heterozygous and homozygous carriers of the polymorphism (black plot). The boxes are bounded by the 75th and 25th percentile and the maximum and minimum values are marked by bars.

Caption 1:

151 AN ITALIAN PROSPECTIVE REGISTRY OF CATHETER-RELATED COMPLICATIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES (CATHEM)

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Background: Central Venous Catheters (CVC) are commonly used in patients receiving chemotherapy. CVC-related thrombosis causes significant morbidity in patients with cancer, but data available in patients with hematological malignancies are scant. At variance with cancer patients, hematologic patients are characterized by more severe neutropenia and thrombocytopenia, which might decrease the risk of thrombosis, but may contraindicate the use of anticoagulant drugs.

Aims: to estimate the rate and the risk factors for clinically overt CVC-related thrombotic and infective complications in patients with hematological malignancies. Moreover, we aimed to assess the feasibility and the hemorrhagic risk of venous thromboembolism (VTE) prophylaxis with anticoagulant drugs.

Methods: we designed a prospective, observational study in 8 Italian Hematology Departments. We enrolled 458 CVC in 412 patients with hematological malignancies (males 50.4%, mean age 50.9 years + 14.7). The more frequent diagnoses were acute leukaemia, multiple myeloma and non-Hodgkin lymphoma. Most patients were thrombocytopenic ($< 50,000 \times 106/L$ in 81.2% of cases, with a mean overall duration of 19.2 days) and many had severe neutropenia ($< 100 \times 106/L$ in 59.8% of cases); 14.2% of patients received antithrombotic prophylaxis (LMWH in most cases). We registered events up to 3 months from CVC insertion. The following clinically overt events were included in the analysis: CVC-related deep vein thrombosis (DVT) and pulmonary embolism (PE) instrumentally proven, CVC-related superficial thrombophlebitis, CVC-occlusion / malfunction of thrombotic origin, major cardiovascular events, bleeding, infections and death. A composite study end-point (catheter-related VTE + superficial thrombophlebitis + CVC occlusion / malfunction) was calculated in order to estimate a possible predictive relationship with previously selected variable. Results (in brackets, percentage of patients receiving antithrombotic drugs): CVC-related DVT was documented in 1.5% of cases (0%); total PE in 1.3% (0%); fatal PE in 0.6% (0%); CVC-related superficial thrombophlebitis in 3.9% (0.2%); CVC occlusion/malfunction of thrombotic origin in 6.1% (1.1%); other major cardiovascular events in 1.7% (0.4%); bloodstream infections (BSI) in 15.6%; CVC-related BSI in 4.6%; serious bleeding in 3.5% (0.4%). At multivariate logistic regression analysis thrombocytopenia was found to significantly reduce the risk of CVC-related VTE. The percentage of cases with thrombocytopenia and experiencing a CVC-related complication was however not negligible (10.2%, vs 17.4% of incidence in patients with platelet counts $> 50,000 \times 106/L$). We were not able to document any correlation between thrombotic and infectious events.

Conclusion: the incidence of clinically overt CVC-related thrombotic and infectious complications in patients with hematological malignancies is not negligible. A platelet count $< 50,000 \times 106/L$ is significantly correlated with a lower rate of thrombotic events, which remain however consistent.

Prophylaxis with anticoagulant drugs is feasible and is non apparently associated with increased hemorrhagic risk.

152 ANALYSIS OF PREDISPOSING FACTORS AND SIX MONTH OUTCOMES OF DVT MANAGEMENT IN 668 PATIENTS TREATED WITH LMWH (TINZAPARIN) AS OUTPATIENTS

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In Swindon, eligible patients with a suspected or proven DVT are treated as outpatients using a once daily low molecular weight heparin (LMWH). Prospective audit using an electronic database has been ongoing since 1998. As a result of this data analysis we can identify patients at greater risk of recurrent thromboembolic disease and bleeding complications. As of August 2003 there was a total cohort of 717 patients: average age was 61 years, with 53% female patients. The major predisposing factors were recorded as immobility (32.5%), previous VTE (21.3%) and surgery (14.8%).

6-month follow-up data were available for 668 patients: 20 (3.0%) patients had confirmed thromboembolic complications, of which 18 (2.7%) occurred subsequent to the initial LMWH treatment phase (late). Bleeding complications occurred in 12 (1.8%), with 4 (0.6%) being classified as major - 3 gastrointestinal bleeds and 1 retroperitoneal haematoma. Of the 20 confirmed thromboembolic complications 2 (10%) had no predisposing factor at initial presentation. Other risk factors were malignancy (6 events), immobility (6 events), surgery/trauma (4 events), oral contraceptive/HRT use (3 events), previous VTE (2 events), family history of VTE (2 events) and a long haul flight (1 event). 6 patients had more than one risk factor. 2 of the patients had 2 events and both of these patients had a malignancy.

Incidence of thromboembolic and bleeding events at 6 months were low (comparable with clinical trial data and other UK audit data) in our centre. The most significant risk factors for recurrent thromboembolic complications were malignancy and immobility. The database has allowed analysis of the data over different time periods enabling us to show that the service has been extended to more patients but with a reduction in complication rates. The service is now beginning to manage selected patients with a confirmed PE on an ambulatory basis.

153 BLEEDING COMPLICATIONS OF ORAL ANTICOAGULANT THERAPY: A PROSPECTIVE AUDIT

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Bleeding is the most serious complication of the use of oral anticoagulation in the prevention and treatment of thromboembolism. We designed a tool to prospectively audit the frequency and severity of over anticoagulation and bleeding in two Anticoagulant services: a District hospital serving a population of 330,000 patients (3,400 anticoagulant patients) and a University hospital with a population of 300,000 patients (4,000 anticoagulant patients), both providing Anticoagulant Practitioner-led inpatient and outpatient anticoagulant dosing. Data was collected over 10 months including INR at time of event, whether complications necessitated hospital attendance and/or admission, investigations carried out and treatment given.

There were 347 events. 133 were asymptomatic high INRs (>8). The majority of these were treated with oral vitamin K as outpatients. There were 214 bleeding episodes, 54 major and 160 minor. The most common sites of bleeding were epistaxis (70/214), GI bleeding (45/214), haematoma (36/214) and haematuria (24/214). 106 patients were admitted to hospital, constituting 0.61% of the total emergency medical admissions over this period. Patients spent a total of 887 days in hospital. 14 patients died, 4 directly as a result of the bleeding episode.

INR stability in the period prior to the event was measured by +/- 1 standard deviation from the mean. 52/347 of patients were new at the time of the event (receiving anticoagulant therapy for less than 4 weeks), 164/347 were unstable, 121/347 were stable and 10/347 were GP monitored patients with no INR data available. At the time of event 68% of patients had an INR above target range (major bleed 72%; minor 66%). The most common factors contributing to the event were concurrent illness (78/347), prescription of potentiating medication (65/347), increase in prescribed warfarin dose (21/347) or patients inadvertently exceeding their prescribed dose (15/347).

154 INITIAL OUTPATIENT TREATMENT OF PULMONARY EMBOLISM WITH FONDAPARINUX (ARIXTRA): THE MATISSE-PE TRIAL

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Background: Continuous intravenous unfractionated heparin (UFH), the current standard initial therapy of pulmonary embolism (PE), does not allow for outpatient treatment. In the MATISSE-PE trial, comparing once-daily subcutaneous fondaparinux, a synthetic selective factor Xa inhibitor, with UFH in the initial treatment of PE, outpatient treatment of fondaparinux was encouraged but left at the investigators discretion.

Aims: We analyzed the data in patients who received fondaparinux on an outpatient basis.

Methods: MATISSE-PE was an open, randomized trial comparing once-daily subcutaneous 7.5 mg fondaparinux (5.0 mg and 10.0 mg in patients <50 kg and >100 kg, respectively) with intravenous UFH in 2213 patients with symptomatic hemodynamically stable PE. All drugs were given for at least 5 days and until anticoagulation with oral anticoagulants was therapeutic. The primary efficacy and safety outcomes were recurrent venous thromboembolism (VTE) during 3 months follow-up and major bleeding (MB) and death during the initial treatment period.

Results: VTE recurred in 3.8% fondaparinux patients versus 5.0% UFH patients, a 1.2% difference in favor of fondaparinux (95% CI: -3.0%;0.5%), a relative risk reduction of 25%. The rates of MB and death were comparable between the groups. The data from the 158 (14.3%) patients who received fondaparinux on an outpatient basis were similar to those from the total fondaparinux population (Table).

Summary/Conclusion: Using fondaparinux, outpatient initial treatment of PE is feasible, effective and safe.

All randomized patients	UFH	Fondaparinux	
		Total patients	Outpatients
n	1110	1103	158
Age, yr (mean±SD)	62±17	63±16	57±16
Male/female	477/633	501/601	82/76
Hospital discharge, days (mean±SD)	10.2±6.8	9.7±7.7	4.4±2.2
≥2 VTE risk factors, n (%)	260 (23.4)	241 (21.8)	26 (16.5)
VTE, n (%)	56 (5.0)	42 (3.8)	5 (3.2)
MB*, n (%)	12 (1.1)	14 (1.3)	0 (0.0)

*: As treated patients

Caption 1: Table

155 FONDAPARINUX IN THE TREATMENT OF DEEP-VEIN THROMBOSIS AND PULMONARY EMBOLISM

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Background: The MATISSE trials investigated whether a single dose regimen of fondaparinux had an efficacy/safety profile comparable to standard therapies in the treatment of venous thromboembolism (VTE).

Aims: Since the MATISSE studies were non-inferiority trials, we present the results of the per-protocol cohort.

Methods: Fondaparinux was administered at a once-daily subcutaneous dose of 7.5 mg (5.0 mg and 10.0 mg in patients <50 kg and >100 kg, respectively). In the MATISSE-DVT trial, fondaparinux was compared with twice-daily subcutaneous enoxaparin (1 mg/kg) in patients with deep-vein thrombosis. In the MATISSE-PE trial, it was compared with adjusted-dose intravenous unfractionated heparin (UFH) in patients with pulmonary embolism. All drugs were given for at least 5 days and until anticoagulation with oral anticoagulants was therapeutic. The primary efficacy and safety outcomes were recurrent VTE during 3 months follow-up and major bleeding and death during the initial treatment period. The per-protocol cohort included all randomised patients who had confirmed symptomatic VTE at entry, had received study drug for at least 5 days or up to the occurrence of a primary efficacy endpoint, had good

compliance to vitamin K antagonists, and who did not use any prohibited medication.

Results: In MATISSE-DVT, VTE recurred in 3.4% (34/988) fondaparinux patients, versus 4.3% (42/976) enoxaparin patients, a difference of 0.9% in favour of fondaparinux (95%CI: -2.6%; 0.8%). In MATISSE-PE, VTE recurred in 2.9% (27/920) fondaparinux patients versus 4.4% (35/794) UFH patients, a difference of 1.5% in favour of fondaparinux (95%CI: -3.3%; 0.3%). The rates of major bleeding and death were comparable between the groups.

156 GIESSEN THROMBOPHILIA STUDY: MANIFESTATION AGE OF THROMBOEMBOLISM IN THROMBOPHILIA

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Introduction: Thrombophilia is diagnosed in about 50 % of patients suffering from thromboembolic events. More than 10 % of thrombosis patients present with combined hereditary thrombophilic defects - for most of them life-long treatment with oral anticoagulants is recommended.

Severity of thrombophilia can be calculated from manifestation age of first thromboembolic event. Therefore, manifestation age of thromboembolism in different thrombophilic states was examined to find out, whether combined defects really are more endangered than single defects and do require life-long anticoagulation.

Patients: 1158 patients (343 male, 815 female) were studied. 811/1158 had experienced thromboembolic events. Average age at presentation was 44 + 15 years.

Methods: In all patients studied, PT, aPTT, fibrinogen, antithrombin, protein C and S, aPC ratio, plasminogen, antiphospholipid antibodies, homocysteine, Factor V Leiden mutation, prothrombin (G20210A)- and MTHFR (677T)-polymorphism were checked.

Results: Thrombophilic defects were detected in 736/1158 patients. Hyperhomocysteinaemia and / or MTHFR polymorphism were found in 233 of them.

180 patients presented with Factor V Leiden mutation. In 46 protein S deficiency, in 23 protein C deficiency, in 25 antiphospholipid syndrome, in 22 prothrombin polymorphism and in 5 patients antithrombin deficiency was detected.

165 patients had a combination of two , 17 patients of three such defects.

Manifestation age of thromboembolism in patients without coagulation defects was 36 + 14 years. In patients with coagulation defects, manifestation age was not significantly earlier - except in patients with antithrombin deficiency (19 + 3 years). Patients with combined defects presented with manifestation age of 36 + 15 years (2 defects) and 35 + 13 years (3 defects).

Conclusion: Based on manifestation age of thromboembolism, combined thrombophilic defects do not seem to be more severe than single defects. Therefore, life-long OAC is not necessary in every patient with combined thrombophilic defect.

157 THROMBOPROPHYLAXIS WITH FONDAPARINUX IN ACUTELY ILL MEDICAL PATIENTS WITH MODERATE RENAL IMPAIRMENT

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Purpose: In the ARTEMIS trial in acutely ill medical patients, fondaparinux significantly reduced the risk of venous thromboembolism (VTE) (risk reduction=46.7%, p=0.029) and the incidence of fatal pulmonary embolism (PE) without increasing the risk of major bleeding compared with placebo. Since renal function may influence the benefit-to-risk ratio of thromboprophylactic drugs excreted by the kidneys, we analyzed the data in patients with moderate renal impairment (creatinine clearance [CCI] between 30 and 50 mL/min).

Materials and Methods: ARTEMIS was a randomized, double-blind trial comparing once-daily 2.5 mg subcutaneous fondaparinux with placebo in 849 medical patients aged 60 years or more hospitalized for acute cardiac, respiratory, infectious, or inflammatory disease; and expected to remain bedridden for 4 days or more. Severe renal insufficiency (CCI <30 mL/min) was an exclusion criterion. Treatment was continued for 6-14 days. The primary efficacy outcome was confirmed VTE up to day 15. The main safety outcome was the incidence of major bleeding.

Results: Among the randomized and treated patients, 281 (33.5%) patients had MRI. The superior efficacy of fondaparinux over placebo was independent of CCI. In patients with moderate renal impairment evaluable for efficacy, the rates of VTE were 12.1% (13/107) in the placebo group versus 7.6% (8/105) in the fondaparinux group; there were 3 PE (2.8%), all fatal,

in the placebo group versus none in the fondaparinux group. Fondaparinux was as safe as placebo, regardless of CCI: in patients with moderate renal impairment, there were no major bleeding in both study groups.

Conclusions: Fondaparinux versus placebo significantly reduced the risk of VTE and of PE without increasing the risk of clinically relevant bleeding in acutely ill medical patients, including those with moderate renal impairment.

158 THE MANAGEMENT OF PATIENTS ON LONG TERM ORAL ANTICOAGULANT TREATMENT (OAT) UNDERGOING ELECTIVE MAJOR SURGERY: A PROSPECTIVE OBSERVATIONAL STUDY

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Background. The perioperative management of patients (pts) on long term OAT is an ever increasing issue in clinical practice for the number of pts on OAT requiring surgery. When surgery is carried out, either in emergency or electively, the problem arises of balancing the thromboembolic risk with the bleeding risk. The issue is unsettled. The therapeutic options are: 1) discontinuation of OAT to obtain an INR value <2.0 at the time of surgery and resumption as soon as possible; 2) replacement of OAT with i.v. unfractionated heparin or low molecular weight heparin (LMWH), initiated at home to reduce the time of the hospitalization. Aims. From November 2001 to August 2003 132 consecutive pts on long term OAT underwent elective major surgery. Modality of treatment and adverse events (major bleeding and thromboembolism) occurring during and within 2 months after the procedure were registered.

Treatment schedule: OAT was discontinued 3-5 days before surgery and substituted, according to the thromboembolic risk, by sid or bid LMWH, initiated at home, when INR values was below the therapeutic range; last dose of LMWH was administered 12-24 h before surgery; resumed 12-24 h after procedure and discontinued when INR was within the therapeutic range. Study population: 132 pts (80 men, 52 females), median age 72 years (29-91). Acenocoumarol in 11 pts, warfarin in 121 pts. OAT indications: atrial fibrillation (AF) 64 pts, prosthetic mechanical heart valves 43; prophylaxis for venous thromboembolism 21; cardioembolic stroke 4. Type of surgery: herniotomy 25 pts; orthopedic surgery 25; cholecystectomy 20 (16 laparoscopy); ileum resection or hemicolectomy 9; urologic surgery 20; gynecologic surgery 10; thyroidectomy 4; mastectomy 4; gastrectomy 4; vascular surgery 4; hematoma drainage 2; plastic surgery 2; anal fistula 3.

Results. (median and range). Nadroparin in 112 pts, enoxaparin in 20. OAT discontinuation: 5 days (1-27); start of LMWH before surgery 5 days (1-28); sid LMWH dose (82 pts) 3800 U (2750-7600 U). bid (50 pts) 4000 U (2750-8000 U); OAT resumption day +1 (1-10); INR value at surgery 1.12 (1.0-2.0). Adverse events: 1 death (cardiac arrest at day 13 after surgery); 2 arterial thromboembolism in AF (at day 3 and 4 post surgery, respectively); 5 major bleedings (at day 1-3) and 9 minor bleedings; age of bleeders and no bleeders 73 years (62-89) vs 72 (29-91).

Conclusions. The rate of thromboembolic events is low, occurring only in patients treated with sid dose. Age of patients was not a risk factor for bleeding; the incidence of major and minor bleeding is higher in patients treated with bid doses of LMWH. LMWH initiated at home is a convenient choice for thrombosis prophylaxis in pts on long-term OAT submitted to major surgical procedures.

	Incidence of thrombosis (%)		Incidence of bleeding (%)		
	Arterial	Venous	Minor	Major	Total
All patients	1.5	0	6.8	3.8	10.6
LMWH sid (82 pts)	2.44	0	4.87	2.44	7.31
LMWH bid (50 pts)	0	0	10.0	6.0	16.0

Caption 1: Table

INFECTIOUS COMPLICATION

159 ANAEROBIC BACTEREMIA IN NEUTROPENIC PATIENTS WITH LEUKEMIA AND LYMPHOMA. OROPHARYNGEAL MUCOSITIS AND STATUS OF HEMATOLOGIC DISEASE ARE THE MOST IMPORTANT PREDISPOSING CONDITIONS.

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Anaerobes are one of the most important components of the normal human skin and oropharyngeal flora and can be a cause of bacterial infections of endogenous origin in immunocompromised host. An increasing number of Anaerobic infections in this setting has been recently reported and often the type of anaerobes isolated suggests an oropharyngeal source of these infections.

Herein we report our experience about Anaerobic Bacteremia (AN). Over a 10-year period (from January 1992 to December 2001) 34 episodes of AB were identified in 34 different patients (16 males, 18 females, median age 35, range 17-69) for a rate of 0,5 AB per 100 patient admissions; it accounted for 4 % of all bacteremic episodes that occurred in our Department during the study period. The majority of pts had a refractory/relapsed leukemia (20/26) or refractory/relapsed lymphoma (5/8); 5/34 (15%) received a BMT procedure. 28/34 (82%) pts had a severe neutropenia at onset of AB with a WBC count less than 100 cells/mm³ and 26/34 (76%) had a severe oral mucositis following intensive chemotherapy (grade III-IV WHO in 20/26 cases). Bacteremic episodes occurred after a median of 16 days of severe neutropenia (range 5-28) and of these 8/34 (24%) were polymicrobial. Pulmonary infiltrates were observed in 4/34 (12%) pts. The *Fusobacterium nucleatum* was the most frequently isolated pathogen (in 18 cases), followed by *Bacteroides* species (in 8 cases), *Peptostreptococcus* species (in 5 cases) and *Clostridium* species (in 3 cases). The most common antibiotic treatment was piperacillin-tazobactam (13 pts), carbapenems (10 pts) and glycopeptide plus ceftazidime (4 pts). The median duration of antibiotic therapy was 9 days (range 3-18). The response rate of first line antibiotic therapy was 72% and the mortality AB related was 5/34 (15%). To identify risk factors for infections a retrospective case-control study was performed (for each case 2 control pts). Univariate analysis revealed that the severe mucositis (WHO III-IV) and the status of refractory/relapsed disease were significantly associated to development of AB. No significant differences were found with regard to chemotherapy regimen, duration of neutropenia, empirical antibiotic treatment, antibacterial prophylaxis, invasive procedures. Conclusions. 1) In our experience *Fusobacterium* sp, *Bacteroides* sp, *Peptostreptococcus* sp, and *Clostridium* sp, are the most common isolates causing anaerobic bacteremia in onco-hematologic patients. 2) Predisposing conditions to AB include severe oropharyngeal mucositis and refractory/relapsed status of oncohematologic disease; in these cases the use of antibiotic therapy with activity against anaerobes should be considered especially in those do not respond to initial empirical antimicrobial treatment

160 CASPOFUNGIN (CANCIDAS) AS FIRST LINE TREATMENT FOR 32 PROVEN OR PROBABLE INVASIVE FUNGAL INFECTIONS IN ONCOHEMATOLOGIC NEUTROPENIC PATIENTS.

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INTRODUCTION. Invasive Fungal Infections (IFI) remain a severe complication for hematologic patients. However, new agents are increasing the choice of therapy and offer the potential to improve clinical outcomes. The Caspofungin (Cancidas-Merk) is a large lipopeptide molecule able to inhibit the Beta-glucan synthesis; this action damages fungal cell walls but not mammalian cells. In vitro and in vivo, Caspofungin (CAS) is active against most *Candida* spp and *Aspergillus* spp. We report on our experience with this drug as a first line therapy for proven or probable IFI in neutropenic oncohematologic patients. **PATIENTS AND RESULTS.** 32 consecutive patients have been treated with CAS (26 AL, 3 NHL, 2 CL and 1 MM), 20 males and 12 females, median age 51 yrs (range 22-72); 14/32 (44%) pts had a relapsed or resistant hematologic disease while 13 pts were in complete remission, other 5 pts were at onset of disease. 9/32 (28%) pts had proven IFI (3 Candidiasis and 6 Aspergillosis) and 23/32 (72%) pts had a probable (defined according to international consensus) IFI. 25/32 (78%) pts had less than 1x10⁹ granulocytes/mm³ at onset of infection and 9/32 (28%) pts developed IFI after a BMT procedure. CAS was given 70 mg/day (day 1) followed by

50 mg/day (from day 2) by 1 hour iv infusion. In 8/32 (25%) pts the drug was administered in combination with amphotericine. The median duration of CAS therapy was 15 days (range 5-62). The overall response rate was 69% (22/32) with 14/22 complete responses and 8/22 partial responses (5/8 very good partial responses). Ten out of 32 pts (31%) were not responders. No adverse clinical effects were reported. Only grade I-II transient increase of alkaline phosphatase and/or transaminases occurred in 3/32 (9%) pts. We underline that 5 pts without response and 4 pts with partial response received, after CAS, a sequential therapy with voriconazole; of them 3/5 and 4/4, respectively, obtained a complete remission of IFI. **CONCLUSIONS.** Our experience suggests a good efficacy of CAS as first line treatment of proven or probable IFI. This drug has been very well tolerated. There were no significant hepatic adverse events neither in leukemic pts nor in the cohort receiving CAS with cyclosporin after a BMT. A significant proportion of not responders or partial responders can be rescued with a sequential therapy including CAS followed by voriconazole. The absence of CAS antagonism with other antifungal agents indicates that the combined or sequential antifungal therapy could be a promising option to treat IFI.

161 CRYPTOCOCCOSIS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES. EXPERIENCE OF GIMEMA-INFECTION

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BACKGROUND: Cryptococcosis is an important cause of morbidity and death in immunocompromised patients. Currently, AIDS is the predisposing factor in approximately 90% of cryptococcal infections but other patients with immunologic defects in T-cell-mediated host defense mechanisms cause an increased risk of cryptococcosis. However, reports on patients with hematological malignancy and cryptococcosis appear only sporadically, and the category more frequently affected by this complication is suggested to be that of patients with Hodgkins disease.

AIMS: To evaluate the clinical, laboratory characteristics and outcome of patients with cryptococcosis complicating haematological diseases.

METHODS: All cases of microbiologically and/or histologically documented cryptococcosis in adult patients (>12 years of age) with hematological malignancies, observed between January 1993, and December 2002 in 21 GIMEMA centers, were retrospectively collected.

RESULTS: The study included 17 patients (m/f 12/5; median age 61 years) with haematological diseases (6 AML, 3 NHL, 2 ALL, 2 MDS, 1 each for CLL, HD, MM, and CML) who developed a cryptococcosis. Among these, only 4 patients underwent stem cell transplantation (2 BMT, 1 aBMT, 1 PBST). Analyzing possible risk factors we recognized that before the onset of infection: 6 patients received steroids for the underlying malignancy; 4 patients suffered from diabetes mellitus; 2 had cutaneous wounds and 1 each had respectively an autoimmune disease, an hepatic cirrhosis, a chronic renal failure and exposure to pigeons. Five patients received an oral prophylaxis, in only 2 cases with fluconazole. Symptomatology was characterized by fever, neurological and respiratory signs reflecting the primary sites of infection (5 blood, 4 CNS and lung respectively, and 1 each gut, skin and mouth). Diagnosis was made with antigen detection in serum or CSF, positive microbiological culture or PCR. All patients started a specific treatment (7 patients with fluconazole and 10 with deoxycolate or liposomal amphotericin B). Only 2 patients died from cryptococcosis within 30 days from diagnosis.

SUMMARY/CONCLUSIONS: Cryptococcosis represents a very rare complication in patients with hematological malignancies. It is characterized by a low lethality if compared to that observed in the other fungal infections in neutropenic patients (i.e. aspergillosis or mucormycosis). A specific treatment, timely started, is able to positively influence the outcome.

162 SCREENING FOR ASPERGILLUS GALACTOMANNAN ANTIGENEMIA : THE ROLE IN A HEMATOLOGY UNIT

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Objectives :

To assess the role of Aspergillus galactomannan (GM) antigen detection in a general hematology unit.

Methods :

Between 06/02 and 12/03, there were 667 admissions corresponding to 244 different patients (pts) in the hematology unit. Overall 151 pts were tested at least twice by the Aspergillus GM ELISA. A total of 1813 serum samples were analyzed (Mean Nr tests/pt: 11.85; range: 2-51; SD: 10.52). The mean period of follow-up (FU) was 100.7 d (range: 3-487, SD: 91.6). GM antigen detection was made in the setting of: routine surveillance after chemotherapy (105 pts), after hematopoietic stem cell transplantation (HSCT) (2 allo- and 3 'mini' allo-HSCT; 27 auto-HSCT) or in the FU of infection (14 pts). Underlying diseases were: lymphoid: 55%; myeloid: 40%; non malignant: 5%. Clinical efficiency of the test was assessed at three different cut-off values (1.5, 1.0 and 0.7).

Results:

Results are summarized in the table. The overall incidence of invasive aspergillosis (IA) was 6.4% (43/667 admissions). Following EORTC definition criteria, the repartition was: 2 Definite IA, 16 Probable IA and 25 Possible IA. The definition of 'probable' IA was substantiated by positive GM antigen tests (8 cases); both by microbiological (positive cultures) and positive GM antigen tests (4 cases) or only by microbiological criteria (4 cases). GM antigen was detected at all different cut-off values in 30 cases corresponding to: 1/2 definite IA, 12/16 Probable IA. Results were considered as false-positives in 17 pts: 4 cases without clinical context; 15 cases with a negative chest CT-scan; 2 pts were even treated as possible IA, one because of a positive chest CT-scan (CMV pneumonia), the other because of past proven IA. All 'false-positive' results were in patients who received antibiotics at time of antigen testing (Pipera/tazobactam 7 pts; Amoxi/clavulanate 9 pts; Cefepime 1 pt).

Conclusion:

Detection of circulating GM antigen may be helpful for the diagnosis of IA, particularly in the absence of microbiological data, but a substantiated number of false-positive results do occur among patients undergoing antibiotic therapy with Pipera/tazobactam or Amoxi/clavulanate. Considering different cut-off values did not improve the sensitivity nor the specificity of the assay.

163 THROMBOCYTOSIS AND TOXOCARIASIS : REPORT OF TWO PEDIATRIC CASES

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Background:

Toxocariasis is a world-wide distributed zoonosis caused by *Toxocara canis* and *Toxocara cati*, intestinal roundworms (nematodes) of dogs and cats. Transmission to humans occurs by direct ingestion of embryonated eggs found in the soil, on unwashed hands or by consumption of contaminated vegetables. One manifestation of this disease is Visceral Larva Migrans (VLM) and is characterized by leukocytosis with persistent eosinophilia. We report two cases of VLM in children almost asymptomatic at diagnosis but with an unusual biological presentation.

Methods / Results:

A six year-old girl and a twenty-one month-old girl presented at diagnosis with a persistent thrombocytosis mimicking an essential thrombocythemia ($> 1\,000 \times 109/l$) and a variable eosinophilia ($> 1 \times 109/l$). Examination of bone marrow showed no fibrosis and revealed a megakaryocytic hyperplasia. These changes suggested a myeloproliferative disorder with an unusual eosinophilia or a reactive process like parasitic infection. All other laboratory tests were normal and cytogenetic study revealed no abnormalities. The diagnosis of toxocariasis was established with serologic findings and the children were treated with adequate medical therapy (either with thiabendazole or albendazole). After anthelmintic therapy, total leukocyte, eosinophil and platelet counts fell promptly.

Conclusion:

Thrombocytosis, with platelet count greater than $1\,000 \times 109/l$, is rare in children and when the elevation persists during several weeks, it is necessary to distinguish reactive thrombocytosis from essential thrombocythemia. In our two cases, biological results revealed an exceptional and persistently elevated platelet count associated with

marked eosinophilia. Bone marrow examination and peripheral eosinophilia suggested initially a parasitic etiology which was confirmed by toxocariasis serology. After treatment, eosinophil and platelet counts returned to normal ranges; consequently we conclude that thrombocytosis was due in both cases to toxocariasis. In literature, the eosinophil count has been reported to be increased in VLM; while thrombocytosis had never been described. To our knowledge, this is the first report of thrombocytosis occurring during toxocariasis.

164 FATAL HEMOPHAGOCYTIC SYNDROME WITH EVIDENCE OF HHV-8 INFECTION IN THREE IMMUNOCOMPETENT SUBJECTS

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BACKGROUND: Infection with human herpesvirus 8 (HHV-8) has been directly involved in the development of Kaposi sarcoma (KS) and of rare lymphoproliferative disorders (primary effusion lymphoma and Castelman disease), particularly in immunodeficient patients. The occurrence of non-malignant disease such as bone marrow failure or hemophagocytic syndrome, in transplant recipients or HIV positive patients with KS, has also been linked with HHV-8 infection in occasional cases.

AIM: We report the occurrence of a fatal hemophagocytic syndrome in three immunocompetent patients seen at our Institution over a 12 months period who were shown to be infected by HHV-8.

PATIENTS:

- 1) A 63-year old woman was admitted with fever, peripheral cytopenia and splenomegaly. One month before she had been started on corticosteroid treatment (6-metilprednisolone 1.5 mg/Kg) for autoimmune hemolytic anemia (AHA) and ten days before she had received nephrectomy for renal carcinoma. There was no evidence of metastatic disease and of bacterial or viral infection. She received eritropoietin, G-CSF and high dose intravenous immune globulin, with no benefit and died with a rapidly evolving multiorgan failure.
- 2) A 69-year old man was admitted for AHA and splenomegaly. He initially responded to 6-metilprednisolone 1.5 mg/Kg, but rapidly developed pancytopenia and fever. He received high dose intravenous immune globulin, acyclovir and broad spectrum antibiotics but died with rapidly progressive multiorgan failure.
- 3) A 66-year old woman was admitted with fever and progressive pancytopenia; no splenomegaly and no signs of hemolysis were detected. She was started on corticosteroid treatment (6-metilprednisolone 2.0 mg/Kg), high dose intravenous immune globulin, and gancyclovir but died in 24 hours because of a massive cerebral hemorrhage.

RESULTS: A bone marrow aspirate or biopsy performed in all three patients showed a normo/hypercellular marrow with myelodysplastic features (cytogenetic alterations not specific for myelodysplasia were detected in two cases) associated with signs of hemophagocytosis, without evidence of lymphomatous infiltration. Biochemical data including high serum ferritin and LDH and low cholesterol level were concordant with hemophagocytic syndrome. In all cases infection by HHV-8 was demonstrated by HHV-8 DNA detection in peripheral blood samples by PCR analysis with primers specific for the viral gene orf 26. In patient 1 immunohistochemical analysis in the bone marrow biopsy showed the presence of the HHV-8 latency-associated nuclear antigen on bone marrow cells. There was no evidence of other infections except for serum CMV DNA positivity with repeatedly negative CMV antigenemia in patient 1 and serum EBV DNA positivity in patient 2.

CONCLUSIONS: Pancytopenia with hemophagocytic syndrome, myelodysplasia +/- autoimmune haemolytic anemia occurring in immunocompetent adults is a clinical picture which should be added to the spectrum of diseases associated with HHV-8 infection. Its frequency may be underestimated and it should be always considered in the differential diagnosis of unexplained peripheral cytopenia. Its prompt recognition and the early institution of effective antiviral treatment may be the only way to avoid its otherwise rapidly fatal evolution.

IMMUNE THROMBOCYTOPENIC PURPURA

165 REMISSION OF REFRACTORY ITP AFTER TREATMENT FOR HELICOBACTER PYLORI INFECTION- A CASE REPORT

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Background :

Infectious etiologies that have been explored in patients with idiopathic thrombocytopenic purpura (ITP), including cytomegalovirus, HTLV1, human immunodeficiency virus-1 and Hepatitis C that antiviral therapy has been associated with an improvement in platelet count in the majority of cases. Helicobacter pylori (H. pylori) has been suggested as one of the possible infectious factors associated with ITP. We report here a patient with refractory ITP who experienced a complete and longstanding normalization of platelet count after treatment for H. pylori infection.

Aims :

Efficacy of Helicobacter pylori treatment in refractory ITP.

Methods :

The patient was a 24- year -old woman, who had been under corticosteroid therapy for ITP, but underwent splenectomy 6 years ago, due to corticosteroid resistance (platelet count < 50 x10⁹/L). After splenectomy, the platelet count was still low(20-30x 10⁹/L); therefore corticosteroid, danazol and different immunosuppressive drugs, including azathioprine, vincristine and vinblastine were tried according to hematology consult. Over this period her platelet count never exceeded 30x10⁹/L. One year ago, the patient had CNS bleeding associated with a platelet count between 5-7x 10⁹/L, and received IVIG and IV methylprednisolone, which increased the platelet count temporarily. Two months afterwards, because of recurrence of thrombocytopenia abdominal radionuclide scan was done, which showed an accessory spleen. Removal of the accessory spleen again raised the platelet count to 100 x10⁹/L. However 1 month later the platelet count decreased and was resistant to corticosteroid, danazol and vincristine. Serologic test was performed for diagnosis of H. Pylori, which was positive (> 100 U/ml titer, normal value less than 15U/ml).

The patient was treated with quadruple anti-H. pylori regimen for 2 weeks. The platelet count increased to above 90-100x 10⁹/L, 2-3 days after beginning of treatment.

Results :

At present, after almost 9 months from H. pylori treatment, the platelet count is within normal range, and the patient is clinically stable. The last platelet count is 321x 10⁹/L.

Conclusions:

Our data suggest the efficacy of H. pylori eradication in increasing platelet count in adult ITP patients. Based on these studies, it is recommended that a search for Helicobacter pylori is appropriate in ITP patients at the time of diagnosis so that treatment can be given. Further studies are needed to substantiate these early observations and longer follow up of the treated patients is required to confirm that the improvement in the patients thrombocytopenia after Helicobacter treatment will persist.

166 RITUXIMAB IN THE TREATMENT OF RELAPSED AND REFRACTORY IMMUNE THROMBOCYTOPENIA

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Idiopathic thrombocytopenic purpura (ITP) is a common disorder characterized by autoimmune platelet destruction. ITP is diagnosed by demonstrating an immune destructive thrombocytopenia and excluding other causes. ITP is caused by the binding of autoantibodies to platelet antigens. Thrombocytopenia is a consequence of the premature destruction of the platelets by the reticuloendothelial system. Rituximab is monoclonal antibody to a particular protein - the CD 20 antigen on the surface of normal and malignant B cells. B lymphocytes represent the main chain in the pathogenesis of autoimmune disorders. Depletion of B-Ly is a logical approach in the treatment of autoimmune thrombocytopenia and Rituximab represent a potential agent.

We present two cases of relapsed and resistant immune thrombocytopenia successfully treated with Rituximab with stable platelet count and no bleeding symptoms. Case 1. J.C., female, (DOB 1969) diagnosed in 1980 and treated with steroids with satisfactory clinical response. Severe relapse of the disease occurred in 1996. The treatment with steroids showed minor response with persistent bleeding episodes. Four doses

of Rituximab were applied in weekly intervals. The satisfactory response (Plt>80-90) was obtained after two months. The maintenance treatment with Rituximab every three months was applied (Plt>110). Case 2.- P.S., female, (DOB1974) diagnosed 1997 as immune thrombocytopenia after the pregnancy. Initial steroid treatment was with minor response. The splenectomy was done 10 months after the diagnosis with clinical remission lasting three years. Relapse of the disease was resistant to steroids. Five doses of Rituximab were applied in weekly intervals with excellent but delayed response (after 4 months). Six doses of Rituximab were given as maintenance in three months intervals. The patient is without bleeding episodes and platelet count 80-110. Our modest experience showed that Rituximab is strong and safe potential treatment in refractory and relapsed cases of immune thrombocytopenia.

167 TREATMENT OF PATIENTS WITH REFRACTORY IMMUNE THROMBOCYTOPENIC PURPURA WITH ANTI- CD20 MONOCLONAL ANTIBODY (RITUXIMAB).

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Background-aims. No standard therapeutic approach exists for the one-third of adult patients with immune thrombocytopenic purpura (ITP) who develop chronic, refractory disease. Treatment is individualized on the basis of lifestyle, bleeding risk and tolerance of therapy. We evaluated the effectiveness and side-effect profile of the anti-CD20 humanised monoclonal antibody rituximab as treatment alternative for patients with recurring or chronic refractory ATP.

Methods. All six patients (3 males, 3 females) suffered from ITP refractory to more than two therapeutic modalities. Their median age was 55 (range 19-70). Three patients had relapsed after prior splenectomy. All six had shown untoward effects from prolonged corticosteroid administration. Median platelet levels before rituximab therapy were 20,000/microliter (μL) (range 2,000/μL-40,000/μL). Rituximab was infused intravenously at a dose of 375 mg/m² of body surface area weekly for four consecutive weeks.

Results. Rituximab therapy was tolerated exceptionally well without any clinically significant acute or late side-effects. At a median follow-up of 4 months (range 2-6), all patients responded to treatment with complete steroid withdrawal. Four patients (60%) maintain platelet levels higher than 100,000/μL whereas two have platelet levels between 50,000 and 100,000/μL. No ITP recurrence has been observed yet.

Conclusion. Rituximab appears as a promising immunomodulatory agent for the management of autoimmune thrombocytopenias with a favourable toxicity profile. Additional research for the evaluation of clinical benefit resulting from in vivo B-lymphocyte depletion in immune cytopenias is warranted.

168 DANAZOL THERAPY IN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA : LONG-TERM RESULTS

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Purpose: Adults with chronic idiopathic thrombocytopenic purpura who have failed standard dose corticosteroids and splenectomy or with contraindications to these therapies often require further treatment due to life-threatening platelet count or bleeding. We studied whether danazol, an attenuated androgen, is useful in this setting.

Subjects and methods: To assess both clinical outcome and tolerance issues, 57 patient with refractory chronic idiopathic thrombocytopenic purpura (n = 27), contraindications to splenectomy or corticosteroids or rejection of these therapeutic options (n = 30) were studied.

Results: Thirty-eight patients experienced a partial or complete response to therapy (67%), among whom 27 (46%) remained in remission with a median of 119 54 months. Treatment tolerance was acceptable, although severe adverse events were reported in 9 (16%) patients.

Conclusions: Our findings suggest that danazol therapy may be beneficial in the management of refractory chronic idiopathic thrombocytopenic purpura or in case of contraindications to splenectomy and/or corticosteroids.

169 TITER OF ANTI-HEPARIN / PF4-ANTIBODIES AND PLATELET ACTIVATION MARKERS IN HEPARIN INDUCED THROMBOCYTOPENIA

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Background: Heparin-induced thrombocytopenia (HIT) is mediated by antibodies directed against the heparin/platelet factor 4 (HPF4) complex. We have previously shown that a titre of anti-HPF4-antibodies of equal/above 4 is of clinical relevance (Am J Med 2003;114:528) and independently predicts an increased in vivo thrombin generation (Thromb Haemost 2004; 91:276). The aim of the present study was to investigate whether the titre of anti-HPF4-antibodies is related to platelet activation and proinflammatory markers.

Methods: We measured anti-HPF4 antibody titres (PaGIA ID-HPF4), soluble P-selectin, beta-thromboglobulin and CD40 ligand (sCD40L) in plasma of 71 patients with suspected HIT, without concomitant infection/sepsis, peripheral artery disease, tumour, renal insufficiency, or surgery in the 48 hours preceding blood sampling. Patients were divided into 4 groups according to the titre of anti-HPF4-antibodies: group 1 = undetectable (n=28), group 2 = titre 1-2 (n=16), group 3 = titre 4-32 (n=21) and group 4 = titre 64-256 (n=6).

Results: Analysis of variance between groups was performed by Kruskal-Wallis test and significant differences were assessed by Dunn's method. Comparison between pairs of groups was performed by Mann-Whitney rank sum test. P-Selectin levels were significantly higher in groups 3 and 4 (median 179.27 ng/ml and 203.10 ng/ml, respectively) than in groups 1 and 2 (63.71 ng/ml and 76.85 ng/ml, respectively; $p < 0.001$). In contrast, beta-thromboglobulin did not show statistically significant changes among the four groups ($p = 0.67$). Finally, group 4 showed significantly higher sCD40L levels (median 0.389 ng/ml) compared to group 3 (0.026 ng/ml), group 2 (0.082 ng/ml) and 1 (0.137 ng/ml; $p = 0.03$).

Conclusion: Consistent with our previous reports, low anti-HPF4-titres do not lead to an increased platelet activation as compared to patients with clinically suspected HIT but without detectable antibodies. Interestingly, patients with very high anti-HPF4-titres have increased levels of sCD40L. This suggests that very high levels of anti-HPF4-antibodies are able to promote a proinflammatory state.

170 DO WE HAVE THE TOOLS TO CURE REFRACTORY ADULT CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA?

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Aim: to evaluate the outcome and therapy of refractory chronic immune thrombocytopenic purpura (ITP) over a period of 17 years, treated in a single centre.

Patients and methods: we collected data from retrospective analysis of 64 patients (pts) affected by PTI, followed in our unit between 1986 and february 2004.

The diagnosis required a platelet (plt) count $< 70 \times 10^9/L$ and exclusion of other haematological disorders, thyroid or liver disease, HIV infections. There were 27 males and 37 females with mean age at diagnosis of 50 years;

36 pts were asymptomatic (56%); 22 pts (34%) had purpura and petechiae; 5 pts (7%) had epistaxis and 2 pts (3%) had gastrointestinal bleeding.

Complete remission (CR) was defined by plt $> 100 \times 10^9/L$; partial remission (PR) plt $> 30 \times 10^9/L$; no response (NR) plt $< 30 \times 10^9/L$.

We defined refractory PTI pts who failed 2 courses of therapy: most failed splenectomy (Sx) or splenic radiotherapy (SRT)

Results:

29 pts had moderate thrombocytopenia (plt count $> 30 \times 10^9/L$ at diagnosis; 21 of these never required treatment, after a median follow up of 49 months (range 2-200); 8 were subsequently treated for worsening thrombocytopenia after a mean time after diagnosis of 21,6 months (3-150). All the 13 refractory pts developed resistance to Sx (10/13) or SRT (3/13). Four elderly pts in whom Sx was not feasible were treated with SRT: one pt entered sustained CR (24+ months) and 2 obtained PR (32 and 19 months) and subsequently relapsed.

Therapy for refractory patients was as follow:

5 pts received cyclosporine A (CyA) at an initially dose of 5 mg/kg per day administered orally twice daily and then reduced to maintain a therapeutic serum level between 200 and 400 ng/mL: 1 pt achieved a sustained CR lasting 36+ months; 2 achieved a PR and 2 were unresponsive; one of them responded to Rituximab at a dose of 375

mg/m2 intravenously once weekly for a total of 4 infusions with a PR of 21+ months.

1 pt received pulse cyclophosphamide (C) 500 mg/m2 weekly for up 6 cycles + CyA, with a sustained CR 38+ months

2 pts received C: one was NR, and one obtained RP (40+ months)

2 pts received a second course of steroid therapy after Sx with RP (108+ and 25+ months)

1 pt was treated with danazol and entered CR (30+ months)

2 pts were treated with rituximab and both entered CR (4+, 8+ months)

There were no major hemorrhagic complication during the follow up of refractory patients.

One patient, treated with C, high-dose dexamethazone and CyA died of invasive pulmonary Aspergillosis.

Conclusions:

Patients with mild thrombocytopenia present a benign course and most never require treatment.

Splenic radiotherapy seems to be a reasonable approach in old patients not suitable for surgery.

Rituximab and CyA can be active agents and should be considered in this subset of patients.

171 ITP IN PREGNANCY - A DIAGNOSTIC AND THERAPEUTIC CHALLENGE

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Therapeutic strategies of ITP in pregnancy are not very well standardized.

We present our one-year experience in 9 cases of ITP in pregnant women. All patients were diagnosed with ITP during pregnancy and received no previous treatment. The clinical status was graded as moderate in 8 patients (petechiae, gum bleeding, echymosis and epistaxis) and severe in one patient (hematuria, purpuric rash, melena and severe epistaxis). All patients had platelet-associated antibodies (PAA) detected by immuno- and flowcitometry. In 3 patients thrombocytopenia was associated with anticardiolipin antibodies. One patient was treated with corticosteroids and aspirin and delivered a healthy child. One patient developed severe thrombocytopenia (of about 1000 plt/mm³) associated with varicella and had a fetal loss despite corticosteroid therapy. The third patient had a spontaneous abortion in the first trimester of pregnancy, with a mean platelet count of 100 000/mm³.

Referring to the other 6 patients, the mean platelet count before therapy initiation was 50 60000/mm³. Therapy was initiated either in the third trimester (9th mo. of gestation) or as the platelet count dropped to 50000/mm³. Two regimens were used: one consisting of low-dose Prednisone (0,5mg/kg/d) for maximum 10 days, followed by Dexamethasone 8-16mg/d before delivery; two patients underwent a second regimen of intravenous immunoglobulin (Octagam). All six patients delivered by cesarean section healthy babies with no haemorrhagic events. All newborns had a low platelet count at birth of about 110000/mm³, which subsequently recovered to the normal levels for age.

Conclusions: ITP in pregnancy is a diagnostic and therapeutic challenge both for the haematologist, obstetrician and neonatologist. The mainstay of treatment relies on prompt recognition and consists of Dexamethasone pulse therapy +/- intravenous immunoglobulin. Prognosis depends not only on therapy, but also on the associated conditions (the presence of anticardiolipin antibodies usually carries out a poor prognosis).

TRANSFUSION MEDICINE AND IMMUNOHEMATOLOGY

172 THE IN VITRO ASSESSMENT OF TNF ACCOMPANYING ABO & RHD INCOMPATIBILITIES

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Objective: In spite of great advances in the field of Immunohematology, there are still some reports of adverse reactions after incompatible blood transfusions due to human mistakes or clerical errors, which in some cases lead to death. Since the clinical manifestations of these reactions are very similar to septic shock syndrome and considering the importance of Tumour Necrosis Factor (TNF) in septic shock, the involvement of TNF in these reactions was also proposed.

Design: Previous in vitro studies with whole blood revealed that subsequent to ABO incompatibility, IgM (Anti-A)-coated donor Red Blood Cells (RBCs), activate complement and the latter will ultimately induce the production of TNF. In vitro studies regarding RhD incompatibilities also revealed that upon exposure of monocyte monolayer with Anti-RhD-sensitized RBCs, TNF appears in culture supernatant.

Methods: In the present study, TNF production following exposure of monocyte monolayer with Anti-RhD (IgG)-coated and Anti-A (IgM)-coated RBCs were compared using TNF bioassay by murine fibrosarcoma cell line, L929.

Results: The obtained results showed that huge amounts (40960 U/ml) of TNF were produced in ABO model, which were significantly more than that of RhD model (1280 U/ml).

Conclusion: Our results suggest that regarding the complete elimination of complement from the culture medium, TNF may be produced by an alternative mechanism, considering the presence of receptor for IgM on the surface of monocytes. Tnf production could be triggered either by cross-linking of Fcγ receptors on the monocytes, or IgM-mediated phagocytosis of RBCs, but the exact underlying mechanism remain to be determined and the two hypotheses are not mutually exclusive.

173 METHYLENE BLUE-PHOTOINACTIVATED PLASMA VERSUS FRESH FROZEN PLASMA AS REPLACEMENT FLUID FOR PLASMA EXCHANGE IN THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Plasma exchange with fresh-frozen plasma (FFP) is the treatment of choice in thrombotic thrombocytopenic purpura (TTP). Methylene blue-photoinactivated plasma (MBPIP) has been proposed as a safer alternative to FFP, but its effectiveness in the treatment of TTP is not well established.

Objective: To investigate whether MBPIP is as effective as FFP in the treatment of TTP by plasma exchange.

Methods: Retrospective analysis of 56 TTP episodes treated by plasma exchange from 1990 to 2003. MBPIP was used for fluid replacement in 27 episodes and FFP in 29. The effect of plasma (MBPIP or FFP) on treatment outcomes was analyzed by multivariate logistic regression.

Results: As compared with patients treated with FFP, those receiving MBPIP had increased risk of dying from progressive TTP (adjusted odds ratio [OR]: 31; 95% confidence interval [CI]: 1.2 - > 100), more recurrences while on plasma exchange therapy (OR: 4.6; 95% CI: 1.2-17), and a lower probability of attaining a sustained remission within 9 days of starting plasma exchange (OR: 5.2; 95% CI: 1.3-20).

Conclusion: MBPIP seems to be less effective than FFP in the treatment of TTP. It is therefore prudent to avoid MBPIP until therapeutic equivalency to FFP is established by randomized, controlled trials.

174 CLINICAL SIGNIFICANCE OF FC-gamma-R IIA AND FC-gamma-R IIIA POLYMORPHISMS IN KOREAN PATIENTS WITH ACUTE AND CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background: Low-affinity immunoglobulin (Ig) Fc receptors, FcγR IIa and FcγR IIIa, are known to be responsible for removal of antibody (Ab) coated platelets in idiopathic thrombocytopenic purpura (ITP). The polymorphisms of these receptors affect the Ig-binding affinity. For this reason, we speculated that the polymorphisms may have an important effect on the pathogenesis or clinical courses of ITP.

Aims: The aim of this study was to identify the relationship between various clinical parameters and FcγR IIa and FcγR IIIa polymorphisms in Korean ITP patients.

Methods: Forty four ITP patients and 28 healthy control, who visited for regular health check-up, were enrolled. Clinical data and whole blood were collected prospectively. After extraction of genomic DNA, allele specific PCR was performed to analyze FcγR IIa and FcγR IIIa polymorphisms. SPSS 11.0 for windows program was used for Chi-square analyses.

Results: The FcγRIIIa polymorphism frequency of the acute ITP group (HH; 7/11, HR 1/11, RR; 3/11) was different from those of the control group (HH; 13/28, HR; 13/28, RR; 2/28) and of the chronic ITP group (HH; 17/33, HR; 15/33, RR; 1/33) (p<0.05). The FcγRIIIa polymorphism frequency of the acute ITP group (ff; 9/11, fv; 2/11, vv; 0/11) was different from those of the control group (ff; 9/28, fv; 14/28, 5/28) and of the chronic ITP group (ff; 9/33, fv; 22/33, vv; 2/33) (p<0.05). To identify the overall effect of FcγRIIa and FcγRIIIa polymorphisms, we analyzed the FcγRIIa and FcγRIIIa polymorphisms together. The most frequent combination of the polymorphisms was HH and fv in the control group (7/28), HH and ff in the acute ITP group (6/11) and HR and fv in the chronic ITP group (12/33) (p<0.05).

Conclusion: We discovered that the polymorphisms of FcγRIIa and FcγRIIIa receptors affect only the pathogenesis of acute ITP. Also, certain combinations of FcγRIIa and FcγRIIIa receptor polymorphisms were more frequently found in acute (HH and ff) and chronic (HR and fv) ITP. However, further study is needed with larger samples.

175 SEVERE CASE OF NEONATAL ALLOIMMUNE

THROMBOCYTOPENIA PROBABLY DUE TO ANTI-HPA-3A

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Background: Maternal antibodies against human platelet antigen (HPA) -3a causing fetal/neonatal alloimmune thrombocytopenia (F/NAIT) are difficult to be verified and documented cases are rare.

Case report: A 25-year-old healthy woman gave birth to a male child in the 38th week of her first pregnancy. Because of a Small for Date Syndrome (1,620g, 44cm) the child was transferred to intensive care unit. Eight hours postpartum the child developed tachypnoe and needed artificial oxygen supply. An antibiotic therapy was started. Furthermore, petechiae and a severe thrombocytopenia (16,000/l) were diagnosed. Four hours later, platelets declined further to 9,000/l. Inflammatory and infection parameters were negative. A random apheresis platelet concentrate was transfused and intravenous immunoglobulin was given (increment 119,000/l). Subsequently, HPA-genotyping of family members was performed. On day (d) 2 platelets (plt) declined to 19,000/l, and plt homozygous for HPA-3b were transfused as a maternal alloantibody against HPA-3a was suspected. During 10d plt-counts ranged between 37,000 and 64,000/l getting normal on d15, without need of further plt transfusion.

Methods and Results: HPA-(-1-5)-genotyping (PCR-SSP, Protrans): mother 1ab, 2aa, 3bb, 5aa, father 1ab, 2aa, 3aa and 5aa, newborn 1ab, 2aa, 3ab and 5aa. No alloantibodies against plt glycoproteins and HLA-class-I-antigens were found in maternal serum using antigen capture ELISA (PakPlus, QuikScreen, GTI). Interestingly, positive reactions were observed in a solid phase assay (Capture-P, Immucor) with HPA-3a plt. Additionally, positive reactions were found with plt of the father and of donors typed HPA-3aa and -3ab but not with plt of HPA-3bb donors using the MASPAT kit (CLB). In MAIPA alloantibodies against HLA-class-I-antigens were observed but could not be confirmed or specified in other tests like lymphocytotoxicity (NIH) and ELISA.

Conclusion: Anti-HPA-3a antibodies occur to be verifiable in assays using intact platelets only. F/NAIT caused by anti-HPA-3a is rare, however, it should be taken into consideration when screening pregnant women and newborns with unsolved thrombocytopenia.

176 ANALYSIS OF IN VITRO FUNCTION OF PLATELETS STORED IN PLASMA OR PLATELET ADDITIVE SOLUTION (PAS III) BY ROTATION THROMBELASTOGRAPHY AND PLATELET AGGREGOMETRY

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Purpose: Platelet function of buffy coat derived platelet concentrates (PCs) resuspended in either plasma or 80 % platelet additive solution PAS III (Macropharma®) was analysed by rotation thrombelastography (ROTEG®, Pentapharm) and platelet aggregometry (BCT, Dade Behring) during 7 days (d) of storage.

Methods: 18 plasma PCs and 6 PAS III PCs were tested on d1, 3, 5 and 7, respectively. Using ROTEG® we analysed Clotting Time (CT), Maximum Clot Firmness (MCF) and Maximum Clot Elasticity (MCE). Using aggregometry, Maximum Aggregation (MA, %) and Maximum Velocity of MA (Vmax, mE/min) were measured. Platelet counts and metabolic markers (Roche Omni) were estimated on d1 and d5, respectively.

Results: In PCs with plasma and PAS III mean concentration of platelets was 2.60.1 and 2.70.6x10E11/unit. pH 7.20.1 and 6.90.1, glucose 257.420.1 and 27.614.9mmol/L and lactate 13.62.7 and 14.92.5mmol/L, respectively.

Using tissue factor as activating agent in the EXTEG® assay of ROTEG®, we observed a significant decrease of MCF and MCE of PCs with PAS III on d7 compared to d5 (38.318.7 vs. 61.03.8 and 76.558.4 vs. 158.426.0, p<0.05, respectively) and to PCs with plasma on d7 (MCF 63.63.1, MCE 176.222.9, p<0.05). No major differences in CT were observed during storage time.

Performing aggregometry, MA decreased significantly on d3, compared to d1 (plasma 69.86.8 % vs. 85.06.7 %, PAS III 17.023.0 % vs. 75.47.4 %, p<0.05). Interestingly, platelets stored in PAS III for 5d could not be stimulated by collagen in contrast to platelets stored in plasma (8.418.3 % vs. 61.010.5 %, p<0.05). Also Vmax of PCs with PAS III declined markedly on d3 compared to d1 (120.0131.5 versus 565.0163.7mE/min, p<0.05) and to plasma PC on d3 (545.3110.5mE/min, p<0.05). (All values as meanSD.)

Conclusion: Platelets stored for 3 days show a reduced disposition for in vitro aggregation independent from storage medium. Remarkably, platelets stored in PAS III seem to be almost resistant against activation by collagen after d3. In contrary, using tissue factor as a strong activator of coagulation, contribution of platelets to clot formation is well maintained during 5d of storage. It is known that in vitro observations cannot predict the functional efficiency of transfused platelets in vivo, as physiological environment is required for optimal platelet function. Therefore, additional studies are required to estimate differences of in vivo platelet function in association to use of variable storage media.

177 HAEMOVIGILANCE: PROPOSAL FOR A COMPREHENSIVE CLASSIFICATION OF TRANSFUSION-RELATED EVENTS

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Background and Aims: Analysis of the transfusion related-events recorded over the last 5 years in the haemovigilance system of a university hospital and proposal of a simple and comprehensive classification of these events in order to facilitate their comparison between different haemovigilance systems.

Material and Methods: Retrospective analysis of all transfusion-related events reported between 1999 and 2003 at the Centre Hospitalier Universitaire Vaudois (CHUV).

Results: During the last 5 years, about 94 100 blood products have been transfused (63 000 erythrocyte concentrates, 7 800 platelet concentrates, and 22 000 fresh frozen plasma). 480 transfusion-related events, corresponding to 5.1 events for 1000 blood products delivered, were reported. Febrile non-haemolytic transfusion reactions were observed in 56% of the cases, whereas allergy was responsible of 21% of the cases. Bacterial contamination (5%) as well as transfusion errors (5%) represented 10% of all cases. Finally, 13% of events were due to isolated signs or symptoms. Platelet concentrates gave rise to statistically significantly (p<0.001) more transfusion reactions (14) than red blood cells (4) and fresh frozen plasma (1.2). Due to the imprecise reporting

of the events, a more detailed analysis of the data was not possible. For instance, the place of near miss events was not clearly defined. Moreover transfusion therapy-related complications were completely ignored, except for some obvious red blood cell allo-immunization (5.5/1000 erythrocyte concentrates transfused), which have been reported since 2003.

Conclusion: In order to facilitate the reporting and analysis of transfusion-related events, a more precise and comprehensive classification should be implemented. Based on our experience, transfusion related events can be divided into two main groups. Group I includes all errors (type IA: transfusion errors and type IB: near miss errors). Group II embraces all events directly related to blood transfusion. Type IIA applies when the event is due to the transfusion of a specific blood product, notably infection, fever and/or shivering and allergy. Type IIB characterizes events happening in the setting of transfusion therapy, such as allo-immunization, metabolic disturbances (hyperkaliaemia, other), volume overload or iron overload. With this simple classification, analysis should be rendered easy and comparison between different haemovigilance systems worldwide made possible, helping rapid identification of transfusion-related events needing investigations and actions.

178 EFFECTIVENESS OF RITUXIMAB IN THE TREATMENT OF ADULT PATIENTS WITH REFRACTORY AUTOIMMUNE CYTOPENIAS

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Background: Chimeric monoclonal antibody anti-CD20 (Rituximab, Mabthera) is highly effective in the management of B cell lymphoproliferative disorders, especially low grade lymphomas. During the last few years, it is also used in less clear indications like autoimmune cytopenias, with promising results. It is considered that the main action of Rituximab is the elimination or depletion of B autoreactive lymphocyte clones that are responsible for the production of autoantibodies.

Patients and Methods: We studied 8 patients with a median age at diagnosis of 33 years (range 17-42) with autoimmune cytopenias refractory to previous therapies or in relapse: 4 patients with ITP (2 male, 2 female), 3 female patients with IgG Coombs (+) AIHA and 1 male patient with cold agglutinin disease. A complete virologic and immunologic examination was negative apart from one patient with ITP who had lupus anticoagulant antibody. Two of the AIHA patients also suffered from homozygous beta-thalassemia, had anti-HCV(+) and were splenectomized in the past. Splenectomy was also performed in two patients with ITP. Most patients had received at least two lines of therapy (corticosteroids, intravenous immunoglobulin, splenectomy, immunosuppressive or immunomodulatory therapy) and had active disease when they started Rituximab. The patients with refractory ITP had less than 25000/ μ L platelets and the patients with AIHA hemoglobin < 8gr/dl and active hemolysis.

The dose of Rituximab was 375 mg/m² every week for four consecutive cycles and then some more cycles every fifteen days. All patients received at least 4 cycles of therapy (range 4-8). The patient with cold agglutinin disease received 6 monthly cycles of Rituximab and then bimonthly as maintenance.

Results: Two patients showed complete response (2/8, 25%). In particular, one patient with ITP responded completely to Rituximab. The duration of his response was 24 months. Then he relapsed and was managed with intravenous immunoglobulin. The patient with cold IgM agglutinin disease also showed complete response (Hb>13.5 gr/dl, normalization of LDH and bilirubin), which lasts over than 20 months. One young non-thalassaemic female patient with AIHA, who didn't respond to Rituximab, was then successfully treated with splenectomy. None of the patients experienced any serious adverse event during the therapy with Rituximab, except from grade I allergic reactions during the Rituximab first infusion in two patients.

Conclusions: The therapy with Rituximab is safe and can lead to complete responses in a subgroup of patients with refractory autoimmune hemolytic anemia or thrombocytopenia. Larger series of more homogenous groups of patients are needed in order to acquire valuable information concerning the identification of patients, which are likely to respond.

179 A STUDY TO DETERMINE THE REASONS FOR NOT TRANSFUSING CROSSMATCHED BLOOD: AN AREA FOR IMPROVEMENT

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Background: It is increasingly recognised that blood is a limited and expensive resource. Effort should be made to maximise usage and minimise wastage. Approximately one third of crossmatched units are not transfused which is an inefficient use of time and resources for blood bank departments. The potential for waste is also increased, as the blood approaches its expiry date. Time expiry is a major cause of wastage for all blood groups at hospitals.

Aims: To determine the reasons why blood was not transfused once requested for crossmatching. In so doing the increased awareness of this potential wastage may modify clinicians behaviour, reducing the number of unnecessary requests and thereby conserve stocks and resources.

Methods: 37 requests for crossmatched blood that were untransfused were randomly selected. The case records were obtained and the reason for blood not being used determined.

Results: There is poor documentation in the case records of the indication for transfusion, as well as why it was not subsequently given. However, the reasons for not transfusing cross-matched blood could be divided into three groups. Group One - the request for the blood is appropriate and for unavoidable reasons is not transfused; Group Two - the request for blood is appropriate, but the blood not given; Group Three - the request for blood is inappropriate. The unavoidable reasons found in Group 1 included patient transfer to another hospital, resolution of a bleeding ulcer, good haemostasis during laparotomy for a case of TTP with a platelet count of eight, and one case found DCT positive. The reasons for not transfusing in Group 2 were avoidable. Often the doctor requesting the transfusion fails to communicate the request so no-one is aware blood is available. Other reasons included differences in clinical opinion, and indecision. Seven of the ten cases had to be re-crossmatched with implications for time and resources. Group 3 requests were inappropriate. Two requests were made for pre-operative patients when Group & Screen is recommended practice. Three requests were made for crossmatch when iron replacement was appropriate. Six cases involved overzealous requesting for crossmatch resulting in transfusion of only some of the units.

Conclusion: Of the cases in which the reasons for not transfusing were avoidable the responsibility mainly lay with the requesting clinicians. It highlighted the lack of education of junior medical staff, and failure of communication between doctors, nurses and blood bank. Education about appropriate blood use would be beneficial. The reason for administration of all transfusions should be routinely documented in patients medical records. The record should also indicate the effectiveness of the transfusion. Consideration may need to be given to more detailed guidelines, or transfusion decisions to be consultant led. By distributing these results we hope to modify clinicians behaviour in ordering transfusions. Ensuring that only appropriate requests for crossmatching blood are made is an area for future improvement.

180 AN OPEN LABEL, MULTI-CENTER STUDY ON THE EFFICACY AND SAFETY OF ANTI-D IMMUNOGLOBULIN IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

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Background: Immune thrombocytopenic purpura (ITP) is a bleeding disorder caused by autoantibodies to platelets. The antibody coated platelets are usually eliminated from the circulation by macrophages. Treatment with anti-D immunoglobulin has shown to be effective in non-splenectomized Rhesus positive patients. The efficacy is attributed to the blockade of macrophage Fc-receptor by antibody-coated red cells.

Aims: The study was designed to show the efficacy and safety of a novel chromatographically purified, solvent-detergent treated and nanofiltered anti-D immunoglobulin in patients with chronic ITP.

Methods: Ninety-eight Rhesus positive, non-splenectomized patients with chronic ITP were included in this prospective, multi-center, open label trial. The platelet count at screening had to be below $30 \times 10^9/L$ and the hemoglobin above 10 g/L. The patients were treated with 50 µg/kg bodyweight anti-D immunoglobulin as a single injection. The study drug was provided in ready to use, pre-filled syringes. Primary efficacy endpoint was the response rate defined by the proportion of patients with an increase of platelet count by

more than $20 \times 10^9/L$ to at least $30 \times 10^9/L$ within 2 weeks following injection. As secondary endpoints the platelet counts at different time points and the regression of hemorrhages were evaluated. Adverse events and laboratory parameters including parameters for hemolysis (hemoglobin, haptoglobin, reticulocytes, bilirubine) were recorded for one month..

Results: Sixty-four female and 34 male patients aged between 12 and 78 years were treated with anti-D immunoglobulin with a total dose between 2400 µg and 5700 µg. The overall response rate was 70%. Mild decrease in hemoglobin was recorded in several patients, but no severe hemolysis or intravascular hemolysis was noted. As expected by the mechanism of action of anti-D, the drop of hemoglobin was transient and patient recovered their hemoglobin within two weeks.

Conclusion: These results confirm previous observations that treatment with anti-D immunoglobulin is effective in chronic ITP. The chromatographically purified anti-D immunoglobulin is well tolerated in ITP patients.

GENETICS LYMPHOID MALIGNANCIES

181 IN MULTIPLE MYELOMA, FISH DOES NOT REVEAL CHROMOSOMAL ABERRATIONS INVOLVING THE DKK1 GENE LOCUS AT CHROMOSOME BAND 10q11

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Introduction: In multiple myeloma (MM), a number of secreted factors, such as RANKL and osteoprotegerin, are involved in the pathogenesis of bone destruction. In a most recent gene expression profiling study, overexpression of DKK1, an inhibitor of osteoblast differentiation, has been demonstrated to be associated with the presence of lytic bone lesions in patients (pts.) with MM (Tian et al., 2003). The mechanisms leading to DKK1 overexpression in this disease remain unclear. The DKK1 gene resides to chromosome band 10q11. In chromosome banding or comparative genomic hybridization studies, chromosome 10 has not been reported to be a frequent site of genomic changes, such as genomic imbalances or reciprocal translocations. However, both techniques have limitations, for instance with respect to their spatial resolution. To our knowledge, the DKK1 gene locus has not been studied yet for chromosomal aberrations using interphase FISH.

Aim: To determine whether or not interphase FISH can reveal chromosomal abnormalities, e.g. high-level amplifications or chromosomal translocations, involving the DKK1 locus in clonal plasma cell disorders.

Material and methods: Bone marrow specimen from 27 pts. (MM, n=20; MGUS, n=7), obtained during routine diagnostic procedures, have been analyzed in this study so far. Among the MM pts., 10 had advanced focal bone lesions as determined by conventional radiographs, while 10 had no or only one osteolysis. FISH and immunocytology were performed as previously described. The BAC probe RP11-346D6 (181 kbp) containing the DKK1 gene was used in combination with various control probes.

Results: Recurring chromosomal gains, especially high-level amplifications involving the DKK1 locus, were not found in this preliminary study. One pt. exhibited four copies of the DKK1 probe. This case was subsequently found to be tetraploid using various control probes. There was no evidence for translocations breakpoints involving the DKK1 locus, especially no disruption of BAC probe RP11-346D6.

Conclusion: Our preliminary data suggests that in clonal plasma cell disorders, the DKK1 locus does not reveal structural alterations detectable by interphase FISH. Data on an enlarged series of pts. will be presented at the meeting.

182 THE HUMAN TUMOR PROTEIN D52 (TPD52): A NEW B-CELL/ PLASMA CELL MOLECULE IDENTIFIED THROUGH A PROTEOMIC APPROACH AND CHARACTERIZED BY A UNIQUE EXPRESSION PATTERN

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Background: Proteomic technologies represent powerful tools to dissect the complexity of protein expression and function in biological systems. Among

their several applications in hematological sciences, they can integrate the hybridoma technology in discovering new antigens of biological and clinical relevance through the systematic characterization of monoclonal antibodies (mAbs) of unknown specificity, that are generated when using whole cells and even purified known proteins as immunogens.

Aim. In this study we aimed at characterizing, by means of a new mAb (B28p), a protein sharing an antigenic determinant with the unrelated molecule IRTA1, which was the immunogen employed in the unforeseen generation of B28p-mAb.

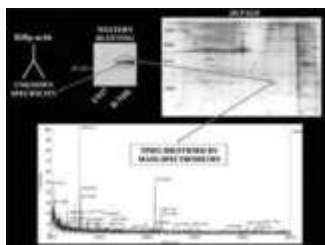
Methods/Results. Among ~1000 hybridomas deriving from mice immunized with the intracytoplasmic portion of IRTA1, one producing an IgG1-mAb (B-28p) was selected for its interesting immunohistochemical reactivity in lympho-hemopoietic tissues. In fact, besides its cross-reaction with IRTA1, it recognized a 28-kDa cytoplasmic protein expressed strongly by all mature B-cell subsets with the highest levels in plasma cells and weakly by tonsil epithelial cells. This pattern clearly differs from that expected for IRTA1, a 70-kDa cell-membrane receptor we previously showed to be selectively expressed in MALT marginal-zone B-cells (Blood 2003;102:3684).

To identify the 28-kDa antigen, the proteome of a B-cell lymphoma sample expressing this molecule was resolved by 2D-PAGE, and the B28p-immunoreactive spot excised and subjected to nano-electrospray ionisation and tandem-mass spectrometry analysis (see Figure). Several peptide spectra were thereby obtained, all matching the human tumor protein D52 (TPD52), previously described to be expressed only in epithelial tissues (Oncogene 1996;12:741). The specificity of B28p-mAb for TPD52 was confirmed by the strong reactivity of the antibody with TPD52-transfected cells at immunocytochemistry and Western blotting, as well as by the identical staining pattern (mature B-cells/plasma cells and, less strongly, epithelial cells) displayed by an anti-TPD52 polyclonal antibody at immunohistology.

To evaluate the usefulness of TPD52 as diagnostic tool in lymphoid malignancies, we then extended the analysis of its expression in routine tumor biopsies, and found it mostly paralleled that seen in their normal counterparts, being observed in all peripheral B-NHL subtypes (except some DLCLs) and most abundantly in multiple myeloma. Notably, TPD52 was also expressed by a proportion of tumor cells in about 40% Hodgkins lymphomas, including cases that had been misdiagnosed as ALK-negative ALCL with null phenotype.

In comparison to other B-cell markers (PAX5, CD19, C79a, CD20), TPD52 represents the first example of a B-cell protein whose expression increases during plasma cell differentiation. Interestingly, by interacting with Annexin-A6 (which we also demonstrated to be strongly expressed in plasma cells) TPD52 has been shown to be involved in the trafficking events essential for digestive enzymes secretion in pancreatic acinar cells, suggesting the hypothesis that up-regulation of TPD52 in plasma cells might play a role in their chief function, i.e. immunoglobulin secretion.

Conclusions. By using a proteomic approach, this study first identifies the epithelium-associated TPD52 protein as a new B-cell/plasma cell molecule characterized by i) unique expression pattern, ii) diagnostic value and, likely, iii) biological importance.



Caption 1: Identification of TPD52 by 2D-PAGE/MassSpec.

183 INCIDENCE AND PROGNOSTIC IMPACT OF CHROMOSOMAL ABNORMALITIES DETECTED BY INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION IN PATIENTS WITH B-CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: B-chronic lymphocytic leukemia (B-CLL) is the most common leukemia of adult people in Western countries. Molecular genetic

characterization of B-CLL made significant progress during the last few years and typical chromosomal anomalies were also identified. The most frequent are deletions 13q14, 17p13, 11q22-23 and trisomy 12. **Aims:** The aim of this study was to establish incidence of chromosomal changes in B-CLL patients and to evaluate their prognostic implications. **Methods:** Interphase fluorescence in situ hybridization (I-FISH) was done on bone marrow and/or blood smears of 217 B-CLL patients (124 males, mean age 59,7 years and 94 females, mean age 61,2 years). Median of follow-up was 29 months. All DNA probes were from VYSIS(tm): CEP12, LSI 13q14, LSI p53, LSI MLL and LSI ATM. Statistical analyses were performed to correlate molecular-cytogenetic findings with disease status (stable versus progressive), Rai stage, CD38/CD19 antigens co-expression, IgVH mutational pattern and other clinical and laboratory parameters. **Results:** Trisomy 12 was found in 35 patients (16%), deletion 13q14 was proved in 112 of 207 patients (54%). Deletion 17p13 was found in 34 (16%) out of 206 examined. Deletion of 11q23 (MLL gene) was analysed in 56 patients and was proved in 7 (12%) of them. Retrospectively, we examined patients positive for deletion 11q23 by LSI ATM probe (region 11q22.3) and deletion of this region was found in six of them. **Conclusions:** No apparent differences in distribution were noted for anomalies +12, del(13)(q14), del(17)(p13) among patients with stable and progressive disease, no consistent pattern in the distribution of type of genomic changes were found among various Rai stages and in CD38/CD19 positive or negative patients. Patients without IgVH mutation had worse prognosis, however distribution of chromosomal abnormalities identified by FISH was the same for patients with and without IgVH mutations.

This work was supported by grant J 13/98 1111 0000 4 MS MT CR.

184 SINGLE NUCLEOTIDE POLYMORPHISMS OF THE BCL-6 PROTO-ONCOGENE: CORRELATION TO CLINICAL FEATURES AND OUTCOME IN FOLLICULAR LYMPHOMAS

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The BCL-6 gene is implicated in the normal germinal center formation and is frequently altered in lymphomas. Genetic modifications are various including translocations, deletions, and somatic mutations of the 5 non-coding region, termed Major Mutation Cluster (MMC), all of them keeping the coding region intact. Three single nucleotide polymorphisms (SNPs) of the MMC have been reported, including 2 substitutions (397G/C, 502G/A) and one point deletion [520(-)]. Frequencies, clinical and biological relevance of these SNPs are unknown in the European population. Based on a French population and case-control study, we assessed the BCL-6 SNPs frequencies in follicular lymphoma (FL) and in 56 NHL with 3q27 rearrangement (including 38 diffuse large B-cell lymphomas). Genomic DNA from lymph nodes biopsies at the time of diagnosis of 105 FL cases with a t(14;18) translocation and control DNA obtained from 106 ethnically matched healthy blood donors were studied. Acquired and constitutional mutations of a 230 bp region encompassing the 3 SNPs were obtained by direct sequencing and were correlated to clinical features and outcome. Analysis performed on 56 matched DNA from PBMCs confirmed the constitutional or the acquired nature of the mutations. The control group was in Hardy-Weinberg equilibrium for the 397C, 397G and 520(+T), 520(-T) alleles but a linkage disequilibrium between the 502A and the 520(-T) variant alleles was observed ($p = .0006$). The C397, 520(-T) and 502A allele frequencies in the FL group were 0.11, 0.08 and 0.02 respectively, as compared to 0.11, 0.15 and 0.00 in controls ($p=NS$). The 520(-T) allele was more frequently observed in 3q27+ NHL compared to t(14;18)+FL (0.19, $p=.01$). The 397 C/C or G/C genotypes ($n = 20$) were significantly correlated to a higher rate of bone marrow involvement ($p=.01$). The 520(+/-+T) genotype ($n = 84$) was correlated to a higher risk of transformation ($p=.07$) when compared to the 520(-/-T) or 520(-/-+) genotypes. No correlation between LDH level, ECOG, overall survival (OS) or disease free survival (DFS) and the different genotypes was observed. Acquired mutations located within the 230 bp region encompassing the SNPs were observed in 15 FL cases (14 %). Recurrent mutations with a clustering effect (9/27 mutations cluster within a 15 bp region) were observed. No correlation was found between mutations and clinical features or OS but a trend for a better DFS in the mutated group was observed (63 months vs. 23 months; $p = .11$). BCL-6 mRNA expression levels measured by quantitative real-time PCR in 2 FLs with a distinct 520T genotype (+/+ and +/-) differ dramatically (40 and 16 in a normalized expression level respectively) but no correlation between genotypes and BCL-6 expression was demonstrated in biopsies samples. Furthermore a gel mobility shift assay using double-stranded DNA probes containing the

520(T) SNP shows representative results for protein/DNA complexes. To conclude, we described a short and highly variable region of the BCL-6 first intron as a consequence of constitutional and/or acquired mutations, which may have biological relevance and clinical impact in FL.

185 GENETIC CHANGES IN LEUKEMIC LYMPHOPROLIFERATION WITH MANTLE CELL PHENOTYPE

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Background: Mantle cell lymphoma (MCL) is recognized as a lymphoma with relatively great morphologic heterogeneity but with a characteristic phenotype (strong IgM, CD5, CD20 positivity, CD23, CD10 negativity of monoclonal B cells), a typical cytogenetic 'the t(11;14)(q13;q32)' and a molecular genetic event (bcl-1/IgH rearrangement) with overexpression of cyclin D1 gene. Additional genetic abnormalities usually with adverse prognostic impact in MCL have been identified in recent years and helped us to explain some different steps in MCL lymphomagenesis. Bone marrow and peripheral blood (PB) are relatively often involved in patients with MCL.

Aims: Description and characterization of genetic changes, survival and prognosis in patients with mantle cell phenotype and leukemic PB involvement.

Methods and results: 57 pts. with MCL phenotype and more than 4.0x10⁹/l lymphocytes in PB were diagnosed at the Department of Hemato-oncology, University Hospital, Olomouc between 1993-2003. The male:female ratio was 39:18, and patient age ranged from 39 to 87 years (median, 66 years). The median of absolute lymphocyte count was 12.3x10⁹/l (range, 4.1-243.0). t(11;14) was detected by FISH in 27/42 pts. (64%), bcl-1/IgH rearrangement by PCR in 13/38 (34%) pts. We divided pts. into 4 groups according to a presence of their genetic changes. In group A, 27/42 (64%) pts. had evidence of t(11;14)FISH (with/or without additional genetic aberration) or bcl-1/IgHPCR or hyperexpression of cyclin D1 PCR at mRNA level. In group B, 6/42 (14%) pts. had complex genetic aberration without evidence of t(11;14)FISH or bcl-1/IgHPCR; cyclin D1 was not examined. In group C, 9/42 (22%) pts. were cyclin D1 negative (6/6), bcl-1/IgH negative (8/8), t(11;14) negative (8/8), without adverse additional genetic changes (4x c12, 1x der14). There were 15 pts. with no material available for analysis in group D. 35 pts. died 1-72 months after the diagnosis (median, 16 months), 20 pts. is alive with median follow-up 13 months (range, 3-82 months). 2 pts. were lost to follow-up.

Conclusion: A poor prognosis and short survival were statistically associated with the presence of t(11;14) or cyclin D1+ (group A) and with complex genetic changes, but without t(11;14) (group B). A better prognosis appeared to be founded in a group of pts. with typical MCL phenotype, but without t(11;14), hyperexpression of cyclin-D1 and complex genetic changes (group C, p=0.005). Most of the pts. in group C had a relatively stable and indolent disease without necessity of an immediate treatment. Further detailed analyses will show if such pts. could be defined as a distinct subtype of indolent non-Hodgkins lymphoma or MCL.

186 SPECTRAL KARYOTYPING REFINES CYTOGENETIC DIAGNOSTICS OF CHROMOSOMAL ABNORMALITIES IN SPLENIC MARGINAL ZONE B-CELL LYMPHOMA (SMBCZL) AND MANTLE CELL LYMPHOMA (MCL)

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BACKGROUND

Mature B-cell neoplasms frequently present complex karyotypes with numerical and structural aberrations. The recent introduction of Spectral Karyotyping (SKY) has made possible the identification of each of the human chromosomes by a different colour, facilitating precise definition of all rearrangements in a tumour karyotype.

Splenic marginal zone B-cell lymphoma (SMZBCL) and mantle cell lymphoma (MCL) are specific low grade small B-cell lymphomas with clinical, morphological, immunophenotypic and histologic characteristics well established by the WHO classification. In SMZBCL the most frequent cytogenetic aberrations are deletions at 7q22-q32, and gains of 3q. Other recurrent chromosomes involved are 1 and 8. Mantle cell lymphoma (MCL), is a relatively aggressive B-cell lymphoma. The t(11;14)(q13;q32) is a consistent chromosome change in mantle cell lymphomas.

AIM

To define by SKY complex karyotypes from patients with SMZBCL and MCL.

METHODS

We present four patients with a SMZBL and four with a MCL in whom the diagnosis was morphologically, phenotypically and clinically confirmed. All samples were studied at diagnosis and were obtained from peripheral blood (4 cases), bone marrow (3 cases) and spleen (1 case). Conventional cytogenetic studies have been performed following standard protocols. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN). The SKY technique was applied according to the manufacturers protocol with minor modifications.

RESULTS

All samples showed a complex karyotype with chromosome aberrations which, in most cases, were not fully characterized by G-banding. The SKY technique was able to refine the designations of over 23 aberrations not well defined by G-banding (Table 1).

Among the eight cases with clonal abnormalities, the G-banded karyotype of four cases remained unchanged after SKY analysis. In the remaining four, SKY provided additional cytogenetic information. G-banding analysis detected a total of 39 aberrations, of which 9 were undefined (4 additions, 3 markers and 1 derivatives). All the undefined aberrations were resolved by SKY. Of the 39 aberrations, SKY confirmed 16 and revised 23.

CONCLUSIONS

In our knowledge, a limited number of Mature B-cell Lymphomas have been studied by SKY.

In some cases, is difficult to define chromosomal aberrations by G-banding because of karyotype complexity or poor morphology. Therefore, SKY provides a useful complementary technique to routine G-banding in the cytogenetic analysis of lymphomas.

In our study, new aberrations have been detected by SKY, der(13)t(3;13)(q21;q32), der(7)t(7;9;13), der(9)t(9;15)(q34;q15), inv(3)(p13q26) and del(10)(q24q26) in SMZBCL, and t(1;1)(p22;p36), i(13)(q10), der(13)t(3;11;13), der(12)t(8;12)(q11;p13), der(15;21)(q10;q10), t(2;11;14)(p21;q13;q32) and t(11;12;15)(q13;q23;q22) in MCL.

New cytogenetic characterisation provides the basis for investigations to identify molecular mechanisms in Mature B-cell Lymphomas.

ACKNOWLEDGEMENTS

This work has been partially supported by the grants SAF-4947, GO3/179, CO3/10 and PI030394 from the Spanish government. We want to thank Carme Melero and Juan Cruz Cigudosa for their help in the SKY technique.



Caption 1:

187 FUNCTIONAL ANALYSIS OF THE PAX5/TEL CHIMERIC PROTEIN

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Background: we previously cloned the PAX5/TEL chimeric gene, originated from the translocation t(9;12)(q11;p13) in an ALL adult patient. Recent data indicate that PAX5/TEL fusion defines the cytogenetic entity dic(9;12)(p13;p13). PAX5/TEL is likely to be an aberrant transcription factor, resulting from joining the 5' region of the partner gene PAX5 (a transcription factor essential for B cell development) to the 3' region of TEL/ETV6 (Ets-family DNA binding domain).

Aim of the study was to investigate the functions of the PAX5/TEL chimeric protein as a potential oncoprotein.

Methods: we have cloned the FLAG-full length chimeric PAX5/TEL cDNA in the retroviral vector pMSCV-IRES-GFP (MigR1). NIH3T3 murine fibroblast and IL-3 dependent murine proB Ba/F3 cells lines were

transduced with the retroviral construct to analyze subcellular localization and transforming activity of PAX5/TEL.

Results: immunofluorescence analysis showed a specific nuclear localization of the chimeric protein in NIH3T3. Soft-agar colony forming assay of NIH3T3 infected with MigR1-PAX5/TEL did not show a significant transforming activity of the chimeric protein. IL-3 dependent murine proB Ba/F3 cells infected with MigR1-PAX5/TEL showed modulation of cellular growth rate; single subclones expressing the chimeric protein showed a decreased growth respect to control subclones. In addition, the expression of PAX5/TEL in Ba/F3 did not induce IL-3 growth independence. In summary, preliminary results did not show oncogenic activity of the PAX5/TEL fusion. Further analysis are needed to evaluate the functional role of the chimeric PAX5/TEL protein in Ba/F3 and other haematopoietic cell lines.

188 GENE EXPRESSION PROFILING OF POST-TRANSPLANTATION LYMPHOMAS

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Background. Transplanted patients may suffer from post-transplantation lymphoproliferative diseases (PTLD). PTLD are often the consequence of uncontrolled growth of B cells infected by the Epstein-Barr virus (EBV) in an immunosuppressed environment. The in vivo steps leading to such lymphoproliferative diseases are poorly defined. Clinical and experimental data support that T helper (Th) type 2 cytokines may promote PTLD development, whereas Th type 1 cytokines could inhibit such a process.

Aim. We defined here molecular signatures associated with the in vivo development of PTLD in a xenochimeric model of aggressive human B cell PTLD. Methods. Severe combined immunodeficient (SCID) mice were intraperitoneally injected with human peripheral blood mononuclear cells (PBMC) from donors with latent EBV infection. Such SCID mice developed intraperitoneal tumors characterized by polymorphic or diffuse large B cell lymphoma morphologies. Gene expression in PTLD was compared to that in PBMC by DNA microarray and real-time PCR.

Results. DNA microarray analysis for about 11 500 genes identified around 300 genes with significant expression differences. Up-regulated genes comprised notably: i) ninety-three genes implicated in cellular metabolism or replication, reflecting the higher proliferative activity of the PTLD cells, ii) ten B cell specific genes, probably in relation with the increased proportion of B lymphocytes in the PTLD as well as changes in B cell differentiation induced by EBV, iii) nine genes having roles in cell-extracellular matrix interactions and iv) 17 genes implicated in signaling or signal transduction. Few cellular genes implicated in oncogenesis (n=5) or in apoptosis inhibition (n=1) were upregulated, which may be explain by genes with such functions already present in the EBV genome. Of the down-regulated genes, most were T lymphocyte and macrophage related reflecting the relative purification of B cells in the material analyzed. Expressions of Th type 1 and 2 cytokine genes were subsequently analyzed by real-time PCR. Preliminary results on 4 PTLD and 3 PBMC indicated that expressions of IL-12, IFN-gamma and TNF-alpha (type 1), and IL-10 (type 2) genes were up-regulated in PTLD, and those of IL-2 and IL-15 (type 1) and IL-4 (type 2) genes were down-regulated.

Conclusion. This large-scale gene expression analysis reveals the complexity of factors associated with EBV-dependent lymphomagenesis. In particular, PTLD can arise in a complex Th type cytokine environment, where B cell proliferating cytokines are both up-regulated (i.e. IL-10, TNF alpha) and down-regulated (i.e. IL-2, IL-4, IL-15). These observations may help to dissect cytokine-mediated mechanisms involved in PTLD development and control, which may be relevant for designing new immune-mediated strategies for PTLD prevention or treatment.

189 TEL/ARG INDUCES CYTOSKELETAL ABNORMALITIES IN 293T CELLS

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Background: the Ets variant gene 6 (ETV6/TEL), a transcriptional repressor, is rearranged in the majority of haematological patients with 12p13 translocations and fused to a number of different partners. We previously identified TEL/ARG, a novel fusion transcript consisting of the oligomerization domain of TEL and the catalytic domain of the tyrosine kinase Abelson-related gene (ARG), in a case of acute myeloid leukaemia carrying a t(1;12)(q25;p13).

Aim of the study was to investigate the transforming activity of TEL/ARG, focusing on interactions with the cytoskeletal elements, as described for the homologue BCR/ABL.

Methods: we fused the FLAG sequence to the TEL/ARG cDNA and cloned this construct in the bicistronic lentiviral vector pRRLPPT.CMV.iresGFPpre at 5' terminal of the IRES sequence.

Results: we analysed the subcellular localization of the chimeric proteins in transduced human 293T fibroblasts by immunofluorescence using an anti-FLAG antibody; we found that TEL/ARG was cytoplasmatic and co-localized with the actin. Moreover, its expression in 293T, monitored by GFP expression and western-blot analyses, induced a morphologic change to fibroblasts, which underwent cell rounding and detachment from the tissue culture plastic.

Conclusion: TEL/ARG transcript seems to induce abnormalities of cytoskeletal function, a potentially important mechanism for its oncogenic activity. It will be interesting to investigate the effects of TEL/ARG expression in haematopoietic precursor cells, where other authors described a role of the homologue BCR/ABL in inducing cytoskeletal abnormalities and altered adhesion to extracellular bone marrow matrix protein.

190 IN MALIGNANT LYMPHOCYTES EXPRESSION OF DAXX

INCREASES APOPTOSIS BY PROMOTING EXTRINSIC SIGNALING

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Background: The role of DAXX, in particular its ability to promote or hinder apoptosis, still remains controversial. Aims and Methods: In order to elucidate the functional relevance of DAXX in the extrinsic signaling of malignant lymphocytes Jurkat T-cells were stably transfected with a DAXX-expressing vector or with the respective DAXX-negative control vector. Results: We thus demonstrate that upon stimulation of the extrinsic pathway by incubation with an agonistic Fas- or TRAIL-antibody overexpression of DAXX increases the rate of apoptosis as well as the disruption of mitochondrial membrane potential by enhancing activation of the initiator caspases-8 and -10, as well as of the executioner caspases. Incubation of Jurkat clones with the pan caspase inhibitor zVAD-fmk abrogates the apoptosis-promoting effects of DAXX, proving the caspase-dependence of the described effects. Noteworthy, expression of DAXX enables Jurkat cells to retain an increased rate of apoptosis and disruption of MMP despite inhibition of caspase-8 or caspase-3 by alternatively activating initiator as well as executioner caspases.

Conclusion: Our study defines the functional relevance of DAXX as a proapoptotic protein in the extrinsic signaling of lymphatic cells, demonstrating its impact on the activation of initiator and executioner caspases, and in particular its ability to enable cells to circumvent inhibition of caspases-8 and -3.

191 G-CSF MOBILIZATION INDUCES SIGNIFICANT CHANGES IN GENE EXPRESSION PROFILE WHICH ARE UNDETECTED THREE MONTHS LATER.

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Background: G-CSF is the most commonly used agent for mobilization hematopoietic stem cells into the periphery in healthy donors for allogeneic transplantation. Thought, it is well known that G-CSF stimulates proliferation and developing of myeloid cell line, many of its effects and their possible repercussion in healthy donors are still unknown.

The possibility of studying thousand of genes in the same experiment by microarrays technology could provide a powerful informations previously unknown.

Aims: To analyse G-CSF effects on peripheral blood cells transcriptome of healthy donors. To evaluate how long these effects are present.

Methods: Seven donors mobilized with G-CSF (5 microg/kg/q12h for 4 days) were included. Peripheral blood samples were collected before starting G-CSF (day 0), the day of collection (day +5) and 3 and 6 months after mobilization.

Methods: Total RNA (5-10 microg) was labeled and hybridized to Human Genome U133A microarray (Affymetrix). Hierarchical clustering algorithm and multidimensional scaling method were used in the unsupervised approach. For supervised learning, Significance Analysis of Microarrays (SAM) was performed.

Results: Two-way hierarchical cluster analysis showed a dendrogram with 2 major branches whitth the samples before G-CSF and on the day

+5, after G-CSF. Supervised analysis identified 939 significant genes that distinguished the two groups: 499 increase their expression level and 430 decrease their expression level after G-CSF. The most affected genes functions categories were adhesion, apoptosis, cellular cycle and extracellular matrix. Hierarchical cluster analysis adding 3 and 6 months after G-CSFs samples, showed a dendrogram with 2 mayor branches: samples on day of collection on one branch, and samples of 3 and 6 months after G-CSF and before mobilization on the other branch. There were no changes in the gene expression before G-CSF and 3 and 6 months after G-CSF on the gene expression profile of peripheral blood cells.

Conclusion: The use of G-CSF as hematopoietic stem cells mobilization agent in healthy donors induces changes in expression levels of multiple genes involved on important mononuclear blood cells functions. This gene expression changes are temporal and returned to the normal profile after three months.

192 TCR REPERTOIRE AND LYMPHOID MALIGNANCIES: A FAMILIAL STUDY

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Background: Genetic predisposition to hematological neoplasms has been supported by some studies and, in particular, a familial aggregation has been demonstrated for chronic lymphocytic leukemia. A significantly increased number of oligoclonalities has been demonstrated in the T cell receptor BV (TCRBV) repertoire of patients with CLL and NHL. Whether or not such distortions in the TCRBV repertoire expression could play a role in the development of lymphoproliferative diseases is at present unknown.

Aims: The purpose of this study was to identify variations in the TCRBV repertoire expression in members of families in which more than one case of lymphoproliferative neoplasms was present.

Methods: Peripheral blood samples were collected from people belonging to nine families in which more than one member was affected by lymphoid malignancies (group A) and to six families in which only one person was affected (group B). We analysed 16 patients (6 NHL, 4 ALL, 4 HD, 2 MM) and 18 healthy relatives in the group A and 6 patients (3 NHL, 1 ALL, 1 HD, 1 MM) and 11 healthy relatives in the group B. Data were compared with those obtained in 30 unrelated healthy controls. The TCR BV repertoire in CD4+ e CD8+ T lymphocytes was analysed by flow cytometry using the following Anti-TcR mAbs: BV1, BV2, BV3, BV5.1, BV7, BV8.1, BV13.1, BV14, BV17, BV20, BV21.3 and BV22. All BV mAbs were FICT-conjugated. Triple staining of BV mAbs with CD4-PE and CD8-PC5 was performed. Statistical comparison between the mean TCRBV expressions determined in distinct groups was calculated through the non-parametric Wilcoxon test. The expansions of BV3 in one family were molecularly investigated by CDR3 spectratyping.

Results: The mean expression of each BV families in CD4+ and CD8+ T lymphocytes did not show any significant difference between the groups A and B, both considering patients and healthy relatives together and comparing patients and relatives separately. Moreover, we did not evidence any significant difference in the number of BV expansions between the two groups. Interestingly, we detected a strong expansion of the BV3 in the CD8+ subset in all members of a family (2 LNH and 2 healthy relatives). By CDR3 spectratyping the expanded BV3 resulted to be clonal only in one person affected by NHL, whereas a polyclonal profile was detected in the other members. Finally, the TCRBV pattern found in group A and B did not differ from that determined in controls.

Conclusions: The TCR repertoire in members of families with high incidence of lymphoid malignancies turned out to be similar to that evidenced in healthy controls. However, a TCRBV3 expansion was present in all the four members of a family in which two cases of NHL were present. This finding could be related to the presence of the well-known BV3 genomic polymorphism, i.e. allele 2. It has been reported that BV3 allele 2 carriers show antigen-driven TCRBV3 high expression. Further analyses, especially regarding the TCRBV genomic polymorphisms, should be performed in families with aggregation for a specific lymphoproliferative disorder.

CYTOKINES / GROWTH FACTORS

193 SYNTHETIC ADENOSINE RECEPTOR AGONISTS MODULATE MURINE HAEMATOPOIESIS: A STUDY EMPLOYING THE CYTOTOXIC ACTION OF 5-FLUOROURACIL

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Background

It has been recognized that adenosine and its analogues exhibit signalling activity in regulation of a variety of functions including cell proliferation, differentiation and apoptosis. Many of these processes are mediated by activation of specific receptors localized on cell surface. In the last twelve years the laboratory of the authors has been engaged in research on modulation of haematopoietic processes by activating adenosine receptors. Haematopoiesis-stimulating effects of elevated extracellular adenosine obtained by combined administration of adenosine monophosphate, an adenosine produg, and dipyrindamole, inhibiting the cellular uptake of adenosine, have been reported by our research group.

Aims

Adenosine cell surface receptors exist in subtypes, denominated A1, A2A, A2B, and A3, which differ in activation of various signalling pathways. The above approach for activating adenosine receptors was non-specific. Nowadays, however, synthetic adenosine analogues are available which are used to define the receptor subtypes involved in the signalling pathways of extracellular adenosine. The aim of this study was to investigate the effects of selected adenosine receptor agonists on murine haematopoiesis by utilizing the model of myelosuppression induced by 5-fluorouracil (5-FU), a cycle-specific cytostatic agent.

Methods

Experiments were performed on (CBAx57BL)F1 mice. Actions of a non-selective agonist 5-(N-ethylcarboxamido)adenosine (NECA) and of selective agonists for A1 (N6-cyclopentyladenosine, CPA), A2A (2-p-(carboxyethyl)-phenethylamino-5'-N-ethylcarboxamidoadenosine, CGS 21680), and A3 (1-deoxy-1-(6-[[[3-iodophenyl]methyl]-amino]-9H-purin-9-yl)-N-methyl-β-D-ribofuranosamide, IB-MECA) receptors were tested. Adenosine receptor agonists were given in single injections in various time intervals before 5-FU administration and the effects were evaluated 4 days later. Bone marrow cellularity of haematopoietic organs was determined and in vitro clonogenic assays for progenitor cells for granulocytes and macrophages (GM-CFC) and erythrocytes (BFU-E) were performed.

Results

The non-selective agonist NECA given at a dose of 200 nmol/kg induced biphasic time-dependent effects, i.e. protection and sensitization, when given 10 hours and 22 hours before 5-FU administration, respectively. The use of isomolar doses of selective adenosine receptor agonists indicated that the protective effects of NECA was mediated by activation of A2A receptors, while the sensitizing action of NECA was mediated via A3 receptors. In addition, it was observed that A1 receptors induced protection when activated by administration of CPA 22 hours before 5-FU.

Conclusions

It follows from these findings that murine haematopoiesis may be modulated by activation of adenosine cell surface receptors both positively and negatively. These results are not only of theoretical importance but they may find also practical implications in treatment of myelosuppression induced by ionizing radiation or cytostatic drugs.

This work was supported by the Grant Agency of the Czech Republic (grant no. 305/02/0423).

194 REGULATORY ROLE OF ADENOSINE SIGNALLING IN HAEMATOPOIESIS: MOBILIZATION AND IN VITRO STUDIES

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Background:

Signalling through adenosine receptors is an important regulatory mechanism modulating cell cycle, differentiation, viability and apoptosis. Experimental evidence suggests that adenosine can act extracellularly in concert with other regulatory molecules to modulate cell proliferation. Previous studies performed in our laboratory have demonstrated that

drugs elevating extracellular adenosine stimulated haematopoiesis in normal and myelosuppressed mice and potentiated the action of granulocyte colony-stimulating factor (G-CSF).

Aims:

The aim of the studies whose results are presented here was 1) to investigate the effect of extracellular adenosine given alone or in combination with G-CSF on mobilization of progenitor cells for granulocytes and macrophages (GM-CFC) and granulocytes into the peripheral blood, and 2) to assess the ability of sera of mice treated with these drugs to stimulate proliferation of GM-CFC in vitro.

Methods:

In vivo experiments were performed on (CBAx57BL)F1 mice. Elevation of extracellular adenosine was achieved by joint administration of dipyradamole (DP), a drug inhibiting the cellular uptake of adenosine, and adenosine monophosphate (AMP), an adenosine prodrug. DP+AMP, G-CSF or all these drugs were administered either singly or repeatedly in a 4-d treatment regimen and the effects were evaluated 6 or 24 h after the last injection. Numbers of granulocytes and GM-CFC in the peripheral blood were determined.

For evaluation of colony-stimulating activity of blood serum of mice given DP+AMP, G-CSF or all these drugs, sera in various time intervals after drug administration were obtained, added to the cultures of normal bone marrow cells, and numbers of GM-CFC were determined 7 days later.

Results:

Elevation of extracellular adenosine has been found to mobilize significantly both GM-CFC and granulocytes after both single and repeated administration of DP+AMP. When the combination of DP+AMP+G-CSF was given under the 4-d regimen, the mobilizing effects of its administration have been observed to be additive when compared with those of DP+AMP alone or G-CSF alone.

In comparison with controls, increased colony-stimulating activities of the sera of mice given DP+AMP, G-CSF or combination of all the drugs were found. When sera of mice given combination of DP+AMP+G-CSF were added to the cultures, significantly higher counts of GM-CFC colonies were found when compared with cultures containing serum of mice given either DP+AMP alone or G-CSF alone.

Conclusions:

The results of in vivo experiments on mobilizing effects of the drugs studied show that elevation of extracellular adenosine obtained by administration of DP+AMP presents a potent mechanism for mobilization of GM-CFC and granulocytes into the blood, and that elevated extracellular adenosine can potentiate the mobilizing action of G-CSF. In vitro studies reveal that serum of mice given drugs elevating extracellular adenosine displays distinct positive action on production of GM-CFC colonies from normal bone marrow cells and that this effect may be intensified if combination therapy of DP+AMP with G-CSF is given. Mobilizing and haematopoiesis-stimulating effects of drugs elevating extracellular adenosine might be probably exploited also in clinical practice.

This work was supported by the Grant Agency of the Czech Republic (grants no. 305/03/D050 and 305/02/0423).

195 EFFECT OF TGF-, INHIBITORS OF MITOGEN-ACTIVATED PROTEIN KINASES AND PROTEASOME INHIBITOR ON TGF-RECEPTORS MRNAS, SMAD MRNAS AND TARGET PROTEIN P21CIP1/WAF1 MRNA LEVELS IN CTV-1 LEUKAEMIA CELL LINE

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Cytokines of the transforming growth factor- β (TGF- β) superfamily exert their cellular effects by binding and bringing together two classes of transmembrane receptors that possess serine/threonine kinase activity. Type II receptor transphosphorylates the type I receptor, which then transduces the signal to Smad proteins. Resistance of tumour cells to TGF- β seems to contribute to the development of many tumour types. The downregulation of transcription of Smad genes in bone marrow mononuclear cells which we found in 10-20% of acute myeloid leukaemia and myelodysplastic syndrome patients could be involved in reduced responsiveness to cytokines of TGF- β superfamily. Inhibitors of mitogen-activated protein kinases (MAPK) and proteasome inhibitors offer a potential alternative to standard chemotherapy in leukaemia treatment. In order to investigate the effect of these drugs on TGF- β receptors, Smad and the target protein p21CIP1/WAF1 mRNA levels we used a human leukaemia cell line CTV-1 established from a patient with relapsed acute monoblastic leukaemia (Chen et al., Gann 75, 660-664, 1984). This line was bought from the DSMZ-German Collection of Microorganisms and

Cell Cultures. Imai et al. (Oncogene 20, 88-96, 2001) described the heterozygous mutation in Smad4 gene in CTV-1 cells. We did not find this mutation and we found that the proliferation of these cells measured by tritiated thymidine incorporation assay is increased by 175% by the treatment with monoclonal antibody to TGF- β 1 (20 μ g/ml). TGF- β 1 (5 ng/ml and 10 ng/ml) reduced proliferation of these cells by 50%. CTV-1 cells were 24 h preincubated with TGF- β 1 or with monoclonal antibody to TGF- β 1, then incubated for 8 h with tritiated thymidine and its incorporation into DNA was analysed. MAPK kinases inhibitors (the p38 MAPK inhibitor-5 μ M SB203580 and the extracellular signal-regulated kinase inhibitor-5 μ M UO126) and proteasome inhibitor (40 μ M MG132) were used in incubation for 8 or 24 h. Total cellular RNA was isolated and the levels of specific mRNA were determined by reverse transcription and polymerase chain reaction amplification (PCR) using specific oligonucleotide primers. Both MAPK kinases inhibitors and proteasome inhibitor decreased TGF- β receptors (T β RII and ALK5) mRNA levels and also Smad1,2,3 and 4 mRNA levels in CTV-1 cells incubated for 8 or 24 h. TGF- β 1 (5ng/ml and 10 ng/ml) also reduced TGF- β receptors and Smad1,2,3 and 4 mRNA levels in CTV-1 cells incubated for 8 or 24 h. However, the levels of mRNA for p21CIP1/WAF1 that is a target for TGF- β action is increased after treatment with TGF- β and the proteasome inhibitor MG132. MAPK inhibitors reduced p21CIP1/WAF1 mRNA levels. These results together with results of other laboratories show that a possible use of these drugs in treatment of leukaemia needs further research. This study was financially supported by the Internal Grant Agency of the Ministry of Health, Czech Republic (NC/7605-3).

196 TUMOR NECROSIS FACTOR, LYMPHOTOXIN- α AND INTERLEUKIN 6 GENETIC POLYMORPHISMS AND RISK OF DEVELOPMENT HEMATOLOGICAL MALIGNANCIES

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Background. Hematological malignancies (HM) are the most common form of cancer in childhood. Nowadays many clinical research efforts have focused on stratification of patients into various risk groups based on the different prognostic features. Tumor necrosis factor (TNF), lymphotoxin- α (LTA) and interleukin 6 (IL-6) are cytokines with pleiotropic biological activities, including the induction of programmed cell death and the regulation of immune cell proliferation and differentiation. The secretion of appointed cytokines is influenced by genetic polymorphisms within their genes.

The aim of this study was to test the 308TNF and +252LTA, -174IL-6 gene polymorphisms for association with hematological malignancies in children from the Bashkortostan Republic.

Methods. PCR-RFLP-based genotyping assays were used to determine the frequency of polymorphisms -308G/A TNF, +252A/G LTA, -174G/C IL-6 genes in a case-control study comprised 120 children patients with hematological malignancies and 167 healthy individuals from Bashkortostan Republic. Among HM patients, 95 had acute lymphoblastic leukemia (ALL), 14 with acute myeloblastic leukemia (AML) and 11 with Non Hodgkins lymphoma (NHL). Ninety-eight patients had first presentation of disease, 22 were with relapse. There were 29 patients with lethal outcome of disease among 120 children.

Results. According to our results, TNF*GG genotype was in association with NHL (OR=4,7) and with relapse form of HM (OR=9,9; p=0,02) in children. We also determined protective role of TNF*GA and LTA*AG genotypes in the development of NHL (OR=0,2). Individuals carrying LTA*AG genotype had a higher risk of development AML (OR=1,8) and lethal outcome of HM (OR=1,4).

We determined the most frequent genotype combinations of TNF and LTA genes in the patients groups, so the combinations TNF*GG/LTA*GG, TNF*GA/LTA*GG were characterized for ALL patient; combinations TNF*GG/LTA*AA - for NHL; TNF*GG/LTA*AG, TNF*AA/LTA*AG - for AML.

The frequency of IL-6*GG genotype was increased in ALL (OR=2,9) and NHL (OR=2,1) patient groups. In the group of survivor after management of HM relapse

IL-6*GG genotype was found in 67% versus 36% in controls (OR=2,9). It is important, that IL-6*CC (low risk genotype) were not found in the AML group.

Summary. We found that genotyping of TNF superfamily genes more important in the group of patients with NHL and AML. Our results suggest a role of the investigated genetic polymorphisms with regard to risk of development HM in children.

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197 EFFECT OF TUMOR NECROSIS FACTOR ON ADHESIVENESS OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECS) IN NON-HODGKINS LYMPHOMAS AND GASTRIC CANCER

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Background. Metastatic spreading of some neoplasms are thought to depend upon the adhesion of tumor cells to vascular endothelium followed by extravasation into surrounding tissues. One of the key regulators of endothelial cells interactions is tumor necrosis factor (TNF) which likely is elaborated in higher concentrations in cancer and non-Hodgkins lymphoma (NHL) patients (pts), can modify the EC properties with increasing of their reactivity to tumor cells adhesion and metastasis. The aim of our investigation was to determine the attachment of labeled by 3H-thymidine some cell lines to monolayer of HUVECs in vitro after stimulation of rhTNF (Sigma, USA), plasma and conditional mediums (CM) of primary cultures of gastric cancer (GC) cells, mononuclear cells from lymph nodes (MNLN) and from affected spleen (MNS) as well as mononuclear cells from peripheral blood (MNPB) of 39 NHL and 21 GC pts, who hadn't been treated before investigation, and to compare these data with endogenous TNF (enTNF) production.

Methods. TNF level was detected by bioassay with measuring its cytotoxicity for sensitive L929 cell line. Endothelial cells were obtained from human umbilical vein by digestion with 0,2% collagenase and were cultivated as described (Jaffe E.A., et al., 1973). GC tumor samples were disaggregated by collagenase and DNase. The tumor cells suspensions were incubated for 24 h and their CM were then removed for the assay. Cell lines K562, L1210, L929 were incubated in RPMI 1640, supplemented with 10% FCS and were not activated before investigation. The attachment of K562, L1210, L929 cells to HUVECs was estimated by the index of adhesion (IA).

Results. Obtained data indicated that plasma as well as CM from NHL pts stimulated an adhesive activity of HUVECs. Furthermore CM of MNLN and MNS from NHL pts, where enTNF levels were 0,1810,066 ng/ml and 0,1460,028 respectively, showed more strong influence on adhesiveness of HUVECs concerning both L1210 and K562 cells, in comparison with CM of MNPB (enTNF = 0,1150,021 ng/ml). Nevertheless enTNF level in plasma of NHL pts was significantly higher (0,9160,127 ng/ml) than in all cells CM ($p < 0,001$), its influence on adhesive activity of HUVECs (IA = 1,900,11 for K562 and IA = 1,720,40 for L1210) was similar to those ones. After influence of rhTNF in concentrations from 0,001 up to 0,024 ng/ml HUVECs demonstrated considerable adhesiveness to L1210 (IA from 1,42 to 3,02). RhTNF in the concentrations from 0,030 up to 0,065 ng/ml slightly diminished the attachment of L1210 (IA < 1,0).

Conclusions. rhTNF in vitro demonstrates mainly stimulative influence on adhesive properties of HUVECs except of its narrow range and reveals this in a dose-dependent manner. On the whole plasma as well as CM of different cells origin, in particular from malignant lymphoid tissues stimulated adhesiveness of HUVECs. On the other hand we didn't find tight correlation between enTNF concentration and adhesive activity of HUVECs in NHL and GC pts. This may suggest that dissemination of these malignancies is an active, multifactorial, versatile and well-controlled process.

198 CLINICAL RELEVANCE OF VASCULAR ENDOTHELIAL GROWTH FACTOR (sVEGF) LEVELS IN CHILDREN WITH ACUTE LEUKEMIAS AND MALIGNANT LYMPHOMAS

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Background: The importance of angiogenic factors i.e. VEGF although clearly established in solid tumors, there is limited information about its role in childhood acute leukemias, Hodgkin's lymphomas and Burkitt's lymphomas. In the present study we wanted to evaluate sVEGF levels in children with acute leukemia and lymphoma at diagnosis and in remission.

Materials and methods: The levels of sVEGF were measured by ELISA in 32 children (6 AML, 5 ALL, 10 Burkitt's lymphoma-BL, 2 T-cell lymphoma, 9 Hodgkin's lymphoma-HL) and 15 healthy controls. Age range was 7 months-15 years (median 8 years).

Results: sVEGF levels of children with AML were 2-1040 pg/ml (median 35 pg/ml) and were 136-1960 pg/ml (median 630 pg/ml) at diagnosis and

in remission respectively ($p < 0.001$). sVEGF levels of ALL patients were 2-640 pg/ml (median 116 pg/ml) and were 116-956 pg/ml (median 136 pg/ml), respectively ($p > 0.05$).

In contrast to leukemia groups, sVEGF levels of children with malignant lymphoma were higher at initial diagnosis than in remission. In BL, sVEGF levels were 2-1200 pg/ml (median 490 pg/ml) and were 136-1960 pg/ml (median 630 pg/ml) at diagnosis and in remission respectively. In HL, sVEGF levels were 6-170 pg/ml (median 140 pg/ml) and were 44-354 pg/ml (median 114 pg/ml) at diagnosis and in remission respectively. The difference was statistically significant ($p < 0.001$). In our study, there were only 2 T-cell lymphomas, interestingly one of those with nasal NK/T cell (angiocentric) lymphoma had the highest level of sVEGF at initial diagnosis (2360 pg/ml) which decreased to after achieving remission (136 pg/ml).

Conclusion: Our preliminary results showed that the changes of sVEGF in hematopoietic malignancies have clinical importance, similar to findings in solid tumors. However, in contrary to the few reports, sVEGF levels were higher in remission when compared to initial diagnosis in our leukemia group. That might be related to the reconstitution of normal hematopoietic cells and healthy microenvironment in the bone marrow. On the other hand, in lymphomas, lower sVEGF levels in remission compared to initial diagnosis might be due to decreased tumor burden and related angiogenesis. We want to conclude that the measurement of sVEGF may be regarded as a useful marker to assess the clinical status of hematopoietic and lymphoreticular malignancies.

199 REQUIREMENT OF ERYTHROID CELL STIMULATING FACTOR DURING ENHANCED ERYTHROPOIESIS

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We have demonstrated earlier the presence of an erythroid cell stimulating factor (ESF) in mouse serum that enhances the erythropoietin-dependent erythroid cell proliferation in vitro. Employing a highly specific monoclonal antibody generated in our laboratory we have also shown that ESF has an essential role in normal erythropoiesis in vivo (Exp Hematol. 31, Supp. 1, 165, 2003). Here we have investigated whether the suppression of ESF by anti-ESF antibody administration during the stimulated erythropoiesis, induced by exposure to hypoxia, can affect erythroid cell proliferation. Female BDF1 mice of 8-9 weeks were exposed to hypoxia of 40% atmospheric pressure for 18 hours a day in a specifically built chamber. On third day of hypoxic exposure some of them were injected (i.p.), with either antibody to ESF (40µg) or isotype-matched immunoglobulin (40µg) and the hypoxic exposure continued for another 48 hours. Erythroid cell formation in these mice was monitored by administering radioactive iron on day 5 and determining its 48 hour incorporation into circulating red blood cells (RBC). Exposing the mice to hypoxia for 5 days significantly increased erythroid cell proliferation, as indicated by radioactive iron incorporation into RBC, to 46.21 ± 1.66% (mean ± SEM) compare to iron incorporation into RBC in normal mice of 33.30 ± 2.60% ($p < 0.01$). Radioactive iron incorporation into RBC decreased significantly to 38.06 ± 2.23% in similarly hypoxia exposed mice injected with anti-ESF antibody on third day of hypoxic exposure ($p < 0.02$). Injection of isotype-matched immunoglobulin at the same interval, on the other hand, did not result in a similar decrease in radioactive iron incorporation into RBC. Decrease in radioactive iron incorporation into RBC was also seen when antibody was injected on day 4 of hypoxic exposure. Thus the administration of antibody to ESF significantly reduced in vivo erythroid cell proliferation in mice even during enhanced erythropoiesis, suggesting the requirement for ESF during stimulated erythropoiesis as well.

200 INCREASED SERUM LEVEL OF MACROPHAGE INFLAMMATORY PROTEIN - 1 (MIP-α/CCL3) BUT NOT RANTES/CCL5 IN PATIENTS WITH B-CELL LYMPHOPROLIFERATIVE DISORDERS (MULTIPLE MYELOMA AND NON-HODGKIN'S LYMPHOMA)

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Background: Interaction between tumour cells and stroma is essential for tumour growth. Tumour cells stimulate the formation of stroma that excretes a variety of growth factors, cytokines, and, among them, chemokines. The chemokines: RANTES (regulated on activation normal T-cell expressed and secreted) and macrophage inflammatory protein - 1 (MIP-1α/CCL3) are the members of the CC-chemokine family.

MIP-1 α /CCL3 plays an important role in chemotaxis and regulates osteoclast differentiation. RANTES has an influence on the biology of many types of cells: T-cell, monocytes, NK cells, dendritic cells, basophils and eosinophils.

Non-Hodgkins lymphomas (NHL) constitute heterogeneous group of lymphoproliferative diseases in both histopathological and clinical presentation, with different presenting features, clinical courses and responses to treatment. In Western countries majority of NHL's are B-cell origin. Multiple myeloma (MM) is a plasma cell malignancy derived from the B-lymphocyte lineage characterised by bone marrow infiltration and the presence of monoclonal protein in serum and/or urine. In MM increased level of MIP-1 α /CCL3 correlates with bone osteolytic lesions

Aim: The purpose of this study was to determine the serum levels of MIP-1 α /CCL3 and RANTES/CCL5 in the NHL and MM patients in active phase of the disease.

Methods: A total of 69 patients with B-cell lymphoproliferative disorders: 35 with nHL, 34 with active MM and 14 healthy subjects were included in this study. nHL patients were in stage IIA-IVB according Ann Arbor classification system (14 females and 21 males, aged 22-75 years), 16 patients were before treatment and 19 in active stage of the disease. MM patients were recently diagnosed (17) and 17 were in progression of the disease. Among them were 19 females and 15 males, aged 31-72 years, there were 17 IgG, 9 IgA and 1 IgM, one with plasma cell leukaemia and 6 with light chain disease. Staging according to Durie and Salmon disclosed: 7 IIA stage, 15 - IIIA and 12 - IIIB. Control group was constituted of 14 healthy subjects - 10 women and 4 men aged 23-61 years. MIP-1 α /CCL3 and RANTES/CCL5 serum concentrations were measured by ELISA using sandwich ELISA kit (R D System Inc., Minneapolis, USA). Statistical analysis was performed using Newman - Keul test ($p < 0.05$).

Results: The mean level of MIP-1 α /CCL3 in nHL patients as well as MM patients was significantly higher than in the control: 55.65pg/ml (64.52 SD), 34.05 (17.54 SD) and 23.64 pg/ml (2.14 SD) respectively ($p < 0.05$). The mean level of RANTES/CCL5 in nHL, MM and in the control did not differ statistically and was: 38.49pg/ml (27.55 SD), 56.26 (43.26 SD) and 48.87 pg/ml (21.82 SD) respectively.

Conclusion: Our data shows that levels of MIP-1 α /CCL3 are increased in active phase of B-cell lymphoproliferative disorders (nHL and MM).

201 A META-ANALYSIS OF FILGRASTIM DOSING DURATION FOR CHEMOTHERAPY-INDUCED NEUTROPENIA IN NONMYELOID MALIGNANCIES

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Background/Aims: A meta-analysis was conducted to study the average number of days of filgrastim dosing within a cycle that were needed to achieve neutrophil (ANC) recovery. Data from 9 multi-cycle oncology studies that contained a filgrastim treatment group were analyzed.

Methods: The analysis utilized individual patient (pt) data from three large phase 3 or phase 4 filgrastim studies and six phase 2 or phase 3 pegfilgrastim studies. The filgrastim studies were conducted from 1988 to 1993 and the pegfilgrastim studies were conducted from 1998 to 2000. In each study, pts received standard doses of chemotherapy for the nonmyeloid malignancy; filgrastim dosing was initiated 24 hours after the last dose of chemotherapy to an ANC recovery of $10 \times 10^9/L$. Total days of filgrastim per cycle were analyzed. Additionally, the number of days of filgrastim required to reach an observed ANC $\geq 10 \times 10^9/L$ was examined to gain information on the possible impact of dosing noncompliance, ie, dosing beyond the target ANC. In this analysis, days of dosing were counted up to the day before an ANC $\geq 10 \times 10^9/L$ was documented, truncating any additional doses of filgrastim beyond that day.

Results: Data were available from a total of 1220 pts who contributed 4897 cycles of chemotherapy. There was a high degree of consistency between cycles in required days of filgrastim dosing. Except for one lung cancer study using carboplatin and paclitaxel, which typically does not produce a high degree of neutropenia, the mean days of filgrastim per cycle across all cycles were 10-12. The mean days of filgrastim to the ANC target were generally 1 day less than total days of filgrastim; therefore dosing noncompliance was not typically observed.

Conclusions: This analysis demonstrated that, when filgrastim was administered in accordance with label, ie, started 24 hours after the last dose of chemotherapy and dosed once daily through an ANC target of $10 \times 10^9/L$, an average of 11 days per cycle was given.

Study	Tumor	No. Pts	No. Cycles	All cycles	
				Mean (SD)	Median
Crawford 1991	SCLC	95	477	11.4 (1.8)	11.0
Trillet-Lenoir 1993	SCLC	64	317	11.6 (1.9)	12.0
Donnelly 2003	Nonmyeloid	739	2938	11.1 (3.2)	10.0
Green 2003	Breast	77	292	10.7 (2.0)	11.0
Holmes 2002	Breast	25	98	10.5 (2.1)	10.0
Holmes 2002	Breast	150	584	10.5 (1.8)	10.0
Johnston 2000	Thoracic	26	83	8.3 (1.6)	8.0
Vose 2003	Lymphomas	31	62	11.2 (1.3)	11.0
Grigg 2003	NHL	13	46	10.5 (2.4)	10.0
Filgrastim dose was 5ug kg/day in all studies, except Crawford and Trillet-Lenoir which used 230ugm/day					
Green 2003	Breast	77	292	10.7 (2.0)	11.0
Holmes 2002	Breast	25	98	10.5 (2.1)	10.0
Holmes 2002	Breast	150	584	10.5 (1.8)	10.0

Caption 1:

202 ACUTE MYELOGENOUS LEUKEMIA (AML) FOLLOWED BY METASTATIC GALL BLADDER CANCER: EFFICACY AND SAFETY OF INTERLEUKIN-2

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Background: Interleukin-2 (IL-2) is a lymphocytotropic cytokine that possesses a much more restricted range of biologic activities than that of other cytokines, with effects on T-cells, which produce it, as well as B, Natural Killer and several non-lymphoid cells. On these basis IL-2 has undergone extensive clinical uses, either as single agent, as well as in combination with other cytokines and/or chemotherapy. The antileukemic effect of recombinant IL-2 in AML patients have been well established. In addition IL-2 have shown clinical benefits in the treatment of several metastatic tumors.

Case Report: a 58-years old male was referred to our Department in June 2002 because of leukocytosis. Bone marrow aspirate specimens revealed an infiltration approximately of 80 % type II blast cells, myeloperoxidase positives and non-specific esterases negatives. As immunophenotype the blast cells resulted CD 33, CD 45, HLA-DR positives and CD 34 negatives. Cytogenetics did not detect abnormalities. Thus the diagnosis of AML FAB M2 cytotype was made. Ultrasound scan revealed a sclerous gall bladder. In July 2002 complete haematological remission (CHR) was obtained with a conventional 3+7 induction schedule including daunorubicin, cytarabine and etoposide, even if the second daunorubicin dose was not given because of atrial fibrillation episode. In September 2002, after consolidation therapy, ultrasound scan and nuclear magnetic resonance confirmed a sclerous gall bladder compatible with chronic cholecystitis. In November 2002, since the patient was considered eligible for high dose chemotherapy followed by autologous peripheral stem cell transplantation, surgical resection of gall bladder was performed. The frozen sections showed a gall bladder adenocarcinoma, thus a successive partial surgical resection of liver and pancreas was performed, while all loco-regional nodes were removed. Histological specimens of nodes and hepatic tissues showed metastatic infiltration. In January 2003 patient underwent IL-2 treatment at dosage of 6×10^6 UI three days for week. No significant side effects, except a flu-like syndrome progressively disappearing, were observed. In February 2004 total body CT scan failed to evidence cancer relapse. To date the patient, continuing IL-2 treatment, remains in first continuous CHR and cancer free status for 19 and 15 months, respectively. To explain the association between AML and cancer it has been argued that secondary neoplasia may be ascribed to immunodeficiency induced by AML chemotherapy. In our case the interval between the diagnosis of AML and gall bladder cancer was 4 months, thus we are induced to consider two neoplasia as concomitant. Probably in this instance the impaired immune surveillance related both to AML per se and chemotherapy might contribute to a faster development of cancer. IL-2 is an useful immunotherapeutic agent in AML treatment and in some advanced cancer but, up to date, there are not systematic studies on IL-2 treatment in gall bladder cancer. Metastatic cancer of the gall bladder is highly lethal disease, in our patient IL-2 treatment has shown efficacy and

safety since it is able to maintain durable CHR and to strengthen immune system against neoplasia.

203 NEUROPROTECTIVE EFFECT OF ERYTHROPOIETIN TREATMENT IN MYELOPATHY PATIENTS

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BACKGROUND: Erythropoietin (EPO) is a pleiotropic cytokine with tissue-protective actions in addition to its important role in red-cell production. Recently, neuroprotective and anti-inflammatory properties of EPO have been reported. Recombinant human EPO (rHuEPO) enters the brain after systemic administration, exerts neuroprotective effect in the damaged brain tissue and may improve traumatic injury of the spinal cord in mice.

AIM: We investigated the hypothesis that rHuEPO treatment may improve the spinal cord function in myelopathy patients.

METHODS: Eight patients, six male and two female, aged 42 to 83 years old with spinal cord myelopathy after traumatic or pressure lesions were studied. rHuEPO was given in a dose of 10,000IU three times per week. Seven patients had had a successful surgical decompression of the spinal cord pressure and one patient had been treated conservatively for central spinal cord syndrome before entry to study. rHuEPO treatment was started one to 18 months after patients initial admission. Clinical evaluation included neurological examination and determination of the Modified Orthopedic Association Cervical Spine Myelopathy Functional Assessment Scale score (JSC). Hemoglobin values and the serum levels of the vascular endothelial growth factor (VEGF) were measured before and after treatment.

RESULTS: All patients further improved the sensor and motor disturbances after rHuEpo treatment. Babinski and Hoffman reflexes disappeared in all patients. JSC before surgery ranged from 2 to 13 (median 9). JSC after surgical decompression increased significantly and ranged from 13 to 14, median 13 ($p=0.027$). An additional increase of JSC occurred with rHuEPO treatment, which ranged from 15 to 18, median 16 ($p=0.026$). Clinical evidence of neurological improvement was noticed after at least 15 days of treatment. All patients referred improvement of their general health condition. Serum VEGF levels before treatment ranged from 191.27pg/mL to 679.61pg/mL (median 462.09pg/mL) and was higher than controls values ($p=0.05$). After rHuEPO treatment VEGF values presented a non-significant decline ($p>0.05$). rHuEpo related toxicity was not observed in any patient.

CONCLUSION: rHuEPO treatment in myelopathy patients with ischemic damage after injury or spinal compression from a mass may have beneficial effects. The general health condition and the motor function improved when received rHuEPO immediately or a long period after surgical or conservative treatment. Pretreatment serum VEGF levels were higher in patients than controls and declined mildly with rHuEPO treatment.

STEM CELLS

204 DC-DEVELOPED FROM CD34+ CELLS USED AS VACCINE FOR POSSIBLE ANTICANCER THERAPY

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The most powerful antigen-presenting cells, specialized to initiate and regulate the immune response, are the dendritic cells (DC). Their clinical use as cellular adjuvants in vaccination strategies is well-known. Based on some published papers, in addition to our previous studies, it is obvious that enriched CD34+ can be expanded ex-vivo to become DC; the serum-free culture procedure provides better conditions for production of DC. We have found that derived in such way, these DC work and act more efficiently as antigen-presenting cells than other DC derived from blood monocytes.

Our present experimental study aims to establish ex-vivo culture conditions for expansion and preparation of DC for clinical use and to test the optimal immunologic properties of these cells.: the authors try to optimize serum-free culture conditions for expansion of CD34+ derived DC and to evaluate phenotypically by flowcytometry and immunohistochemistry matured DC.

Materials and methods: a) cell sources - leukapheresis harvest samples are obtained from cancer patients (The Norwegian Radium Hospital, Oslo) undergoing peripheral blood stem cell mobilization. The clinical models are leukemia cases. The mean purity of 19 samples is 89%; b) media - Dulbecco's Modified Eagle Medium supplemented with 10% FCS; alternatively - serum-free CellGro/SCGM (CellGenix) supplemented with 25% Albumin; c) DC cultures - CD34+ enriched cells in culture bags (CellGenix); d) recombinant human cytokines and growth factors - GM-CSF, TNF-alpha, IL-4, flt-3, SCF; e) monoclonal antibodies - FITC- or PE-labelled mAbs against CD1a, CD14, CD33, CD34, CD40, CD80, CD83, CD86, HLA-DR.

Our results show that the serum-free culture conditions with appropriate combination of cytokines allow to generate large amounts of DC from CD34+ progenitors. The differentiation of CD34+ cells into mature DC is analyzed flowcytometrically. The DC phenotyping confirms a high expression of CD86, CD83, CD40, CD80 and HLA-DR, whereas CD14 and CD33 are less actively expressed. The overall evaluation of the achieved results allow the authors to continue with the next steps (stimulatory capacity and immunologic properties of ex-vivo cultured DC, by T-stimulation, transfection and ability of cultured DC to induce T-cell proliferation in MLR), which will give a green light for clinical application. The results are rather encouraging for use of such vaccine therapy on cancer patients.

205 PTH TREATMENT INDUCE ALTERATIONS IN MURINE LONG-TERM BONE MARROW CULTURE

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Parathyroid hormone (PTH) stimulates bone formation and resorption and is used in clinical trials for treatment postmenopausal women with osteoporosis (Neer, et al., 2001, N Engl J Med, 344, 1434). Osteoblasts upon the PTH induced activation are increased in number and produce hematopoietic growth factors. An increase in the number of stem cells was observed in mice after PTH injection, that provide immediate support to osteoblasts as direct regulators of hematopoiesis (Calvi, et al., 2003, Nature, 425, 841). Long-term bone marrow culture was used as a model for study the influence of PTH on hematopoiesis. Rat PTH (1-34) (final concentration, 10-7M) was added at the moment of establishment and with each media change to long-term bone marrow culture (LTBMC). Cell number, CFU-GM and CAFC were measured during 10 weeks of cultivation. Gene expression was detected by semi quantitative RT-PCR. Expression of cellular markers and extracellular matrix was studied by immunofluorescence. The treatments with PTH decreased total cell production in LTBMC per 20% and CFU-GM per 30-40%, but the number of CAFC -7 remain the same as in untreated cultures. At the same time the proliferation rate of CFU-GM was higher in PTH LTBMC (21% versus 13% in untreated cultures). BMI-1 is required for maintenance and proliferation of hematopoietic stem cells. The expression of BMI-1 increased in adherent cell layer (ACL) of PTH LTBMC after 3 weeks of cultivation and was still revealed after 6 weeks; in untreated cultures BMI-1 expression was no longer detected. In suspension fraction of PTH LTBMC the expression of c-kit was increased whereas SCF decreased,

what indirectly indicate the increase in the number hematopoietic stem cells. In ACL PTH LTBMC the expression level of some differentiation markers such as for cartilage (COMP and IBSP) and endothelial (PECAM and FVIII) decrease, while the level of bone markers (osteocalcin and osteopontin), FGF1 and VEGF increased. There was no difference in expression of surface antigens that include fibronectin, collagens type I and IV, CD44, integrin b1 (CD29) in ACL of treated and untreated cultures, while collagen type II (cartilage matrix molecule) formed spread network in PTH ACL after 3 weeks of treatment. Our findings demonstrated that PTH treatment increases the expression of some cartilage and bone differentiating markers in stromal cells of ACL. At the same time differentiated precursor cells and mature cell production reduced insignificantly. The hematopoietic cells proliferation rises. PTH as potential drug for stem-cell-based therapies and osteoporosis did not reveal any drawbacks in vitro.

206 THE COMPARISON OF GENE EXPRESSION OF UMBILICAL CORD VEIN AND BONE MARROW DERIVED MESENCHYMAL STEM CELLS

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Background: Mesenchymal stem cells (MSC) give origin to the marrow stromal environment that supports hematopoiesis. These cells present a wide range of differentiation potentials and a complex relationship with hematopoietic stem cells (HSC) and endothelial cells. In addition to bone marrow (BM), MSC can be obtained from other sites in the adult or the fetus. Aims: To compare MSC obtained from the human umbilical vein with MSC obtained from the bone marrow. Methods: We isolate MSC from the umbilical cord vein (UC) that are morphologically and immunophenotypically similar to MSC obtained from the BM. In culture, these cells are capable of differentiating in vitro into adipocytes, osteoblasts and chondrocytes. The gene expression profiles of BM-MSC and of UC-MSC were compared by serial analysis of gene expression (SAGE), validated by RT-PCR of selected genes. Results: The two lineages shared almost all of the first thousand most expressed transcripts, including vimentin, galectin 1, osteonectin, collagens, transgelins, annexin A2 and MMP2. Nevertheless, a set of genes related to anti-microbial activity and to osteogenesis were more expressed in BM-MSC, whereas higher expression in UC-MSC was observed for genes that participate in pathways related to matrix remodeling via metalloproteinases and angiogenesis. Finally, cultured endothelial cells, CD34+ HSC, MSC, blood leukocytes and bulk BM clustered together, separated from other 7 normal non-hematopoietic tissues, on the basis of shared expressed genes. Conclusion: MSC isolated from UC vein are functionally similar to BM-MSC, but differentially expressed genes may reflect differences related to their sites of origin: BM-MSC would be more committed to osteogenesis whereas UCV-MSC would be more committed to angiogenesis.

207 HEMATOPOIETIC STEM CELLS AND MESENCHYMAL STEM CELLS IN LONG-TERM BONE MARROW CULTURES OF TNF KNOCKOUT MICE

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Hematopoiesis in long-term bone marrow culture (LTBMC) of TNF-deficient mice (TNFko) is supported about 10-fold longer (more than 200 weeks) in comparison with LTBMC of wild type (WT) mice (15-25 weeks). Total cell production, number of CFU-GM, CFU-S, CAFC-7 and CAFC-28 increased 10-100 fold in suspension fraction (SF) of TNFko LTBMC after 30-35 weeks in culture and stayed on this level for about 70 weeks. Then the hematopoietic precursor and mature cells decreased significantly, but CD45+ cells, CFU-GM and CAFC were still detected. Mesenchymal stem cells forming adherent stromal cell lines were revealed in SF of TNFko LTBMC after 70 weeks in culture and more than hundred cell lines from LTBMC of different age were established. These cell lines could maintain hematopoiesis (CFU-S, CFU-GM and CAFC production) for 10 weeks. The comparison of the expression of differentiation markers in SF and cloned from single cell stromal cell lines derived from the SF at the same period of time was done. Gene expression was detected by semi-quantitative RT-PCR. bActin was used as a reference expression marker. All cell lines and cells from SF expressed high level of integrin b1 and Sca-1 (tested by immunofluorescence). Bone marker BMP4 was expressed in SF in most cultures since at least 90 weeks of cultivation. Some of

cell lines also expressed BMP4. Another bone marker osteomodulin was expressed in 20% of cell lines and never in SF. Cartilage marker COMP was detected on low level in most SF and was pronounced in 27% cell lines. Mostly its expression intersects with osteomodulin. Endothelial marker PECAM1 was detected in SF of 3 out of 4 cultures tested from 60 till 200 weeks of cultivation. It was revealed in cell lines established from 3 of these 4 cultures. PECAM1 expression in cell lines was much higher than in SF. We never observed the expression of smooth muscle cell marker desmin in cell lines, but high level of its expression was revealed in SF of all cultures. The nerve cell marker tubulin beta 3 was expressed in SF after 60 weeks of cultivation and in 72% of cell lines. All tested cell lines expressed SDF1; its expression was observed on the lower level in 44% of SF independently of time of cultivation. Most of cell lines expressed considerable level of SCF, VEGF and FGF1, on the contrary expression of c-kit and CXCR4 in SF was more pronounced. These data support a model of lineage specification in which unilineage commitment is prefaced by a 'promiscuous' phase of multilineage locus activation. We cannot distinguish between hematopoietic and mesenchymal stem cells in SF; the results demonstrate their survival in SF of LTBMC in the absence of TNF.

208 UNMANIPULATED ADULT LIVER STEM CELLS FAILED TO SUSTAIN HEMATOPOIETIC RECONSTITUTION AFTER TRANSPLANTATION IN NONHUMAN PRIMATES

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Adult murine tissues outside bone marrow have been demonstrated to exhibit a hematopoietic potential. This conducted our team to evaluate this concept in nonhuman primates that are relevant models to humans. The present study was focused on the search for hematopoietic potential in the adult macaque liver (both autologous and allogeneic settings). Hepatic mononuclear cells (HMNC) were isolated after partial hepatectomy by perfusing and digesting liver samples with collagenase A. Hepatocytes and clumps were sedimented out. HMNC count and viability was determined. HMNC were characterized for immature and hematopoietic markers using flow cytometry and clonogenic assays. Less than 1% of HMNC exhibited a side population (SP) phenotype after incubation with 15 mcg/mL (optimal concentration) of Hoechst 33342. Liver SP cells were CD45neg and about 30% expressed CD34 antigen; the higher the CD34 expression, the greater the ability to efflux the dye. Low levels of clonogenic progenitors (less than 5 total CFU for 1x105 fresh HMNC plated) were scored after culturing unseparated HMNC for 14 days in methylcellulose medium supplemented with hematopoietic growth factors and BMP-4 (25 ng/mL).

Four adult macaques underwent a left hepatic lobectomy under general anesthesia, their HMNC were isolated as indicated above and then were cryopreserved. One month later, the animals were lethally irradiated (10 Gy 60Co gamma) and transplanted the day after with thawed unmanipulated HMNC (121 12 x 106 cells). Two of them (one autologous hepatic graft, one allogeneic) were co-grafted with mesenchymal stem cells (MSC) in order to enhance hematopoietic reconstitution. All animals displayed a transient neutrophil recovery from day 27 after transplantation (ANC higher than 1x109/L for 14 days on average) but only the MSC co-grafted animals exhibited improved platelet counts. This study suggests that the occurrence of stem cell plasticity in primates may require in vitro or in vivo cell manipulation as well as cell cooperation.

209 BIOLOGY OF THE HEMOGENIC MICROENVIRONMENT: THE OSTEO-HEMATON

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Background: During vertebrate ontogenesis hemogenic cells emerge at distinct allocation territories prior to formation of the definitive bone marrow. Stem cells are the cornerstones of organogenesis, tissue renewal and compensatory regeneration. The adult BM encloses a variety of multipotential stem cells, too, that may differentiate toward non-hemogenic cell fates. These data imply that despite their powerful attributes, stem cells are not autonomous tissue units. Indeed, emerging data support the concept that the fate and potentiality of stem cells is under the dominant control of tissue-specific microenvironment, or stem-cell-niches.

Aim: The cellular and molecular mechanisms how definitive stem cells and specific niches emerge during ontogenesis and how a few primordial cells evolve organ-specific functional subunits remain enigmatic. We investigated the origin, spatial-temporal emergence and functional competence of stem cell niches in mouse and human hemogenic tissues.

Methods and Results: Time-laps video imaging in gut/liver primordium cultures and microangiography showed intrinsic emergence of a pulsative myogenic, vasculohemogenic meshwork around nascent hepatic cords, providing direct visual evidence for the involvement of cardiomyogenic mesoderm in liver morphogenesis. Quantitative colony assays mapped primitive, hemogenic cells in liver from day 8.75 of embryogenesis (E8.75), clearly prior to colonization of definitive stem cells (LTC-ICd35) at E12 and their robust expansion until E16. Gain of hemopoietic competence in the liver primordium, spleen and ultimately in the BM required the association of stem cells and niche-forming stroma cells into compact, 3D structures, that we named the hematons. In the liver, these isolatable niches enclosed both hemopoietic, cholangio-hepatic stem cells and vasculogenic cells guided by the mesoderm that together evolved typical hepatic subunits in long-term organotypic culture. Next, we investigated the hematons in the adult human BM, recovered and purified from the filter-retained material immediately after BM graft conditioning. Video image analysis, quantitative colony assays and immunocytochemistry showed that the hematons enclose stem-cell-niches, mesenchymal stem cells, preadipocytes/adipocytes, contractile myogenic cells, a fine vascular meshwork and hemopoietic stem/progenitor cells. They enclosed guardian macrophages and might chaperon progenitor cells from the neighborhood. Many hematons enclosed tiny bone spicules indicating for the intimate, physical contact between the hematons and spongy bone. Immunocytochemistry and biochemical analysis document that a hematons plays the role of a morphogen signaling center that accumulates retinoids, 1 α ,25-dihydroxyvitamin D3 and emits growth factors, chemokines and putative veto signaling substances.

Conclusion: We conclude that: i. the ontogenic emergence of stem cell niches predisposes the gain of definitive hemopoietic competence both in the liver and BM, ii. a stem cell niche/hematons plays the role of a morphogen signaling center conditioning stem cells for a tissue specific fate, and iii. during organogenesis the spatial-temporal expansion of stem-cell-niches is guided by the arborizing vasculogenic endothelium and limited by the surrounding mesoderm. The intimate, physical association of hematons and spongy bone suggests that they form many structural-functional units in the adult BM that we suggest to designate, the osteo-hematons.

210 PROPERTIES OF IMMATURE HUMAN MESENCHYMAL STEM CELLS AS PUTATIVE COMPONENT OF THE IN VIVO NICHE OF HEMATOPOIETIC STEM CELLS

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Background: Depending on the microenvironment, bone marrow-derived stem cells have the potential to differentiate into mature cells of various tissues. We have recently demonstrated the vital role of cell-cell contacts, especially between hematopoietic stem cells (HSC) and the stromal environment, as regulative factor for self-renewal and differentiation.

Aims: In this study we have quantified and compared the affinity of HSC towards immature human mesenchymal stem cells (MSC) and the unique murine stem cell supporting cell line AFT024.

Methods and Results: CD34+/133+ HSC were cocultured for up to 24h either with human MSC or murine AFT024. By means of a time-lapse camera system we have continuously monitored the behavior of HSC. Independent of the type of feeder layer, HSC displayed a uniform behavior with podia formation of 75.3 $\pm$ 7.2% of the cells, rapid migration across the feeder layer, and continuously seeking for homotypic and heterotypic cell contacts. Determination of adhesion kinetics revealed no significant differences between the two feeder layers. In coculture with AFT024, maximum adhesion of HSC occurred after 2h with 37.6 $\pm$ 12.7% adherent cells. When cells were cocultured with MSC, maximum adhesion was achieved after 30min. with 31.7 $\pm$ 6.1% adherent cells. In both cases maximum adhesion was followed by a plateau phase. HSC adhered firmly (re cocultured with MSC, maximum adhesion was achieved after 30min. with 31.7 $\pm$ 6.1% adherent cells. In both cases maximum adhesion was followed by a plateau phase. HSC adhered firmly (re cocultured with MSC, maximum adhesion was achieved after

30min. with 31.7 $\pm$ 6.1% adherent cells. In both cases maximum adhesion was followed by a plateau phase. HSC adhered firmly (cant differences. For comparison of the stem cell supporting properties, a long term culture initiating cell assay in limiting dilution was performed that revealed no differences between MSC and AFT024 in maintaining progenitor cells.

Conclusion: These data demonstrate comparable homing and stem cell supporting properties of human mesenchymal stem cells versus the unique stromal cell line AFT024. Immature marrow stromal cells might represent a component of the in vivo niche of HSC.

211 COMPARATIVE ANALYSIS OF CHONDROCYTES ISOLATED FROM HUMAN CARTILAGE AND DERIVED FROM HUMAN MESENCHYMAL STEM CELLS

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Background. Mesenchymal stem cells (MSC) are multipotential progenitors that give rise to vascular smooth muscle cells, skeletal and cardiac myocytes, adipocytes, but also to osteoblasts and chondrocytes. Engineering human chondrocytes derived from MSCs with the appropriate scaffold could be useful to repair cartilage defects due to degenerative diseases or trauma.

Aim. The purpose of the study was to compare the in vitro behavior of chondrocytes derived from human MSC with that of chondrocytes isolated from human knee cartilage.

Method. Human mesenchymal cells from bone marrow were isolated by adherence on plastic. The cells were cultured in DMEM supplemented with FBS10% for 3 weeks, the medium being replaced every 3 days. After this period of time, the cells were harvested by trypsinization, centrifuged and the pellet mass was resuspended in DMEM medium, supplemented with TGFbeta and dexamethasone for 6-8 weeks. Human cartilage fragments were harvested by arthroscopic biopsy. Chondrocytes were isolated by enzymatic digestion using collagenase (12 to 24 hours), and cultivated in DMEM with human albumin, for 8 weeks. Human albumin was used instead of fetal bovine serum. After one week, Insulin-like Growth Factor I (IGF-I) was also added to the medium. Finally, the cells were resuspended by trypsinization. In both experiments, cells viability was assessed using the trypan blue test. Hematoxylin-eosin, Papanicolaou, Giemsa and Alcian Blue stainings were performed in order to evaluate the morphology of cultivated cells. Immunohistochemical analysis for collagen II was also performed. We tested the capacity of chondrocytes from both experiments to adhere to nickel-titanium 3D-scaffolds.

Results. After 6 to 8 weeks of culture with TGFbeta and dexamethasone, 20-25% of mesenchymal stem cells became rounded, compact, rather large, with a 3D architecture, aspect similar to that of freshly isolated chondrocytes. These cells were positive for collagen II. In case of human chondrocytes culture, supplementation of the medium with human albumin was extremely helpful, increasing their proliferation rate. Freshly isolated chondrocytes, as well as chondrocytes derived from human mesenchymal stem cells, gradually settled in isogenous groups around the 3D scaffold until they almost covered it (60-80%).

Conclusions: Human mesenchymal stem cells harvested from bone marrow represent a good source of chondrocytes in order to be used for autologous transplantation. Their in vitro behavior seems to be similar to that of chondrocytes isolated directly from human cartilage. Supplementation of the medium with human albumin may be a solution for fetal bovine serum replacement.

212 EVALUATION OF CORD BLOOD FOR QUANTITATION OF HAEMATOPOIETIC PROGENITOR CELLS

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Human cord blood has been used to treat several types of haematological conditions, due to its content of haematopoietic progenitor cells. In most cases the source has been postnatal cord blood. In this study the presence of haematopoietic progenitor cells were evaluated in prenatal (intra uterus) and in postnatal cord blood (obtained immediately after delivery). Materials and Methods. We studied 25 prenatal blood samples, obtained by cordocentesis through ultrasound guided intrahepatic vein puncture, from

pregnancies with 17-32 weeks of gestational age, in which conditions such as congenital fetal malformation, sensitization due to Rh incompatibility, maternal age over 35 years in the first pregnancy or congenital rubella were diagnosed or suspected; 30 cord blood samples were obtained immediately after delivery from normal pregnancy placentas. The following exams were done: Hb, haematocrit, Hb electrophoresis, flow cytometer immunophenotyping studies with the following monoclonal antibodies: HLA-Dr, CD2, CD4, CD7, CD8, CD10, CD11b, CD13, CD14, CD19, CD20, CD33, CD34, and CD45. To differentiate subtypes of CD34+ the following reagents were used: CD34PE/CD33-FITC and CD34PE/HLA-Dr-FITC. The cord blood cells were cultured in semisolid medium in the presence of erythropoietin and IL-3. Results: Increased values of Hb and haematocrit were observed in fetal blood obtained from pregnancies of 17 to 32 weeks of gestation. VCM showed severe macrocytosis (135 fl. at 17-20 weeks). Immunophenotyping of blood cells from 17-32 weeks-old fetuses allowed the identification of leukocytes (CD45+ and HLA-Dr+ cells), T lymphocytes (CD2+, CD7+, CD4+, and CD8+ cells), B lymphocytes (CD10+, CD19+, CD20+ cells), cells expressing myeloid markers (CD13, CD14, CD33), and haematopoietic progenitor cells (CD34+ cells); this last group showed a maximal value of 4.12% around the 17th week, with a tendency to decrease as the pregnancy progressed. In postnatal cord blood the value of CD34+ cells was 0.89%, most of them of the CD34+/CD33- phenotype. Both, prenatal and postnatal cord blood, when cultured in methylcellulose, generated erythroid and granulocytic-macrophagic colonies after 7 days of culture; the colonies were most abundant with the earlier samples. Conclusions: Haematopoietic progenitor cells and mature leukocytes can be easily identified in fetal blood obtained from 17-32 week-old pregnancies. The higher value of CD34+ progenitor cells was detected in samples from the 17th week, and the lowest in samples of postnatal cord blood.

213 THE ROLE OF ONCOSTATIN M IN FETAL HAEMOPOIESIS

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The fetal liver (FL) ceases to be the main site of haemopoiesis in the third trimester of pregnancy. Since oncostatin M (OSM) promotes the maturation of hepatocytes and stroma in FL with the loss of ability to support haemopoiesis in mice, it has been suggested that OSM is the key cytokine involved in suppressing FL haemopoiesis. The cells producing OSM and the role of OSM in human fetal haemopoiesis are unknown. The aim of this study was: 1. to determine whether human FL MSC produced OSM; 2. to determine the effect of OSM in human third trimester haematopoietic progenitor cells. We tested the ability of FL MSC to produce OSM by RT-PCR. FL MSC from all gestation tested (8+0 to 14+4 weeks of gestation, n=4) were positive. To test the specificity of OSM production by FL MSC we also studied MSC from first trimester FB (8+4 to 12+2 weeks, n=5) and FBM (9+4 to 16+4 weeks, n=4). All FB and FL MSC produced OSM also. To test the effect of OSM on third trimester HSC we isolated MNC from cord blood and established methylcellulose-based clonogenic cultures (n=21) with a range of concentrations of OSM (0 to 100 ng/ml). OSM was found to significantly inhibit the growth of fetal erythroid and myeloid progenitors in a dose dependent manner (CFU-E: 289.8 26.7 colonies/10⁵ MNC versus 188.4 22 colonies/10⁵ MNC at 0 and 100 ng/ml respectively, p=0.0001; BFU-E: 545.5 49.9 colonies/10⁵ MNC versus 337.4 28 colonies/10⁵ MNC at 0 and 100 ng/ml respectively, p=0.0001; CFU-GM: 224.3 24.8 colonies/10⁵ MNC versus 144.9 16.6 colonies/10⁵ MNC, p=0.0002 at 0 and 100 ng/ml respectively). In contrast, OSM was found to stimulate the growth of multipotent progenitors (CFU-GEMM: 0.29 0.15 colonies/10⁵ MNC versus 7.2 0.13 colonies/10⁵ MNC at 0 and 100 ng/ml respectively, p=0.0002) in a dose dependent manner. In conclusion OSM is produced by FL MSC and does inhibit committed progenitors in a dose dependent manner; however, as OSM is also produced by both FB and FBM MSC and stimulates multipotent progenitors it is unlikely that OSM production causes switching off of the FL haemopoiesis in humans towards term.

214 GENERATION AND CHARACTERISATION OF A NOVEL MULTIPOTENTIAL MURINE HEMATOPOIETIC PROGENITOR CELL LINE WITH DC-DIFFERENTIATION POTENTIAL USING NOTCH-1 GENE TRANSFER

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The generation of hematopoietic progenitor cell lines using gene transfer of defined genetic elements would greatly facilitate molecular and

developmental studies of stem cell differentiation. In an attempt to dissect the control mechanism governing dendritic cell (DC) differentiation, we have generated and characterised a novel murine IL3-dependent cell line (CR1-mix). C57BL/6 derived Sca1⁺lin⁻ hematopoietic stem cells were transduced using a VSV-G pseudotyped bicistronic retrovirus expressing Notch1 and eGFP. Upon selection of eGFP positive cells, we determined growth and differentiation potential in the presence of various hematopoietic cytokines. eGFP-transduced Sca1⁺lin⁻ cells served as controls. Notch1- transduced hematopoietic stem cells, but not control-transduced hematopoietic stem cells, could be maintained as pluripotent immature cells in the presence of recombinant mu-IL3 (10 ng/ml). The cell line, named CR1-mix, was kept in culture for more than 12 months without loss of viability or reduction of proliferative potential. Spectral karyotyping of 10 metaphases revealed a normal diploid chromosome pattern without any evidence of clonal numerical or structural aberrations. Upon exposure to selective hematopoietic cytokines (mu-IL3, mu-IL6, mu-EPO), we were able to document their differentiation potential into myeloid but not erythroid cells, as assessed in CFU-assays. Interestingly, CR1-mix cells can also efficiently differentiate into both myeloid and plasmacytoid DCs in vitro: more than 95% of the DCs generated in the presence of GM-CSF/IL4 showed morphological and immunophenotypic features of myeloid DCs (expression of the cell surface markers MHCII, CD11c, CD40, CD80 and CD86), whereas 70% of DCs selectively grown in the presence of Flt3L co-expressed CD11c and B220, a phenotype characterising plasmacytoid DCs. In functional studies, CR1-mix-derived myeloid DCs compared favorably to primary bone marrow derived DCs, as tested by their capacity to stimulate allogeneic T cells and to secrete IL12 upon TNF- exposure (10-fold induction of IL12p70-secretion in both primary and CR1-mix-derived DCs). To confirm their differentiation potential in vivo, we also transplanted CR1-mix cells into CD45.1 congenic recipient mice and analyzed the hematopoietic system five weeks later by FACS. We found CR1-mix derived cells (CD45.2) in bone marrow (7-12%), spleen (11-13%), and thymus (32-35%), differentiating into T-cells (CD3), NK-cells (NK1.1), DCs (CD11c), macrophages (CD11b, F4/80) and granulocytes (GR-1). In summary, we provide evidence that retroviral transduction of murine hematopoietic stem cells using Notch1-expressing retroviruses is a useful tool to generate a genetically stable IL3-dependent multipotential progenitor cell lines.

215 IMMUNOPHENOTYPE OF CD34+ CELLS FROM PLACENTAL/ UMBILICAL CORD BLOOD AFTER CULTURE

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Identification and enumeration of human hematopoietic stem cells remains problematic, since in vitro and in vivo stem cell assays have different outcomes. We tested if altered expression of adhesion molecules during stem cell expansion could be a reason for this. CD34⁺CD38⁻ and CD34⁺CD38⁺ cells from umbilical cord blood were analyzed before and after culture with thrombopoietin (TPO), FLT-3 ligand (FL) and kit ligand (KL) in different combinations. Cells were immunophenotyped by four-color fluorescence using antibodies against CD11c, CD31, CD49e, CD61, CD62L, CD117 and HLA-DR. Low-density cord blood contained 1.40.9% CD34⁺ cells, 2.62.1% of which were CD38-negative. CD34⁺ cells were isolated with magnetic beads and cultured for up to 7 days. The combination TPO+FL+KL presented the best condition for maintenance of stem cells. The total cell number increased 4.31.8 fold, but the number of viable CD34⁺ cells decreased by 4625%. The absolute number of CD34⁺CD38⁻ amplified 1512 fold. The expression of CD62L, HLA-DR and CD117 was modulated after culture, particularly with TPO+FL+KL, explaining differences between the adhesion and engraftment of primary and cultured candidate stem cells. In conclusion, culture of CD34⁺ cells with TPO+FL+KL results in a significant increase in the number of candidate stem cells with the CD34⁺CD38⁻ phenotype.

GENETIC LEUKEMIA I

216 CHROMOSOMAL ABERRATION OF THE 11Q22-25 BANDS WITH AND WITHOUT MLL GENE REARRANGEMENT: ANALYSIS OF 378 ACUTE LEUKEMIA PATIENTS

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Structural abnormalities of the 11q23 band (11q23+) bearing MLL gene translocation (MLL+) are a recurrent chromosome change observed in 3-7% of acute lymphoblastic leukemias (ALL) and in 3-4% of acute myeloblastic leukemias (AML). 11q23/MLL is a highly promiscuous region: more than 50 chromosomal loci have been described as 11q23 partners and over 30 MLL partner genes have been characterised. The resolution of conventional cytogenetics (CC) is limited when the translocative partner has a telomeric location and not infrequently reciprocal 11q23 translocation are referred to as del(11)(q23) or add(11)(q23). Moreover CC can hardly discriminate between truly 11q23+/MLL+ and rearrangements clustering within the 11q22-25 region without MLL involvement (MLL-). We characterised a series of 378 consecutive adult acute leukemia (AL) patients with CC, FISH and Multiplex Karyotyping (M-FISH) analysis, with respect to 11q22-25 rearrangements and MLL+. As expected FISH was more sensitive than CC as it allowed to detect 4 more cases of MLL+ (26%). Rather unexpectedly 9 out of 20 (45%) patients with 11q22-25+ were not MLL+. Five of these patients showed 5 different reciprocal translocations cytogenetically indistinguishable from truly 11q23+/MLL+ cases, while the remaining 4 had 11q22-25 unbalanced rearrangements. Leukemia with 11q22-25+/MLL- are heterogeneous at the biological and clinical level. A better characterisation of these entities is relevant for the identification of new recurrent translocations, cloning of genes and elucidation of pathogenic mechanism involved in AL. Moreover these - patients should be readily distinguished from 11q23+/MLL+ cases. In light of this heterogeneity, we recommend that karyotypic analysis of chromosome abnormalities involving 11q22-25 bands should be always complemented by molecular or FISH methods to unravel MLL involvement.

217 GEOGRAPHICAL DIFFERENCES IN THE INCIDENCE OF T(15;17) AND T(8;21) IN SPAIN.

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AIMS: In order to analyze if the incidence of chromosomal abnormalities differs according to geographical distribution we have investigated a large series of patients from three different regions of Spain.

METHODS: A total of 1271 AML patients, diagnosed between 1995 and 2002, from three different regions of Spain: northern, central and southern have been included in the study. They were classified according to WHO criteria. Cytogenetic studies were carried out after 24 and 48 hours unstimulated culture. Data was compared using Pearson's chi-squared test for categorical variables and ANOVA for continuous variables.

RESULTS: There were 624 males (55%) and 505 females (45%). Age ranged between 1 month and 94 years with a median age (interquartile range) of 61 years (42-72). Abnormal karyotypes were observed in 64% of cases. Numerical abnormalities were detected in 15% of patients, while structural aberrations were present in 28% of cases and both abnormalities were found in 22% of patients. Complex karyotypes defined according to SWOG criteria (>2 unrelated cytogenetic abnormalities) were observed in 20% of cases, while according to MRC criteria (>4 unrelated cytogenetic abnormalities) they were detected in 13% of cases. The overall frequency of karyotypic subgroups was: t(15;17) 15%, inv(16) 3%, t(8;21) 3%, del(5)(q) 9%, del(7)(q) 9%, +8 11%, +21 3%, +11 2%, abn(3)(q) 2%, abn(11)(p) 2%, abn(11)(q23) 3% and iso(17)(q) 1%. The incidence of other abnormalities was less than 1%. A significantly higher proportion of t(15;17) was found in the south of Spain than in the central or northern parts (22%, 17% and 13%, p 0.007), by contrast patients from the south of Spain had lower percentage of t(8;21) (0% compared to 2% and 4% in central and northern parts, respectively, p 0.01). It should be noted that these latter figures are lower than the incidence of t(8;21) reported from other countries. Trisomy 8 was more frequent in the central

region (15% of cases) and the south (14%) than it was in the north (10%) (p 0.03). Interestingly, 11p abnormalities were detected in 5% of AML cases in the south, 3% in the north and only 1% in the middle (p 0.04). Other karyotypic abnormalities were evenly distributed.

CONCLUSIONS: Geographical differences regarding cytogenetic abnormalities are evident in this sample of Spanish AML patients: t(15;17) is significantly more common among AML patients from the south of Spain, while the opposite pattern is observed for t(8;21) with significantly lower occurrence than in central or northern Spain.

218 FISH ANALYSIS OF 26 PATIENTS WITH HEMATOLOGICAL NEOPLASIAS AND 1P36 REARRANGEMENTS ALLOWS TO DEFINE A CLUSTER OF 2.5 MB INCLUDED IN THE MINIMAL REGION DELETED IN THE 1P36 DELETION SYNDROME

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Rearrangements in the distal region of the short arm of chromosome 1 (1p36) are recurrent aberrations in a broad spectrum of human neoplasias, and have been correlated with a shorter overall survival. However, the location of the breakpoints (BP) on 1p36, and the candidate genes involved have not been fully determined. We have characterised by FISH the BP in 26 patients with haematological neoplasias and 1p36 rearrangements, defining a region of 2.5 Mb where the BP clustered. Sixteen patients had myeloid and 10 lymphoid neoplasias. FISH assays were performed using 51 BAC and PACs located between 1p32.3 y 1p36.33, designed according to the current mapping data, and a commercial probe for the subtelomeric region of 1p (Vysis); 65 BAC and PACs were used to characterise the BP in the partner chromosomes. FISH allowed the characterisation of all the samples analysed. Ten cases (38.5%) showed reciprocal translocations, and 16 (61.5%) unbalanced translocations, two of them recurrent: 2 cases with MDS showed a der(1)dup(1)(p32.3p34.1), and 2 cases with NHL and one with ALL a der(1)t(1;1)(p36.33;q21). In 18 cases of the 26 analysed the BP were located in the band 1p36, that covers 25.5 Mb. In 14 of these cases (78%, 14/18), without distinction between myeloid and lymphoid neoplasias, the BP clustered in a 2.5 Mb region located between 1p36.32 and the telomere. Interestingly, this region is contained in the 10.5 Mb cluster on 1p36.22-1pter defined in cases with the 1p36 deletion syndrome. The 2.5 Mb region located on 1p36.32-pter, has a higher frequency of occurrence of certain genomic repeats (such as tandem and inverted repeats) if comparing with the 25.5 Mb of the complete 1p36 band. The local percentage of sequence covered by these repeats, considering contiguous windows of 100 kb of genomic DNA, reaches values of 20%, while analogous measures for nearby sequences located in the rest of the 1p36 band are around 5%. These repeat sequences have been associated with the location of breakpoints in chromosomal aberrations. Further enough, more than half of the sequences located at the first 2 Mb of 1p36 matches to duplicons larger than 1 kb. Duplicons have been recently found to mediate the genesis of recurrent chromosomal translocations. In conclusion, the characterisation by FISH of 26 patients with 1p36 rearrangements allowed us to define a 2.5 Mb cluster region on 1p36.32 where the BP of 78% of the cases analysed are located. This region shows characteristics, as the presence of genomic repeats, that could explain its proneness to get involved in chromosomal rearrangements.

219 ACTIVE MUTATIONS OF C-KIT IN T(8;21) ACUTE MYELOID LEUKEMIA; DISTINCT CHARACTERISTICS, PROGNOSTIC SIGNIFICANCE, AND RESPONSE TO STI571

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Constitutive activation of KIT tyrosine kinase by KIT mutation is found in systemic mastocytosis. Active mutation of KIT are mostly detected in tyrosine kinase domain; juxtamembrane (exon 11) and second intracellular kinase (exon 17) domains. Mutation of KIT are also reported among acute myeloid leukemia (AML), especially core binding factor leukemia, however, cellular and clinical characteristics of AML with KIT mutation are still unclear. In this study, we examined for KIT mutation in 61 adult patients with de novo AML M2 subtype: 30 cases of AML with t(8;21) and 31 cases without t(8;21). Mutation in exon 11 (Met541Leu) was found in one AML case without t(8;21) (1.6%). On the other hand,

exon 17 mutation was detected at Asp816 in 6 out of 61 cases (9.8%) and all these six cases had t(8;21) (20% in t(8;21) AML). Asp to Tyr change (Asp816Tyr), Asp816Asn and Asp816Val mutations were found in four, one, and one case respectively. Western blot showed stem cell factor-independent KIT activation in primary AML cells with Asp816 mutations. Among t(8;21) AML, patients with mutation were significantly associated with leukocytosis ($27.7 \times 10^9/L$ vs $7.8 \times 10^9/L$; $P=0.04$) and poor overall survival ($P=0.0001$; 5 year survival, 0% vs 73%). t(8;21) AML cells with KIT mutations highly expressed CD18 ($P=0.01$) and CD56 ($P=0.005$). KIT kinase inhibitor, STI571, inhibited the survival of primary t(8;21) AML cells with KIT mutation but not without mutation in vitro. Furthermore, when Asp816 mutation of KIT was transfected into MO7e cells, the growth of these cells was also significantly suppressed by STI571. This growth inhibitory effect of STI571 was not found on wild type of MO7e cells. These results suggest t(8;21) AML with Asp816 mutations could be a distinctive subtype of t(8;21) AML and that STI571 would be effective for the treatment of t(8;21) AML patients with KIT mutations.

220 ARRAY CGH STUDY IN PATIENTS WITH ACUTE LEUKEMIA WITH NORMAL KARYOTYPE

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Background: Cytogenetic and molecular analyses of leukemias have now enable the identification of nonrandom genetic changes with diagnostic, prognostic and therapeutic significance. Nevertheless, for over 10-45% of all leukemia patients with normal karyotype a very little is known about the genetic abnormalities that characterize and may contribute to the origin and development of leukemia.

Aim: To find out whether the patient with AL with normal karyotype have quantitative gene changes, from which some of them can be determined using array based comparative genomic hybridization (array CGH).

Methods: We used array CGH with microarray GenoSensor300 (Abbott Vysis) in 10 AL patients (7 AML, 4 ALL) with normal karyotype and in one ALL patient with abnormal karyotype as a control. All but one patients were examined at the time of diagnosis. All patients were examined by cytogenetics, FISH for recurrent translocations and by PCR for detection of FLT3 mutation. Two patients were examined by CGH. FISH with locus specific probes was used to confirm some array CGH results.

Results: The control hybridization with DNA from ALL patient confirmed all changes detected by cytogenetics and FISH. As a new aberration the losses of two genes on 9p were detected and confirmed by FISH.

In group of 7 AML patients at least 3 to 23 gains and losses were detected. In three patients the same loss at 6q13 and in two of them identical loss at 19q13.1-13.2 were found. Other detected gains and losses varied and involved genes on chromosomes 1, 2, 7, 11, 16, 17, 19, 20 and 22.

In 3 patients with ALL at least 5 to 9 gains and losses were recognized. In two patients the same loss of p16 gene was revealed and confirmed by FISH.

Summary: Our first experience with array CGH shows, that it is a sensitive method, which provides valuable insight into the molecular events underlying leukemogenesis and might provide new information about role of these gene changes in leukemias.

221 SUPERIORITY OF COMBINED BROAD MOLECULAR SCREENING AND CYTOGENETICS IN DIAGNOSIS OF ACUTE LEUKEMIAS

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Introduction: Acute leukemias are classified today (WHO) by morphology, surface markers, genetic characteristics and based on clinical and prognostic significance. In view of cost containment the need for multiple investigations is questioned. We tested the impact of combined broad molecular screening for 28 fusion transcripts and conventional cytogenetics in patients with acute leukemia.

Patients and Methods: In addition to standard morphology and immunophenotyping, 186 patients [104 males (56%), 174 adults (94%)]

12 children (6%), 155 AML (83%)/31 ALL (17%)] were prospectively screened by multiplex RT-PCR and conventional cytogenetics (160 at first diagnosis, 2 with persistent disease and 24 at relapse). The multiplex RT-PCR assay is a two step nested RT-PCR which allows the simultaneous detection of 28 fusion transcripts (Hemavision).

Results: Molecular genetics revealed a fusion transcript in 49 (26%) patients and cytogenetics an abnormal karyotype in 120 (65%) patients. Molecular and cytogenetic results were concordant in 105 (56%) patients. In 9 (5%) patients molecular analysis revealed a cryptic fusion transcript corresponding in 4 of them to AML with recurrent abnormalities and good prognosis. One was a PML-RAR fusion transcript allowing definitive diagnosis of promyelocytic leukemia. The other 5 cryptic fusion transcripts represented recurrent rearrangements (1x BCR/ABL in an AML; 1x MLL rearrangement, 1x TEL/AML1, 1x E2A/PBX, 1x SIL/TAL in ALL) not taken into account by the WHO classification but with prognostic and therapeutic consequence. In 72 (39%) patients with normal PCR, cytogenetics revealed various and complex abnormalities indicating bad prognosis.

Discussion: Both molecular and cytogenetics are needed for correct diagnosis of acute leukemia, and reveal complementary prognostic information. While cytogenetics show various and complex defects, broad multiplex RT-PCR reveals cryptic anomalies. Revision of the WHO classification should consider both elements.

222 UNREARRANGED MLL GENE AMPLIFICATION IN THREE CASES OF ACUTE MYELOID LEUKEMIA (AML)

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The mixed-lineage leukemia gene (MLL, HTRX1, HRX, ALL1), located at 11q23, is frequently rearranged in acute leukemias. Recently, overrepresentation or amplification of MLL has been also recognized as a recurrent aberration in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Previous reports indicate that 11q23/MLL overexpression affects mainly elderly patients and is associated with poor prognosis, previous treatment with alkylating agents and karyotype complexity. From January 2000 to February 2004 we have analysed 173 consecutive non-promyelocytic AML patients with an MLL flanking dual-color fluorescence in situ hybridization (FISH) probe (Vysis, Downers Grove, IL). Among them, we detected three patients (1.7%) with multiple FISH signals reflecting genomic amplification of this chromosomal region. **Case description:** 1) 45 years old man with a de novo AML, M4 FAB subtype, complex karyotype, primary resistant to three different induction courses and short survival (5 months). FISH disclosed the presence of six copies of the MLL gene, four of them assorted in a small metacentric marker chromosome. 2) 74 years old woman with a secondary AML to breast cancer treated with chemotherapy plus radiotherapy, presenting with a highly hyperdiploid karyotype and who was not treated because of early death. FISH showed a number of copies of MLL from three to a cluster composed of multiple hybridization spots. 3) 72 years old man with a de novo AML, M0 FAB subtype, complex karyotype and who did not received therapy because of poor performance status. FISH results showed MLL overexpression related to homogeneous staining region (hsr) observed by conventional cytogenetics. Comparative genomic hybridization (CGH) results demonstrated, among other aberrations, gain of large parts of 11q in the three patients. In our study, patients with supernumerary copies of MLL were in general older and had a shorter survival compared with other patients without MLL overexpression. Moreover, overexpression was associated with a complex karyotype in all cases. The significance of MLL overexpression and mechanisms by which it could play a role in leukemogenesis and/or disease progression remain to be clarified.

This work was supported in part by Grant for Research Groups No. 03/225 from Generalitat Valenciana.

223 DER(17)T(5;17) AND P53 GENE MUTATIONS IN SECONDARY ACUTE LEUKEMIAS

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Background: Secondary leukemias represent more than 40% of all patients with a myeloid leukemia (AML) or myelodysplastic syndrome

(MDS). A clinical history of exposure to alkylating agents or radiations is often present in these patients; however many older patients are diagnosed with leukemia with no antecedent exposure and show a similar phenotype to classic therapy-related leukemias.

Complete or partial deletions of the long arm of chromosome 5 are found in AML and MDS, in particular when the disorder is related to previous cytotoxic therapy (t-AML, t-MDS); however unbalanced translocations involving chromosomes 5 and 17 have been observed: dic(5;17), der(5)t(5;17), or der(17)t(5;17). These translocations are associated with drug resistance and worse prognosis. Several studies reported a strong correlation between cytogenetic rearrangements leading to a 17p deletion, a specific type of dysgranulopoiesis combining pseudo Pelger-Huet hypolobulation and vacuoles in neutrophils and p53 mutations.

Aim: We examined chromosome and molecular aberrations in four cases of secondary acute myeloid leukemia with abnormalities of 17p to evaluate the incidence of p53 mutations and its correlation with cytogenetic findings.

Methods and Results: At conventional cytogenetic all patients showed abnormal karyotypes with chromosomal loss or deletion of chromosome 5 and an alteration of chromosome 17(17p+); in one patient a trisomy 8 was also present. FISH with painting for chromosome 5 and with probe for 17p13 showed the presence of a der(17)t(5;17) in three out of four cases. Interphase FISH analysis performed with 5q31/5p15 and 17p13 probes showed the deletion of long arm of chr. 5 and of p53 in all three cases with translocation. The patient with 17p+ and +8 didn't show p53 deletion.

Since in three cases we have found a p53 deletion by FISH analysis, we also performed a molecular analysis to investigate if the remaining expressed p53 could present some mutations in the 'hot spot' regions. We analyzed RNA and we performed a sequencing analysis. Two patients presented the same mutation GCC→AGC GLY→SER at codon 245 in hemizygous state meanwhile the other patient deleted by FISH was normal.

Conclusions: Unbalanced 5;17 translocations represent recurring chromosomal abnormalities in myeloid disorders which result in the loss of genetic material from both 5q and 17p.

An alteration in the level of expression of the genes located at the breakpoints of this translocation, hemizygosity for genes on 5q and 17p and/or loss of function of critical genes can be considered the first event of a genetic instability and so the juxtaposition of genomic sequences as result of the (5;17) translocation seems to be a secondary step in the progression of the disease.

In 50% of our cases we demonstrated that cytogenetic abnormalities are accompanied by the same mutations of p53 on the cytogenetically normal homologous chromosome. The inactivation by mutations and deletions of p53 in addition to loss of tumor suppressor genes on 5q probably play a basic role in the pathogenesis of these malignant diseases.

224 A NOVEL CHROMOSOMAL TRANSLOCATION T(3;7)(Q26;Q21) IN MYELOID LEUKEMIA RESULTING IN OVEREXPRESSION OF EVI1

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Background. The EVI1 proto-oncogene encodes a nuclear zinc finger protein that acts as a transcription repressor factor. In myeloid leukemia it is often activated by chromosomal rearrangements involving band 3q26, where the gene has been mapped. Various mechanisms. Although the exact mechanism by which EVI1 exerts its leukemogenic effect is not clear, it is postulated that aberrant expression of the gene in myeloid progenitor cells may contribute to block granulocytic differentiation through the abnormal repression of genes necessary for cellular maturation. **Aims.** We analyze two leukemia cases, a chronic myeloid leukemia blast crisis (CML-BC) and an acute myeloid leukemia (AML)- M4 showing a t(3;7)(q26;q21) translocation in a balanced and unbalanced form, respectively, to verify the involvement of the EVI1 gene in these rearrangements. **Methods.** The bacterial artificial chromosome (BAC) clones used in this study were selected according to the physical and genetic mapping data on chromosomes 3 and 7 reported in the National Center for Biotechnology Information (NCBI, Ensembl database and the latest update of the University of California Santa Cruz (UCSC)

Human Genome Browser. Whole chromosome paintings (WCPs) for chromosomes 3 and 7 were obtained in collaboration with the Sanger Center. To isolate new clones, human primer sequence tagged site (STS) SHGC-87096 was selected and used to prepare probes for library screening. These probes were used to screen high-density filters of the entire RPCI-11/2 human male BAC library. We investigated the expression of human EVI1, CDK6, and EVI1/CDK6 fusion transcripts was investigated using a RT-PCR approach.

Results. Fluorescent in situ hybridization (FISH) analysis revealed that both patients showed a breakpoint on chromosome 3 inside the clone RP1133A1 containing the EVI1 oncogene and, on chromosome 7, inside the clone RP11322M5, partially containing the CDK6 oncogene which is a D cyclin-dependent kinase gene, observed to be overexpressed and disrupted in many hematological malignancies. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis showed overexpression of EVI1 in both cases, but excluded the presence of any CDK6/EVI1 fusion transcript. CDK6 expression was also detected.

Conclusion. The absence of the EVI1/CDK6 chimeric transcript in the two patients under investigation, together with the overexpression of EVI1, strongly suggest a deregulation of the EVI1 transcription as a consequence of a position effect. EVI1 expression might be activated by its closeness to a putative enhancer region in band 7q21, in the vicinity of CDK6.

225 DETECTION OF A CRYPTIC EVI1 GENE REARRANGEMENT USING A VALIDATED PROBE SET FOR INTERPHASE FISH

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Background:

The EVI1 gene, located on chromosome 3q26, is a proto-oncogene with leukaemogenic potential in myeloid malignancies. Translocations and inversions involving the EVI1 gene result in EVI1 expression. Ectopic EVI1 expression is correlated with highly aggressive disease, unfavourable treatment outcome and unfavourable karyotypes (-7/7q-, -5/5q- or complex karyotypes). Moreover, high EVI1 expression occurs with high frequency in patients without 3q26 abnormalities, suggesting other mechanisms elicit EVI1 expression.

Purpose:

In an attempt to explain aberrant EVI1 expression in AML, MDS and CML patients without cytogenetically visible chromosomal 3q26 aberrations, we initiated a screening of bone marrow samples of patients with myeloid malignancies using a validated interphase FISH assay for detection of cryptic 3q26 aberrations.

Methods:

BAC clones were carefully selected at the centromeric (RP11-694D5) and telomeric (RP11-362K14) end of a 1.3 Mb EVI1 contig besides a centrally located clone covering the EVI1 locus (RP11-82C9) (B. Poppe et al. in preparation). A set of two probe combinations, one probe covering the EVI1 gene (RP11-82C9) with either the telomeric or centromeric probe were hybridized to each sample. Normal interphase cells show two fusion signals corresponding to co-localisation of the two probes on the EVI1 locus.

Results:

A cryptic 3q26 rearrangement resulting from a t(3;17)(q26;q24.3) was detected in a 7-year-old boy with AML. Split signals were evident in 85 % of the cells using the probe combination of the centromeric probe and the probe covering the EVI1 locus. Analysis of G-banded metaphases showed monosomy 7 and additional material on (17)(q24.3) in 18/20 metaphases. Subsequent M-FISH analysis indicated that the add(17)(q24.3) resulted from a reciprocal t(3;17)(q26;q24.3). Careful re-analysis of the G banded metaphases (850 band resolution, ISCN 1995) however did not show any detectable aberration at chromosome band 3q26. The breakpoint on chromosome 17 was located at band q24.3. Bone marrow aspirate showed dysmegakaryopoiesis, a characteristic morphologic feature observed in association with 3q26 rearrangements.

Conclusion :

Our preliminary results point to the necessity to prospectively screen patients with myeloid malignancies for EVI1 gene rearrangements and EVI1 and MDS1-EVI1 gene expression using real-time quantitative PCR, in particular patients with monosomy 7 or other chromosome 7 aberrations.

226 CRYPTIC INSERTION LEADING TO NUP98/NSD1 CHIMERIC TRANSCRIPT IN MYELOID MALIGNANCY

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Background: The t(5;11)(p15;q35) is a cryptic rearrangement which results into the fusion between NUP98 at 11p15 and NSD1 at 5q35. Cytogenetic counterparts are both a normal karyotype or the presence of a del(5)(q). It is typically found in acute myeloid leukemia (AML) of both children and adults.

Aim: The aim of our study was to apply fluorescence in situ hybridization (FISH) in hematological malignancies with 11p15 rearrangements in order to pick up involvement of NUP98.

Methods: We report on a 55-year old man referred for anemia, leucopenia, and thrombocytopenia. A diagnosis of refractory anemia with excess of blasts (RAEB) was done on bone marrow aspirate. Cytogenetic analysis showed a 46,XY,add(11)(p15) karyotype in all 15 metaphases. Multicolor-FISH (MetaSystem[®] 24Xcyte, Metasystem, Altussheim, Germany) was done in order to characterize the add(11). Metaphase-FISH with probes for 11p13-15 (centromere- PAC 74J1- YAC 896B12- BAC 345G15- PAC 1173K1- PAC 143G7- PAC 908H22), 5q35-qter (centromere- YAC 925F9- BAC 549A4- PAC 240G13), and 15q22-qter (centromere- cosmid 15-11, 22, 5- PAC 124O5- telomere) was performed.

Results: Multicolor-FISH identified the extramaterial on add(11)(p15) as belonging to chromosome 15. Metaphase-FISH revealed a complex three way rearrangement between chromosomes 5, 11, and 15. The 11p15 breakpoint was identified by PAC 1173K1 (NUP98) which gave three fluorescence signals on normal 11 and on apparently normal 5 and 15. Double color experiments with PAC1173K1 and BAC 549A4 (NSD1/5q35) demonstrated that NUP98 was inserted at NSD1/5q35 locus. 3' RACE-PCR identified an in-frame fusion between NUP98 and NSD1. RT-PCR confirmed the presence of NUP98/NSD1 chimeric transcript, and showed two different spliced forms. The reciprocal NSD1/NUP98 product was not detected.

Conclusion: This is the first case of RAEB with a cryptic NUP98/NSD1 rearrangement, which results from an insertion of NUP98 at the 5q35/NSD1 locus. No accompanying 5q deletion was present at karyotypic level.

Expression of NUP98/NSD1 transcript, without the reciprocal NSD1/NUP98, emphasizes the relevant role of this chimeric protein in the leukemogenic pathway.

227 BREAKPOINT RESTRICTION AND BONE MARROW LINEAGE AFFILIATION OF A COMPLEX CHROMOSOME REARRANGEMENT INCLUDING THE ETV6-PDGFRB FUSION GENE

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Background: PDGFR β rearrangements identify a distinct subgroup of Ph-negative chronic myeloproliferative disorders.

Aims: The aim of our study was to characterize a Ph-negative myeloproliferative disorder by fluorescence in situ hybridization (FISH) and to investigate lineage affiliation of clonal bone marrow precursors.

Methods: A 43-year-old man with increased leukocytosis and eosinophils (WBC: 40.300 /mm³; Eo: 3200/mm³) was referred to our Hospital. Patient underwent treatment with imatinib mesylate (400 mg/daily). After three months complete hematological, cytogenetic, and molecular remission was achieved.

Cytogenetic analysis was done on bone marrow metaphases after short-term cultures, and karyotypes were described after G-banding with Wright stain.

Multicolor-FISH was performed as suggested by manufacturer (MetaSystem 24Xcyte, Metasystem, Altussheim, Germany).

Probes for 1p36 (telomere- PAC 163G9- BAC 193J6- PAC 1156D13- PAC 785P20- PAC 187A9- 863N7- PAC 967O8- cosmid 12A2- YAC 922E6- YAC 68G9- centromere), 5q33 (cosmid 9-4 and cosmid 4-1, for the 3' and 5' of PDGFR β , respectively), and 12p13 (cosmid 148B6 and cosmid 179A6 for the 3' and 5' of ETV6, respectively) were applied. Cytospins with mononuclear bone marrow cells were used in FICTION investigations (monoclonal antibodies+interphase FISH) for the bone marrow lineage affiliation of the ETV6-PDGFR β rearrangement. The following monoclonal antibodies were used: anti-CD13, anti-CD14, anti-CD3, anti-CD19, anti-CD20, anti-Glycophorin C (Dako, Hamburg, Germany) and anti-CD34 (Becton Dickinson, Franklin Lakes, NJ USA).

FICTION methodology was adapted from Weber-Matthiesen et al. (J Histochem Cytochem 1992;40:171-5). Immunostaining was performed with a three-step technique: Cy3-conjugated polyclonal goat anti-mouse, rabbit antigoat, and donkey antirabbit (Jackson-ImmunoResearch/Dianova, Hamburg, Germany).

Eosinophils were identified by autofluorescence of cytoplasmic granules.

Cosmids for ETV6 were used for FISH. In each experiment one control bone marrow slide was added from a typical CML in which each antibody was simultaneously tested with a BCR-FISH probe. Slides were analysed under a fluorescence microscope (Olympus) equipped with a CCD camera (Sensys) run from an image analysis software (Vysis, SmartCapture). For each antibody 10 to 50 cells were checked.

Results: Abnormalities of chromosomes 1p36, 5q33, and 12q24 were found at karyotypic level. Multicolor-FISH identified a four-break translocation i.e. t(1;12;5;12)(p36;p13;q33;q24). Metaphase FISH revealed a fusion between 5'ETV6 and 3'DGFR β at 5q33 while the 3'ETV6 was kept at 12p13 and the 5'PDGFR β was translocated to 12q24. The 1p36 breakpoint was identified by cosmid 12A2 which was split between 1p36 and 12p13. FICTION showed ETV6-PDGFR β rearrangement in 3/30 CD34 positive cells and 3/30 glycophorin positive erythroid precursor. Instead all granulomonocytic (CD13+ and CD14+) and all eosinophilic elements showed the rearrangement.

Conclusion: With this study we definitively identified eosinophils as part of the malignant clone in the PDGFR β -positive myeloproliferative disorders. Moreover striking differences emerged between bone marrow cells affected by the ETV6-PDGFR β lesion in present case and by BCR rearrangement in the typical CML used as control in this study.

GENETIC LEUKEMIA II

228 INCREASED RISK FOR ACUTE LEUKEMIA IN INDIVIDUALS WITH GLUTATHIONE M1 (GSTM1) AND T1 (GSTT1) GENE DEFECT

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Background: Environmental or occupational exposure to carcinogens as well as individuals treated with chemotherapy may increase the risk of developing acute leukemia (AL). The glutathione S-transferases are a group of enzymes involved in the detoxification of different substances, including carcinogens. The GST M1 (GSTM1) and theta 1 (GSTT1) genes are polymorphic in humans and the phenotypic absence of enzyme activity (null phenotype) may affect the risk of AL. **Aim:** In this case control study, we tested whether the homozygous null genotype of GSTM1 and GSTT1 altered the risk of AL.

Methods: We analyzed GSTM1 and GSTT1 genes simultaneously by a multiplex PCR method in 55 patients with AL (25 patients with secondary leukemia and 30 patients with de novo leukemia: 16 patients had acute myeloid leukemia and 14 acute lymphoblastic leukemia) and 147 cancer free controls of similar age, race and sex. Patients and controls originated from the same area-district. x2 test was used for comparisons between the two groups. P-values less than 0.05 were considered statistically significant.

Results: The frequency of the GSTM1 null genotype was significantly higher among patients with AL (60%) than among controls (33.3%) (p=0.0007 and O.R.=3). The GSTT1 null genotype was also associated with increased risk of AL (patients: 25.45% and controls: 6.66%) p=0.0003 and O.R.=4.8 (Table).

Conclusions: Individuals with the homozygous deletion of the GSTM1 and GSTT1 genes may have enhanced susceptibility to AL. The mechanism might involve decreased detoxification of environmental or endogenous carcinogenesis.

229 AML1 GENE REARRANGEMENTS IN RADIATION-ASSOCIATED ACUTE MYELOID LEUKEMIA AND MYELOYDYSPLASTIC SYNDROME

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Background: Several studies suggested a link between the AML1 gene rearrangements and radiation-induced acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Hromas et al. (Blood 95:

4011, 2000) described 3 patients with AML after exposure to nuclear explosions who had novel translocations in the AML1 gene. Harada et al. (Blood 101:67, 2003) found high incidence of AML1 point mutations in MDS patients among atomic bomb survivors in Hiroshima. Aims. The aim of the study was to check whether the AML1 gene alterations are of importance in the leukemogenesis of AML and MDS patients with the history of radiation exposure. Methods. Forty-six patients for this study were initially diagnosed with AML or MDS at the Research Centre for Radiation Medicine, Kyiv, Ukraine. Of these patients, 24 had experienced radiation exposure due to the Chernobyl accident in 1986, and 22 had spontaneous AML and served as controls. Interphase FISH using dual-color AML1/ETO probe (Vysis, Downers Grove, USA) and/or reverse transcription polymerase chain reaction (RT-PCR) detection of AML1/ETO translocation was performed on the samples of 43 AML patients. AML1 mutations were screened in 5 Chernobyl victims with MDS or AML following MDS by direct sequencing of genomic DNA of the coding region after PCR amplification of each single exon using intronic primer pairs. Results. In contrast to the study of Hromas et al., we did not find any AML1 rearrangements except of AML1/ETO translocation in the group of radiation-associated AML. AML1/ETO translocations were found in 7 (31,8%) of 22 spontaneous AML, but only in 1 (4,8%) of 21 radiation-associated AML cases. The prevalence of AML1/ETO translocations was significantly higher ($P < .03$) in the control group. In contrast to the study of Harada et al., we did not detect any point mutations in MDS patient but a hexanucleotide duplication of CCGCAT inserted in exon 6 after base position 1547, causing an additional Glycine and Isoleucine. To our knowledge, intragenic duplications in AML1 gene have never been described in MDS before. Moreover, all of the AML1 mutations in atomic bomb survivors were found in the DNA-binding Runt homology domain. It was stated that mutations in the carboxy (C)-terminal region strongly correlated with sporadic MDS (Harada et al., Blood 102: 913a, 2003) whereas, the mutation in our MDS patient survived acute radiation syndrome in 1986, was detected in C-terminal transcriptional activation domain next to the residue 368. Conclusions. We conclude, that recurrent chromosomal translocations of the AML1 gene characteristic for the disease de novo and therapy-related AML after treatment with topoisomerase II inhibitors are not common among the AML patients exposed to ionizing radiation due to the Chernobyl accident. These results preclude the importance of the AML1 gene translocations in radiation-induced leukemogenesis. In spite of some differences to those of Harada et al., our results do not conflict with the idea that radiation may contribute to the development of MDS through AML1 gene mutation.

230 COMPARISON OF A MULTIPLEX RT-PCR ASSAY WITH CYTOGENETIC ANALYSES IN ADULT HEMATOLOGIC NEOPLASMS

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A multiplex RT-PCR assay for the simultaneous detection of 29 chromosomal aberrations was developed (Pallisgaard N. et al.: Blood 92(2) 1998: 574-588). To examine its usefulness samples from 118 patients were tested and results were compared with those obtained by cytogenetic analysis: 62 AML (37 abnormal), 22 ALL (11 abnormal), 11 CMPD (4 Ph+, 7 Ph-), 18 MDS (6 abnormal) and 5 without leukemia. Out of 58 patients with karyotype abnormalities, 30 had translocations also covered by the multiplex RT-PCR assay: one each with t(1;19), t(4;11), and inv(16), 2 t(9;11), 3 t(6;9), 4 t(8;21), 6 t(15;17) and 12 t(9;22). All but one rearrangements were also detected by PCR. The only exception was an AML patient with a cytogenetically identified t(9;11) translocation that was PCR negative. On the other hand, 28 patients had abnormalities that are not covered by the multiplex RT-PCR assay, 26 of them were PCR negative. In one AML patient a MLL duplication was detected, which, however, was not identifiable on the cytogenetic level. In addition, a masked inv(16) was detected in a AML patient by multiplex PCR and confirmed by FISH analysis. In 2/17 patients with no mitoses a t(9;22) and a dupMLL was detected, all others were PCR negative. In both patients the specific translocations were confirmed by FISH analysis. In one patient with normal karyotype a dupMLL rearrangement was detected by multiplex PCR. Of note, PCR did not detect any rearrangement in either of the remaining 42 patients with normal karyotypes. In addition, the respective split-out reactions of the multiplex RT-PCR assay were used for long-term follow up MRD in cases that were positive for a specific rearrangement.

In conclusion, the multiplex RT-PCR assay is a useful and fast supplement to cytogenetic analysis. Results are typically achieved within

48 hours without the need of dividing cells and translocation specific split-out reactions may be used for MRD detection.

231 PROGNOSTIC SIGNIFICANCE OF STRUCTURAL CHROMOSOMAL REARRANGEMENTS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND HIGH HYPERDIPLOIDY

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Background: Hyperdiploidy with more than 50 chromosomes is one of the most reliable predictors of a favourable clinical outcome in childhood ALL. Despite of generally favourable prognosis of high hyperdiploidy, around one fourth of children will suffer an adverse event within 5 years after diagnosis with current therapy. One of the possible explanations of this phenomenon could be that in approximately half of the cases with high hyperdiploidy, translocations and other structural chromosomal abnormalities can be present as well. Aims: The aim of the study was to evaluate the significance of structural and/or complex chromosomal rearrangements for prognosis of children with ALL and high hyperdiploidy. Methods: To detect hyperdiploidy in interphase cells we used I-FISH with combinations of alpha-satellite and/or locus-specific probes (200 nuclei analysed per probe-mix, cut-off level 2.5% tested on controls, standard deviation $\geq 0.5\%$). The patients were divided into prognostic groups according to molecular-cytogenetic results and level of ploidy. Structural and/or complex chromosomal aberrations in hyperdiploid cells were analysed by mFISH. Results: We examined prospectively or retrospectively 125 children with ALL (79 boys, 46 girls; mean age 7.4 years). Cells with high hyperdiploidy were found in 56 patients (44,8%). The mean follow-up of high hyperdiploid cases was 32.2 months, during that time eight patients (14,3%) suffered an adverse event and five of them (8,9%) died (three for leukemia and two for treatment complications). Structural and/or complex chromosomal rearrangements together with hyperdiploidy were ascertained in bone marrow of 12 patients from this group (21,4%). t(9;22) was detected in one patient, two patients had dup(1q) and one patient del(6q). Other structural rearrangements found in our patients were random. Analysis of event-free survival (EFS) revealed significantly shorter survival in patients with structural chromosomal rearrangements in hyperdiploid cells in comparison to those with numerical changes only ($p=0,007$). Conclusions: Hyperdiploidy can be associated with structural chromosomal abnormalities (deletions, translocations, insertions), which can be proved by M-FISH. In our cohort of patients with high hyperdiploidy, karyotypes with structural and/or complex aberrations were connected with poor prognosis.

Supported by grants GACR 301/04/0407 and IGA MZ CR NE 6472-3. zuze@vfn.cz

232 DYNAMICS OF TELOMERASE ACTIVITY AND EXPRESSION OF THE HTERT GENE IN MYELODYSPLASTIC SYNDROMES AND ACUTE LEUKEMIA

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Background: Telomerase is ribonucleoprotein with reverse transcriptase activity synthesizing repetitive sequences onto telomeres - specialized structures on chromosome ends that keep chromosome integrity and protect them from fusion and degradation. In normal somatic cells telomerase is detectable only in tissues with self-renewal capacity. On the other hand, for almost all of tumors, including hematological malignancies, is characteristic reactivation or enhancement of telomerase activity leading to cell immortalization. The hTERT gene encoding a catalytic subunit of telomerase represents one of the main regulators of enzyme activity.

Aims: To study activity of telomerase and expression of the hTERT gene in patients with myelodysplastic syndromes (MDS) and acute leukemia (AML, ALL) with the intention to find out their potential as molecular marker of leukemic cells on the base of their association with clinical outcome and disease progression.

Methods: The study was performed on 53 samples of 48 patients with MDS, 49 samples of 41 patients with AML, 14 patients with ALL and 32 healthy BM/PBPC donors (Me=32 years, range 3-60) serving as negative controls. Peripheral blood or bone marrow mononuclear cells

from samples taken at the time of diagnosis and during the course of the disease were used for preparation of protein extracts and RNA. Protein extracts equivalent to 1 000 cells were analyzed for telomerase activity using modified TRAP Assay - TeloTAGGG Telomerase PCR ELISAPLUS kit. Expression of the hTERT gene was assayed by quantitative real-time PCR with specific Tag-Man probe.

Results: In MDS enhancement of telomerase activity was detected in 50% (12/24) of patients with RA and RARS (TA=0.18990.1746) and in 75% (12/16) of patients with advanced forms (RAEB, RAEB-t; TA=0.021640.1656). In RAEB/RAEB-t average telomerase activity was higher, but not significantly different when compared to early stages of MDS (0.2164 vs. 0.1899). In acute leukemia, increased telomerase activity was observed in roughly 80% of patients, both with ALL (11/14; TA=2.09720.5277) and with AML (32/40; TA=3.49177.9708). We found no correlation between level of telomerase activity and different subtypes of AML. We confirmed a decrease of telomerase activity after induction treatment in six AML patients, four of them became telomerase negative in remission. We detected normal level of telomerase activity in almost all of AML patients investigated after treatment only (78%; 7/9). Expression of the hTERT gene was significantly increased in 20% (1/5) of patients with MDS and in 44% (8/18) of AML patients.

Summary/conclusions: As we showed, telomerase activity seems to be a common feature of acute leukemia found in the majority of patients before treatment. Decrease of telomerase activity during the course of AML has confirmed a correlation of enzyme level with clinical outcome, especially with blast cells in BM/PB. Our results indicate that telomerase activity could serve as molecular marker of acute leukemia. Although expression of the hTERT gene indicates positive correlation with telomerase activity, evaluation of its clinical significance and validity for disease prognosis requires more extended study.

The study was supported by the grants of the MHCR NC/7606-3 and CR 0023736001.

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233 INFLUENCE OF THE GSTM1 NULL GENOTYPE IN THE RISK OF ACUTE MYELOID LEUKAEMIA AND NEUTROPENIA DUE TO CHRONIC EXPOSURE TO CHEMICAL AGENTS

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Background: Glutathione S-transferases (GST) modulate the effects of exposure to various cytotoxic and genotoxic agents, including those associated with increased risks of acute myeloid leukaemia (AML) and neutropenia due to chronic exposure to chemical agents. Cancer is the second most common cause of death in south-eastern Brazil and occupational related diseases resulting from exposure to chemical agents have been described as a serious health problem in this region of the country. Both the GST mu1 (GSTM1) and GST theta1 (GSTT1) genes have a null variant allele in which the entire gene is absent. Aims: In this study, we tested whether null genotypes for the GSTM1 and GSTT1 genes altered the risks for diseases. Methods: The diagnosis of AML was reached in 38 patients according to the criteria of the French-American-British Cooperative Group. The diagnosis of neutropenia due to chronic exposure to chemical agents was established in 55 patients with no medical problems, who presented consistent history of chemical exposition, neutrophil < 1.4x103/ul in peripheral blood, and hypocellular bone marrow. The control group consisted of 300 consecutive blood donors from the same university hospital in order to provide a representative group of the general population that seeks medical assistance in this region. DNA from AML and neutropenic patients and controls was analysed by multiplex polymerase chain reaction. Results: The frequencies of GSTM1 and GSTT1 null genotypes and combined null genotypes were significantly higher in AML patients than in the controls (73.6% vs 44.7%, 34.2% vs 17.3% and 26.3% vs 8.0%, respectively; $P < 0.03$). In contrast, the frequency of the GSTM1 null genotype in neutropenic patients was significantly lower than that found in controls (27.3% vs 44.7%, $P = 0.03$). The frequencies of GSTT1 null genotype and combined null genotype were similar in the same groups of individuals (25.4% vs 17.3% and 12.7% vs 8.0%, respectively, $P > 0.50$). Our observation of a 3.5 (95% CI: 1.63-7.40) and 2.5-fold (95% CI: 1.20-5.17) increased risk associated with the GSTM1 and GSTT1 null genotypes, respectively, and a 8.2-fold (95% CI: 2.85-23.68) increased risk associated with the combined null genotype presents evidence that the inherited absence of this carcinogen detoxification pathway may be an important determinant of AML. Surprisingly, our observation of a 0.43 (95% CI: 0.20-0.94) decreased risk associated with GSTM1 null genotype

in neutropenic patients presents evidence that the inherited absence of this carcinogen detoxification pathway may confer protection against neutropenia. Conclusions: Taken results together, we hypothesize that among individual chronically exposed to chemical agents, those with the GSTM1 null genotype may develop AML, whilst those with the presence of the GSTM1 genes may present a more benign form of this occupational disease.

Supported by FAPESP

234 GENE EXPRESSION PROFILE OF ACUTE MYELOID LEUKEMIA (AML) CASES WITH T(8;16)(P11;P13) AND MYST3/CREBBP REARRANGEMENT

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Background: Translocation t(8;16)(p11;p13) is an infrequent but recurrent chromosomal abnormality found in both de novo and secondary AML and leading to fusion of MYST3 (MOZ) and CREBBP (CBP) genes. This leukemia variety displays an unique clinico-biological profile characterized by myelomonocytic phenotype, frequent extramedullary involvement, and severe coagulation disorders.

Aim: To analyze the gene expression profile of a series of AML patients with MYST3/CREBBP rearrangement in comparison to other well-defined leukemia subtypes.

Patients and methods: Genotypic analyses using oligonucleotide U133A arrays (Affymetrix) were performed in sixteen AML samples. The series included AML with t(8;16) (n=3), t(15;17) (n=3), t(8;21) (n=2), inv(16) (n=2), t(9;11) involving MLL rearrangement (n=2), M4 (FAB subtype) with normal karyotype (n=3), and one case of M0 with t(16;16).

Results: After unsupervised hierarchical cluster analysis, cases of AML with t(8;16) clustered together, displaying a differential expression profile. Supervised software methods of analysis were performed to identify the top 200 up- and down-regulated genes of t(8;16) AML cases. The up-regulated genes included the proto-oncogene RET, genes involved in cell adhesion (lectin LGALS3), transcription regulators such as homeobox HOXA9, HOXA10, MEIS1, and the tyrosine kinase FLT3. Of note, HOXA9, HOXA10, and MEIS1 have been described to be up-regulated in MLL-rearranged myeloid leukemias. Interestingly, one of the t(8;16) cases studied had a concomitant amplification of the MLL gene.

Conclusions: AML with t(8;16) and MYST/CREBBP rearrangement represents a subgroup with singular clinical and biological features and a specific gene expression profile, which shares some similarities with MLL-rearranged leukemia.

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235 ANALYSIS OF THE EXPRESSION OF 11Q23/MLL ABNORMAL FUSION TRANSCRIPTS IN ACUTE LEUKEMIA: MULTI-CENTRE PERFORMANCE EVALUATION OF THE MLL FUSIONCHIP™

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Background: Rearrangements involving the MLL gene at 11q23 are highly heterogenous, with more than 50 partner genes having been described to date. These abnormalities are collectively associated with an unfavorable prognosis in acute lymphoblastic leukemia (ALL) and an intermediate risk in acute myeloid leukaemia (AML). However, the prognostic significance of specific fusion transcripts is unclear. Diagnostic testing of patients with acute leukemia includes cytogenetic analysis confirmed by FISH, RT-PCR, and/or Southern blotting. Molecular screening can be cumbersome, since identification of the fusion partner involves multiple and time-consuming analysis. Aims: We present the results of an international multi-center study to assess the clinical performance of a new biochip based molecular device (MLL FusionChip kit) designed to confirm the presence of an 11q23 abnormality and identify the MLL fusion gene partner. The analytical performance of

this device has been validated in a previous study. Methods: A range of ALL and AML samples with known MLL rearrangements, identified by cytogenetics, FISH, and/or RT-PCR, were available and tested in nine laboratories from seven countries. Following assessment of RNA quality, 127 MLL positive and 23 control samples were analysed with the MLL FusionChip™. Results: From the analysis of 150 samples, nine different partners were accurately identified. These included the rare partners: AF17, AF10, MSF, and P300. Furthermore, in two cases, the MLL FusionChip detected partners which had not been identified by RT-PCR. The overall agreement between the diagnostic analysis and the MLL FusionChip™ was >90%. Conclusions: These results demonstrate that the MLL FusionChip™ provides accurate results when compared with those obtained by alternative methods, and that it functions reliably in different laboratory environments. Accumulated data from a larger sample number may improve the stratification of MLL acute leukemia, which may lead to the development of individual therapeutic approaches for the different MLL partners, based on their unique molecular targets.

236 INCIDENCE AND SIGNIFICANCE OF MLL AMPLIFICATION DETECTED BY FISH IN ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid Leukemias (AML) carrying MLL rearrangements are characterized by a high degree of clinical and immunologic heterogeneity, as demonstrated by the variability in their immunophenotype, consistent with lymphoid or myeloid/monocytic derivation, as well as their occurrence in distinct age groups from infancy to adulthood.

Recently, amplification of 11q23 encompassing the MLL gene has been observed in AML patients and it has been identified as a new consistent cytogenetic mechanism of MLL activation, mediated by the upregulation of a number of other genes. The gene expression profile in patients with MLL amplification is similar to what observed in MLL-rearranged acute leukemia. A possible common pathogenetic mechanism in the two groups of disease may rely on the gain of function of the MLL gene which has been observed also in patients with three copies of MLL. Also in patients with trisomy 11, the presence of a MLL low-copy amplification may have an etiologic role based on MLL gain of function.

The introduction of FISH with MLL-specific probe have led to the identification of this new patient subgroup with 11q23 overrepresentation or amplification, consistently associated with an increased MLL copy number.

Aim: We studied the involvement of the MLL gene and in particular the presence of MLL amplification in 111 cases of de novo AML diagnosed following FAB criteria and treated according to the GIMEMA protocol.

Results: FISH analysis performed with MLL probe showed MLL involvement in 13/111 patients. Five patients were rearranged (4.5%); 8 cases were amplified (7.2%): four presented a trisomy 11. The trisomy 11 was isolated in one patient that had MLL tandem duplication, in another case it was associated with complex karyotype and in two cases it was associated with other trisomies (+2,+4,+8). The remaining four showed a number of copies of MLL varying from three to undefined number of signals: one of these had a t(8;21), one had a hyperdiploid karyotype and two a complex karyotype at cytogenetic analysis.

Conclusion: It was observed that the MLL copy number varied considerably from 3 up to a undefined number of copies in samples containing homogeneously staining regions or double minute chromosomes. Based on the number of MLL copies we divided the patients into 3 different groups (Poppe B., Blood, 2004). Group A (3 pts) included samples with 3 to 5 copies of the MLL gene due to retention of MLL on abnormal chromosome 11 or on the third chr.11; group B (1 pt) comprised samples with MLL amplification (5-10 MLL copies), not evidenced by banding analyses; and group C (4 pts) contained samples with a high number of MLL signals that could not be reliably counted, resulting from the presence of markers and double minute chromosomes. The presence of MLL amplification is not rare in de novo AML and the incidence in our study (7.2%) is similar or even superior to the frequency of MLL rearrangements.

237 ADDITIONAL CHROMOSOMAL ABNORMALITIES IN CML PATIENTS TREATED WITH GLIVEC (CYTOGENETIC AND MOLECULAR CYTOGENETIC STUDY OF 72 CASES)

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Background: Additional chromosomal abnormalities have been reported in patients with chronic myeloid leukemia (CML) in the chronic phase and accelerated phase during treatment with interferon and, more recently, with Glivec. Additional cytogenetic abnormalities occur in Ph-positive as well as in Ph-negative cells. It is not clear whether some of these changes represent true clonal evolution of CML. Therefore, the significance of these additional changes need to be explored.

Aim: To determine frequency and clinical importance of additional chromosomal changes in patients treated with Glivec.

Methods: Cytogenetics, FISH with painting probes and M-FISH, interphase FISH (iFISH) with BCR/ABL ES, BCR/ABL Dual Fusion and centromeric probes were used to determine the frequency of chromosomal changes in 72 CML patients treated with Glivec.

Results: Total 41 male and 31 female, median age 52 (range 17-71), were treated in an average of 18 months (range 1-39 months). At the start of treatment, 50 patients showed a standard Ph translocation, while in 22 patients a variant Ph translocation and/or additional changes were observed. Most frequent additional changes were deletion of 9q in 8 patients, trisomy 8 in 3 patients and additional Ph chromosome in 2 patients. Other 9 patients displayed different additional aberrations. Cytogenetic and/or molecular cytogenetic responses to Glivec were analyzed after 6 months of treatment. Nine patients died within the first 6 months of treatment, 2 patients were treated for less than 6 months, and therefore only 61 patients were analyzed. Cytogenetic and molecular cytogenetic responses occurred in 49 (80%) patients (complete response in 6, major response in 37, minor response in 6), 12 (20%) patients were cytogenetic non-responders. Six of the 12 non-responders had additional changes. Thus, the response to Glivec after 6 months of therapy was not influenced by additional chromosomal abnormalities. All patients with deletion of 9q responded to treatment, one of them achieved complete cytogenetic remission. Five patients developed new chromosomal aberrations, two of them in the Ph-clone. Out of 72 CML patients treated with Glivec, 12 patients have died; five of them had additional changes.

Summary: Cytogenetic and molecular cytogenetic study of CML patients treated with Glivec showed that 80% of patients responded within 6 months of treatment. The response did not differ with respect to the patients with or without additional abnormalities including patients with deletion of 9q. However, the long-term follow-up data will still be required in assessing the unambiguous importance of additional changes for prognosis and treatment of CML patients.

Grants IGA NC7490 and GACR 301/04/1239 supported this work.

238 ASSESSMENT OF ETV6 GENE INVOLVEMENT BY FLUORESCENCE IN SITU HYBRIDIZATION IN THREE NOVEL CHROMOSOME 12P REARRANGEMENTS IN HAEMATOLOGICAL MALIGNANCIES

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Translocations involving the short arm of chromosome 12 are frequent events in various haematological malignancies. In approximately half of the patients, the breakpoints are clustered within the ETV6/TEL gene on band 12p13, leading to its fusion with a variety of partner genes on different chromosomes. In the remaining cases, breakpoints occur telomeric or centromeric to ETV6, but no critical gene has been assigned to either one of those chromosome locations yet. Using fluorescence in situ hybridization (FISH) analysis with cosmid, BAC and YAC clones mapping to 12p, we characterized the chromosomal breakpoints in malignant cells of 3 patients with haematological malignancy, including 2 cases with precursor B lymphoblastic leukemia (B-ALL) and 1 with myelodysplastic/myeloproliferative disorder, unclassifiable (MDS/MPD, U). Cytogenetic analysis of bone marrow cells performed at diagnosis disclosed a t(5;12)(q12;p13), dic(9;12)(p;p?) and t(12;15)(p13;q22) as

single structural abnormalities in the karyotypes of the 2 B-ALL patients and the MDS/MPD patient, respectively. In the patient with t(5;12), the 12p13 breakpoint was localized between exons 5 and 8 of ETV6 using cosmids. However, clones mapping distally to exon 5 of ETV6 hybridized on chromosome 21 instead of on chromosome 5, revealing the presence of a three-way translocation involving chromosomes 5, 12 and 21 while suggesting the presence of a variant t(12;21) with fusion of the ETV6 and AML1 genes. FISH analysis with ETV6 and AML1 probes and reverse transcription ' polymerase chain reaction analysis confirmed the presence of an ETV6/AML1 fusion in this patient. In the patient with dic(9;12), hybridization of several clones mapping proximal and distal to ETV6 and the PAX5 gene at 9p13 yielded signals on the normal chromosomes 12 and 9 only, respectively, indicating that most part of the short arm was deleted in the rearranged chromosomes. In contrast with previously reported patients with dic(9;12)(p13;p13) and ETV6/PAX5 fusion, the present case suggests a different molecular rearrangement since both genes were shown to be deleted on the dic(9;12). In the MDS/MPD patient with t(12;15), the 12p13-pter region was shown to be translocated to the terminal part of the short arm of chromosome 6 instead of chromosome 15, revealing the presence of another three-way translocation. In addition, a breakpoint distal and proximal to ETV6 was found, revealing a 1.2 Mb deletion including ETV6 and 7 other genes. This report demonstrates the importance of FISH analysis in the characterization of variant 12p translocations with involvement of ETV6 and in the localization of novel breakpoints within band 12p13, indicating that other genes in the vicinity of ETV6 may also be involved in malignancy (This work was supported by Ncleo Regional do Sul ' Liga Portuguesa Contra o Cancro).

ANEMIA - CONGENITAL

239 HAPLOTYPES LINKED TO THE BETA S GENE IN SICKLE CELL ANEMIA PATIENTS FROM SOUTHERN IRAN

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1. Background: The clinical severity and hematological manifestations of sickle cell anemia are variable and influenced by a number of factors including haplotype background linked to the β S gene, variation in Hb F level and the simultaneous presence of α -thalassemia.
2. Aims: The main aim of the present investigation was to study the haplotype backgrounds of sickle cell disease in Southern Iran.
3. Methods: Haplotype of the β -globin gene cluster was determined in 52 sickle cell anemia (SS) patients by PCR-RFLP procedure. Denaturing gradient gel electrophoresis (DGGE) of A, B, and G fragments of the β -globin gene was carried out and the profile of G fragment allowed the determination of β -globin gene framework (Fw).
4. Results: Seventy out of 104 (67.5%) chromosomes of the SS patients were in linkage disequilibrium with the Arab-Indian haplotype. Twelve chromosomes (11.5%) were of Benin haplotype with the characteristic absence of Taq I site upstream of the Ay gene and Fw 2 of G-fragment of β -gene. Sixteen chromosomes (15.4%) were linked to the typical and atypical Bantu haplotypes. Five chromosomes (4.8%) were associated with Senegal haplotype and one chromosome with the Cameroon haplotype (1%). Six atypical haplotypes accounted for 14.4% of all chromosomes. It was noticeable that all 5 typical haplotypes were encountered in SS patients, Arab-Indian being the most and Cameroon being the least frequent haplotype.
5. Summary/conclusion: The β S gene in Southern Iran was found to be associated with multiple mutational events. The high prevalence of Arab-Indian haplotype linked to the β S gene in Southern Iran may explain the mild clinical presentation and high Hb F (around 15%) in SS patients. The presence of Benin haplotype may denote a gene flow from Africa and the high prevalence of atypical Bantu A2 haplotype may indicate a genetic admixture of Iranian population with Central African Republic (Bantu speaking).

240 MICROANGIOPATHIC HAEMOLYTIC ANAEMIA IN SICKLE CELL DISEASE

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Background: A syndrome resembling thrombotic thrombocytopenic purpura (TTP) has been rarely reported in sickle cell disease (SCD) patients presenting in crises. These are mostly isolated case reports. In this report we present 6 patients with SCD who showed microangiopathic haemolytic anaemia (MAHA) with thrombocytopenia and variable organ failure.

Aims: To analyse clinical and laboratory features of SCD who develop MAHA.

Methods: A retrospective study was conducted at Salmaniya Medical Complex, Bahrain. Patients with SCD in crises with thrombocytopenia whose blood smears showed significant numbers of fragmented RBC and who presented during the period January 2002 to December 2003 were selected. Clinical and laboratory data were documented from hospital case-files.

Results : There were 6 adult patients who presented with painful crises with features of an acute chest syndrome in two. All had jaundice and developed respiratory distress with low oxygen saturation, bilateral pulmonary infiltrates and required ventilator support. Three of these patients also manifested a TTP-like syndrome with fever, neurological complications and renal failure. The peripheral blood film showed burr cells, 'blister' cells and spherocytes in addition to schistocytes and thrombocytopenia. Clinical recovery was usually dramatic after TPE was instituted. TPE was continued until 2 consecutive platelet counts were ≥ 100 . Seven to ten sessions were required with 1.5 plasma volumes exchanged in each session. One patient who failed to respond showed pancytopenia and likely had marrow infarction in addition to systemic infection.

Conclusion: Recognition of a microangiopathic component in sickle cell crises is important because prompt institution of TPE is life-saving. The platelet count is a reliable guide for deciding the duration of the therapy.

241 XMN I SITE POLYMORPHISM 5' TO GAMA G IN SICKLE CELL DISEASE IN SOUTHERN IRAN

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1. Background: Molecular genetic factors regulating hemoglobin F (HbF) expression are important modifiers of the severity of sickle cell anemia. HbF is coded by γ genes of the β -globin gene cluster that are capable of binding α -chains. It has been suggested that the polymorphism C $\rightarrow$ T 5' to the γ gene in position '158 (recognized by XmnI restriction enzyme), is related to high G expression, high levels of HbF and delayed switching from fetal to adult hemoglobin.
2. Aims: The aims of the present investigation were to study the prevalence of Xmn I polymorphic site, the Hb F level, and the γ : α ratio in various haplotypes of 52 Sickle cell anemia (SS), 18 Sickle/ β Thalassemia (S/ β Thal) and 17 Sickle cell trait (AS) patients from Southern Iran.
3. Methods: Cellulose acetate electrophoresis at alkaline and acid pH and solubility test were used for diagnosis of sickle cell anemia. DNA was extracted from white blood cells by phenol-chloroform. The XmnI polymorphic site 5' to the γ gene and seven polymorphic sites through the β -globin gene cluster including 5' to ϵ gene by Hind II, within IVS 2 of the γ and α genes by Hind III, 5' to the α by Taq I, 3' to ψ by Hind II, IVS 2 of the β gene by Ava II, and 3' to the β gene by Hinf I were determined by PCR-RFLP procedure.
4. Results: The prevalence of Xmn I (+/+) site in SS patients was 53.8% which was higher than that for any of S/Thal (23.5%), AS (22.2%) and also normal Iranian individuals (7.5%). There was a correlation between the presence of Xmn I site and high γ : α ratio in Arab-Indian homozygous or heterozygous haplotypes (Contingency coefficient=0.43, P=0.002). However, this correlation existed only in SS and S/Thal patients but not in AS patients. The Hb F level was significantly higher (P=0.01) in SS patients with one or two Arab-Indian haplotype as compared to Bantu, Benin and Cameroon. However, the Hb F level was significantly higher in

S/Thal patients having two Xmn I sites carrying Arab-Indian and Senegal haplotypes as compared to Bantu, Benin and Cameroon haplotypes ($P=0.009$). The increasing effect of the presence Xmn I site on Hb F levels appears only when hemolytic stress is present as in SS and S/Thal patients (Contingency coefficient=0.35, $P=0.01$).

5. Summary / conclusion: It was concluded that the presence of Xmn I site 5 to the Gy gene which is observed only in Arab-Indian and Senegal haplotypes is the strong factor in predicting high Gy gene expression and elevation of Hb F level, especially in the presence of hemolytic stress. The wide variations in the Hb F levels among Arab-Indian and African haplotypes could be attributed to the presence other genetic mechanisms linked or non linked to the β S haplotype in the modulation of Hb F level.

242 A NEW VARIANT OF GLUCOSE PHOSPHATE ISOMERASE (GPI) (G145C/C921A) ASSOCIATED WITH SEVERE HEMOLYTIC ANEMIA

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Glucose-6-phosphate isomerase (GPI, EC 5.3.1.9) is a dimeric enzyme that catalyses the reversible isomerisation of glucose-6-phosphate (G6P) to fructose-6-phosphate (F6P), the second reaction step of glycolysis. GPI deficiency is the second most common erythro-enzymopathy of anaerobic glycolysis, after pyruvate kinase. So far about 50 cases of GPI deficiency have been described, and 29 mutations have been reported at the nucleotide level.

The aim of this study is to describe the haematological, biochemical and molecular characteristics of a new defective GPI variant associated with severe haemolytic anaemia. The propositus was a 1 yrs old boy of Northern Italian origin with chronic haemolytic anaemia and need for blood transfusion in concomitance of infectious episodes. At the time of the study Hb was 7.6 g/dL, reticulocytes 231x10⁹/L, total bilirubin 1.4 mg/dL. Screening tests for unstable and abnormal haemoglobins, red cell osmotic fragility, autohaemolysis and direct antiglobulin test were negative. SDS-PAGE analysis of red cell membrane proteins gave normal results. The study of the most important red cell enzymes did not reveal any abnormality except for a very low GPI activity (12.5% of normal). The parents were not consanguineous, and displayed intermediate GPI activity (66% of normal in both). The sequence of complete erythrocyte GPI gene revealed the presence of two new missense mutations: G145C (Gly49Arg) and C921A (Phe307Leu). Since both these aminoacids are conserved in eukaryotic AK1 enzymes, the mutations G145C and C921A are likely to impair the enzyme function or stability.

243 DIAGNOSTIC AND ANEMIA-ASSOCIATED ABERRATIONS OF RED BLOOD CELL MEMBRANE PROTEINS IN HEREDITARY SPHEROCYTOSIS AND MILD THALASSEMIA

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Background and Aims: Defects in red blood cell (RBC) membrane proteins may cause congenital hemolytic disorders like hereditary spherocytosis (HS). HS is manifesting as anemia, recurrent jaundice and splenomegaly. To elucidate the membrane protein aberrations that may play a pathophysiologic role in the observed hemolysis, we examined 12 HS patients (primary defects in spectrin, ankyrin or band 3), in comparison with a group of mild thalassemic cases (heterozygous alpha thalassemia and beta thalassemia minor) and several normal subjects of Greek origin. Methods: General hematological parameters and osmotic fragility test were performed by routine procedures. Peripheral blood RBC, total ghosts, membrane skeletons (MSk) and protein extracts were analyzed by SDS-PAGE scanning and immunoblotted against a variety of erythroid-specific antibodies. Results: The majority of the HS-ghosts were characterized by IgG binding above the normal range, pathological formation of band 3 dimers under non-reducing conditions, pallidin deficiency and a sharp increase in the amount of band 8 (21KD). The latter, is a mainly Triton-soluble component of cytoplasmic origin that is found in the normal RBC membrane as a minor electrophoretic zone. Contrary to the band 3-deficient HS cases, the majority of the skeletal protein-deficient ghosts and MSk were also found enriched in bound globin. By Western blot analysis, several HbA-positive aberrant bands represented globin multimers and cross-linkings with other membrane

proteins like spectrin (255 KD) were revealed. In a striking contrast, the supernormal globin load of healthy control ghosts was exclusively in the form of globin monomers. In the 4.1(-) hereditary elliptocytosis the MSk contained no globin. The amount of MSk bound globin was not proportional to the ghost-bound hemoglobin and was not aggravated after splenectomy, as usual in thalassemic MSk. Moreover, the HS globin oligomers and crosslinkages were more sensitive to the effect of reducing agents in comparison to those found in the thalassemic samples. The mild thalassemic samples were characterized by disturbed electrophoretic pattern of glycophorins, a sharp increase in the amount of ankyrin 2.1 and band 8, mild deficiency in band 3 and, in some cases, aberrant electrophoretic zones and tryptic peptides of spectrin. Conclusions: We conclude that the high affinity of the membrane for specific cytoplasmic proteins and IgGs and, possibly, the aberrations of many membrane components are associated with the observed anaemia and may be useful especially for the diagnosis in HS. In this small sample, the formation of globin multimers and cross-linkings seems to be associated with the pathophysiology of skeletal protein deficiency HS and is possibly indicative of oxidative defects of the membrane, that further promote the peripheral hemolysis events shown in HS.

This study was supported by the 'Empeirikeion Foundation' and the Special Account for Research Grants of the University of Athens to I. S. Papassideri.

244 IMPAIRED PROLIFERATION AND TRANSLATION IN DIAMOND-BLACKFAN ANEMIA PATIENTS

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BACKGROUND: 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 translation and proliferation rates at a basal state and after activation in DBA patients, especially in those having a mutation in RPS19.

METHODS: Peripheral blood mononuclear cells were isolated from DBA patients and healthy controls using Histopaque gradient centrifugation. Cells were divided into two groups and maintained in RPMI-1640 medium. In one group, phytohemagglutinin (PHA) was added to a final concentration of 2g/ml to activate proliferation. After two days of cultivation, proliferation was evaluated by the amount of [3H]-thymidine incorporated by the cells, and translation was measured by the amount of [3H]-Leu incorporated by the cells after 5 and 10 minutes of incubation. We analyzed 6 patients with no mutation in RPS19 (N-group), 3 patients with a RPS19 mutation (M-group), and 8 healthy controls.

RESULTS: The average level of basal translation (no PHA) was significantly lower in both the N-group and M-group in comparison with controls ($p<0.05$). The level of translation in the M-group was lower than in the N-group, non-significantly however. After the addition of PHA, the level of translation in both DBA groups remained lower than in controls (M-group, $p<0.005$), however the difference in the N-group was not significant ($p<0.07$). Interestingly, both DBA groups showed a higher activation of translation after PHA addition than did controls.

The average level of basal proliferation in both DBA groups was only 44% of controls ($p<0.05$). Following PHA addition, the level of proliferation improved, reaching 84% and 76% of the control level in the N-group and M-group, respectively (non-significant difference). Interestingly, PHA caused a nearly two-times higher activation of proliferation in N- and M-groups in comparison with controls ($p<0.01$).

The translation dynamics in the basal state (10min/5min ratio, no PHA) in the N-group was nearly equal to controls, while the translation dynamics in the M-group was significantly lower in comparison with both the N-group and controls ($p<0.05$). Interestingly, PHA addition led to a normalization of the translation dynamics in the M-group.

SUMMARY/CONCLUSION: Based on our results we conclude that 1) basal levels of proliferation and translation are significantly lower in DBA patients irrespective of mutation in RPS19; 2) patients with RPS19 mutation show more impaired translation than patients without RPS19 mutation; 3) DBA cells after PHA stimulation can activate proliferation and translation significantly more efficiently than controls. In summary,

hypoproliferation and significantly lower translation might be the mechanisms underlying DBA pathogenesis, and therapeutics generally increasing translation might be beneficial for DBA patient treatment.

245 CHARACTERIZATION OF A PYRIMIDIN 5' NUCLEOTIDASE MUTANT FORM (G241R) CAUSING HEREDITARY HEMOLYTIC ANEMIA.

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Background. Pyrimidine 5'-nucleotidase (P5'-I), also known as cytosolic 5'-nucleotidase III (cN-III) dephosphorylates preferentially pyrimidine 5'-monophosphates to corresponding nucleosides and inorganic phosphate. The enzyme is highly expressed in red blood cell, where it participates to the degradation of RNA during erythrocyte maturation. The enzyme also exhibits a phosphotransferase activity. P5'-1 deficiency is thought to be the third most common red blood cell enzymopathy causing hemolysis after G6PD and PK deficiency; the disorder is transmitted as an autosomal recessive trait, and it is associated with mild-to-moderate hemolytic anemia. The gene coding for the P5'-I has been cloned, and 13 different mutations have been so far reported. More recently we have expressed, purified and characterized the recombinant wild-type enzyme, and we have performed the first in depth biochemical analysis of N190S recombinant mutant form (Chiarelli et al, Blood 2003, abstract).

Aims. To increase our understanding of the molecular basis of the hemolytic anemia caused by P5'-I deficiency, we have undertaken the expression, purification and biochemical characterization of an additional mutant form of the enzyme. We focused our attention to the Gly241 to Arg (nt G721→C) that is the most common mutation described in Japanese P5N-1 deficient patients.

Methods. After site-directed mutagenesis, the recombinant mutant form was expressed in *E. coli* following induction with 0.5 mM IPTG at 30°C for four hours. The maximal expression level was in the range of values observed for the recombinant wild-type P5N-I (10-12 mg/L). The G241R enzyme was purified to homogeneity following the procedure developed for recombinant wild-type protein.

Results. A drastic reduction in the catalytic efficiency (2 orders of magnitude) and a significant reduction in thermostability ($t_{1/2}$ at 46°C about 5 min compared to 1 hour for the wild-type enzyme) were observed, whereas K_m values vs both substrates CMP and UMP resulted essentially unchanged.

Conclusion. The Gly241 is conserved in all known P5N-I enzymes, thus the mutation G241R probably affects an amino acid residue crucial for the catalytic mechanism and the bulky charged residue introduced is likely to impair the enzyme stability. Our data strongly suggest that the decreased enzyme activity observed in patients homozygous for the G241R mutation is due to altered kinetic properties as well as to lowered level of P5N-I as a consequence of protein instability.

This work was supported by grants from University of Pavia (FAR) and MIUR (FIRB and Progetto Genomica Funzionale, legge 449/97)

246 THE EFFECT OF PROLACTIN ON THE PROLIFERATION AND DIFFERENTIATION OF ERYTHROID PROGENITORS FROM HEMATOPOIETIC STEM CELLS IN DIAMOND-BLACKFAN ANEMIA.

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Background

Diamond-Blackfan anemia (DBA) is a rare congenital disorder of erythropoiesis characterized by reticulocytopenia and macrocytic anemia. In vitro colony assays of bone marrow cells of patients with DBA, colony formation of erythroid progenitors; erythroid colony-forming units (CFU-E) and erythroid burst-forming units (BFU-E) is usually defective or significantly impaired. Recently, Abkowitz et al (Blood 2002;100:2687) reported results of clinical pilot study of metoclopramide for patients with DBA. They reported the results of a clinical trial which studied the effect of prolactin (PRL), via metoclopramide treatment, on the proliferation of erythroid progenitor cells in patients with DBA. They stated that PRL increased red blood cell counts in some patients but they did not find the stimulating effect of PRL on erythroid progenitor cells from DBA patients in vitro.

Aims

We have studied, in vitro, the effect of PRL on the proliferation and differentiation of erythroid progenitor cells from CD34+ cells derived from two patients with DBA.

Methods

We obtained CD34+ cells, by using immunoreactive magnetic beads, from the bone marrow cells of six-month old (Patient 1) and one-year old (Patient 2) Japanese girls diagnosed with DBA, after obtaining written, informed consent from their parents. CD34+ (1x10³) cells were cultured in serum-free methylcellulose containing 3 U/ml rhEPO, 100 ng/ml rhSCF, and 30 ng/ml rhIL-3, with or without 100 ng/ml rhPRL for 14 days.

Results

Hemoglobinized erythroid colonies (BFU-E) were formed from CD34+ cells from each subject with a combination of SCF, IL-3, and EPO (Patient 1; 63; Patient 2; 113; controls; 478). Erythroid colony formation was enhanced by adding PRL to the mixture of SCF, IL-3, and EPO in a statistically significant manner (Patient 1; 172; Patient 2; 315; controls; 10311). However, we failed to see any clinical effect of PRL in Patient 1 when we used metoclopramide.

Summary

The discrepancy between our results and those of Abkowitz et al may be due to differences in the cells used in the respective culture systems; we used CD34+ cells whereas they were using mononuclear cells. Additionally, our starting cells contained comparatively more CD34+ cells whereas their cultures consisted of predominantly lineage committed cells. Furthermore, Bellone et al (J Cell Physiol 1995;163:221) previously reported that CD34+ cells expressed PRL receptors which are not expressed in the erythroid lineage committed cells employed in the Abkowitz study. In conclusion, our in vitro results support the results of the clinical study by Abkowitz et al and therefore suggest that metoclopramide is a potentially effective treatment for patients with DBA derived from two patients with DBA.

247 A FAMILY WITH SECONDARY AND CONGENITAL POLYCYTHEMIA: PROBABLE INVOLVEMENT OF O2 SENSOR PATHWAY

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BACKGROUND: Secondary polycythemia is characterized by sustained elevation of red cell mass due to excess of exogenous factors that promote erythropoiesis. In most cases, serum erythropoietin (Epo) is elevated. Conditions causing elevated Epo include hypoxia, O₂ hemoglobin (Hgb) hyperaffinity, decreased 2,3 DPG or inappropriate Epo secretion without a known pathogenesis. Recently, a mutation in the von Hippel Lindau (VHL) gene (Arg200Trp) has been described in patients with Chuvash polycythemia, an endemic autosomal recessive disorder which shares features of both primary (hypersensitivity to Epo) and secondary polycythemias (high sEpo levels). The underlying molecular defect consists on impaired interaction of mutated pVHL with HIF-1 alpha, reducing the rate of HIF 1alpha degradation, and as a consequence, increased Epo secretion. Here we present a novel family with secondary and congenital polycythemia. **CASE REPORT:** Five of 6 siblings (2 women, twins, and 3 men) display elevated Hgb levels since first years of life. In 1998 (age range: 18-29 years), Hgb ranged between 20.7-24 g/dL and hematocrit 61-73.4 %. No additional affected relatives were found, included their parents and all offsprings. Blood red cell mass was high (65 mL/Kg) in the propositus, in whom organic lesions have been ruled out. All 5 affected individuals have normal leucocyte counts and, curiously, all display low to moderate thrombocytopenia, (62 - 102 x 10⁹/L), inversely proportional to Hgb levels. PRV-1 expression was normal. We also excluded mutations in the cytoplasmic domain of the Epo-receptor gene by sequencing. All but one affected individual had elevated sEpo levels (range ELISA: 9-68 U/L), and all had normal pO₂ and O₂ saturation, p50, Hgb electrophoresis, 2,3 DPG and 2,3 DPG mutase. BFU-E cultures showed a normal pattern of Epo-dependent growth. Four patients have hyperviscosity symptoms and are being treated with phlebotomies. Twenty four hours after phlebotomies, in all 3 men there was an elevation of sEPO levels (5-70 mU/mL over basal values), suggesting hypersensitivity of O₂ sensing. Since these findings are similar to Chuvash polycythemia, we have performed linkage analysis and found co-segregation with markers spanning the VHL locus. Surprisingly, we found no mutations in the protein coding sequence of VHL. Analysis of the promoter and non-coding regions are under way. **CONCLUSIONS:** High sEpo levels appear to be caused by an as yet unknown defect in the O₂ sensor pathway. Inheritance of this familial polycythemia is compatible with an autosomal recessive trait and linkage analysis suggests involvement of the VHL locus. To our knowledge, there are no reports of familial polycythemia displaying thrombocytopenia at the same time.

248 THE FREQUENCY AND MOLECULAR GENETICS OF ERYTHROCYTE G6PD DEFICIENCY IN ZANJAN CITY (NORTHWEST IRAN)

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Background: Glucose-6-phosphate dehydrogenase (G6PD) is an X-linked enzymopathy affecting about 400 million people worldwide. Neonatal jaundice, drug-induced hemolysis and infection-induced hemolysis may happen in some deficient individuals and lead to considerable morbidity and mortality. The distribution of G6PD deficiency and the molecular genetics of this enzyme vary widely among different ethnic groups in different countries.

Aim: To find out the frequency of G6PD deficiency and characterize the molecular type of G6PD in deficient individuals in Zanjan city (northwest Iran).

Subjects and Methods: One thousand and five hundred unrelated normal male individuals were screened for G6PD deficiency by fluorescent spot test. DNA was extracted from peripheral white blood cells of individuals who were deficient for G6PD. Polymerase chain reaction (PCR) was used to amplify flanking regions of exons six and seven of this gene using primers 91 and 92. The PCR products were digested by the enzyme MbolI, then the digested products electrophoresed on a 2.5% agarose gel.

Results: Thirty-three out of 1500 individuals (2.2%) was shown to be deficient for G6PD enzyme. Twenty-four out of 33 (75.3%) showed a mutation at nt 563 of G6PD gene which is characteristics of Mediterranean type of mutation. Nine individuals (24.7) were negative for this mutation.

Conclusion: A strong correlation has been shown between G6PD deficiency and malaria in regions in that malaria is prevalent. Since the frequency of malaria is very low in Zanjan city, therefore, this rate of G6PD deficiency was predictable for this region. Despite different frequencies reported for deficiency of G6PD in central, north, northwest and southeast regions of Iran the results of this study and other studies carried out by us and others have shown that the incidence for Mediterranean type of G6PD in all the above regions are approximately the same. This may indicate that the predominant G6PD mutation in Iran is of Mediterranean type. ymort@yahoo.com

249 MOLECULAR ANALYSIS OF FERROCHELATASE GENE IN ITALIAN PATIENTS WITH ERYTHROPOIETIC PROTOPORPHYRIA

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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 anemia and, in 5-10% of the cases, progressive hepatic failure. Diagnosis of EPP can be supported by the presence of fluorescent erythrocytes 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 75 different mutations responsible for EPP, showing a high genetic heterogeneity. Phenotypic expression of EPP required coinheritance of a null FECH allele and a wild-type low expressed allele carrying IVS3-48 C polymorphism.

Aims and Patients: In this study we searched for molecular defects in FECH gene, in order to identify the prevalent mutations in Italian patients affected by erythropoietic protoporphyria. We investigated fourteen unrelated Italian EPP patients and their relatives. The diagnosis of EPP was based on skin photosensitivity, over-normal fluoroxytes count and abnormal fluorescence emission in plasma and red blood cells.

Methods: We applied a two-step screening strategy for all 11 exons plus intron-exon boundaries of FECH gene. Denaturing gradient gel electrophoresis (DGGE) was followed by direct sequencing of PCR fragments showing abnormal migration patterns compared to control.

Results: Seven different molecular defects in FECH gene have

been identified in fourteen patients. Four were previously reported in caucasians (IVS1+5 G>A, 213insT, 843delC, 899delTG) and two of them were the most common in our EPP group. Four mutations are new findings: a 14 bp deletion in exon 5 (488-501) causing a protein truncation at aminoacid 170; a missense mutation (791 C>T) in exon 7 resulting in a 264 Ser to Leu amino acid substitution; two nonsense mutation (892 C>T) in exon 8 and (930 G>A) in exon 9 responsible for creation of a stop codon at amino acid 298 and 310, respectively. These mutations were also identified in five symptomatic relatives and two asymptomatic carriers. All symptomatic subjects displayed on the other allele the polymorphism IVS3-48C, responsible for allele low expression.

Summary: This is the first molecular study aimed to identify FECH molecular defects in Italian EPP patients. These results confirmed the high heterogeneity of mutations responsible for EPP phenotype and allowed the identification of four novel mutations, so far limited to Italian population. Among our relatives we identified symptomatic and asymptomatic carriers, confirming the usefulness of molecular analysis for EPP carriers detection.

250 DIAGNOSTIC TOOLS IN CRYOHYDROCYTOSIS DEMONSTRATED IN A PATIENT WITH A SPONTANEOUS MUTATION

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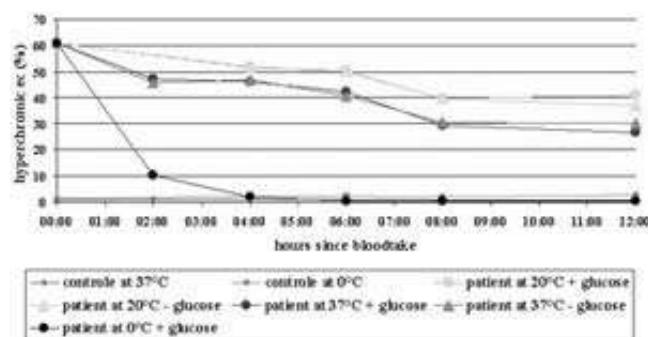
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Background: Cryohydrocytosis is a stomatocytic disorder with abnormal permeability of the red cell membrane to sodium and potassium. This leak has its minimum at about 20-23°C and is typically greatly enhanced at 0°C (Miller et al. Blood 38(1971)184-203; Coles et al. Br. J. Haematol. 105(1999)1055-1065). Patients show generally a mild hemolytic condition, pseudohyperkalemia and splenomegaly. Hemolysis does not resolve completely after splenectomy. Diagnosis of cryohydrocytosis is in principle simple, but remains often unnoticed, because of a mild, well compensated anemia and variable red blood cell (RBC) shapes ranging from stomatocytes to 'atypical spherocytes'. **Aims:** A new case of spontaneous mutation to cryohydrocytosis has prompted us to design a scaling up of diagnostic tools to either exclude or confirm cryohydrocytosis. **Methods:** Routine hematology tests, including reticulocyte counts and erythrocytic histogram were performed on a Bayer Advia 120. To assess altered cation permeability of red cell membrane we measured plasma potassium concentration [K⁺], mean cellular volume (MCV), mean cellular hemoglobin concentration (MCHC) and percentage of hyperchromic erythrocytes in whole heparinized blood (WHB) stored for the given times at different temperatures (0°C, 20°C and 37°C).

Results: Our patient showed a mild hemolytic condition: hemoglobin 14.2g/dl (normal range 13.5-17.2), reticulocytes 44 (6-17), MCV 82.6fl (80-100), MCHC 37.8g/dl (31-36), hyperchromic erythrocytes 60.9% (0-1.5). Both parents showed normal readings. In normal individuals plasma [K⁺], MCV and percentage of hyperchromic RBC remain virtually the same on storage of WHB at 0°C. WHB of our patient revealed upon a 2h incubation at 0°C a 7-fold increase in plasma [K⁺], a 83%-decrease of the percentage of hyperchromic RBC and a 10%-increase in MCV. With this simple test we could easily document a greatly altered red cell membrane cation permeability. Further investigations, including percentage of hyperchromic RBC, plasma [K⁺], and MCV in WHB stored in the presence or absence of glucose at 0, 23 and 37°C (figure 1 for hyperchromic RBC) and flux measurements confirmed the type of disturbance. The intracellular electrolytes on fresh blood showed a high [Na⁺] and a low [K⁺]. The isotopic flux studies at 37°C showed an increased ouabain+bumetanide-resistant influx of potassium compared to control RBC. The temperature dependence of this leak showed a U-shaped profile with a minimum at about 23°C and a maximum at 0°C.

Conclusion: The normal hematological parameters were not conclusive, but the data from cold stored WHB were typical for cryohydrocytosis, with a pronounced potassium leak of the RBC. The potassium leak showed a minimum at 23°C and a very marked enhancement at 0°C. This is consistent with the findings in our simple analysis with stored blood at 0°C, where maximal changes of plasma [K⁺], MCV and hyperchromic erythrocytes occurred. Hence, we recommend this test in cases where hematological parameters and RBC shape determinations yield inconclusive results. This test will give a tentative diagnosis that can be strengthened with measurements as shown in figure 1. Flux measurements will further allow to assign the patient to the known variants of cryohydrocytosis.



Caption 1: Figure 1

ANEMIA - THALASSEMIAS

251 PRELIMINARY REPORT ON THE STUDY ON HEPATITIS C TREATMENT USING RIBAVIRIN AND PEGYLATED INTERFERON IN THALASSEMIA PATIENTS (SCRIPT)

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BACKGROUND: Treatment of thalassemia patients with HCV using combination interferon and ribavirin is limited to few case series.

DESIGN: Multicenter randomized single-blinded trial comparing PEG-IFN α -2a +/- ribavirin in thalassemia major patients with chronic HCV.

METHODS: 20 patients with HCV genotypes 1 and 4 were randomized into Group A (PEG-IFN 180 mg SC once weekly) and B [PEG-IFN and ribavirin (10.6 mg/kg/d)] for 48 weeks. Baseline studies included liver biopsy (histology and iron concentration), quantitative PCR, and HCV genotyping. The primary end point was a SVR 24 weeks post-treatment. Secondary end points were transfusion requirements, EVR, ETR, and aminotransferases.

RESULTS: Baseline characteristics of both groups were comparable. The mean age was 21.6 $\pm$ 11 years in group A and 16.5 $\pm$ 3.9 in group B. Fibrosis grade >1 was present in 42% in group A and 50 % in group B. Mean viral load was 137,570 IU/ml and 178,087 IU/ml respectively. Genotype 1 was found in 66% in group A and 87% in group B. EVR occurred in 8/12 in group A and 6/8 in group B (67 and 75% respectively). Transfusion requirements increased by 55% in group B versus 21% in group A ($p=0.08$). Adverse events were not significantly different between treatment groups.

CONCLUSIONS: This interim report suggests that combination PEG-IFN and ribavirin is effective at achieving EVR in thalassemics, is associated with an increase in transfusion requirements but no other significant side effects. Additional follow up and patient recruitment is needed to evaluate long term safety and efficacy.

252 THE -195 C>G MUTATION IN THE A-GAMMA-GLOBIN GENE AMELIORATES THE HETEROZYGOUS BETA-THALASSAEMIA PHENOTYPE

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Haemoglobin F (Hb F) is the main Hb in foetal period. After birth, its synthesis normally decreases to about 75% of total Hb, and continues to fall to less than 2% by 2 years of age, being simultaneously replaced by the synthesis of adult Hb A. Non-deletional Hereditary Persistence of Foetal Haemoglobin (nd-HPFH) is a condition characterised by a continuous expression of gamma-globin genes during adult life. This increase is associated with specific single-base mutations in protein binding motifs of the gamma-globin gene promoters, which prevent the binding of repressors or enhance the binding of transcriptional activators, interfering with gene expression.

In the present study, we characterised the genetic factors associated with very increased Hb F levels in two beta-thalassaemia carrier families (Hb F between 19-50%) and in two isolated healthy individuals with moderately raised Hb F (6.4-10.8%).

Red blood cell indices were obtained with an automated cell counter. Hb analysis was performed by isoelectrofocusing. Hb A2 was quantified by low pressure cation-exchange chromatography (LPLC) and Hb F by alkali denaturation. The G-gamma/A-gamma chain ratios were measured by reversed-phase high performance liquid chromatography (RP-HPLC). Genomic DNA was isolated from white blood cells. The beta-thalassaemia mutations were identified by Amplification Refractory Mutation System (ARMS). The gamma-globin gene promoters were analysed by sequencing of the appropriate PCR-amplified fragments and beta-globin gene cluster haplotypes were characterised by PCR and restriction enzyme analysis of nine biallelic polymorphic sites.

The studied individuals (carrying or not a beta-thalassaemia mutation) presented HbF mainly of A-gamma type. The molecular basis of this condition was identified as being the -195 C>G mutation in the A-gamma-globin gene promoter, presented in heterozygosity. Moreover this nd-HPFH determinant was in linkage disequilibrium with base C at -158 of G-gamma gene and with haplotype I or Vb. This nd-HPFH allele when in trans with a beta-zero-thalassaemia allele [Cd 39 C>T (one case) and IVS-1-1 G>A (two cases)] was responsible for very high Hb F levels (up to 50%), and ameliorated the red blood cell indices and Hb A2 level.

A-gamma globin gene -195 C>G mutation was already found in some healthy Brazilian individuals, closely associated with moderately Hb F level. We describe, for the first time, this nd-HPFH determinant in the Portuguese population and its effect when co-inherited with beta-thalassaemia.

The modulation effect of the -195 C>G A-gamma mutation on the haematological phenotype of heterozygous beta-thalassaemia is discussed and we highlight the importance of these kind of determinants in the understanding of phenotypic variation in beta-thalassaemia. paula.faustino@insa.min-saude.pt

253 NTBI VALUES IN THALASSAEMIA PATIENTS ON COMBINED CHELATION (DFO+L1) TREATMENT OR ON MONOTHERAPY (DFO OR L1)

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Background: Chelation therapy is required to reduce the morbidity and mortality associated with secondary iron overload due to chronic transfusion therapy. Several studies have shown in iron overloaded patients a significant increase of serum toxic low-molecular-weight iron (NTBI). Subcutaneous infusion of desferoxamine (DFO) remains the first choice treatment although deferiprone (L1) has been used as oral chelating agent in patients not compliant to DFO. Moreover it has been recently suggested that combined chelation with DFO and L1 could be more effective than monotherapy.

Aim: In order to evaluate if the combination of DFO and L1 may better chelate iron than monotherapy, we measured NTBI in thalassaemia major (TM) patients.

Methods: We studied the NTBI in 21 regularly transfused TM patients treated with DFO 50 mg/Kg/day 5 days/week for at least 10 years; in 15 TM on L1 (75 mg/Kg/day) for 12 months and in 17 TM on combined therapy (DFO 40 mg/Kg/day 3 days/week + L1 75 mg/Kg/day) for one year. Serum iron and transferrin saturation % were evaluated by standard procedures; serum ferritin was determined by immunoenzymatic assay (EIA). NTBI was assayed in serum by HPLC after nitrilotriacetic acid chelation (NTA). Briefly, serum 450 μ L was added with 0.8 M NTA and allowed to stand for 20 min to remove both the iron (Fe) nonspecifically bound to serum proteins and low molecular weight ligands. The NTBI scavenged was quantitatively converted into Fe-NTA complex. The mixture was then ultrafiltered using a microconcentrator and the clean solution containing Fe-NTA complex was directly injected into the HPLC system.

Results: No differences were observed between the 3 groups for the iron status parameters (ferritin and transferrin saturation %). NTBI was significantly lower in patients on combined treatment DFO+L1 (1,66 $\pm$ 0,62 μ mol/L) compared to patients treated with L1 (2,80 $\pm$ 2,06 μ mol/L) ($p=0,037$) or DFO (2,34 $\pm$ 1,87 μ mol/L) alone. No correlation was observed between NTBI and pretransfusional haemoglobin, ferritin or transferrin saturation % in any of the groups.

Summary: It has recently been reported that combined chelation treatment with DFO and L1 results more effective in removing iron from heart, being the L1 considered as a possible shuttle uptaking iron from the cell and making it available to DFO chelation. Our preliminary results show that combined therapy is more effective in removing toxic free iron than both drug alone.

254 COMPARISON BETWEEN DESFERRIOXAMINE AND PROSPECTIVE COMBINED THERAPY WITH DESFERRIOXAMINE AND DEFERIPRONE IN BETA THALASSEMIA MAJOR PATIENTS WITH IRON OVERLOAD.

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Background: Desferrioxamine[DFX] has been the major iron-chelating agent used extensively worldwide for treatment of transfusional iron overload. However, compliance has been a major hindrance in achieving optimal therapeutic results. The availability of safe, oral iron chelation with deferiprone [L1] was welcome but its efficacy when used alone is inferior to DFX.

Aim: To compare DFX and prospective combined therapy with DFX and L1 in beta thalassemia major patients with iron overload.

Methods: We studied 63 patients with beta thalassemia major (Mean age + SD, 15.9 + 6; Range 5-30 years) attending the Day Care unit for regular transfusional support. They received packed red cells every 3-4 weeks to maintain pre-transfusion hemoglobin concentration above 9 g/dl. They had been receiving DFX at a daily dose of 40mg/kg/day by subcutaneous infusion for 8-10 hrs on 4-5 nights each week for past several years. However, due to various reasons, they had developed considerable transfusional iron overload. These patients were allocated to prospectively receive additional therapy with oral iron chelator L1 at 75mg/kg body weight in three divided doses with meals after informed consent.

Results: Of the 63 patients, 2 developed severe GI upset, 1 developed persistently raised liver enzymes and 1 agranulocytosis and did not continue in the study. In the remaining 59 patients, the mean serum ferritin(+SD) fell dramatically from 3894(+1690) ng/ml[pretherapy with L1] to 1904(+1178) ng/ml at 24 months post combination therapy [n=13 patients, p<0.0001]; to 2742(+1709) ng/ml at 18 months[n=17;p<0.001]; to 3608(+1748) ng/ml at 12 months [n=41;p<0.001]; to 3478(+1586) ng/ml at 6 months post therapy [n=59;p=0.0014]

Summary/Conclusion: The study emphasizes that beta thalassemia major patients with transfusional iron overload can be successfully treated with a combination of DFX and L1. Our results also demonstrate a significant statistical improvement at 6 months of combination therapy.

255 STUDY OF IRON OVERLOAD MEASURED WITH MAGNETIC RESONANCE IMAGING (M.R.I.) IN PATIENTS WITH B-THALASSAEMIA COMPARED TO CHELATION THERAPY

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Background .The iron chelating therapy with desferrioxamine (DFO) changed dramatically the outcome of the patients with b-thalassemia. However, the inconvenience and painful subcutaneous administration leads to low compliance. The last few years, the oral chelator deferiprone (L1) improved the quality of life and ameliorated therapy compliance. The combined use of DFO and L1 seems to have synergistic action with better chelation efficacy. The aim of this study is to compare the iron deposition in the myocardium and the liver, measured with magnetic resonance imaging (M.R.I.) in thalassemia patients chelated with DFO and patients chelated with both DFO and L1.

Methods. Forty one patients were studied, aged 12 to 25 yr (average: 19.8). The patients were divided into two groups. Group A consisted of 24 patients who were receiving DFO and group B consisted of 17 patients non compliance to DFO who were receiving combined therapy with DFO and L1. Patients were examined by heart and liver M.R.I. at the beginning of the study and 18 months after.

Results. Regarding myocardial iron deposition twenty nine patients (70%) from both groups, showed a statistically significant improvement (p=0,0001). Comparing the two groups, Group B patients showed

better results than Group A patients (0.0006 vs 0.0011, respectively). In contrary, liver M.R.I. showed a statistically significant increase of iron load (p=0,022) almost in all patients. All patients of Group A presented a statistically significant increase in liver siderosis (p=0,016). The increase was lower in Group B patients (p=0,28).

Conclusions. Combined chelation therapy (DFO and L1) seems to have satisfactory results in reducing the iron stores of the myocardium and delay the iron overloading of the liver from its early use.

256 RARE NEUTRAL BETA CHAIN HAEMOGLOBIN VARIANTS DETECTED AND CHARACTERIZED BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN THE PORTUGUESE POPULATION

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The haemoglobin (Hb) detection methods currently used in biochemical laboratories are electrophoresis and chromatography. These techniques separate the Hb fraction by their specific electric charge or chromatographic behaviour. However they fail in the detection of different abnormal Hbs showing similar electric charge. Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) could be used to detect these kinds of Hb variants that display only differences in their hydrophobicity.

During a screening programme for haemoglobinopathies prevention in the Portuguese population some rare neutral variants, beta-structural Hb mutants were detected and characterized by RP-HPLC and identified by DNA sequencing.

Patients haematological phenotype was characterized using an automated cell counter (Coulter MaxM). The separation and quantitation of globin chains by RP-HPLC was carried out in a Gold Beckman Liquid Chromatograph using a Merck Lichrospher 100 RP-8 column. In order to identify these Hb variants molecular analyses were performed in patients DNA. The codificant regions of the beta-globin gene were amplified by polymerase chain reaction (PCR) followed by direct sequencing.

In the present study we describe three neutral beta chain Hb variants identified for the first time, in the Portuguese population:

-Hb Yaound [beta134(H12)Val>Ala] in heterozygosity with Hb C, in a Caucasian family. It is a silent and asymptomatic variant that was recently described in a black man from Cameroon in association with Hb Kenitra and was subsequently found, and described as Hb Mataro, in a Sub-Saharan child.

-Hb Loves Park [beta 68(E12)Leu>Phe] in heterozygosity in a child of two years old, with microcytic and hypochromic anaemia. It was reported only once in an American family of Italian origin, associated with mild anaemia and cyanosis.

-Hb City of Hope [beta69(E13)Gly>Ser] in association with beta-zero-thalassaemia (cd 39 C>T) and Hb J-Paris-I (alpha12(A10)Ala>Asp] in a Portuguese women presenting mild microcytic and hypochromic anaemia, with mildly increase of Hb foetal. It was previously described in individuals from different geographic origin, in heterozygosity and in combination with beta-zero-thalassaemia (the last one associated to thalassaemia intermedia).

Analysis of the globin chains by RP-HPLC became an additional tool for characterizing Hb variants and has led to the discovery of new variants that display only differences in its hydrophobicity. Until now the presence of such neutral variants in the Portuguese population was unknown and this chromatographic procedure was extremely useful for the detection and characterization of these neutral variants and allowed to understand the haematological and clinical manifestations in these patients.

257 COINHERITANCE OF BETA-0 MUTATIONS AND TRIPlicated ALPHA-GLOBIN GENES: PHENOTYPICAL EXPRESSION IN ADULT LIFE.

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Background: The pathophysiology and clinical severity of beta-thalassaemias are dependent on the degree of globin chain imbalance. beta-thalassaemia trait (beta-heterozygosity) varies from a picture with none or mild haematological abnormalities to phenotypes characterized by microcytic anaemia and mild splenomegaly. The main explanations for the haematological variability observed in beta-heterozygous subjects are the iron deficiency or the coinheritance of different number of alpha-globin genes that causes a greater degree of globin chain imbalance.

Patients: From 2000 to 2003, 59 subjects with a previous diagnosis of beta-thalassemia trait have been referred to our Centre for a reevaluation because of worsening anaemia during different circumstances (i.e: pregnancy, infection, surgery) or for splenomegaly.

Methods: In all the subjects the common haematological parameters (Hb, Ht, RBC, MCV, reticulocytes), the iron status (serum iron, % transferrin saturation, ferritin) and bilirubin levels have been evaluated. The Hb fractions (Hb A, Hb A2, Hb F) have been measured by HPLC.

Results: All the subjects had microcytic anaemia (Hb 9.911.96) and increased Hb A2. Thirtythree out of 59 subjects (52.5%) (12 M/21 F) showed a more marked microcytic anaemia (Hb < 10.5 g/dl) with increased Hb A2 associated with slight increase of Hb F, and slight increase of unconjugated bilirubin. In these subset of subjects we performed the molecular analysis of the beta- and alpha-globin genes. A single beta0-thal mutation was identified in each subject associated to a triplicated alpha gene. The coinheritance of the two defect turned out to be the cause of the haematological mild thalassemia intermedia phenotype. The mean values of haematological parameters are summarized in table 1. Data are expressed as media SD.

Conclusions: We conclude that subjects heterozygotes for beta-thalassaemia, with haemoglobin levels < 10.5g/dl and slight increase of Hb F, without any other cause of anaemia, have to be considered potential carrier of triplicated alpha-globin genes. In these subjects a proper diagnosis could prevent hospitalization and further search for their anaemia. Moreover, although they are apparently well being, they deserve a clinical follow-up as they may develop transfusions requirement in some circumstances or splenomegaly.

N. cases	Age (yrs)	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	HbA2 (%)	HbF (%)	Uncon. Bil. (mg/dl)
33	38±13	9.68±1.44	69±11	22±3	32±1	4.77±0.96	2.8±2.42	1.67±1.43

Caption 1: Table 1

258 HAPLOTYPE DIVERSITY OF BETA-GLOBIN GENES IN BETA-THALASSEMIA CARRIERS OF SERBIAN DESCENT

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Thalassemia syndromes are less frequent in Serbia (a part of former Yugoslavia) than in adjacent Mediterranean countries (the overall frequency is 1.9%). A systematic survey of patients with thalassemia-like phenotype in Serbia, conducted since 1998, revealed that three beta-thalassemia mutations (Hb Lepore, codon 39, IVS-I-110) account for more than 70%.

In order to determine the chromosomal background on which these mutations arose, as well as their geographical pattern, a detailed survey of beta-globin gene haplotypes was performed in beta-thalassemia carriers of Serbian descent. We have studied eight linked polymorphic restriction enzyme sites along beta-globin gene cluster (HincII/ε, XmnI/5Gy, HindIII/Gy, HindIII/Ay, HincII/ψβ, HincII/3ψβ, AvalI/β, BamHI/3β) and intragenic beta-globin gene sequence diversity (frameworks) using PCR-RFLP and direct sequencing analysis.

Four different haplotypes associated with β-thalassemia mutations were detected: two were typical of Mediterranean countries (I and V), and two were autochthonous haplotypes, specific for Serbian population: haplotype A (+--+--+) and haplotype B (+--+--+). All four haplotypes were found in normal betaA/betaA individuals as well.

Hb Lepore Boston Washington (BW), a thalassemic Hb variant, is the most frequent mutation causing thalassemia syndrome in Serbia (more than 30%). A novel haplotype (haplotype A) associated with Hb Lepore BW gene has been identified in Serbian population. The high frequency of Hb Lepore BW hemoglobin variant in the population, the homogeneity of Hb Lepore BW haplotype (no haplotype V, typical for Eastern Italy and former Yugoslavia, was detected), as well as its uniqueness, suggest its independent origin in Serbia. These data support the hypothesis of the multicentric origin of this mutation.

Haplotype analysis revealed that codon 39 thalassemia mutation was mostly associated with haplotype I and framework 1. Chromosomal background of codon 39 mutation suggests Western Mediterranean (Italian) origin of this mutation in Serbian population. However, in one family, codon 39 chromosomes are found to be associated with framework 2. To the best of our knowledge, this is the first report of the association between framework 2 and codon 39 mutation.

Two different haplotypes were associated with IVS-I-110 thalassemia mutation: haplotype I and haplotype B. Mutation IVS-I-110 is previously reported to be of Eastern Mediterranean (Turkish) origin and associated with haplotype I. The diversity of haplotypes could have been generated by recombination events (crossing-over or gene conversion) between the haplotype I and haplotypes A or B, common in the population of Serbia.

Our data have shown that two most common Mediterranean mutations, codon 39 and IVS-I-110, have probably been introduced into Serbian population from Italy and Turkey, respectively, through historically documented migrations and settlements, while Hb Lepore BW detected in Serbian thalassemic population originates from Serbia.

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259 TOLERABILITY AND EFFICACY OF LONG TERM COMBINED THERAPY OF DEFERIPRONE AND DESFERIOXAMINE IN PATIENTS WITH THALASSEMIA MAJOR

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Previous studies have shown enhanced iron excretion with the simultaneous administration of deferiprone (DFP) and desferrioxamine (DFO). Combined therapy with these two chelators has not undergone long term, extensive evaluation. We report our experience in combining DFP and DFO for the treatment of iron overload in patients with β-thalassemia major. **PATIENTS METHODS:** Seventy eight patients (46 females) of 15 to 39 (mean 27.3 4.8) years of age were treated with an individualized regimen of combined administration of DFO and DFP. Eligibility criteria included poor compliance with or poor efficacy of DFO, documented by either ferritin levels > 3000 g/L or cardiac toxicity or significant liver hemosiderosis. Combined therapy consisted of daily DFP (Ferriprox, Apotex, Canada) at a dose of 25 mg/kg/ dose x 2-3 doses/day along with subcutaneous infusion of DFO (Desferal, Novartis, Switzerland) at a dose of 2.5 -3 g/day for 2 -3 days per week. Efficacy was followed by serial evaluation of ferritin levels. Mean baseline ferritin was 3395 (2164) g/L. Mean duration of treatment was 16.8 8.5 months. **RESULTS:** Ferritin levels decreased sharply on the first 12 months of treatment (mean change = -997910 g/L, p<0.05), and stabilized thereafter. Six patients attained normal levels of ferritin (< 250 g/L) after 7 to 15 months of treatment and interrupted therapy. Twenty patients (25.6%) interrupted treatment, after 2 to 33 months because of either side effects (8 patients), or poor compliance (4 patients), or ferritin levels reaching normal range (6 patients), or interferon therapy (1 patient), or transferring care to another center (1 patient). Four patients developed agranulocytosis (neutrophil count 0-0.5 x 10⁹/L) (incidence of 3.6 cases/ 100 patient-years), while 2 patients developed mild neutropenia (neutrophil count 0.5-1.5 x 10⁹/L) (incidence of 1.8 cases/ 100 patient-years). Agranulocytosis occurred 6 to 14 months after starting therapy and lead to cessation of DFP. It resolved in all cases either spontaneously or after the use of granulocytic stimulation factor (3 patients). One patient developed alloantibodies reaction after 26 months of therapy. One patient with marked somnolence and increased appetite discontinued therapy. Transient elevation of ALT (>3 times the upper normal limits) was noted in 23 patients (14 with hepatitis C seropositivity), but in 12 of them ALT elevation existed before treatment with DFP. Five patients developed somnolence and 2 increased appetite. Two patients experienced gastrointestinal discomfort. **CONCLUSIONS:** Combined therapy with DFO and DFP resulted in a decrease of ferritin levels, which was more pronounced in the beginning of the treatment. In contrast to both our and others experience on DFP monotherapy, we observed a high incidence of agranulocytosis during combined therapy of DFO and DFP. This may be due to either the fact that this was a selected group of patients of limited size or that the simultaneous use of the two chelators do have higher marrow-suppressive effect than DFP alone. Further studies on the use of the combined therapy are warranted.

260 THE FIRST IVS2 SPLICE DONOR SITE MUTATION IN THE ALPHA2 GLOBIN GENE (IVS-II-2 T>A) ASSOCIATED WITH AN ALPHA-THALASSEMIA PHENOTYPE.

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A father and a son of Caucasian descent, aged 58 and 36 years respectively, presented with chronic border line Hb (13.5 and 13.4 g/dl) and microcytic (MCV 72 and 71 fl) hypochromic (MCH 23.7 and 24.2 pg) parameters. Iron deficiency was excluded and Gap-PCR revealed none of the 7 common alpha-thalassemia deletion defects. The beta/alpha globin chains synthesis measured in vitro was however unbalanced (1.4 : 1.2) indicating a reduced expression of the alpha-globin genes. No abnormal hemoglobin fractions were observed on HPLC (Bio-Rad Variant-I beta-short program) or on starch gel electrophoresis at pH 8.7. Direct sequencing of the alpha globin genes revealed a T>A transversion at the IVS-II-2 position of the alpha2 gene in heterozygous state. Non-deletion alpha-thalassemias are considered rare, however the list of point mutations affecting the expression of the alpha globin genes is still growing. Seven similar defects are already listed, described in Mediterranean, Middle East, Indian, Thai, Argentinian and Northern European cases. This is the first case described involving the IVS-II splice donor site of the alpha2-gene sequence and the first case in a Dutch patient. The two carriers both present with an alpha+-thalassemia phenotype. The reduction in expression due to point mutations in the alpha2-globin gene are generally considered to be more severe than those in alpha1- or deletion types of alpha+-thalassemias (-a/aa). It seems therefore likely that the combination of the presently described mutation with alpha0-thalassemia alleles, may result in an intermediate or severe HbH disease.

261 MAGNETIC RESONANCE IMAGING (MRI) IN THE EVALUATION OF IRON OVERLOAD IN PATIENTS WITH BETA THALASSEMIA AND SICKLE CELL DISEASE

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Background: Magnetic Resonance Imaging (MRI) is a new non-invasive technique which appears useful for the monitoring of iron overload but has not yet been adequately validated.

Material and Methods: We have evaluated 106 patients with thalassemia (42M /64 F, age 19-65 y). Of these, 80 were transfusion dependent (thalassemia major, TM) while 26 were not transfused or transfused sporadically (thalassemia intermedia, TI). All transfused patients had been on chelation therapy with deferoxamine or deferasirox over several years (1 to 25). Additionally, we studied 35 patients with SCD (15 M/20 F, age 18-61 y); these patients were transfused sporadically and none of them was on chelation therapy. The study considered the following parameters: (a) serum ferritin, (b) Liver and myocardial T2 relaxation times (obtained with a Phillips Intera CV, 1.5 T apparatus using a 16. echo CPMG spin echo sequence with respiratory and cardiac triggering (E1,2,...16, 5,10,...80ms, TR 1000 ms and 2 beats respectively) (c) liver iron content (LIC) obtained by biopsy in 29 patients (4 IT, 10 TM and 15 SCD patients) and measured by atomic absorption. Normal liver and heart T2 values were established in 15 age-matched volunteers (48,01±9,34 ms for the liver and 51,20±4,50 ms for the myocardium).

Results and Discussion: Ferritin levels were higher in patients with TM than in patients with TI (p<0.0003) and SCD (p<0.0001). Similarly, TM patients had reduced values of both liver and heart T2 values compared to patients with TI (p<0.001 and <0.003 for liver and heart respectively), to patients with SCD (p<0.001 and <0.002 for liver and heart respectively), and to controls (p<0.00001 and <0.00001 respectively). Ferritin levels showed a significant negative correlation with the liver T2 values in all three groups of patients (p : 0.0001 for TI; p : 0.00001 for TM, and p : 0.00001 for the SCD patients). Similarly, a significant negative correlation was found between serum ferritin levels and heart T2 values in TM patients (p : 0.0004), but not in those with TI and SCD (p : 0.1). Heart and liver T2 values showed a significant correlation in TM patients (p : 0.002), but not in patients with TI and SCD (p : 0.41 and p : 0.56, respectively). LIC

values showed a strong positive correlation with ferritin levels in both groups of patients (p : 0.02 for IT and TM and p : 0.00001 for SCD) and an impressive inverse correlation with the respective liver T2 values in all patients (TM and TI, p : 0.0003 ; SCD, p : 0.00001), thus suggesting that liver T2 values reflect accurately the hepatic iron deposition. Heart T2 values showed a positive correlation with LVEF in TM and SCD patients (p : 0.03, respectively), but not in patients with TI (p : 0.40).

Conclusions: The MRI technique is a reliable non-invasive method for the assessment of the deposition of iron in the liver; on the contrary, this technique does not provide reliable data concerning the iron deposition in the heart.

262 PAMIDRONATE TREATMENT IN THALASSEMIA-INDUCED OSTEOPOROSIS

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Background: The complications such as anemia, hypoxia, chronic iron loading, desferrioxamine toxicity may develop in thalassemic patients. One of the complications is also osteoporosis.

Aim: In this study, we determined osteoporosis by Dual Energy X-Ray (DEXA) and the efficiency of pamidronate treatment in 24 thalassemic patients in Pediatric Hematology Unit of Erciyes University Faculty of Medicine.

Methods: Twenty-four thalassemic patients with an age range of 6-22 years (14 girl, 10 boy and mean age: 11.20 ± 3.9) were included in the study. Height, weight, bone age and body mass index were evaluated. Calcium, phosphate, alkaline phosphatase and parathyroid hormone levels in the serum were also measured. Bone mineral densities (BMD) of lumbar vertebrae L1-L4, femur neck, trochanter major and ward triangle regions were measured by DEXA method. Pamidronate treatment (15 mg/dose for patients at age below 15, 30 mg/dose at age above 15, per three months for one year) was given to 23 osteoporotic (Z score below -2.5) and osteopenic (Z score -1 to -2.5) patients with bone pain and history of bone fracture.

Results: Z score of BMD of vertebrae L1-L4 was below -2.5 in 9 patients (37.5 %), -1 to -2.5 in 14 patients (58.3%) and normal in one patient. The mean Z score of L1-L4 was -2.34 ± 0.77 (Mean BMD: 0.555 ± 0.106) at the beginning and -2.17 ± 0.88 (Mean BMD: 0.587 ± 0.107) after one year. There was statistically significant difference between results of BMD at the beginning and after 12 months.

Conclusion: Treatment of pamidronate is effective in thalassemia-induced osteoporosis.

263 THALASSEMIA MAJOR VERSUS THALASSEMIA INTERMEDIA: A COMPARATIVE CARDIAC ASSESSMENT

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Background: Heart disease represents the main determinant of prognosis and survival in beta-thalassemia, but its particular features in the two clinical forms of the disease, namely thalassemia major (TM) and thalassemia intermedia (TI), are not completely clarified. The differences in genetical background along with the highly variable therapy applied in different patient groups have led to conflicting evidence with respect to the form and the frequency of cardiac complications. The significant prolongation of survival achieved over the past years in TM renders nowadays a comparative analysis of the two forms feasible.

Aim: To compare cardiac condition between two homogeneous groups of typically treated TM and TI patients.

Methods: We studied 202 regularly transfused and highly compliant with chelation therapy TM patients and 74 age-matched, non-transfused TI patients. Assessment included history taking, clinical examination and complete resting Doppler echocardiography.

Results: Congestive heart failure was encountered in 5 patients with TM (2.5%), aged 31.84 ± 2.2 years (range, 26-38 years) and in 2 with TI (2.7%), aged 37.53 ± 5.5 years (range, 35-40 years). History of pericarditis was present in 5.0% of TM patients and in 6.7% of TI patients. Cardiac chamber dimensions, left ventricular (LV) mass, LV shortening and ejection fractions and cardiac output were significantly higher in TI. Systolic LV dysfunction was found in 14 patients with TM (6.9%) and in none with TI. Peak systolic tricuspid pressure gradient (TG), which estimates systolic pulmonary artery pressure, was higher in TI, both overall (22.7 ± 6.0 versus 31.4 ± 11.8 mmHg, p<0.001) and within different

age groups. A $TG > 30.0 \text{ mmHg}$ (upper normal limit) was observed in 9.9% TM cases (range 30.1–41.9 mmHg) and in 59.5% TI cases (range 30.1–97.2 mmHg, $p < .001$). Severe pulmonary hypertension ($TG > 45.0 \text{ mmHg}$) was present in 5 patients with TI (6.8%) and none with TM. Conclusion: Standard lifelong transfusion and chelation therapy in TM prevented premature heart disease and pulmonary hypertension and

reduced the rate of pericarditis; however, LV dysfunction still occurred and led to heart failure, which began to appear in the third decade of life. The absence of transfusions in TI, in contrast, prevented iron-induced LV dysfunction, but allowed the development of pulmonary hypertension, which also led to heart failure but with a later onset than that observed in TM.

Simultaneous Sessions

264 IN VITRO SEPARATION OF GVL EFFECT AND GVHR FROM ANTI-LEUKEMIA CTL LINES: A PERSPECTIVE FOR ADOPTIVE IMMUNOTHERAPY AFTER ALLOGENEIC HSCT

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Background. Adoptive transfer of ex vivo generated donor-derived anti-leukemia CTL lines could be a successful approach in preventing or treating leukemia relapse after allogeneic HSCT. However, a major risk is that of generating cells potentially able to promote the development of GVHD. This risk could be particularly relevant in pairs where donor and recipient are HLA-disparate. We previously demonstrated the possibility of generating and expanding in vitro CTL lines directed towards different types of leukemia blasts (LB), using both HLA-matched and partially matched donors as source of effector cells. Some of the anti-leukemia CTL lines displayed a sizeable cytotoxicity against patient non-leukemia cells, even though this alloreactivity was lower than that observed against patient LB, especially at the lowest effector/target ratio. The exact antigen specificity of these CTL lines is unknown, even if our data suggest that, at least part of anti-leukemia T cells, recognizes an antigen only present or up regulated on LB.

Aims. The aim of present study was to verify the possibility of separating in vitro T cells able to mediate GVL effect to these involved in the development of GVHD.

Methods. To this purpose, one alloreactive anti-leukemia CTL line derived from a partially matched family donor was cloned and T cell clones (TCC) we obtained were tested for their capacity to lyse patient LB or patient non-malignant cells, such as T- lymphoblastoid cell line (T-LCL) or fibroblasts (FB). TCC selectively able to lyse only LB were further tested for surface phenotype, TCR repertoire and intracellular cytokine production.

Results. 420 TCC were obtained. 281/420 TCC were cytotoxic against both LB and T-LCL, while 90/281 lysed also patient FB. 16/420 lysed LB and FB, while 64/420 were selectively able to lyse only LB (LB-directed TCC). 37/64 LB-directed TCC were CD3+/CD8+ cells, while the remaining were CD3+/CD4+ cells. Evaluation of expression of TCR Vbeta chains revealed that at least 14 different families of Vbeta TCR regions chains were represented among LB-directed TCC. Expansion of LB-directed TCC with OKT3, allogeneic feeder cells and IL-2 allowed us to obtain a great number of anti-leukemia cells maintaining their specificities. Experiments are in progress to determine the pattern of cytokine production and HLA-restriction.

Summary. These data suggests that i) different effector cells can be differentiate in vitro in response to LB, ii) among an alloreactive anti-leukemia T cell line it is possible to distinguish and separate T cells expressing different specificities in order to reduce the risk of inducing GVHD after in vivo infusion of these cells.

265 ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANT VS. BONE MARROW TRANSPLANT IN THE MANAGEMENT OF HEMATOLOGICAL MALIGNANCIES: AN INDIVIDUAL PATIENT DATA META-ANALYSIS OF 9 RANDOMIZED TRIALS

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Background: Considerable uncertainty exists with respect to the relative effects of allogeneic peripheral blood stem cell transplant (PBSCT) vs. bone marrow transplant (BMT) on the outcomes of patients with hematological malignancies. **Methods:** To provide the totality of research evidence related to the effects of PBSCT vs. BMT, we identified all trials that randomized patients with hematological malignancies to allogeneic PBSCT vs. BMT. The two trials that used T-cell depletion or primed bone marrow were excluded from the analysis. Ten trials enrolling 1,141 patients were identified. Individual patient data were available on 1,111 patients from nine trials and were analyzed using standard meta-analytic techniques.

Results: When compared with BMT, PBSCT lead to faster neutrophil [OR=0.39 (0.33-0.45); p<0.00001] and platelet engraftment [Odds ratio (OR)=0.53 (0.46-0.61); p<0.00001], a significant decrease in relapse [OR=0.72 (95%CI 0.56-0.94); p=0.01] and improved disease-free survival [OR=0.83 (0.69-0.98); p=0.03]. PBSCT was associated with significant increase in the development of chronic GVHD [OR=1.47 (1.15-1.88);p=0.002], but not in acute GVHD [OR=1.16 (0.95-1.43) ; p=0.1]. There was no evidence that the type of allogeneic transplant significantly affected overall survival [OR=0.87 (95% CI 0.72-1.05);p=0.1], or non-relapse related mortality [OR=0.86

(0.68-1.10); p=0.2]. When analyzed according to prognostic features, there was a suggestion that PBSCT was associated with improved survival in the patients with poor prognostic features [OR=0.66 (99% CI 0.67-0.99) vs. 0.97 (0.71-1.33); test for interaction, p=0.06]. No such differences in the effects on relapse and disease-free survival were seen. Further pre-specified subgroup analysis indicated that PBSCT may result in reduction of relapse in patients with chronic myelogenous leukemia [OR=0.36; (0.22-0.6); p<0.0001]. **Conclusion:** PBSCT led to decreased relapse and disease-free survival; it may also improve overall survival in patients with unfavorable prognostic features. Because there is a trade-off between benefits and harms related to stem cell source, the choice of treatment should also be guided by the patient preferences toward different outcomes (e.g. decrease in relapse vs. increase in chronic GVHD).

266 LONG TERM OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR CML

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Background: Stem cell transplantation (SCT) is an established treatment for selected patients with CML and a high proportion of survivors can be regarded as cured of their leukaemia. However, the incidence of late relapse and late mortality are not well defined.

Aim: We aimed to determine overall survival, disease-free survival and relapse rates for patients who were alive and in continuous remission at 5 years after allogeneic SCT for CML.

Methods: We studied a total of 6548 recipients of allogeneic SCTs performed between 1978 and 1997 to determine long-term rates of overall survival, disease-free survival and relapse. Their median age was 35 yr. The phase of disease at time of SCT was chronic 71%, accelerated 21%, and blastic 7%. The donors were HLA-identical sibs 69%, unrelated donors 20% and other donors 11%.

Results: As of December 2002, there were 2807 survivors; of these 2710 (41% of the 6548) had survived for >5 yr, 1926 for >7 yr, 1044 for >10 yr, 212 for >15 yr and 7 for >20 yr. 3741 patients had died; the causes of death included CML (22%), new malignancy (1%), GvHD (14%), pneumonitis (22%), infection (16%) organ failure (8%), other causes (10%) and unknown (7%). 2234 patients were alive and in continuing remission 5 or more years post-SCT. Of these, 60% had received TBI as part of their conditioning; 67% had received CsA and MTX for GvHD prophylaxis. The cumulative incidence of relapse at 15 yrs post-SCT in patients in remission at 5 years was 17%, 15%, 12% and 7% for recipients of sibling transplants in first CP, recipients of sibling transplants not in first CP, recipients of alternative donor transplants in CP and recipients of alternative donor transplants not in CP respectively. The latest relapse occurred at 16 yr post SCT. Among patients alive and in remission at 5 years post SCT, survival at 15 years was 85%, 83%, 80% and 75% for the same patient cohorts. A total of 174 patients died more than 5 years post-SCT; the causes of death were CML (22%), GvHD (7%), second cancer (11%), infection (28%), organ failure (19%) and other (13%).

Conclusions: Remissions after allogeneic SCT are generally durable. After a high risk period there is a low but constant risk of relapse. Both donor lymphocyte infusions and imatinib can reinduce remission in patients who relapse. -

267 SIROLIMUS AND TACROLIMUS AS GRAFT-VS.-HOST DISEASE PROPHYLAXIS IN ALLOGENEIC STEM CELL TRANSPLANTATION:

THE DANA-FARBER CANCER INSTITUTE EXPERIENCE

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Background: Sirolimus is a novel immunosuppressant similar to tacrolimus, however, sirolimus inhibits T cell function uniquely via FKBP12/mTOR and impairs dendritic cell function. Sirolimus acts synergistically with tacrolimus and has non-overlapping toxicities, making their use in combination attractive. We began employing sirolimus as GVHD prophylaxis in 2000, and herein describe our experience with this compound.

Methods: Three clinical trials were performed using sirolimus (target serum level 3-12 ng/ml) and tacrolimus (target serum level 5-10 ng/ml) as primary GVHD

prophylaxis. Trials 1 and 2 used myeloablative conditioning with Cytoxan/TBI. Trial 1 enrolled 41 patients who received HLA-matched, unrelated or single HLA Class I antigen mismatched related or unrelated bone marrow. Abbreviated methotrexate (20 mg/m² total) was routinely given post-transplant. Trial 2 enrolled 38 patients who received HLA-matched, related peripheral blood stem cells. No methotrexate was given post-transplant. Trial 3 enrolled 39 patients who received non-myeloablative conditioning (Busulfex/Fludarabine) and bone marrow from HLA-matched related and unrelated donors. Abbreviated methotrexate (20 mg/m² total) was routinely given in Trial 3.

Results: The median times to neutrophil engraftment (>500) after transplantation were 18 (range 11-32), 14 (range 11-17), and 13 (among patients with neutropenia) days for trials 1, 2 and 3 respectively. The median times to platelet engraftment (>20,000) after transplantation were 29 (range 14-98) and 13 (range 10-47) days for trials 1 and 2. Seven patients in trial 1 (17%) and one patient in trial 2 (3%) did not survive to first hospital discharge (median time to discharge 33 and 19 days from transplantation for trials 1 and 2). Patients in trial 3 were treated predominantly as outpatients. Grade II-IV acute GVHD occurred in 26%, 16% and 6% of patients in the 3 trials. Severe (grade III-IV) acute GVHD occurred in 13%, 5% and 0% of patients in the 3 trials. Chronic GVHD occurred in 44%, 31% and 48% of patients in the 3 trials to date. Treatment-related mortality at 100 days was 15%, 5% and 0% for the three trials. At two years, the relapse-free and overall survival estimate for Trial 1 is 44% (Median follow-up: 12 months). One year relapse-free and overall survival estimates for Trial 2 are 69% and 72% (Median follow-up: 11 months). In Trial 3, one year relapse-free and overall survival estimates are 68% and 74% (Median follow-up: 6 months). Transplant-related toxicity was modest. Among ablative transplantation patients (n=79), VOD occurred in 12 patients (15%), idiopathic pneumonia syndrome occurred in 5 patients (6%) and thrombotic microangiopathy occurred in 6 patients (8%).

Conclusions: Sirolimus and tacrolimus after allogeneic stem cell transplantation is effective GVHD prophylaxis. Engraftment is prompt, GVHD incidence and severity is reduced and transplant-related morbidity and mortality is reduced, regardless of conditioning, stem cell source, and methotrexate use. Early survival estimates are excellent due to reduced GVHD and transplant-related toxicity. Sirolimus is worthy of broader study in allogeneic transplantation.

	Trial 1	Trial 2	Trial 3
Sample Size	41	38	39
Median age	42	41	57
HLA Match			
MRD		30	22
UBD	29		17
Mismatch	12		
Stem Cell Source			
BM	✓		✓
PBSC		✓	
Conditioning			
Ablative	✓	✓	
Non-Myeloablative			✓
Mtx use	✓		✓
ANC recovery (days)	18	14	13
Plt recovery (days)	29	13	NA
GVHD (%)			
Acute Gr. II-IV	26	16	5
Acute Gr. III-IV	13	5	0
Chronic	44	31	48
Survival (%)			
100 day TRM	15	5	0
1 Yr RFS	46	69	68
1 Yr OS	51	72	74

Caption 1: Summary of Clinical Trial Results

268 G-CSF INCREASES THE NUMBER OF PERIPHERAL BLOOD DENDRITIC CELLS CD16+ AND THE EXPRESSION OF THE COESTIMULATORY MOLECULE CD86+. IMPLICATIONS IN ALLOGENEIC TRANSPLANTATION.

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Background. Dendritic cells (DCs) play a critical role in the regulation of alloimmune responses. The two main peripheral blood DC populations are myeloid DCs (DC1) and lymphoid DCs (DC2). A myeloid DC subset

expressing CD16 has been recently identified. Material and Methods. We have analyzed the number of DC1, DC2, and DC CD16+ subsets, and their CD86 expression, in peripheral blood before and after five days of G-CSF 10mg/kg/d (n=18), and also compared the DCs quantity administered in peripheral blood transplants (PBT) (n=70) and bone marrow transplants (BMT) (n=20). Immunophenotypic analysis was performed in a single center. DCs were identified as positive for HLA-DR and negative for lineage markers (CD3, CD14, CD19, CD56). The expression of CD33, CD123 and CD16 was used for identification of DC1, DC2 and DC CD16+ subpopulations, respectively. CD86 was analysed in DC1 and DC CD16+ subpopulations. Results. Median number of DC1 in peripheral blood was scarcely modified with G-CSF treatment (1.4x10⁴/mL vs 1.9x10⁴/mL; p=0.04). G-CSF increased the median number of DC2 (1.3x10⁴/mL vs 6.2x10⁴/mL; p<0.001) and of DC CD16+ (4x10⁴/mL vs 3.2x10⁵/mL; p=0.02). Of note, G-CSF administration was associated with a decrease of CD86 expression on DC1 (p=0.05) and an increase on DC2 (p=0.02). Recipients of PBT received similar quantities of DC1 and higher doses of DC2 than recipients of BMT (3.2x10⁷ vs 1.2 x10⁸; p=0.5 and 1.1 x10⁸ vs 4.2 x10⁷; p=0.0001, respectively). The most prominent difference between both types of transplants was the infused number of DC CD16+ (1.1x10⁹ in PBT vs 3.2x10⁷ in BMT; p<0.0001). Conclusion. Infusion of higher numbers of DC CD16, with high expression of the costimulatory molecule CD86, might explain some of the differences observed in allogeneic reactions after PBT with respect to BMT.

MYELODYPLASTIC SYNDROMES

269 A NEW DISEASE DEFINITION OF LOW GRADE MDS BASED ON THE EXPRESSION OF CYTOPENIA AND DYSPLASIA IN ONE OR MORE LINEAGES AT DIAGNOSIS IMPROVES ON THE FAB AND WHO CLASSIFICATION

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Background: Most 'refractory anemias' have varied degrees of abnormalities in concentration or morphology of leukocytes and platelets. The FAB classifies low grade MDS as refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS). The WHO proposal subdivides this group of patients into six disease subsets.

Aim: To study the distribution of dysplasia and cytopenia and its impact on the classification of MDS.

Methods: We studied 187 MDS patients, classified as 136 RA and 51 RARS according to the FAB. A detailed analysis of PB and BM included 100 nucleated erythroid, myeloid and megakaryocytic cells to establish the extent and distribution of dysplasia.

Results: Erythroid dysplasia showed nuclear budding/lobularity (p=0.005) and vacuoli (p=0.043) associated with survival. Total myeloid dysplasia correlated with survival (p=0.001). Monolobar pseudo Pelger-Hut (PPH) forms, clumped non-segmented forms and small forms were significant, but not bilobar PPH. Myeloid agranularity was related to survival (p=0.002). The presence of total megakaryocytic dysplasia conferred poor outcome (p<0.0001). The FAB classification showed no difference in OS (p=0.236) and TT-AML (p=0.077). After reclassification according to the WHO 17 patients (9%) had anemia and unilineage dysplasia: 6 RA (OS not reached) 11 RARS (OS 79 months). Eight patients had the 5q-syndrome (OS 101 months). According to the criteria of cytopenias and multilineage dysplasia 86 patients (46%) could be reclassified as RCMD and 18 patients (10%) as RCMD-RS. OS 44 and 66 months respectively. 58 patients (31%) were grouped as MDS-unclassified (OS 78 months). Good prognostic categories were extremely small. RA versus RARS and RCMD versus RCMD-RS were not distinguishable regarding outcome. We therefore employed a simplified score for the WHO classification by utilizing the dysplastic parameters shown to be easily detectable and significant. Patients fell readily into three categories (p<0.0001). The first category consisted of 35 patients with unilineage cytopenia and unilineage dysplasia, whether occurring in the same lineage or not (OS 109 months). Due to more stringent dysplastic criteria, only 78 patients remained in the category with multilineage cytopenia and multilineage dysplasia (OS 38 months). An intermediate group of 74 patients was defined by either an unilineage cytopenia/multilineage dysplasia or multilineage cytopenia/unilineage dysplasia (OS 61 months).

Discussion: The premise of the FAB and WHO that ringed sideroblasts divides low risk MDS into different disease subsets could not be validated. The supposed survival advantage of ringed sideroblasts is shown to rely on the concomitant occurrence of cytopenias and BM dysplasia. Our simplified scoring system divides low grade MDS into three readily recognisable categories behaving differently. Although no category was exempt from developing additional cytopenias or leukemic evolution, those with less cytopenias and less dysplasia at diagnosis, had a superior outcome and smaller chance of disease progression. Based on simple criteria from PB and BM, low grade MDS can be subdivided into three groups that are superior to the FAB and WHO for predicting disease evolution.

270 NRAS, FLT3 AND TP53 MUTATIONS IN PATIENTS WITH MDS AND A DEL(5Q)

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The myelodysplastic syndromes (MDS) are an heterogeneous group of acquired clonal disorders of haematopoiesis characterised by blood cytopenias, ineffective haematopoiesis and a hypercellular bone marrow. Approximately 30% of MDS cases transform into AML. Clonal karyotypic abnormalities occur in approximately 50% of cases of primary MDS, the del(5q) is the most frequently reported karyotypic abnormality and is observed in 10-15% of patients. The del(5q) occurs as the sole karyotypic abnormality in the 5q- syndrome, and also in more advanced MDS subtypes and AML. RAS, FLT3 and TP53 genes play important roles in the regulatory processes that govern proliferation, differentiation and apoptosis. Abnormalities in these three genes have been implicated in the pathogenesis of MDS and AML. The most common molecular abnormality in MDS is activation of the RAS proto-oncogenes, which has been reported in 4-48% of patients. TP53 point mutations are detected in 5-10% of MDS, and occur predominantly in the poor risk FAB-subtypes. FLT3 ITD mutation has been reported in approximately 3% of MDS patients. We sought to determine the incidence of NRAS, FLT3 and TP53 mutations in patients with MDS and a 5q deletion and to investigate whether the frequency of these mutations differed between patients with the 5q- syndrome and patients with a del(5q) and more advanced stages of MDS. Peripheral blood samples were obtained from 40 patients with MDS, including 20 patients with the 5q- syndrome. All patients had a del(5q). Sequences spanning codons 12 and 13 and codon 61 of NRAS, exons 5-8 of TP53 and exon 14, intron 14 and exon 15 of FLT3 were amplified by PCR. PCR products were purified and sequenced. ITD of FLT3 was not observed in any of the 40 MDS patients. This finding is consistent with the published low frequency. 5 different mutations of TP53 were observed in 3/40 (7.5%) patients. The reported incidence of TP53 mutations in MDS is 5-10%, and again our data are consistent with these reports. The most interesting observation was the absence of NRAS mutations, no mutations of codons 12, 13 or 61 were observed. Various studies have reported very different RAS mutation rates, ranging from 4% to 48%. The striking absence of any RAS mutations in this study is strong evidence that the mutation plays no role in either initiation or transformation of these leukaemias. We speculate that other biochemical pathways, rather than the aberrant RAS pathway, may play a role in leukaemogenesis in MDS patients with the del(5q). None of the 20 5q- syndrome patients had mutations of the three genes. A recent study investigated tumour suppressor gene deletion by FISH in a similar group of leukaemias, and again no abnormalities in the 5q- syndrome group were observed (Crescenzi et al, 2004). Taking these data together, it does seem that the stability of the 5q- syndrome may be due to the absence of other additional abnormalities. The transformation to AML of some MDS patients may well be due to TP53 mutations, but clearly there are still additional genetic abnormalities to discover.

271 FLOW CYTOMETRIC EVALUATION OF MARROW DYSPLASIA IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background. Recent evidence suggests that flow cytometry immunophenotyping might be useful in the management of MDS patients. However, there is no agreement so far on either the antibody panel or the modality of cytometric analysis to be employed.

Aims. We prospectively evaluated the clinical utility of quantitative flow cytometric analysis of marrow dysplasia in 103 consecutive, unselected

MDS patients, and studied at the same time 27 pathologic controls and 19 healthy bone marrow donors for comparison.

Methods. Six-parameter, 4-color flow cytometry was performed with a FACSCalibur flow cytometer (Becton Dickinson, San Jos, CA) and at least 50.000 events were acquired for each sample. Data were collected and analyzed with CellQuest software. Immunophenotypic data were expressed as percentage of cells positive for one or more antigens. Discriminant function analyses were performed to identify erythroid and myeloid dysplasia, with the aim of differentiating MDS and controls, and of classifying, together with flow cytometric blast percentage, MDS into WHO subgroups.

Results. Erythroid compartment analysis showed that the percentage of erythroblasts was significantly higher in MDS than in controls ($p<0.001$), and positively correlated with the degree of morphological marrow erythroid dysplasia ($r=0.53$, $p<0.001$). The quantitative analysis of the GlyA/CD71 pattern showed a lower percentage of erythroblasts in MDS than in healthy subjects ($p=0.02$). In the myeloid lineage, a lower proportion of CD10+ and an increase in CD56+ granulocytes and in CD56+ monocytes were observed in MDS in comparison with controls ($p<0.001$). The quantitative analysis of CD33/CD16, CD11b/CD16, CD45/CD16, CD13/CD16 patterns showed an increased percentage of immature cells and a reduction of mature granulocytes in MDS patients. For each pattern, we calculated a ratio of immature on mature cells, which resulted significantly higher in MDS than in controls (p values 0.02-0.001), and positively correlated with the degree of morphological myeloid dysplasia (r values 0.30-0.67, $p<0.002$). An erythroid classification function based on CD34+ cells (%), myeloid/lymphoid ratio, erythroblasts (%) and GlyA/CD71 co-expression (Table 1), and a myeloid function based on CD34+ cells (%), myeloid/lymphoid ratio, CD10 and CD56 expression on myeloid cells, and CD33/CD16 immature on mature myeloid cell ratio (Table 2) were defined, and integrated with the immunophenotypic blast count. The combined use of these functions correctly classified 95% of MDS, with correspondence between cytometric and morphological diagnosis in 90% of patients. Two subjects with megaloblastic anemia were improperly classified as MDS. In 20 patients of our series who showed a non-conclusive bone marrow morphology at the diagnosis, the use of the above discriminant formulae allowed a diagnosis of MDS in 12/20 cases.

Conclusion. Based on these findings, we conclude that quantitative immunophenotypic analysis provides an accurate evaluation of marrow dysplasia in MDS patients. A cost-effective panel of 10 monoclonal antibodies provides an accurate classification in most cases with indeterminate morphology, and might be effectively employed in the diagnostic workup of all MDS patients.

Table 1 - Erythroid dysplasia classification functions^a

Cytometric variables	Non MDS	MDS
CD 34 (%)	-1.5008	1.7352
Myeloid to lymphoid ratio	7.8563	14.879
Erythroblast%	26.8259	39.1115
GlyA/CD71	29.2252	29.4377
Constant	-40.2426	58.3147

^aParameters: $X = \log(\text{CD34}\%)$, $Y = \log(\text{Myeloid/Lymphoid ratio})$, $Z = \log(\text{Erythroblast}\%)$, $W = \log(\text{GlyA/CD71})$
 $X_{\text{cut}} = 1.7352\% + 16.879\% + 39.111\% + 29.437\% + 58.3147$
 $Y_{\text{cut}} = 1.5008\% + 8.5863\% + 26.8259\% + 29.2252\% + 40.2426$

In order to classify a subject, both classification functions must be evaluated: the subject will be assigned to the group corresponding to the classification function having the highest value.

Table 2 - Myeloid dysplasia classification functions^a

Cytometric variables	Non MDS	MDS
CD 34 (%)	11.2766	20.4565
Myeloid to lymphoid ratio	5.1171	9.5824
CD 10+ PNH (%)	20.8305	25.5959
CD 56+ PNH (%)	4.2865	7.3012
CD 33/CD16 immature/mature myeloid cells	11.7119	22.5228
Constant	-16.4760	-33.5813

^aParameters: $X = \log(\text{CD34}\%)$, $Y = \log(\text{Myeloid/Lymphoid ratio})$, $Z = \log(\text{CD10+PNH})$, $W = \log(\text{CD56+PNH})$, $V = \log(\text{CD33/CD16 immature/mature cells})$

$X_{\text{cut}} = 20.4565\% + 5.5824\% + 25.5959\% + 7.3012\% + 22.5228\% - 33.5813$

$Y_{\text{cut}} = 11.2766\% + 5.1171\% + 20.8305\% + 4.2865\% + 11.7119\% - 16.4760$

In order to classify a subject, both classification functions must be evaluated: the subject will be assigned to the group corresponding to the classification function having the highest value.

Caption 1: Tables

272 DETECTION OF MYELOBLASTIN (MBN) GENE OVEREXPRESSION IN PATIENTS AFFECTED BY MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKAEMIAS

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Background: Myeloblastin (MBN) gene codes for a serine protease with a broad spectrum of proteolytic activity. MBN is probably involved

in the control of proliferation of myeloid leukemia cells and it confers factor-independent growth to hematopoietic cells when abnormally expressed. Aims: The aim was to study the role of MBN gene in the setting of acute myeloid leukaemias (AML) and Myelodysplastic syndromes (MDS) and to verify whether MBN could represent a marker of leukemic and myelodysplastic hematopoiesis. Methods: we analyzed the expression levels of MBN in 113 BM samples collected at diagnosis from AML patients. The FAB distribution was as follows: M0=5, M1=12, M2=38, M3=12, M4=37, M5=5, M6=4. 19 out of 38 FABM2 patients were characterized by t(8;21) and 16 out of 37 FABM4 cases by inv(16). Moreover we analyzed the expression levels in 57 BM and 42 Peripheral Blood (PB) samples from 88 MDS patients 44 RA, 32 RAEB and 12 secondary-AML). As control, we analyzed the MBN expression in 15 BM and 40 PB samples from healthy volunteers. The expression level of MBN was established using quantitative RealTime PCR based on a specific set of primers and probe (Assays-on-Demand, gene expression products, Applied Biosystems). The values obtained were normalized using ABL as housekeeping gene and the final results were expressed using the DeltaDelta Ct method. The final numerical values are expressed as 2E-DDct. Results: We found that MBN gene is significantly overexpressed in BM samples from AML patients. The mean value of 2E-DDct in AML cases analyzed was 540, range 1,5-5043. We found that MBN is expressed at lower levels in AML FABM0 and FABM1, (mean value of 2E-DDct :13 and 35 respectively) it increases in AML FABM2 (mean value of 2E-DDct : 1051) and FABM3 (mean value of 2E-DDct :251) and in AML FABM4 (mean value of 2E-DDct :510) and it decreases again in FABM5 (mean value:218) and FABM6 (mean value: 49). Interestingly, patients affected by AML FABM2 and FABM4 with the presence of the t(8,21) and inv(16) showed significantly higher values of MBN respect to the same FAB subtypes with normal karyotypes. The mean value of 2E-DDct obtained in BM samples from patients with AML1/ETO-positive FABM2 AML compared to AML1/ETO-negative FABM2 cases was 1522 vs 164 (p=0,0001). Similar differences were detected by analyzing FABM4 cases with and without the CBFbeta/MYH11 fusion transcript (mean value of 2E-DDct:961 vs 150) (p= 0,0002). The MBN overexpression was detected in 60 % of PB samples from RA patients (mean value: 10, range 3-268), and in all the cases of RAEB (mean value 201: range:128-803) and secondary AML (mean value 589, range 207-7131). Moreover, in these patients we could demonstrate a good correlation between the percentage of blast cells and MBN transcript amount. Conclusions: MBN gene may probably play an important role in the leukemic transformation. The quantitative assessment of MBN expression can be a useful marker of leukemic and myelodysplastic hematopoiesis. Finally, MBN could probably represent a good leukemia antigen for an immunotherapeutic approach.

273 MITOCHONDRIAL ABNORMALITIES IN LOW-RISK MYELODYSPLASTIC SYNDROMES: ABERRANT MT FERRITIN ACCUMULATION IS A VERY EARLY EVENT IN RARS ERYTHROPOIESIS

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The myelodysplastic syndromes (MDS) subgroups refractory anemia (RA) and RA with ringed sideroblasts (RARS) are characterized by ineffective erythropoiesis, and enhanced bone marrow apoptosis. We have shown that the apoptosis of early erythroblasts is mediated through a constitutive cytochrome c release from mitochondria, and that G-CSF strongly inhibits this process (Tehranchi et al, 2003). Moreover, we have demonstrated that late erythroblasts in RARS, but not RA show mitochondrial accumulation of aberrant ferritin (MtF) (Cazzola et al, 2003). The aim of this study was to assess the kinetics of cytochrome c release and MtF accumulation during erythroid maturation in MDS, as well as the effects of G-CSF. Secondly, we wanted to test the hypothesis that the inhibitory effect of G-CSF on cytochrome c release is mediated through an enhancing effect on mitochondrial respiratory chain (MRC) activity. We assessed day 0 (CD34+ cells) to day 14 erythroblasts from RARS, RA and normal bone marrow (NBM) cultures for MtF expression and cytochrome c release according to previous publications. We developed a technique to measure MRC activity normalized to citrate

synthase activity in CD34- cells. Freshly separated CD34+ cells from all individuals were negative for MtF. NBM showed only few positive cells (0-4%, day 4-14), and RA erythroblasts a median of 3% (0-8%) positive cells. RARS erythroblasts, on the contrary, showed an early increase in MtF positive cells and a continuous increase during the culture period (day 4: 10%, day 7: 17%, day 11: 13%, day 14: 19%). There was no correlation between MtF expression and cytochrome c release except at day 14. There was no significant difference in either total MRC activity and complex IV (cytochrome c containing) activity, in terms of ATP production, between RARS, RA and NBM. Interestingly, G-CSF significantly reduced the cytochrome c release in RARS and to a more moderate extent in RA, but showed no effect at all on MRC activity. Moreover, G-CSF tended to increase MtF expression in some RARS erythroblast cultures. RARS morphology is characterized by ringed sideroblasts, but in the erythroblast cultures, this feature develops late, day 14-17. We here show that the expression of MtF occurs at a very early stage of erythroid differentiation, in cells that are still CD34+ and without any visible iron accumulation. This iron accumulation, whether or not a result of stem cell apoptosis in RARS, or of a primary defect in iron metabolism, may cause further damage to the mitochondria and result in cytochrome c release and apoptosis in RARS. Alternative mechanisms may be involved in RA pathogenesis, since these cells show cytochrome c release but very moderate MtF expression. Interestingly, the addition of G-CSF seems to inhibit apoptosis by upregulating compensatory mechanisms for cell survival rather than affecting mitochondrial iron metabolism or MRC activity.

CHRONIC MYELOPROLIFERATIVE DISORDERS

274 A NOVEL 53BP1-PDGFRB FUSION AS TARGET FOR IMATINIB IN A PATIENT WITH BCR-ABL NEGATIVE CHRONIC MYELOID LEUKEMIA AND A T(5;15)(Q33;Q22)

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Characterisation of chromosomal translocations in atypical myeloproliferative disorders has led to the identification of a network of tyrosine kinase fusion genes that are believed to function in a manner analogous to BCR-ABL in chronic myeloid leukaemia (CML). We have identified a patient with BCR-ABL negative CML and a novel translocation between chromosomes 5 and 15. PDGFRB involvement was determined by FISH and RACE PCR was used to identify a novel fusion between 53BP1 and PDGFRB. 53BP1 is a p53 binding protein that participates in the cellular response to DNA damage. The fusion protein is predicted to retain the kinetochore-binding domain (KBD) of the 53BP1 protein fused to the transmembrane and intracellular tyrosine kinase domains of PDGFRB. The reciprocal fusion, which would contain the 53BP1 BRCA-1 C-terminus (BRCT) domains that bind the p53, was not detectable by nested PCR. The presence of the fusion was confirmed by two colour FISH, RT-PCR and by characterising the genomic breakpoints. Sensitivity towards imatinib was determined by in vitro analysis of patient CFU-GM. Following systemic imatinib treatment, a partial response and transient transfusion independence was achieved prior to relapse and subsequent death with imatinib-resistant disease. We conclude that 53BP1-PDGFRB is a novel imatinib target in atypical CML.

275 PEGYLATED INTERFERON ALPHA (PEGINTRON) FOR THE TREATMENT OF HIGH-RISK ESSENTIAL THROMBOCYTHEMIA: RESULTS OF A PHASE II STUDY

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In high risk essential thrombocythemia (ET) there is a clear indication for cytoreductive therapy. However, especially in younger patients the

use of alkylating agents or hydroxyurea is a matter of concern because of the potential risk of a secondary malignancy or leukemia. The use of conventional interferon alpha, which is a non-leukemogenic agent is restricted by frequent side effects. Modification of the pharmacokinetic profile of interferon alpha through addition of a polyethylenglicol (pegylation) has resulted in slower absorption and lower elimination rate, thus enabling a weekly application with potentially increased compliance.

In this phase II study we evaluate the safety, tolerability and efficacy of pegylated Interferon alpha (PegIntron, Essex Pharma) in high risk essential thrombocythemia. PegIntron is administered subcutaneously at a starting dose of 50 g weekly. In patients not achieving a platelet count < 500 G/l after 8-12 weeks, the dosages can be increased by 25 g/week up to 150 g/week.

Between 12/01 and 09/03 a total of 36 high risk ET patients (either platelets > 1500 G/l, or age > 60 years, or previous ET related complications) were enrolled by 16 centers. There are 16 male and 20 female, the median platelet count at diagnosis was 900 G/l, the median age at diagnosis was 54 years (range 24-72 years). Treatment was indicated because of at least one of the following reasons: Age > 60 years (38%), platelet count > 1500 G/l (36%) or previous ET related complications (50%). 10 patients (28%) were previously treated (hydroxyurea: 8 patients, anagrelide: 2 patients).

In this analysis there are 35 of 36 patients evaluable, one patient was lost to follow up. Of these 35 patients, 27 are still on therapy with PegIntron after a mean observation time of 15 months (SD 6 months). The individual dosages of PegIntron are shown in the table. A platelet count < 500 G/l was achieved in 57% of the patients after 3 months and in 75% and 89% after 6 and 12 months, respectively.

In 6 patients (17%) therapy was stopped because of drug related side effects (2 patients with alopecia, 2 Patients with arthralgia, one patient with exacerbated depression, one patient with diarrhoea). In one patient the platelet count couldn't be lowered sufficiently and an alternative cytoreductive therapy was initiated. One patient suffered from a cerebral stroke and subsequently was taken of PegIntron application.

After a mean observation time of 15 months, we observed only one potentially ET related complication (stroke) during PegIntron therapy.

These preliminary data indicate the efficacy of PegIntron in the treatment of high risk ET at relatively low doses and reasonable toxicity and safety compared to conventional interferon alpha.

<25 µg per week			3,57%	5,56%
25 µg per week		6,66%	3,57%	5,56%
50 µg per week	100%	66,67%	66,71%	66,67%
75 µg per week		6,66%	7,14%	5,56%
100 µg per week		2,12%	14,29%	5,56%
125 µg per week			3,57%	5,56%
150 µg per week		6,66%	7,14%	5,56%

Caption 1: Dosages of PegIntron after 3, 6 and 12 months

276 HMGA2 DYSREGULATION IN MYELOFIBROSIS WITH MYELOID METAPLASIA (MMM) VERSUS OTHER PH-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS AND MYELODYSPLASTIC SYNDROMES

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Background and aims : Dysregulation and overexpression of the tumorigenic gene HMGA2 has been demonstrated in the blood mononuclear cells of 12 consecutive MMM patients and thus appears a hallmark of the disease [Andrieux al. Genes, chromosomes and cancer, 2004]. Apart from pathogenetic considerations, the consistency of this finding raises the question of its specificity and usefulness as a differential diagnosis tool to distinguish proliferative forms of MMM from other Ph-negative myeloproliferative diseases (MPD) and on the other

hand cytopenic form of MMM from myelodysplastic syndromes (MDS).

Methods : We assessed the level of HMGA2 expression in 30 MMM patients (the former series and 18 additional), 7 healthy volunteers (controls), 17 patients with Polycythemia vera (PV), 5 having turned to spent phase with myelofibrosis, 12 patients with Essential Thrombocythemia (ET), 3 of whom having also developed myelofibrosis, 1 patient with a MMM-like picture due to extensive systemic mastocytosis and 15 patients presenting with various types of myelodysplastic syndromes (MDS), 3 of whom having marked myelofibrosis. RNA was extracted from peripheral blood leukocytes separated through standard Ficoll centrifugation and QT-PCR was performed on cDNA with TaqMan technology.

Results : While controls were all negative, a substantial expression of HMGA2 was confirmed in 28/30 MMM patients, significantly higher than in any other situation. PV and ET patients were negative except for those having subsequent myelofibrosis ; despite his fibrosis, the patient with mastocytosis was negative. Among the short and heterogeneous series of MDS, a weak expression was occasionally found with no obvious correlation with the subtype of MDS nor the presence of myelofibrosis.

Conclusions : Dysregulation and overexpression of HMGA2 characterize MMM and allow a definite discrimination from other Ph-negative MPD ; in PV and ET, the gene is never expressed during the chronic proliferative phase but its expression seems to start when the disease evolves towards myelofibrosis. These findings, along with the evidence of transcripts in some MDS patients, suggest that a reactivation of this embryonic gene is not simply related to the myeloproliferation nor to the myelofibrosis itself but is rather associated with a dysplastic hematopoiesis either primitive or depending from the fibrotic network which alters the hematopoietic micro-environment.

277 RISK FACTORS FOR PROGRESSION TO ACUTE LEUKEMIA IN PATIENTS WITH POLYCYTHEMIA VERA ENROLLED IN THE ECLAP STUDY

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Background: Progression of Polycythemia Vera (PV) into Acute Leukemia (AL) is estimated to occur in 5-15 % of patients at 10th year of the disease, and, once AL develops, the prognosis is extremely poor. Its relation to radiation and alkylating agents is recognized, whereas the leukemogenicity of non-alkylating agents such as hydroxyurea is controversial. To date, there are not applicable ways to predict which patient is likely to develop AL.

Aim: To identify potential risk factors as well as markers of progression of PV into AL.

Methods: In the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) study, 1638 patients with new and old diagnosis of PV, made according to the criteria established by the PVSG or by Pearson and Messinezy, were recruited and prospectively followed for a mean time of 2.7 years (SD 1.3). The median time from diagnosis of PV to recruitment was 3.5 years. Patients who developed AL or myelodysplasia during follow-up, based on the FAB Cooperative Group Criteria, were evaluated for risk factors and markers, by fitting a multivariate Cox proportional hazard model adjusted for age, gender, disease duration, splenomegaly, packed cell volume (PCV) level, platelets and white blood cell (WBC) counts, blood cholesterol values and treatments at recruitment.

Results: Twenty-one cases of AL and 1 case of myelodysplasia were diagnosed during the follow-up period, after a median time of 2.5 years from the recruitment and 8.4 years from the diagnosis of PV. All AL cases were fatal; no patient survived more than 6 months after the diagnosis of the complication.

According to the results of the multivariate analysis, females, patients aged more than 70 years, with high WBC counts (>12 x109/L) and low cholesterol levels (≤ 150 mg/dl) had a significantly increased risk of AL.

With respect to treatments, patients receiving alkylating agents (busulphan, chlorambucil or pipobroman) or P32 at recruitment had a significantly higher risk of AL, while no increased risk was observed in patients receiving hydroxyurea as compared to patients treated with phlebotomy or interferon. (Table)

Summary / Conclusions: Age > 70 years, high WBC counts and low blood cholesterol levels were associated with an increased risk of leukemia in PV patients enrolled in the ECLAP study.

Although the use of alkylating agents and P32 conferred an augmented risk of AL, patients using HU did not have a higher risk compared to patients treated with phlebotomy or interferon.

	AL/MDS N=22	No AL/MDS N= 1616	HR (95% CI)	P
Age (%) < 50 years	3 (13.64)	470 (29.06)	1.00	
60-70 years	5 (22.73)	487 (30.14)	1.67 (0.38-7.32)	0.4944
> 70 years	14 (63.64)	659 (40.78)	3.99 (1.08-14.67)	0.0375
Gender (% females)	14 (63.64)	682 (42.20)	3.04 (1.23-7.50)	0.0180
Diagnosis > 3.5 y (%)	17 (77.27)	802 (49.63)	1.84 (0.65-5.20)	0.2524
Splenomegaly (%)	15 (68.18)	934 (57.80)	0.91 (0.34-2.40)	0.8450
PCV > 0.45 l/l (%)	11 (52.38)	864 (53.28)	1.19 (0.48-2.93)	0.7109
Platelets < 400.10 ⁹ /l (%)	15 (68.18)	921 (57.60)	2.42 (0.94-6.25)	0.0671
WBC > 12 .10 ⁹ /l (%)	8 (40.00)	411 (26.08)	2.65 (1.00-7.04)	0.0499
Cholesterol < 150 mg/dl (%)	7 (31.82)	171 (17.41)	6.24 (1.99-19.62)	0.0017
No therapy, only phlebotomy or IFN- α (%)	5 (22.73)	864 (41.09)	1.00	
Only hydroxyurea (%)	8 (27.27)	736 (45.54)	0.82 (0.25-2.77)	0.7525
Alkylating drugs/P32 (%)	11 (50.00)	216 (13.37)	5.15 (1.72-15.41)	0.0033

Caption 1:

278 ITALIAN COOPERATIVE STUDY ON IDIOPATHIC HYPEREOSINOPHILIC SYNDROME (HES): FIP1-L1/PDGFR α REARRANGEMENT CAN BE EFFECTIVELY TREATED WITH IMATINIB

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Idiopathic hypereosinophilic syndrome (HES) is a rare haematological disorder characterized by persistent eosinophilia with organ involvement. We studied 33 patients with HES, defined as a peripheral blood eosinophilia greater than 1,500 cells/ L for longer than 6 months, absence of other apparent aetiologies for eosinophilia and symptoms of organ involvement. All patients were studied by conventional cytogenetic analysis: in one patient we found evidence of a t(5;12)(q33;p13) translocation, which usually fuses the ETV6/TEL gene to the platelet-derived growth factor receptor-beta (PDGFRB), a receptor tyrosine kinase which maps to 5q33; however, RT-PCR analysis failed to show any fusion transcript. In five patients, we found clonal cytogenetic abnormalities: 45, X0 in two male patients; 47, XX, +8, del 11q2.1, in another one; t(1;15) and 46XY +17q in the remaining two. We sought also for PDGFRB-TEL and FGFR1-BCR transcripts generally associated with HES/CMML/MDS with hypereosinophilic syndrome. All patients resulted negative for these fusion chimeric mRNAs. We also sought for the recently reported involvement of PDGFR α , cryptically translocated with FIP1L1 in some HES patients responsive to Imatinib therapy. We found only three patients positive for the FIP1L1-PDGFR α rearrangement, with previously unreported, abnormal-sized fusion transcript. We treated twelve patients, including the three FIP1L1-PDGFR α positive ones, with imatinib mesylate (400 mg/daily). Median follow up of treatment was 4,5 moths (range 2-17). Complete hematological responses (CHR) to imatinib therapy were recorded in 5 patients (12%): the three positive FIP1L1-PDGFR α HES patients (in one cases also with complete molecular response obtained after 16 moths of therapy) and in two other cases without evidence of FIP1L1-PDGFR α rearrangement in which CHR was also reported. Detailed analysis of our HES patients will be presented.

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This work was supported by grant from COFIN 2002 and 2003, AIRC, FIRB 2001 and ATENEO 60% target projects by Hairshow A.I.L. and Fondazione del Monte di Bologna e Ravenna grants.

279 THE QUANTITATIVE ASSESSEMENT OF WT1 ALLOWS TO IDENTIFY THE HYPEREOSINOPHILIC SYNDROME PATIENTS AND TO PREDICT RESPONSE TO IMATINIB THERAPY

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Background: Several HES patients have been demonstrated to be responsive to low doses of Imatinib, and the rational of the response is, at least in some cases, the presence of the fusion gene FIP1L1/PDGFR α . Aims: The aim of the study was the molecular and cytogenetic characterization of HES patients and the identification of a molecular marker of HES able to predict response to Imatinib therapy. Methods: We analyzed 58 cases of hypereosinophilia, 34 out of 58 were secondary eosinophilic syndromes. Molecular and cytogenetic characterization of the HES patients were performed. Gene sequences of the FIP1L1-PDGFR α positive patients was carried out. Using a RealTime quantitative PCR (TaqMan) assay we analyzed WT1 expression levels in all the 58 patients. As control group we analyzed 52 PB and 15 BM from healthy volunteers. In addition we test the possibility to predict the response to Imatinib therapy using an in vitro assay that has been previously tested for CML patients. We incubated the BM cells of HES patients with or w/o 1 microM Imatinib for 18 hours and we evaluated WT1 expression after incubation. Results: 12 patients out of 24 HES patients were positive for the translocation FIP1L1-PDGFR α with different types of breakpoints as demonstrated by gene sequence analysis. 11 out of 12 respond to low doses of Imatinib therapy and 1 was resistant after treatment with 600 mg of Imatinib. Moreover two patients who were negative for the presence of the fusion transcript were characterized by the presence of a cytogenetic marker. FISH analysis using the probe for PDGFR α and beta were negative in both cases. One of the two patients was sensitive to Imatinib therapy. Among the 10 patients with normal karyotype and negative for the fusion transcript 2 cases were sensitive to Imatinib therapy. The quantitative assessment of WT1 transcript showed an increased expression in both BM and PB in all the HES cases with a median value of WT1 copies /104 ABL of 254 and 56 respectively. By contrast, in all the cases of secondary hypereosinophilia WT1 was found within the normal range. In HES patients who later revealed to be respondent to Imatinib, WT1 expression was significantly downmodulated after in vitro incubation with Imatinib. By contrast no significant changes were observed in the 10 resistant patients. Conclusions: The detection of FIP1L1-PDGFR α transcript does not allow neither to identify all the cases of HES, nor to predict response to Imatinib, since a number of HES are negative for the fusion gene. Moreover, we detected one case of FIP1L1-PDGFR α positive patient who was resistant. These data allow to identify a molecular marker of HES and to validate an in vitro assay which allows to identify the patients sensitive to Imatinib independently on the underlying molecular mechanism.

THROMBOSIS & HEMOSTASIS I

280 IS FACTOR V LEIDEN A RISK FACTOR FOR THROMBOTIC MICROANGIOPATHIES WITHOUT SEVERE ADAMTS-13 DEFICIENCY ?

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Background: Thrombotic microangiopathies (TMA) are microvascular occlusive disorders characterized by platelet aggregation, thrombocytopenia and microangiopathic hemolytic anemia. Two principal groups of TMA are distinguished: The hemolytic-uremic syndrome (HUS) is characterized by platelet-fibrin thrombi in the renal microcirculation, and is often linked to infections with enterohemorrhagic E. coli., especially O157:H7. In contrast, thrombotic thrombocytopenic purpura (TTP) presents with systemic microvascular platelet clumping leading to organ dysfunction such as neurological abnormalities, renal failure and fever. Acute TTP is often associated with a severe deficiency of ADAMTS13, the von Willebrand factor-cleaving protease (<5% of normal). The sensitivity of this finding for the clinical diagnosis of TTP remains equivocal with reported prevalences between 33% and 100%. Recently, Raife (Blood 2002;99:437-442) concluded from a small case series, that the factor V Leiden mutation (FVL) might represent a pathophysiologic risk factor in the subset of TMA patients without severe ADAMTS-13 deficiency. The consequence could be a more individualized treatment

regimen, including anticoagulant drugs in this subset of patients.

Aims: Investigation of FVL in a large patient series referred for ADAMTS-13 activity determination. Risk estimation for FVL carriers in relation to different clinical TMA categories and ADAMTS-13 activity levels.

Methods: Between January 2001 and July 2003 ADAMTS-13 activity was determined in plasma samples of 396 consecutive patients referred to our laboratory for diagnostic purposes. ADAMTS-13 activity was determined by immunoblotting assay and assigned to one of 5 arbitrary categories corresponding to the activity levels. After exclusion of 131 patients (no clinical information available [45]; no acute TMA: other hematological disease [21] or follow-up samples in remission after acute TMA [29]; no plasma left [26]; non-Caucasians [3]; various reasons [7]) the remaining 265 patients were assigned to three clinical categories according to the information provided by the referring clinician: TTP (80); HUS (124); and other TMA (61). FVL carrier-ship was determined by a commercial aPTT-based APC resistance assay using patient plasma diluted 1:5 (v:v) with FV deficient plasma and in cases with prolonged base-line aPTT (>42s), factor V:C <50% or equivocal APC sensitivity ratio by genotyping using DNA purified from patients plasma.

Results: 21 of 265 patients (7.9%) were carriers of the FVL mutation consistent with the prevalences reported for Europe (0.9-14%), especially for Germany (4-8.9%) and Switzerland (3-7%), who referred most patients (176 [66.4%] and 52 [19.6%], respectively). The prevalence of FVL within the different ADAMTS-13 activity categories was as follows: severe ADAMTS-13 deficiency (<5% of normal) = 6.8% (4/59 patients); borderline severe deficiency (ADAMTS-13 activity 5-9%) = 25% (3/12); moderately decreased ADAMTS-13 activity (10-25%) = 0% (0/21); mildly decreased ADAMTS-13 activity (26-50%) = 5.7% (3/53) and normal ADAMTS-13 activity = 9.2% (11/120). Similar frequencies were observed among patients diagnosed as TTP (5/80; 6.3%), HUS (11/124; 8.9%) or other TMA (5/61; 8.2%).

Conclusion: We conclude that FVL is not more prevalent in TMA patients without severe ADAMTS-13 deficiency than in those with ADAMTS-13 activity <5%. Therefore, it is unlikely, that the FVL mutation is involved in the pathogenesis of acute TMA without severe ADAMTS-13 deficiency.

281 IN VITRO EXPRESSION STUDY OF A NEW MUTATION (R1308L) FOUND IN A FAMILY WITH TYPE 2B VON WILLEBRAND DISEASE CHARACTERIZED BY ALL SET OF MULTIMERS IN PLASMA AND NO THROMBOCYTOPENIA AFTER DESMOPRESSIN
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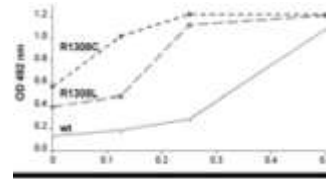
Background. Type 2B von Willebrand disease (VWD) is due to the increased affinity of an abnormal von Willebrand factor (VWF) for the platelet receptor glycoprotein Ib (GPIb); it is characterized by enhanced ristocetin-induced platelet agglutination (RIPA) and, in most cases, by loss of the high molecular weight multimers (HMW) in plasma. Only a few patients have been shown to be associated with a normal multimeric distribution (2B Malmo and New York). We report here expression data derived from a novel mutation (4173G>T R1308L) found in a family of 4 affected members with RIPA (0.3-0.4 mg/mL), VWF:Ag (27-51 U/dL), VWF:RCO (23-49 U/dL), bleeding time (6-11 min), all set of multimers in plasma and normal platelet count before and after desmopressin (DDAVP). We have also compared these data with those observed in 4 cases carrying the common VWD type 2B mutation (R1308C) which is characterized by loss of HMW, low platelet count and RIPA (0.7-0.8 mg/mL), VWF:Ag (26-48 U/dL), VWF:RCO (13-39 U/dL), bleeding time (10-27 min).

Aims. To better understand the molecular mechanisms of these 2B variants, we decided to express both mutated VWF molecules (rVWF-R1308L and rVWF-R1308C) and to analyze the expressed mutants for the ability to bind GPIb receptor along with rVWF-WT.

Methods. Plasmide pSV-VWF, containing full-length wild-type human VWF cDNA was used to generate by mutagenesis vector pSV-VWF-R1308L and pSV-VWF-R1308C. Mutant rVWF molecules were transiently expressed in COS-7 cells along with rVWF-WT. The rVWFs were evaluated by ELISA (VWF:Ag) and by multimeric analysis using low resolution SDS-agarose (0.9%) gel electrophoresis under non-reducing conditions. Binding of rVWFs to the GPIb platelet receptor was tested by an ELISA method recently described (Federici et al. Haematologica 89:77, 2004). VWF:Ag concentrations of the 3 rVWFs were normalized before the test. Binding of VWF to a recombinant GPIb was evaluated at increasing concentrations of ristocetin (0, 0.125, 0.25 and 0.5 mg/mL). The rVWF bound to GPIb, at each ristocetin concentration, was revealed by anti-VWF Antibody-HRP reading O.D. 492 nm.

Results. All set of multimers were present in vitro in rVWF-R1308L, rVWF-R1308C and rVWF-WT. The concentration of 0.125 mg/mL of ristocetin could discriminate between the 2B variants, with an O.D. of > 1.0 versus an O.D. < 0.5 for rVWF-R1308C and rVWF-R1308L, respectively. However, both 2B variants showed an enhanced binding to GPIb in comparison to the rVWF-WT (Figure).

Conclusions. Our data demonstrated that the new mutation (R1308L) can enhance the VWF capacity to bind to GPIb receptor, but with lower efficiency compared to the R1308C variant. This might explain why these patients have normal VWF multimers in plasma. The increased binding capacity of VWF-R1308L for GPIb does not cause its spontaneous interaction with platelets, or at least, not as efficiently as VWF-R1308C does. The enhanced RIPA of 0.4 mg/mL, observed in patients with VWF-R1308L, versus 0.7 mg/mL of VWF-R1308C is probably due to the presence of all set of multimers, that strongly compensate for the lower binding capacity to GPIb of this new variant.



Caption 1: Figure

282 UNLIKE HEPARINS, FONDAPARINUX DOES NOT ADVERSELY AFFECT THE METABOLISM OF CULTURED HUMAN OSTEOBLASTS
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Background: Heparin-induced osteopenia is a side effect of heparin therapy. This may partly reflect its negative effect on the metabolism of osteoblasts. Fondaparinux (Arixtra®) is a new synthetic, selective antithrombotic agent that selectively inhibits factor Xa.

Aims: We compared the effects of fondaparinux with those of unfractionated heparin (UFH) and 2 low-molecular-weight heparins (LMWH), enoxaparin and dalteparin, on the metabolism of cultured osteoblasts.

Methods: Osteoblasts were obtained from bone material acquired during knee endoprosthetic interventions. They were cultured for 9 days without (control) or with UFH, LMWH, or fondaparinux. The drugs were added to osteoblast cultures at concentrations of 0.1, 1, 10, and 100 IU/mL for UFH and LMWH and 0.1, 1, 10, and 100 g/mL for fondaparinux.

Results: None of the drugs were cytotoxic, as demonstrated by the measurement of lactate dehydrogenase (LDH) in the culture supernatant. In the presence of UFH and LMWH, the mitochondrial activity of osteoblasts was maximal at the concentration of 0.1 IU/mL (+963% as compared with control cells $P<0.001$), then declined at increasing drug concentrations. In contrast, fondaparinux increased mitochondrial activity in a dose-dependent manner, up to +2144% at 100g/mL ($P<0.001$). Likewise, protein concentration in the medium significantly increased in the presence of 0.1 IU/mL of UFH and LMWH (+9425%, $P<0.01$), but it significantly ($P<0.01$) decreased at higher concentrations of UFH (-422%) or LMWH (-568%). In contrast, protein concentration in the medium continuously increased in the presence of increasing concentrations of fondaparinux (+1204% at 100 g/mL, $P<0.001$). Compared with control cells, the calcium concentration in the medium of cells cultured in the presence of UFH and dalteparin significantly increased (up to +22%, $P<0.01$), regardless of drug concentrations. In contrast, with fondaparinux, the calcium concentration in the medium was below that observed in control culture medium (0.750.03mmol/L vs 1.250.05mmol/L, respectively), suggesting that fondaparinux induced the calcification of the extracellular matrix. Non of the drugs investigated significantly altered the phosphate concentration of the medium.

Summary/Conclusion: Unlike UFH and LMWH, fondaparinux did not inhibit the mitochondrial activity or protein synthesis of human osteoblasts, either at therapeutic or up to 100 times higher concentrations. These results suggest that the clinical use of fondaparinux will not result in osteoporosis, a finding especially relevant to patients requiring long-term treatment.

283 SPECIFIC ROLES OF TISSUE FACTOR-EXPRESSING CELLS AND PLASMA-ASSOCIATED COAGULATION REACTIONS IN REGULATING SPATIAL GROWTH OF FIBRIN CLOT

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Background: Transmembrane glycoprotein tissue factor (TF) is the principal initiator of blood coagulation in vivo. Exposure of TF bearing cells to blood results in formation of the extrinsic Xase complex that activates factor IX and factor X, ultimately leading to generation of thrombin and fibrin on the cell surface. Generated activated coagulation factors diffuse from the cell surface thus contributing to enlargement of thrombus in size. Efficient thrombin generation can also be supported by the intrinsic, factor VIII-dependent, Xase complex assembled on plasma phospholipid surfaces. The roles of TF bearing cell-mediated and cell-independent mechanisms in spatial growth of fibrin clot have not been experimentally addressed.

Aim: The present study was designed to explore the specific roles of TF bearing cells and plasma-associated coagulation reactions in growth of fibrin clot in size.

Methods: Cultured cells expressing different TF levels (human aortic endothelial cells ECs; smooth muscle cells, SMCs; macrophages, MPs; and lung fibroblasts, LFs) were used to initiate coagulation in contact pathway-inhibited platelet-poor human plasma. Procoagulant activity of cell monolayers was assessed in thrombin- and fibrin-generation microplate assays. Parameters of spatial clot growth were studied using a real-time video microscopy system, which allows visualization of spatial propagation of clot from cell monolayers into non-flowing plasma.

Results: Cell types differed in their ability to support thrombin generation: peaks of free thrombin concentration were 6053 nM for ECs, 13557 nM for SMCs, 21855 nM for MPs and 40759 nM for LFs (mean $\pm$ SD) and the clotting times inversely correlated with thrombin-generating activity. Different procoagulant activity of cell monolayers was manifested as different lag times of clot formation in spatial experiments. However, the steady-state clot growth rate, which characterized propagation of clotting from cell surface to plasma, was largely independent of initiator cell type (<30% difference). Rates of clot growth remained unaffected by variation of fibrinogen level, excluding its inhibitory effect on the propagation of clotting. Pre-incubation of cells with increasing concentrations of factor VIIa and experiments with plasmas congenitally deficient in factor VII, factor X, factor VIII or factor XI revealed that (1) the cell surface-associated extrinsic Xase was critical for initiation and contributed to propagation of clot; (2) the intrinsic Xase regulated only the propagation phase and was determinant of the similar growth rates for all cell types. Blocking of phospholipid surfaces with Annexin V in plasma or removal of cell-derived vesicles by ultracentrifugation resulted in a 50% inhibition of clot growth rate revealing a predominant role of plasma-associated phospholipid surfaces in clot enlargement.

Conclusions: Our results provide direct experimental evidence that TF-bearing cells determine only the onset of coagulation. Plasma-associated intrinsic Xase provides sustained and far-ranging propagation of the clotting process from cell monolayer and is the major mechanism of spatial clot growth. From clinical perspective, inhibition of the intrinsic pathway of blood coagulation may be an efficient and selective way to prevent formation of occlusive thrombus.

284 APTT BIPHASIC WAVEFORM PERSISTENCE IN SEPTIC PATIENTS IS ASSOCIATED WITH A HIGH MORTALITY RISK

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Background: The aPTT biphasic waveform (aPTT BW) has been described to predict DIC, according to the ISTH standardisation committee criteria, with a sensitivity and a specificity of respectively 97,6% and 98,0%. The presence of an aPTT BW in the first hour of intensive care unit (ICU) can assign risk of DIC development that is superior to the D-Dimer (DD) measurement. On the basis of these observations, we performed a preliminary study on 20 septic patients hospitalised in ICU of our hospital. All the patients who had an aPTT BW at least 2 consecutive days or DD

level higher than 5000 ng/ml were dead. In no ICU hospitalised patients, the presence of an aPTT BW has uncertain clinical significance.

Aim: We tried to define the adverse clinical outcome associated with the presence of an aPTT BW for all hospitalised patients.

Methods: We studied all patients that presented with an aPTT BW measured with a MDA II coagulometer and Platelin LS (Biomrieux ®) from 02/12/03 to 23/12/03 (22 days). These patients were followed for 6 consecutive days. Data recovered included: diagnosis, complications, treatment and evolution (favourable or unfavourable). The Score SOFA (Sequential Organ Failure Assessment) was determined (D0) to assess the severity of the process. aPTT BW, Prothrombin time (PT), Thrombin time (TT), Fibrinogen (FG), DD, Platelets and C-reactive protein (CRP) were performed during all 6 days. Antithrombin III (AT-III), protein C (PC) and free protein S (PS) were performed only the first day (D0).

Results: 21 patients (1%) presented an aPTT BW from a total of 2335. Three patients were excluded because coagulation tests were not performed every day. Patients came from a wide range of locations: medical (22%) and surgical (28%) hospitalisation and ICU (50%). The majority of patients had infection 14 (78%): 1 (5%) septic shock, 7 (39%) severe sepsis and 6 (28%) non severe sepsis. Four patients (22%) had cardiac surgery without demonstrated infection. None of the patients presented overt DIC from D0 to D5. DD were elevated (>500 ng/ml) in all patients. Two patients among the 18 died: patient who was in septic shock died the day of admission and one patient who had an aPTT BW two consecutive days with DD > 5000 ng/ml died because a severe sepsis. The other 16 patients presented a good evolution. All presented the aPTT BW only at D0. None of them had DD>5000mg/ml at D0.

Conclusions:

1. Only 1% of all the aPTT measured in a general hospital demonstrated a BW aPTT.
2. The most frequently associated diagnosis of an BW aPTT is sepsis in about 80% of the cases.
3. No clear association was demonstrated between BW aPTT and overt DIC in general hospitalized patients.
4. The persistence of an aPTT BW at least for 2 days was confirmed as a highly lethal factor since all the patients with this persistence died in both the previous and the current studies.

Parameter	Unit	Day 0 (Mean $\pm$ SD)	Day 1 (Mean $\pm$ SD)	Day 5 (Mean $\pm$ SD)
aPTT BW	seconds	32 $\pm$ 11.19	33 $\pm$ 6.31	31 $\pm$ 7.64
PT	%	35 $\pm$ 18.44	41 $\pm$ 20.62	45 $\pm$ 22.63
TT	seconds	27 $\pm$ 46.41	14 $\pm$ 1.30	13 $\pm$ 1.94
FG	mg/dl	392 $\pm$ 193.05	423 $\pm$ 130.40	354 $\pm$ 120.16
DD	mg/dl	4600 $\pm$ 3164.46	3439 $\pm$ 3040.05	4343 $\pm$ 2885.33
Platelets	10 ⁹ /pl	213 $\pm$ 130.14	209 $\pm$ 136.01	239 $\pm$ 161.00
AT-III	%	73 $\pm$ 15.76	—	—
PC	%	37 $\pm$ 23.95	—	—
PS	%	49 $\pm$ 36.75	—	—
CRP	mg/dl	30 $\pm$ 13.15	21 $\pm$ 8.97	10 $\pm$ 5.95

Caption 1: Table 1: mean $\pm$ SD of parameters measured

285 THERAPY WITH HIGH-DOSE DEXAMETHASONE IN PREVIOUSLY UNTREATED PATIENTS AFFECTED BY IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP). FROM THE GIMEMA ITP STUDY GROUP

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Background. The treatment with pulsed high-dose dexamethasone (HD-DXM) in ITP is currently still a controversial issue, both as first-line therapy and in refractory disease.

Aims. We evaluated the efficacy, safety, feasibility and patient compliance of pulsed HD-DXM, given as first-line therapy, in a series of ITP patients (pts), consecutively enrolled in a 'GIMEMA' pilot study.

Methods. Previously untreated pts with newly diagnosed ITP and a platelet (plt) count $\leq$ 30x10⁹/L or $>$ 30x10⁹/L in presence of bleeding symptoms, were the subjects of our study. Exclusion criteria were: pregnancy, hypertension, cardiovascular diseases, diabetes, liver and kidney function impairment; HCV, HIV, HBsAg seropositive status. Oral or intravenous HD-DXM was given at single daily doses of 40 mg (or 20 mg/m² in pts under 18 years) for 4 consecutive days. Four courses were scheduled (on days: 0-14-28-42). Response was evaluated at day 60 from the treatment onset, according to the following criteria: plt count $\geq$ 150x10⁹/L complete response (CR), $\geq$ 50x10⁹/L partial response (PR), $>$ 30x10⁹/L minimal response (MR), $\leq$ 30x10⁹/L or presence

of bleeding symptoms, non response (NR). Persistent response (pCR, pPR, pMR) was defined as stable plt counts without therapy for at least 2 months.

Results. Ninety-five pts (M34/F61; median age 20 years, range 2-70; median plt count $9 \times 10^9/L$; range $1-35 \times 10^9/L$) were enrolled. Ninety pts, who completed 4 therapy courses, were evaluable for response at day 60 (3 patients were lost to follow-up) (FU); 2 stopped treatment early: 1 for gastric toxicity after the 3rd cycle, 1 for medical decision after the 2nd cycle). The responder pts were 77/90 (86%); CR 58, PR 18, MR 1. Unresponsive pts were 13 (14%). The overall response rate was the same in pts under (42 pts) and over (48 pts) 18 years (< 18 yrs 85.7%; > 18 yrs 85.4%). Total response rates (CR+PR+MR) at the end of the 1st, the 2nd and the 3rd cycle were: 69.5% (66/95 pts), 76% (72/95 pts), 89% (81/91 pts) respectively. A statistically significant increase concerning the overall response rate was found after the 3rd cycle ($p = 0.018$), while no further rise after the 4th one was recorded. At the last control (median F.U. 10 months, range 4-24), among the 77 responder pts, 67 (87%) showed a persistent response, without any therapy: pCR 53, pPR 11, pMR 3. Ten/77 (13%) responder pts relapsed after a median F.U. of 6.5 months from response achievement (range 3-10). Treatment with HD-DXM was well tolerated.

Conclusions. Pulsed HD-DXM therapy courses given at short time intervals seem to be feasible and well tolerated by adults and children with ITP. In fact, only one patient stopped treatment early for toxicity. Efficacy is proven by the response rate (86%) being higher than the one obtained with conventional treatments. Moreover, it is noteworthy that we obtained also a high rate of persistent responses (67/90=74%). Thus, HD-DXM would be proposed, in a next randomized 'GIMEMA' clinical trial, versus conventional corticosteroid treatment, for previously untreated adults and children affected by ITP.

LYMPHOPROLIFERATIVE DISORDERS: MOLECULAR BIOLOGY

286 BIASED USAGE OF VH3-72 AND IGVK GENES AND HOMOLOGOUS IGVH AND IGV L CDR3S IN HIGHLY STABLE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: IMPLICATIONS FOR ANTIGEN RECOGNITION IN LEUKEMOGENESIS

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Background: B-cell chronic lymphocytic leukemia (B-CLL) is a molecularly heterogeneous disease that may be dissected into prognostic variants based on somatic hypermutation (SHM) and biased usage of immunoglobulin variable (IgV) genes. **Aims:** characterize usage, CDR3 sequences and mutation distribution of both IgVH and IgVL in a group of highly stable B-CLL. **Methods:** highly stable B-CLL ($n=25$) who never progressed and never required treatment over 10-23 yr period were identified among a consecutive series of 1299 Binet A stage B-CLL. Data were compared to a total of 1,140 IgV genes from various B-cell malignancies: 805 nonselected B-CLL (432 mutated, 373 unmutated); 230 indolent B-cell lymphoproliferations; and 105 aggressive B-cell lymphomas. SHM occurred in 24/25 highly stable B-CLL. **Results:** the VH gene most frequently encountered was VH3-72 (6/25; 24.0%), which, conversely, was rare in nonselected (6/805; 0.74%; $p < 0.001$) or somatically mutated (5/432; 1.16%; $p < 0.001$) B-CLL and was exceptional among indolent (1/230, 0.435%; $p < 0.001$), aggressive lymphomas (0/105; $p < 0.001$) or non-neoplastic B-cells (<1%). VH3-72 genes of highly stable B-CLL disclosed a mutational hotspot at codon 84 of FR3 causing the substitution of isoleucine for threonine, whose frequency was significantly higher than in non-neoplastic B-cells ($p < 0.05$). Because this mutation substitutes a hydrophobic aminoacid for a polar residue, it is expected to cause a conformational change of FR3 proximal to CDR3 that might affect antigen binding. Three out of six VH3-72 B-CLL utilized the same Vk gene B3, whose usage did not occur outside VH3-72 cases and 2/6 VH3-72 B-CLL used the same Vk gene L12. Two VH3-72 B-CLL rearranged the same D segment (D2-2) and JH gene (JH3) and displayed virtually identical VH CDR3 aminoacid sequences, differing only for conservative changes. The CDR3 of these

two B-CLL was characterized by the Cys-XXXX-Cys domain, suggesting formation of a disulphur bridge, that has been also found associated with rheumatoid factor antibodies. Both these VH3-72 B-CLL also utilized germline B3 Vk genes and displayed a virtually identical VL CDR3, differing only for one conservative change. The estimated pI value of VH CDR3 of these two cases is basic (> pH 8.00), whereas that of most B-CLL is acidic (<5.50), possibly reflecting selection for specific structural motifs. Combined analysis of available VH and VL sequences showed a tendency to conserve FR sequences and maintain antigen binding in 5/6 VH3-72 cases. A higher than expected number of CDR replacement mutations, suggesting selection for high affinity antigen binding, occurred in 3/6 VH3-72 cases. **Conclusion:** VH3-72 biased usage in highly stable B-CLL may reflect an antigen driven expansion of VH3-72 encoding B-cells, as suggested by several observations: i) occurrence of SHM and significant FR/CDR clustering of mutations in virtually all VH3-72 B-CLL; ii) high grade of homology in VH and VL CDR3 in two cases; iii) selection of a 'strong' aminoacidic substitution at codon 84 within the FR3; iii) a trend toward a biased usage of VL genes. Finally, because VH3-72 is virtually restricted to highly stable B-CLL, its detection may contribute to prognostic scores predicting for prolonged survival.

287 LIPOPROTEIN LIPASE (LPL) IS A PREDICTOR OF IGVH MUTATIONAL STATUS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: We investigated the role of lipoprotein lipase (LPL) expression in B-cell chronic lymphocytic leukemia (B-CLL).

Methods: LPL mRNA levels were measured by real time PCR in 55 well characterized patients and controls.

Results: LPL expression was absent in CD19+ sorted normal B-cells, low in the CD19- cell fraction, but high in CD19+ sorted B-CLL cells. LPL mRNA levels in peripheral blood mononuclear cells (PBMNC) from CLL patients differed by 2 logs between cases with mutated ($N=28$) or unmutated ($N=27$) IGVH gene status (median: 1.62 vs. 100.43 compared to normal PBMNC). A strong correlation of individual LPL expression with IGVH mutational status was found ($R=0.78$; CI: 0.67, 0.87; $P=10^{-12}$). At a threshold of 20.2-fold (defined from ROC curve analysis) LPL was able to discriminate between mutated and unmutated IGVH status in 51 of 55 patients (93%). High LPL expression predicted unmutated IGVH status with an odds ratio of 108.12 (CI: 20.99, 892.78; $P < 0.0001$). Significance was retained after adjustment for Rai and Binet stages, the percentage of CD19+/CD38+ cells and ZAP-70 mRNA expression indicating that LPL is an independent marker. LPL expression was high in patients with VH1-02, VH1-69, and VH3-21 as well as high risk cytogenetics (loss of p53, deletion in 11q).

Conclusion: This study establishes LPL as a novel and reliable diagnostic target in B-CLL discriminating between IGVH mutated and unmutated cases. LPL is restricted to the leukemic CD19+ cell fraction. Thus, assessment of LPL levels constitutes a reasonable and reliable alternative for ZAP-70 determination by FACS analysis.

288 GENE EXPRESSION PROFILES IN FOLLICULAR LYMPHOMA IN RELATION TO PREDICTION OF RESPONSE TO CHOP CHEMOTHERAPY

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Background: Prediction of response to multiagent chemotherapy remains a clinical challenge in patients with follicular lymphoma. Currently, there is no available method to determine whether a patient will respond to treatment or not.

Aim: Our aim was to elucidate if there was a difference in gene expression pattern in tumours from patients with a diverse response to chemotherapy and to investigate the possible prognostic impact of such expression patterns.

Methods: We compared gene expression (Affymetrix expression array u95Av2) profiles of 21 tumours from patients with good (CR) or poor (PD) response to CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone). Comparison of gene expression levels were made using comparison analysis with Micro Array Suite 5.0. The data was then

imported into GeneSpring 6.0 for unsupervised hierarchical clustering. Interphase-FISH was performed with Vysis LSI® TOP2A probe. Results: Using stringent comparison criteria nine genes were up-regulated in the CR group compared to PD group, but no genes were found to be significantly up-regulated in PD group. The expression levels of these nine genes were used to perform hierarchical clustering in a cohort of 57 follicular lymphoma patients. The cluster tree revealed two main groups, that showed a significant difference in cause-specific survival (log rank test $p=0.027$). The five-year survival was 82% and 60%, respectively. The nine investigated genes were involved in cell cycle, DNA modulation and mitotic mechanisms and thus, could contribute to the differences in response to CHOP chemotherapy. One of the genes, TOP2A (topoisomerase II; believed to be the main target of doxorubicin and essential for chromatide packing and separation), was further analyzed on a genomic level with interphase-FISH. All analyzed samples showed two signals, implying that the observed difference in expression of TOP2A was post-translationally regulated.

Conclusion: We have identified nine genes differently expressed in tumour samples from patients with follicular lymphoma that may be of use in prediction of response to treatment with multiagent chemotherapy.

289 FUNCTIONAL IMPAIRMENT OF THE ATM-P53 PATHWAY IN CHRONIC LYMPHOCYTIC LEUKAEMIA: SUPPRESSION BY BASIC FIBROBLAST GROWTH FACTOR AS AN ALTERNATIVE TO P53/ATM MUTATION

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Background: Inactivating mutation/deletion of the p53 tumour suppressor remains the most powerful adverse prognostic factor in chronic lymphocytic leukaemia (CLL), underlining its central role in co-ordinating the cellular response to DNA damage. Although the defect occurs in only 10-15% of patients, we have previously shown that a similar proportion of cases have p53 dysfunction due to mutation of ATM, a kinase required for the activation of p53 in response to double-strand DNA breaks (Blood 2001;98:814-22). Such cases, which are readily detectable owing to their impaired p53 response to ionising radiation (IR), also have an aggressive form of the disease (Blood 2002;100:1404-9).

Aims: p53 and its main negative regulator MDM2 are subject to post-translational modification by a number of signalling proteins that are activated through cell-surface receptors. We therefore postulated that the ATM-p53 pathway might be suppressed in a further subset of CLL patients due to extrinsic signals emanating from the tumour-cell microenvironment. Our aim was to test this hypothesis.

Methods: Irradiated and non-irradiated CLL lymphocytes were cultured in the presence or absence of basic fibroblast growth factor (bFGF) and examined for p53 accumulation. bFGF was employed because of its established importance in the pathophysiology of CLL and because it has been reported to inhibit the killing of CLL cells by fludarabine, a process which is known to be partly p53 dependent. Experiments were confined to cases that were known to have a normal p53 response to IR.

Results: p53 levels were measured by FACS in 30 cryopreserved samples from 26 patients. In 11 samples from 9 patients, bFGF produced more than 30% inhibition of IR-induced p53 accumulation. Among these, 7 samples from 7 patients showed greater than 50% inhibition. One sample was tested on 5 separate occasions, and 2 samples from 1 patient were each tested twice. A high level of consistency was always observed between separate tests performed on the same samples. Nine samples from 8 patients in which bFGF produced a marked p53-suppressive effect were further tested by Western blotting and very similar results obtained. To explore the underlying mechanism, the latter samples were also examined by Western blotting for levels of phosphoserine 166 MDM2 (the active form of MDM2). bFGF produced a marked increase in phosphoserine 166 MDM2 in 4 of these samples, whereas levels were not increased in the remaining 5 samples. This suggests a possible role for MDM2 in mediating the p53-suppressive effect of bFGF in some but not all cases. Summary/conclusions: To our knowledge, this is the first study in any neoplasm to directly demonstrate that p53 activation can be suppressed by extrinsic stimuli from the tumour-cell microenvironment. Further work is in progress to elucidate the mechanisms responsible for this inhibitory effect, together with its cellular and clinical consequences.

RED BLOOD CELLS / IRON METABOLISM

290 MUTATIONS IN THE VON-HIPPEL-LINDAU (VHL) GENE AND VHL- HAPLOTYPE ANALYSIS IN PATIENTS WITH APPARENTLY CONGENITAL ERYTHROCYTOSIS

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Background: Congenital erythrocytoses represent rare and heterogeneous clinical entities. In 2002, a mutation in the VHL gene (C598T) was identified as the underlying defect in the endemic Chuvash polycythemia. Subsequently, this mutation and other mutations of the same gene in several patients of different ethnic origin with sporadic or familial congenital erythrocytosis were reported. A haplotype analysis of the VHL gene suggested a common origin of the Chuvash mutation.

Aim: The study was aimed to answer the questions 1. whether VHL gene mutation represents a cause of so far etiologically unexplored congenital erythrocytosis also in patients of German ancestry, and 2. whether these patients would have the same haplotype as those described.

Methods: 22 patients aged 4.3-69.2 years with apparently congenital erythrocytosis with so far unknown underlying disorder were examined for VHL gene mutations by PCR and automatic sequencing of the three VHL gene exons. Haplotype analysis were based on six single nucleotide polymorphisms within the 5' and 3' untranslated regions and the two introns of the gene. Analysis was performed by PCR and automatic sequencing.

Results: Three patients (14%) were homozygotes for the Chuvash mutation C598T. One patient had a heterozygous C598T mutation. Another patient showed a heterozygous mutation (G311T) within the first exon not described before. Haplotype analysis was in agreement with recently published data for patients with homozygous or heterozygous Chuvash mutation. However, a haplotype suggesting heterozygosity for this mutation was also found in two individuals who were not affected by a Chuvash mutation.

Conclusions: Mutations in the VHL gene are responsible for a relevant part of former unexplored sporadic or familial congenital erythrocytoses. Nevertheless, in a significant number of patients the underlying disorder remains unclear. Erythrocytoses due to a Chuvash mutation are not geographically restricted. Although affected patients share a common VHL haplotype, the haplotype analysis based on these previously suggested SNPs is not able to distinguish clearly heterozygotes and those without VHL gene mutation.

291 DARBEPOETIN ALFA; SAFETY AND LONG-TERM SURVIVAL DATA FROM 2 LARGE RANDOMISED PHASE 3 STUDIES

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Background: Anaemia is frequent in cancer patients (pts) receiving chemotherapy (ctx) and can be resolved by erythropoietic therapy. Darbepoetin alfa (Aranesp®; DA) is a unique erythropoietic protein that is well tolerated and effective for the treatment of chemotherapy-induced anaemia when given every 1, 2, or 3 wks (Vansteenkiste 2002; Glaspy 2002; Kotasek 2003).

Aims: The objective of this analysis was to evaluate safety and long-term survival data in patients with chemotherapy-induced anemia treated with darbepoetin alfa vs placebo.

Methods: Two double-blind, randomised, phase 3 studies compared the safety and efficacy of weekly DA vs placebo (pbo) for 16 wks (12 wks therapy, 4 wks follow-up) in anaemic (Hb<11.0 g/dL) cancer pts on ctx. One study evaluated 314 pts with lung cancer (155 DA, 159 pbo), whereas the other evaluated 344 pts with lymphoproliferative malignancies (LPM) (175 DA, 169 pbo). Tumour status and survival data were also collected during a long-term safety follow-up period.

Results: In both trials, DA had an overall safety profile similar to pbo and consistent with that expected for pts on ctx. The incidence of serious adverse events (defined as fatal, life-threatening, permanently disabling, or resulting in hospitalisation) during the study was also similar for DA and pbo: lung, n=60 (39%) vs 58 (36%) respectively; LPM, 51(29%) vs 63(37%), respectively. None of the deaths during the 16 wk study was considered related to study drug; the most common cause of death was disease progression. No antibodies to DA were detected. DA pts were hospitalised for fewer mean (SE) days than pbo pts in both studies

(lung: 10.3[1.1] days vs 13.0[1.4] days, respectively; LPM: 5.3[0.8] days vs 7.2[1.0] days, respectively).

Median length of follow-up for time to disease progression or death (progression-free survival) was 20 months for both groups in lung cancer, and 10 months for DA and 11 months for pbo in LPM. The incidence of disease progression or death through follow-up was similar for DA and pbo (lung:131[85%] vs 145[91%], respectively; LPM: 82[47%] vs 76[45%], respectively). Although progression-free survival was longer for DA vs pbo in the subset with small-cell lung cancer (hazard ratio [95% CL], 0.58 [0.3, 0.91]), Kaplan-Meier estimates for the entire group suggest that the median duration of progression-free survival was similar for DA vs pbo in both studies (hazard ratio [95% CL]: lung (0.80)[0.63,1.02]; LPM, (1.09)[0.80,1.50]). Pts are being followed for a min of 1 yr after completion of treatment phase.

Conclusions: Long-term safety and overall survival in pts with lung cancer or LPM on ctx is comparable in pts treated with DA and pbo.

292 CARDIAC OUTPUT IN REGULARLY TRANSFUSED PATIENTS WITH BETA-THALASSEMIA

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Background: High output state is a well-recognized compensatory reaction in chronic severe anemia. High output failure represented the leading cause of death in poorly or non-transfused patients with beta-thalassemia. Nowadays, regular transfusions in thalassemia major (TM) maintain the hemoglobin above 9.0 g/dl. At this hemoglobin level, cardiac output is not increased, as shown by studies in non-thalassemic anemic patients; chronic mild anemia is known to lack structural or functional cardiac consequences in the absence of heart disease. The adaptation of cardiac output levels to regular transfusion therapy are not properly assessed in beta-thalassemia.

Aim: To assess cardiac output and the extent of its contribution to thalassemia heart disease in a large group of TM patients who have been regularly transfused since their early infancy and who lack overt cardiac disease.

Methods: We studied 188 regularly treated TM without symptoms or signs of heart failure and with normal left ventricular (LV) contractility, aged 27.16.4 years, along with 75 aged-matched healthy controls by means of Doppler echocardiography. Echocardiographic measurements were indexed to body surface area to correct for differences in body constitution.

Results: Mean hemoglobin and serum ferritin levels in the patient group were 9.50.3 g/dl and 1866518 ng/dl, respectively. Cardiac index was significantly higher in patients than in controls (4.40.9 versus 3.80.8 l/min/m², p=.001) and was higher than the upper 95% confidence limit of controls mean value in 56.1% of patients. Left atrial, right ventricular and LV diameters, LV volumes and LV mass were also significantly higher in the patient group. Left ventricular dilatation was encountered in 32.4% of patients. Relative LV wall thickness did not differ between patients and controls, while peripheral vascular resistance and LV afterload were significantly lower in the patient group (p<.001). Shortening and ejection fractions were significantly lower in patients (p<.001) although within normal limits. Regarding LV diastolic function, E and E/A indexes were significantly higher in patients and restrictive LV filling was observed in 31.9% of patients. Pulmonary hypertension was practically absent.

Conclusion: Despite the maintenance of high hemoglobin levels, high output state was a permanent finding in these regularly transfused TM patients. The incomplete suppression of bone marrow expansion, the reduced 2,3 DPG levels of the transfused blood, a chronic thalassemia-related inflammatory process and a coexistent hepatic damage due to iron overload and viral infections may represent some of the implicated pathogenetic mechanisms. High output state was followed by cardiac dilatation and LV hypertrophy, although with a favorable remodeling, decreased LV afterload and analogous impairment of diastolic LV indexes. The significantly decreased, although normal, mean LV shortening and ejection fraction values as well as the occurrence of LV dilatation and restrictive LV filling in one third of patients each may represent early signs of cardiac disease.

293 MUTATIONS OF HEMOJUVELIN AND HEPCIDIN IN JUVENILE HEMOCHROMATOSIS

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Background. Juvenile or Type 2 Hemochromatosis (JH) is a rare recessive disease that leads to severe iron overload with clinical complications before 30 years. The disease has a uniform clinical phenotype characterized by early onset of hypogonadism and cardiomyopathy, but the disorder is genetically heterogeneous. The most common type is due to mutations in HJV gene on chromosome 1q, which encodes hemojuvelin, a protein with a proposed crucial role in iron metabolism. A second, rare type of JH, is due to inactivation of hepcidin (HAMP) gene on chromosome 19, which encodes a key regulator of iron homeostasis.

Aim. To define the proportion of HJV and HAMP associated mutations in a large series of JH patients.

Methods. HJV and HAMP coding sequences were amplified by PCR and screened for mutations by direct sequencing in 37 patients worldwide collected. Northern Blot of different tissues was performed by standard methods using PCR developed probes.

Results. We identified 17 different (16 novel) HJV mutations both at the homozygous and at the compound heterozygous state. Mutations were both nonsense and missense substitutions, affecting highly conserved residues, relevant to the protein structure and/or function. We identified two new HAMP mutations: C70R and 25G>A. Northern Blot analysis of human and mouse tissues indicate that HJV and HAMP have a similar expression in liver, skeletal muscle and heart.

Conclusions. Our findings indicate that most JH patients have mutations in HJV and a minority in HAMP. The gene expression in the same tissues and the identical clinical phenotype that follows inactivation of HAMP and HJV indicate a clear relationship between the two proteins in regulation of iron homeostasis.

294 EXPRESSION OF THE MOUSE ORTHOLOG OF HEMOJUVELIN GENE DURING IRON OVERLOAD, INCREASED ERYTHROPOIESIS AND FETAL LIVER DEVELOPMENT

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Background:

Hemojuvelin is a newly described protein whose synthesis is defective in 1q-linked juvenile hemochromatosis. In this respect, hemojuvelin resembles hepcidin, whose mutations also cause juvenile hemochromatosis. Whereas hepcidin levels respond to changes in iron homeostasis, no information has yet been published about expression of the hemojuvelin (HJV) gene during iron overload or during states of increased erythropoiesis.

Aims:

Expression of the mouse ortholog of the hemojuvelin transcript (NM 027126) was investigated in livers from iron-treated, erythropoietin-treated, phenylhydrazine-treated and fetal mice. Obtained data were compared with expression levels of murine hepcidin (Hamp and Hamp2) genes.

Methods:

Gene expression was determined by real-time PCR using SYBR Green protocol. Results were expressed relative to β -actin mRNA.

Results:

A single dose of iron (600 mg/kg s.c. three weeks prior to euthanasia) significantly increased hepatic Hamp and Hamp2 expression, but did not affect the expression of HJV. Application of erythropoietin (50 U/mouse for 4 days) decreased Hamp and Hamp2 expression, again without effects on HJV. Treatment with phenylhydrazine (25 mg/kg twice weekly for 4 weeks) slightly increased Hamp expression, while HJV levels remained unchanged. Thus, in contrast to both Hamp and Hamp2, HJV expression is not influenced by manipulation of hepatic iron stores or changes in iron homeostasis.

Published data on tissue-specific HJV expression indicate high levels of HJV transcripts in fetal liver. Since liver is the major site of erythropoiesis

in fetal mice, we examined the changes in HJV expression from embryonic day 12.5 to embryonic day 17.5. During this interval, HJV mRNA (relative to β -actin mRNA) content increased from 0.0005–0.0004 at day 12.5 to 0.0038–0.0046 at day 14.5 and 0.0274–0.0161 at day 17.5 ($n=5$). A similar trend was seen in the expression of murine Hfe gene (from 0.021–0.008 at embryonic day 12.5 to 0.171–0.059 at day 17.5). As already reported, dramatic changes were observed in HAMP mRNA content, which increased from 0.0005–0.0007 to 6.04–6.75 between embryonic day 12.5 and 17.5.

Conclusions:

A growing body of evidence indicates that hemojuvelin plays a role in iron homeostasis, however, the exact function of this protein is at present not known. It has been proposed that hemojuvelin could act as a component of the hepcidin-activating pathway, since hepcidin levels are low in patients with hemojuvelin deficiency. Our results demonstrate that hepatic HJV mRNA levels are not changed by manipulation of iron stores or erythropoietin administration, while hepcidin mRNA levels are. In absolute terms, the amount of hepatic HAMP mRNA is much higher than the amount of HJV mRNA. During fetal liver development, the content of both HAMP and HJV mRNA gradually increases, with HAMP mRNA levels always exceeding HJV mRNA levels. Overall, these results appear to support the concept that hemojuvelin could be a component of the hepcidin-activating cascade.

IMATINIB IN CML & MPD

295 IMATINIB MODULATES ITS OWN UPTAKE AND EFFLUX EARLY IN THE TREATMENT OF CHRONIC MYELOID LEUKAEMIA, VIA UPREGULATION OF THE TRANSPORTERS HOCT1 AND MDR1

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Several studies have established that mutations in BCR-ABL are important in conferring resistance to imatinib in chronic myeloid leukaemia (CML). It is likely that imatinib may select for the emergence of pre-existing infrequent mutations, but nothing is known of the mechanisms whereby this may occur. It is possible that suboptimal intracellular concentrations of imatinib may contribute to the emergence of BCR-ABL mutations. We have been interested in the role of drug transporters, since it is clear that altered expression confer resistance to anti-epileptic drugs and in cancer therapy.

To investigate drug transporters of imatinib, the uptake of ¹⁴C-labelled imatinib was measured in the leukaemic cell line CCRF-CEM, and in its sub-line VBL100, which has high expression of MDR1. Uptake was found to be temperature dependent, demonstrating that imatinib uptake is an active process. A panel of broad and specific inhibitors of various organic cationic transporters (hOCT) established that hOCT1 but not other hOCT was responsible for imatinib uptake. Studies on transepithelial drug transport in an MDR1 transfected MDCK cell model also showed that imatinib was actively excreted, and that the specific MDR1 inhibitor PSC-833 increased the intracellular level of imatinib. We then studied the expression of hOCT1 and MDR1 in 6 CML cases (4 in first chronic phase; 2 in accelerated phase). Expression of hOCT1 and MDR1 mRNAs was measured using real-time quantitative RT-PCR. GAPDH was used as internal control gene, and expression of hOCT1 and MDR1 was normalised to expression levels in VBL100 cells. Peripheral blood leukocytes were collected at the start and after 4 weeks, 3 months and 6 months of imatinib treatment. For all 6 patients, hOCT1 expression increased by up to 40 fold after 4 weeks and/or 3 months of treatment, compared with pre-treatment levels. Similarly, MDR1 expression was also increased in all 6 patients by up to 32 fold after 4 weeks and/or 3 months treatment.

The results indicate that 1) imatinib uptake is actively mediated by hOCT1, and that efflux is mediated by MDR1; 2) that both hOCT1 and MDR1 are expressed in primary CML cells, and that 3) both hOCT1 and MDR1 expression are dramatically up regulated in blood leucocytes, within a few weeks of commencing imatinib treatment in CML. The relative expression of influx (hOCT1) and efflux (MDR1) transporters may be a critical determinant of intracellular drug levels, which is likely to be important in the emergence of BCR-ABL mutations.

296 ABL MUTATIONS DETECTED BY A NOVEL D-HPLC-BASED ASSAY IN LATE-CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS RESISTANT TO IMATINIB ARE STRONGLY ASSOCIATED TO SUBSEQUENT PROGRESSION TO BLAST CRISIS

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Background: despite strong efficacy of the BCR-ABL kinase inhibitor Imatinib mesylate for treatment of chronic myeloid leukemia (CML), resistance has been observed in a proportion of patients, especially those in more advanced-stage of disease. Mutations within ABL kinase domain seem to be the most frequent mechanism for reactivation of kinase activity within the leukemic clone. Regular monitoring for emerging mutations should enter the clinical practice in order to assure a rational therapeutic management of CML patients.

Aims: aims of our study were: a) to set up and evaluate a novel, high-throughput method for screening and detection of Imatinib resistance-associated mutations based on denaturing-high-performance liquid chromatography (D-HPLC); b) to investigate the frequency, distribution and prognostic relevance of ABL mutations in 47 Imatinib-resistant CML patients enrolled in two multicenter trials of the Italian Cooperative Study Group on CML.

Methods: this study was performed on 86 bone marrow and/or peripheral blood samples from 47 patients showing upfront cytogenetic resistance to Imatinib. Seven patients were enrolled in the CML011/STI571 trial (newly diagnosed, early-CP CML patients treated with Imatinib 400 mg/d and pegylated-interferon) while the remaining 40 were enrolled in the CML002/STI571 trial (late-CP CML patients resistant/refractory to alpha-interferon, treated with Imatinib 400 mg/d). Mutational analysis was done: a) at the twelfth month of therapy for those patients who, though never reaching a major cytogenetic response, completed the first year of therapy ($n=36$); b) immediately before Imatinib discontinuation for those patients who prematurely exited the protocol for progression ($n=8$) or adverse events ($n=3$). A nested RT-PCR was set up and, for each sample, two partially overlapping fragments of 393 and 482 bp corresponding to ABL kinase domain were analysed in parallel by D-HPLC and sequencing.

Results: in 22/47 (47%) patients, D-HPLC showed an abnormal elution profile suggesting a nucleotide change. Direct sequencing confirmed the presence of a mutation in all but three cases, where mutations were revealed only after cloning. Conversely, all the samples scored as wild-type by D-HPLC did not show evidence of mutations by sequencing. Two out of 7 (29%) resistant patients enrolled in the CML011 trial had mutations, thus confirming that the latter are rare in early-CP patients. Among resistant patients enrolled in the CML002 trial, 19/40 (48%) had mutations, two of whom (F311I, E355D) previously unreported. P-loop mutations were detected in 11 patients, of whom 8 subsequently progressed to blast crisis (BC) and 6 died of their disease. By contrast, among the 8 patients without P-loop mutations, 3 progressed to BC and 1 died. In the remaining 21 patients with no mutations, only one progressed to BC after 22 months from Imatinib onset and none died.

Conclusions: in late-CP patients, presence of a point mutation was strongly associated with subsequent progression to BC (Fisher's exact test: $p=0.003$); P-loop mutations seemed to confer a particularly poor outcome. D-HPLC proved to be a rapid, reliable and sensitive tool for large-scale mutational screening of patients undergoing Imatinib treatment. Study supported by COFIN2003 (Baccarani), AIRC, AIL, Fondazione Del Monte di Bologna e Ravenna.

297 INVOLVEMENT OF T-CELL PROTEIN TYROSINE PHOSPHATASE (TC-PTP) IN THE RESISTANCE TO STI571 THROUGH DOWN-STREAM SIGNALS OF BCR-ABL IN CML CELLS

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Background and Aims: The acquired resistance to STI571 in chronic myelogenous leukemia (CML) patients has become a serious clinical problem. We established previously STI571-resistant sublines

(designated KTR cells) from CML cell line KT-1. Interestingly, T-cell protein tyrosine phosphatase (TC-PTP) was markedly down-regulated in all KTR cells compared to parental KT-1 cells. We have demonstrated that KTR cells were more sensitive to interferon- α via prolonged phosphorylation of STAT1 by the loss of TC-PTP (EHA8 No. 0847). To clarify the mechanisms for the STI571-resistance in KTR cells, we have analyzed p-glycoprotein expression, the number of bcr-abl fusion gene and the sequence of ATP binding site of ABL kinase domain in the previous investigation. However, these were not responsible for STI571 resistance in KTR cells. Therefore, we examined whether the suppression of TC-PTP expression might contribute to STI571-resistance in KTR cells. Methods: We transduced the nuclear isoform of TC-PTP (TC45) and catalytically inactive TC45-D182A cDNA into KTR cells by retroviral gene transfer. Subsequently, we analyzed the sensitivity to STI571 by MTT proliferation assays. We also studied the signaling pathways in all transduced cells by Western blottings. Results: The expression level of transduced TC-PTP was assessed by Western blotting and KTR cells successfully expressed TC45 and TC45-D182A protein (designated KTR-TC45 and KTR-D182A cells, respectively). In MTT proliferation assays, proliferation of KTR-TC45 cells was suppressed by 37.0%, 74.2% and 89.3% with 0.2, 0.5 and 1 microM STI571, respectively, which seems to be similar suppression levels in parental KT-1 cells. In contrast, proliferation of KTR-mock cells was suppressed by 5.8%, 39.9% and 75.6% with 0.2, 0.5 and 1 microM STI571, respectively. Proliferation of KTR-D182A cells was almost similar to that of KTR-mock cells, indicating that transduced TC45 restored the sensitivity to STI571 in KTR cells. These results suggested that sensitivity to STI571 was modulated by the quantity of TC-PTP expressed. In parental KT-1 cells, phosphorylation of STAT5 was abolished with the treatment of 0.5 microM STI571 for 1 hour. In contrast, STAT5 phosphorylation in KTR cells was stronger than that of KT-1 cells and only slightly suppressed upon exposure to 0.5 microM STI571. In KTR-mock and KTR-D182A transduced cells, STAT5 phosphorylation was augmented compared to KTR-TC45 transduced cells. Upon treatment with 0.5 microM STI571 for 1 hour, phosphorylation of STAT5 was abolished in KTR2-TC45 cells whereas it remained elevated in KTR-mock and KTR-D182A cells. The expression of TC-PTP had no effect on the phosphorylation of the JAK2 or BCR-ABL in KTR cells. These results indicate that the loss of TC-PTP could enhance tyrosine phosphorylation of STAT5 and was involved in the acquired resistance to STI571 in KTR cells. Conclusion: We demonstrated that forced expression of TC-PTP in STI571-resistant KTR cells restored the sensitivity to STI571. Although it will be necessary to ascertain the relevance of our studies in vivo, we would like to propose that the loss of TC-PTP may represent a novel mechanism by which CML cells can acquire STI571-resistance.

298 MOLECULAR MONITORING OF CHRONIC PHASE CML PATIENTS ENTERED IN THE INTERNATIONAL PROSPECTIVE STUDY (IRIS) COMPARING IMATINIB (IM) AND INTERFERON/CYTARABINE (IFN/ARA-C)

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Background: Imatinib mesylate (IM) has proved highly effective in the management of patients with CML in chronic phase (CP) in the short term.

Aims: The IRIS study was designed to establish whether IM prolongs the life of newly diagnosed CP patients in comparison with standard therapy. The level of residual disease was monitored at different time points by assaying BCR-ABL transcripts.

Methods and Results: 1106 patients with CML-CP from 177 centres in 16 countries were randomly allocated to receive either IM (400 mg/day) or the combination of IFN/Ara-C during the period June 2000 to Jan 2001. After 6, 12 and 24 months of follow-up the incidence of complete cytogenetic remission (CCyR) for patients who received IM as primary therapy was 50%, 68% and 76% respectively. Of the initial 553 patients randomised to IFN/Ara-C, 341 (62%) crossed over to IM after varying periods of their initial treatment. After 6 and 12 months of IM as secondary treatment the incidence of CCyR was 39% and 51% respectively. All patients who achieved CCyR were monitored serially by RT-PCR for BCR-ABL transcripts in peripheral blood. A standardised

protocol coordinated between 3 different collaborating laboratories used BCR transcripts as an internal control; results for individual patients were compared to a mean value for untreated patients and expressed as a ratio of BCR-ABL/BCR transcripts on a log scale. For the 333 evaluable patients who achieved CCyR on IM as first line treatment the median log reduction at 6, 12 and 18 months after start of treatment was 2.8, 3.2 and 3.8 respectively. 72% of these patients had achieved ≥ 3 log reduction at 24 months, which represents 55% of the total patient cohort. When the IM-treated patients were classified by degree of transcript reduction after 12 months of IM, the estimated progression-free survival at 30 months from start of treatment for those who had achieved (1) no CCyR, (2) CCyR but <3 log reduction, and (3) ≥ 3 log reduction was 81%, 93% and 100% respectively. For patients receiving IM as secondary therapy who achieved CCyR the median log reduction in transcript numbers at 6 and 12 months after start of IM treatment was 2.9 and 3.1 respectively.

Summary: More than half of patients who received IM for 24 months as primary treatment achieved ≥ 3 log reduction and this predicts for both long duration of CCyR and high progression-free survival. Patients who crossed over to IM as second-line therapy had a lower overall incidence of CCyR at equivalent time points but for those who achieving CCyR the degree of reduction in BCR-ABL transcripts was similar to that of patients receiving IM as primary therapy.

299 A RISE IN BCR-ABL TRANSCRIPT LEVEL OF MORE THAN 2-FOLD IN IMATINIB-TREATED CHRONIC MYELOID LEUKEMIA IDENTIFIES PATIENTS WITH BCR-ABL KINASE DOMAIN MUTATIONS

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Background

The selective inhibitor of the BCR-ABL tyrosine kinase, imatinib mesylate has demonstrated significant therapeutic benefit for patients with CML. However, resistance to imatinib can occur in all phases of the disease. Mutation within the BCR-ABL kinase domain is now recognized as the main mechanism of acquired resistance and monitoring patients for mutations provides an important guide for clinical management and may have prognostic relevance. It is currently unclear how frequently patients should be monitored for mutations or when the frequency of monitoring should be increased.

Aim

We sought to address the question of whether molecular monitoring of BCR-ABL transcript levels can guide the frequency and timing of sequencing analysis for the early recognition of BCR-ABL mutations. We performed a retrospective examination of serial BCR-ABL measurements, mutation analysis and clinical outcome in 214 imatinib-treated patients to evaluate the hypothesis that a rise in BCR-ABL level can be used as a trigger to screen for mutations.

Methods

Of the 214 patients, 5 were in blast crisis at the start of imatinib therapy, 27 in accelerated phase, 48 in late-chronic phase and 134 in early-chronic phase (defined as less than 12 months since diagnosis). Quantitative PCR of BCR-ABL mRNA was performed using real-time TaqMan technology. Patients were tested at 1 to 6 month intervals for up to 39 months (median 15 months of imatinib therapy). A total of 1,529 samples were analyzed (median 5 per patient, range 2 to 26). The patients were also screened for mutations at least every 6 months by PCR isolation of the BCR-ABL allele and direct sequencing. Mutation analysis was performed on 797 samples.

Results

The 214 patients were grouped according to whether a rise of greater than 2-fold in BCR-ABL occurred in consecutive samples at any time during ongoing imatinib therapy. BCR-ABL mutations were detected in 32 of 54 patients (59%) with a rise in BCR-ABL of greater than 2-fold (median rise 3.0-fold, range 2.1 to 380). This compared to 1 of 160 patients (0.6%) with stable or decreasing BCR-ABL, $P < 0.0001$. In 29 of 32 patients (91%) with mutations and a rise of greater than 2-fold in BCR-ABL, the mutation was detectable at the time of the rise. In the remaining 3 patients, the mutation was detected by 3 months after the rise. The magnitude of the rise was not a better discriminator of mutations since there was no significant difference in the incidence of mutations in patients with a rise between 2 and 3-fold and those with a greater than 3-fold rise. Patients with a greater than 2-fold rise also had a higher incidence of progression to accelerated phase or blast crisis, 22 of 54 patients (41%) compared to 2 of 160 patients (1.3%) with stable or decreasing BCR-ABL, $P < 0.0001$.

Summary

A rise in BCR-ABL of more than 2-fold indicates a high probability that mutations within the BCR-ABL kinase domain will be detected and can be used as a primary screening strategy.

300 PHASE-II STUDY OF HIGH-DOSE IMATINIB IN PATIENTS WITH CHRONIC IDIOPATHIC MYELOFIBROSIS; MODEST ACTIVITY, BUT ESTABLISHMENT OF THE UTILITY OF F-18 FLUOROTHYMININE PET SCANNING IN DISEASE ASSESSMENT

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Background: Chronic idiopathic myelofibrosis (CIMF) comprises a proliferative cellular component, and reactive fibrotic stroma. There are no specific effective therapies. Imatinib mesylate (Glivec), an inhibitor of BCR/ABL, also inhibits c-KIT, c-ARG, c-ABL, PDGFR- α and - β , although at higher concentrations. The c-KIT and PDGF actions may be useful in CIMF as PDGF may have a role in the development of fibrosis. Prior studies have shown modest activity, but used doses that suboptimally inhibit PDGF. Also, non-invasive tracking of the disease has been impossible and marrow biopsies subject to sampling bias.

Aim: We hypothesised that prolonged treatment with high-dose Imatinib may lead to regression of marrow fibrosis. The novel imaging modality F-18 3-deoxy-3-fluorothymidine (FLT; a thymidine analogue) positron emission tomography (PET) scanning was evaluated for assessment of the metabolic activity and anatomic distribution of proliferative marrow.

Methods: Pts were treated on Imatinib Target Exploration Consortium (ITEC) study 2225. Assessment included baseline and 3 monthly measurement of blood counts, serum LDH, Dupriez score (Blood 1996), severity score (JCO 2004), marrow biopsy and fibrosis grading, measurement of spleen and liver span by Tc-99m colloid and FLT-PET scanning. Treatment began at 800mg/day and was planned to continue indefinitely in the absence of progressive disease or prohibitive toxicity.

Results: From 4/02 - 3/03, 7 pts (1 secondary to PRV) were entered. Median age 48yrs (42-77), 2 had prior chemotherapy, and median time from diagnosis 4.4 months (0.6-146). 5 pts have discontinued therapy (median 8.6 months; range 0.6-10.6) due to reversible toxicity (1 = nausea, 2 = skin rash) or progressive disease (n = 2), and 2 pts continue on full-dose therapy at 15.3 and 22.2 months. Mean delivered daily dose during months 1-3 was 604 mg, 4-6 was 565 mg, 7-9 was 710 mg. 5 pts had a reduction in spleen span (by 1.5-4.9 cm), but there were no significant changes in Dupriez or severity scores at 3-9 months (each $P > 0.3$). Haemoglobin values fell, and platelets rose on treatment (see table).

There has been no definitive regression of fibrosis seen to date, but 5 pts had clear reductions in marrow hypercellularity and 2 had substantial restoration of marrow cellular architecture. One pt died from progressive disease off therapy and the 18-month actuarial survival rate is 86%. There were 28 FLT-PET scans performed. At baseline in all cases these showed marked increased splenic activity with reduced axial marrow uptake and variably expanded appendicular skeletal uptake. At 3 months, the 5 pts with splenic shrinkage showed reduced splenic tracer localisation. Significantly, the 3 pts with reduced marrow cellularity on biopsy displayed relative normalisation of FLT bio-distribution.

Summary: High-dose Imatinib has definite modest activity in CIMF and merits evaluation in combination therapy. FLT-PET scanning accurately

images the activity and anatomic distribution of haematopoietic proliferative cellular mass and is a powerful tool for the imaging and assessment of pts with CIMF.

Parameter	Baseline (n = 7)	3 Mo. (n = 6)	6 Mo. (n = 6)	9 Mo. (n = 4)
Haemoglobin (g/L)	106 (89–156)	103 (89–122)*	107 (89–121)*	109 (101–129)
LDH	1006 (678–2710)	833 (557–3446)	923 (516–2975)	737 (510–908)
Platelets	216 (75–848)	372 (111–1265)*	358 (93–1430)*	437 (157–1380)
WBC	5.0 (3.8–11.3)	6.2 (2.8–10.5)	4.9 (3.8–10.9)	5.4 (2.9–7.8)*
Spleen size (cm)	19 (13–29)	17 (12–25)	17 (10–24)*	14 (10–24)*
Liver size (cm)	16 (13–18)	17 (13–18)	16 (13–18)	15 (15–17)

*: $P < 0.1$ vs baseline for within pt comparisons versus baseline measurement.

Caption 1: Median (range) values on therapy

MULTIPLE MYELOMA: THERAPY

301 ORAL MELPHALAN, PRENDNISONE AND THALIDOMIDE FOR PREVIOUSLY UNTREATED MYELOMA PATIENTS

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Background:

Thalidomide is an useful drug for treatment of myeloma. It represents the first new active agent since the introduction of Melphalan and glucocorticoids more than 30 years ago, inducing 30-70% partial response rate in previously treated patients and 35-95% partial response rate in untreated patients. These differences in response rate depend on use of Thalidomide as single agent or in association with steroids and chemotherapy. No data are available on the association of Thalidomide with standard oral Melphalan and Prednisone (MP) that is still considered the best available standard treatment for elderly myeloma patients.

Aims:

To address this issue, we evaluated the potential additive and synergistic effect of the combination Melphalan, Prednisone and Thalidomide (MPT) in newly diagnosed multiple myeloma patients.

Methods:

Between January 2002 and June 2003, 49 consecutive patients, (aged > 65 or younger who refused transplantation for personal reasons) with newly diagnosed symptomatic myeloma were enrolled in randomized trial GISMM2001 comparing MPT vs MP. After the first 100 patients completed six months of therapy, an interim analysis was planned. The data reported here are related to the MPT arm. Melphalan was administered at 4 mg/m², Prednisone at 40 mg/m² both for 7 days every month for a total of 6 courses. Thalidomide was started and maintained at daily of dose 100 mg continuously until relapse or any Common Toxicity Criteria grade III toxicity.

Results:

According to the EBMT/IBMT criteria, 24.5% of patients achieved immunofixation negative complete remission (CR), 14.3% immunofixation positive near complete remission (nCR), 6.1% very good partial response (VGPR), 34.7% partial response (PR), with myeloma protein reduction 50-89%, 8.2% no response (NR) and 2% progressive disease (PD). The median time to maximum response was 4 months. Five patients relapsed after a median follow-up of 13 months.

The major acute adverse events (Common Toxicity Criteria grade III-IV) included: thrombosis (20.5%), infections (12.2%), constipation (6.1%), hematologic (22.6%) and neurologic (8.2%) toxicities. Hospitalisation for intravenous antibiotic therapy was required in 5 patients. One patient died of pulmonary thromboembolism and 1 of pneumonia.

Summary / conclusion:

These data suggest that MPT induces a fast tumor response with a CR rate similar to those observed after transplant. Prophylactic anticoagulation is needed to prevent thromboembolism. Its effect on remission duration and survival remains to be elucidated.

302 THERAPY OF MULTIPLE MYELOMA PATIENTS WITH DISEASE PROGRESSION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION, IN THE ERA OF THALIDOMIDE AND REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION

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Autologous stem cell transplant (autoSCT) has become the gold standard therapy for patients (pts) with multiple myeloma (MM). However this is not a curative approach and the prognosis for pts with post autoSCT progression is poor. Thalidomide (Thal) and reduced intensity allogeneic SCT (RIC SCT), are novel approaches in this setting.

Methods: We applied the following treatment strategy for post autoSCT relapse: 1. Pts with aggressive relapse were given Thal Dexamethasone (Dexa), followed, at response, by RIC SCT with the combination of Mel/Flu/ATG or a 2nd autoSCT, depending on eligibility and donor availability. DTPACE combination or intermediate dose Melphalan was added to Pts that failed to respond to Thal prior to SCT.

2. Pts with smoldering post autoSCT progression were followed with a watch and wait policy. Thal Dexa was administered upon a clinical indication. A second SCT procedure was offered in this group at escape from response (for pts with = / >PR) to Thal, or following DTPACE/intermediate dose Melphalan for no responders to Thal.

Results: Seventy-five pts (median age 55 y) have been treated with Thal according to this strategy. Twenty eight pts subsequently underwent RIC SCT (25 related, 3 unrelated donors), 10 pts had a subsequent 2nd autoSCT and 37 pts continued Thal therapy. The overall response rate (= or >PR) to Thal Dexa was 53%. Treatment related mortality was 8% (6 pts, all after RIC SCT, giving transplant related mortality rate of 21% for RIC SCT in this setting). With a follow up of 3-62 months from progression (median 37 m) the overall survival rate is 58% and the progression free survival (PFS) rate is 50%, for the entire group. In 19 patients (25%), the duration of response exceeded the first progression free period (from first autoSCT until progression). In a multivariate analysis, the duration of first progression free period, the response to Thal and low risk score (b2MG, albumin level and performance status) at relapse were associated with improved prognosis.

Conclusion. The introduction of thalidomide and reduced intensity SCT in MM patients seems to significantly improve the ability to salvage pts relapsing after autoSCT. This will probably affect the overall prognosis of MM patients in the era of these novel methods. The precise timing and scheme of therapy that should be followed, as well as the role of experimental therapies such as proteasome inhibitors and new iMids in this setting, should further be explored.

303 MULTIPLE MYELOMA: POPULATION BASED RELATIVE SURVIVAL IN SWEDEN 1973-2001

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Background:

Over the last decades there have been great advances in the treatment of patients with multiple myeloma (MM). Overall, prognosis has improved with newer treatment strategies, however few studies have addressed which patients benefit most from the changes over the years.

Aims:

To evaluate survival in all diagnosed MM patients in Sweden in relation to the Swedish population over a 28-year period of time.

Methods:

All patients with MM notified to the Swedish Cancer Register in 1973-2001 were followed up by record linkage to the nationwide Cause of Death Register. Survival analyses were performed by obtaining relative survival (RS) defined as the ratio of observed versus expected survival. The study period was divided arbitrarily to four calendar periods: 1973-1979, 1980-1986, 1987-1993, and 1994-2002. Patients were grouped according to age (0-40, 41-50, 51-60, 61-70, 71-80, and 80+) sex, and hospital category. RS was estimated using SAS (Cary, NC, USA) and modelled using Poisson regression.

Results:

A total of 13,376 patients (7,114 males and 6,262 females, mean age 69.8) were diagnosed with MM in Sweden between January 1st 1973 and December 31st 2001. The overall one-year RS estimates were 73%, 78%, 80%, and 81%, respectively, for the four calendar periods. The overall five-year RS was 31%, 32%, 34%, and 36% and the ten-year RS was stable at 12%, 11% 13% in the first three periods whilst ten-year follow-up could not be obtained for the last calendar period.

Increase in one-year RS was observed over the four calendar periods in all age categories, whilst only increase in five-year RS was observed in patients up to 70 years. No increase was recorded with regard to ten-year RS. Younger age at onset was associated with an improved prognosis in all calendar periods. Differences in survival by age at diagnosis and calendar period were highly statistically significant ($p < 0.0001$).

After adjusting for age, sex, and period, there was evidence of a difference in survival according to hospital category, longest survival being in the university hospitals ($p = 0.007$).

Summary/conclusions:

The present series shows an improved prognosis in over 13,000 MM patients over almost 30 years of follow-up in a population-based study. Of interest is that even one-year RS has improved in all age groups over the whole study period. Increase in five-year RS was only recorded in patients <70 years. The ten-year RS did not improve over the first 20 years in our study but ten-year RS estimates for the latest period could not be obtained. The present study shows that there is a significant difference in survival according to type of hospital, with patients diagnosed at a university hospital surviving longer. Whether this is due to differences in diagnostic/treatment procedures, supportive care or referral-bias remains to be explored.

304 SURVIVAL, DURATION OF RESPONSE, AND TIME TO PROGRESSION WITH BORTEZOMIB IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA: AN UPDATE WITH ADDITIONAL FOLLOW-UP

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Background: The proteasome inhibitor bortezomib (VELCADE®) has been approved for the treatment of patients with relapsed and refractory multiple myeloma (MM) who have received 2 prior therapies and are progressing on their last therapy. A major phase 2 trial supporting this approval was SUMMIT, which examined the efficacy and toxicity of bortezomib in patients (N=202) with relapsed and refractory MM. Responses were determined using modified EBMT criteria, with the addition of a near CR (positive M-protein by immunofixation but negative

electrophoresis). The overall response rate (CR+PR+MR) was 35% after up to 8 cycles of therapy. Median values for the overall survival (OS), duration of response (DOR), and time to disease progression (TTP) at the time of the report were 16, 12, and 7 months, respectively. Patients with PD or SD were permitted dexamethasone (20 mg on the day of and day after each bortezomib dose) after 2 or 4 cycles of treatment, respectively. An extension trial was designed to allow continuation of or retreatment with bortezomib for patients who achieved a response in the original trial (objective response or other tangible benefit) and who, in the investigators opinion, might benefit from additional therapy.

Aim: To determine the OS, DOR, and TTP following additional bortezomib therapy or with long-term follow-up in the SUMMIT population.

Methods: Patients received bortezomib 1.3 mg/m² 2x/week x2 q3 weeks for up to 8 cycles in SUMMIT. The dosing regimen for the patients who entered the extension trial was based on patient dose, response, and tolerability in SUMMIT. The time-to-event analyses (OS, DOR, TTP) were performed using combined data from patients receiving treatment in the extension trial (N=46) and follow-up of patients not receiving extended therapy (N=156) (cut-off date 17 November 2003). The time-to-event parameters in the subpopulation of patients who achieved a CR or PR while on the SUMMIT protocol with bortezomib alone (n=53) were also examined.

Results: The median duration of follow-up was 19.1 months. Overall median survival for all treated patients was 17.2 months. Median survival for non-responders (n=87) was 8 months. The median DOR for patients who achieved a CR or PR with bortezomib alone was 14.1 months. The median TTP for all patients treated with bortezomib alone was 7 months. For the subset of patients who achieved a CR or PR, the TTP with bortezomib alone was 15.3 months.

Conclusion: These data suggest that response to bortezomib in the setting of relapsed and refractory multiple myeloma may result in meaningful long-term survival benefits.

305 BORTEZOMIB VERSUS DEXAMETHASONE FOR THE TREATMENT OF PATIENTS WITH RELAPSED MULTIPLE MYELOMA: A RANDOMIZED PHASE 3 TRIAL. THE APEX STUDY GROUP.

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Background: Bortezomib (VELCADE®) is a novel, selective, and reversible proteasome inhibitor that is effective for the treatment of refractory multiple myeloma.

Aim: The objective of this international randomized phase 3 study was to evaluate the efficacy and safety of bortezomib compared with dexamethasone in patients with relapsed multiple myeloma.

Methods: A total of 669 patients who had relapsed following previous treatment with 1 to 3 prior therapies were randomly assigned to receive either bortezomib 1.3 mg/m² by intravenous bolus administration on days 1, 4, 8, and 11 every 3 weeks for 8 cycles followed thereafter by 1.3 mg/m² on days 1, 8, 15, and 22 every 5 weeks for 3 cycles or oral dexamethasone 40 mg on days 1, 4, 9, 12, and 17, 20 every 5 weeks for 4 cycles followed thereafter by 40 mg on days 1, 4 every 28 days for 3 cycles. 657 patients were included in the interim analysis of efficacy, 327 assigned to bortezomib and 330 assigned to dexamethasone. Responses were assessed using EBMT criteria. Time to progression (TTP) was the primary endpoint. Overall survival, time to skeletal events (TSE), and incidence of infection grade 3 or higher were secondary endpoints. In a companion study, patients who progressed on dexamethasone were eligible to receive bortezomib. The study was conducted at 95 sites in 12 countries, which included 9 European countries, the United States, Canada, and Israel.

Results: No apparent differences in baseline characteristics were observed between the 2 study arms. The median age was 62 years. At the time of the interim analysis, 254 patients had experienced progressive

disease. A highly significant benefit in TTP was observed among patients receiving bortezomib. Median TTP was 5.7 months (95% CI: 5.0, 7.9) among patients in the bortezomib arm compared with 3.6 months (95% CI: 3.2, 4.8) among patients in the dexamethasone arm (P < 0.0001). Bortezomib also provided survival benefits: there were 13 deaths among patients randomized to bortezomib versus 24 deaths among patients randomized to dexamethasone (P = 0.038). Median survival had not been reached in either arm at the time of the analysis. There was no difference in TSE between the 2 treatment groups (P = 0.954). The incidence of infections graded ≥ 3 was 22 (6.7%) with bortezomib versus 35 (10.6%) with dexamethasone (P = 0.096), a trend that favored bortezomib. No other major safety differences were observed. Following the interim analysis, the Data Monitoring Committee recommended that the study be halted and that patients randomized to dexamethasone be allowed to receive bortezomib.

Conclusions: Treatment with bortezomib was significantly more effective than treatment with dexamethasone for patients with relapsed multiple myeloma at the interim analysis. Studies are ongoing to evaluate potential differences between the 2 groups in other parameters, including response rate, response duration, survival, and safety.

306 THE USE OF BORTEZOMIB (VELCADE) IN PATIENTS WITH PREVIOUSLY UNTREATED MULTIPLE MYELOMA

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Background: The proteasome inhibitor bortezomib has produced clinically meaningful response rates with manageable toxicities in patients with relapsed and/or refractory disease in the SUMMIT and CREST trials. Although there are several first-line therapeutic options and overall survival is improving, MM remains incurable and more effective treatments are needed.

Aim: The current study examines the efficacy and toxicity of bortezomib in previously untreated patients with MM.

Methods: Patients aged 18-66 years with measurable, newly diagnosed MM, no overt cardiac or pulmonary decompensation, creatinine clearance > 20 mL/min and an ECOG performance status ≤ 2 were eligible. Bortezomib 1.3 mg/m² was administered 2x/week x2 q 3 weeks for a maximum of 6 cycles. Dexamethasone 40 mg on the day of and day after each bortezomib dose was permitted after 2 cycles for patients who achieved < PR, and after 4 cycles for patients who achieved < CR. Responses were defined by modified EBMT criteria, with the addition of a near CR classification (100% disappearance of M protein by electrophoresis but positive immunofixation with normal bone marrow). Potential candidates for stem cell transplantation will have cells harvested at their physicians discretion.

Results: Nineteen patients have been accrued to this study: 47% male, median age 63 years, median Karnofsky Performance score 90 (50/100). The majority of patients (58%) exhibited IgG disease, whereas 32% presented with IgA and 10% with light chain disease. The majority of patients (55%) exhibited Durie-Salmon Stage III disease. Twelve patients were evaluable for response. By the completion of the second cycle of therapy, 50% of patients achieved a PR with bortezomib alone, 8% exhibited an MR, while the remaining 42% exhibited stable disease. By the completion of the fourth cycle, 25% exhibited near CR and 33% PR after receiving bortezomib alone, and an additional 25% demonstrated PR and 8% MR following combination bortezomib and dexamethasone therapy. Because no patient achieved an immunofixation-negative CR by the completion of cycle 4, all patients received dexamethasone. By the end of cycle 6, 33% showed near CR, 42% PR, 8% MR, and 17% PD. There were 2 discontinuations, 1 due to progression in an existing bone lesion, and 1 due to grade 3 peripheral neuropathy. One patient who received stem cell transplantation has exhibited complete hematologic recovery. The most common toxicities (grade 13) were fatigue (67%), diarrhea (58%), constipation (42%), nausea (42%), peripheral neuropathy (33%) and vomiting (33%). Grade 3 events included abdominal pain, diarrhea, dizziness, dyspnea, fever, neuropathic pain, neutropenia, syncope, and vomiting. No grade 4 toxicity was encountered. Dose modification was required in 1 (8%) patient.

Conclusion: Initial results in 12 patients indicate a response rate of 75% (33% near CR + 42% PR) by modified EBMT criteria. This suggests that

bortezomib in combination with dexamethasone has encouraging activity in newly diagnosed MM with manageable toxicities, although peripheral neuropathy was observed. Stem cell harvesting is feasible following bortezomib. Recruitment is ongoing, and the projected 42 patients should be accrued quickly.

THROMBOSIS & HEMOTASIS II

307 A NEW FAMILY WITH PREKALLIKREIN (PK) DEFICIENCY CAUSED BY TWO MISSENSE MUTATIONS (G104R,N124S) IN THE APPLE DOMAIN 2 OF PK HEAVY CHAIN (PK SEKI).
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Background: Hereditary prekallikrein (PK) deficiency is a rare clotting disorder and only about thirty families have been reported. Although genetic studies of PK deficiency are so scanty, some abnormalities in the light chain have been reported. The light chain of the molecule is associated with catalytic domain of PK whereas the heavy chain is composed of four apple domains which thought to be the binding sites for high molecular weight kininogen (HMWK). In this report we describe a new family of PK deficiency caused by abnormality of PK heavy chain.

Aims: The proband is a 60-year old man who came to our hospital for a complete coagulation evaluation because pre-operation screening revealed a severely prolonged APTT (135sec, control: 35.0-44.6sec). He had no history of bleeding diathesis and the family history was also negative. To observe the hereditary background, the complete clotting studies and molecular biological studies were performed in his family members and relatives. His parents were known to be distant relatives came from small village in Seki City, Japan.

Methods: The APTT and PK clotting activity (PK:C, control: 70-150%) assays using PK-deficient plasma were carried out. PK amidolytic activity (PK:AM, control: 73-127%) was assayed photometrically. SDS-PAGE was performed to detect plasma PK antigen (PK:Ag) level. Genomic DNA was extracted from peripheral blood leukocytes of the patients for genetic analysis. Each of the exons (1 to 15) of PK gene were amplified using PCR method and cloned into pBSII vector. The nucleotide sequences of the PCR products were analyzed by dye-primer method.

Results: The proband and two sisters had severely prolonged APTT (125-135sec), very low PK activities by both PK:C (0.9-3.0%) and PK:AM (5-6%). His mother and two daughters had normal APTT (37.2-41.5sec) and normal PK:C activities (74-100%) but moderately low PK:AM activities (18-47%). Plasma PK:Ag level was normal in the proband and also in other family members and relatives. The sequence analysis of PK gene revealed the point mutation at G104R and N124S in Exon 5. The proband and his sisters were homozygous for this mutation, whereas his mother and daughters were heterozygous.

Summary: The majority of patients with PK defects described to present are of true deficiency caused by nonsense mutation in PK gene. No patients with a normal antigen level, but low activity have been described to date. New PK deficiency named PK Seki, showed normal antigen level of non-functional PK. The apple domain (A1 to A4) which is unique structure of PK bears the most important role among PK activities. Renne et. al (J Biol. Chem., 274, 25777, 1999) reported the apple domain 2 (A2) of PK is especially important for binding to HMWK to make a heterodimer complex. PK Seki has genetic abnormalities in A2 domain of PK heavy chain. This is the first family with PK deficiency caused by abnormality of A2 domain. The finding of the mutations is important to investigate for understanding the structure-function relationship between apple domain of PK and HMWK.

308 ITP AND HELICOBACTER PYLORI NO INCREASED INFECTION PREVALENCE AND POOR PLATELET RESPONSE TO ERADICATION THERAPY
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Background. An association between *Helicobacter pylori* (HP) infection and immune thrombocytopenic purpura (ITP) has been suggested by several groups. **Aims.** We investigated the prevalence of HP infection in a large cohort of ITP patients and analysed the effect of HP eradication on platelet response in HP-positive individuals. **Methods and results.** A total of 76 ITP patients (mean age 57 yrs, range 19-86 yrs) were

screened for HP infection using 14C-urea breath test (Heliprobe, Noster System, Stockholm). Infection was diagnosed in 12 (15%) patients and successfully eradicated in 11(91%) patients using proton pump inhibitor and two antibiotics (one patient refused treatment). Comparing HP-positive and -negative patients, there were no significant differences regarding age, gender, gastrointestinal (GI) symptoms and history of ulcer or GI bleeding. Platelet counts were assessed at 3 and 6 months after eradication. Improvement of platelet counts, lasting for at least 6 months, were seen in two of 11 treated patients (platelet count increased from 60 to 97 x10⁹ /L and from 50 to 79 x10⁹/L). No major responses, with clinically evident reduction of bleeding symptoms, were observed. **In conclusion.** The prevalence of HP infection in our ITP cohort was lower than that of the general population in Sweden, being 31%; eradication of HP was not associated with any major improvements in platelet count or bleeding symptoms in HP-positive patients. Thus, unlike several previous reports our study does not support a role for HP in the pathogenesis of ITP.

309 THROMBOPROPHYLAXIS WITH FONDAPARINUX IN ACUTELY ILL MEDICAL PATIENTS AGED 75 YEARS OR MORE
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Purpose: In the ARTEMIS trial in acutely ill medical patients, fondaparinux, a selective factor Xa inhibitor, significantly reduced the risk of venous thromboembolism (VTE) (risk reduction=46.7%, p=0.029) and the incidence of fatal pulmonary embolism (PE) without increasing the risk of major bleeding compared with placebo. Since age may influence the benefit-to-risk ratio of thromboprophylactic drugs, we analyzed the data from the subpopulation of patients aged 75 years or more.

Material and Methods: ARTEMIS was a randomized, double-blind trial comparing once-daily 2.5 mg subcutaneous fondaparinux with placebo in 849 medical patients aged 60 years or more hospitalized for acute cardiac, respiratory, infectious, or inflammatory disease; and expected to remain bedridden for 4 days or more. Treatment was continued for 6-14 days. The primary efficacy outcome was confirmed VTE up to day 15. The main safety outcome was the incidence of major bleeding up to 2 calendar days after the last injection.

Results: Among randomized and treated patients, 435 (51.8%) patients were aged 75 years or more. In both study groups, the incidences of VTE and major bleeding tended to increase with age, but the superiority of fondaparinux over placebo was independent of age. In patients aged 75 years or more evaluable for efficacy, the rates of VTE were 11.3% (18/160) in the placebo group versus 6.2% (10/162) in the fondaparinux group; there were 4 PE (2.5%), all fatal, in the placebo group versus none in the fondaparinux group. Fondaparinux was as safe as placebo, regardless of age: in patients aged 75 years or more, the rates of major bleeding were 0.5% in both study groups.

Conclusions: Compared with placebo, fondaparinux significantly reduced the risk of VTE and PE without increasing the risk of clinically relevant bleeding in acutely ill medical patients aged 75 years or more.

310 HAPTOGLOBIN PHENOTYPES AND THE RISK FOR IDIOPATHIC VENOUS THROMBOEMBOLISM
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Background:

Three major phenotypes of haptoglobin have been described (Hp1-1, Hp1-2 and Hp2-2) according to two distinct alleles in the haptoglobin gene. Hp2-2 is associated with lower haptoglobin plasma levels and shows a minor capacity to form complexes with free haemoglobin. Haptoglobin-bound haemoglobin is rapidly cleared from plasma, while free haemoglobin catalyses the generation of reactive oxygen species, which results in endothelial activation and inflammation. Significant associations between Hp2-2 and the risk for restenosis after peripheral and coronary angioplasty have been found. Since results of numerous in vitro studies suggested that inflammatory processes might play an important role in the development of venous thromboembolism (VTE), the Hp2-2 phenotype might be associated with an increased risk for VTE. At present no data on haptoglobin phenotypes or haptoglobin levels and the risk for VTE have been published.

Aim:

The aim of our study was to investigate haptoglobin phenotypes and plasma levels in patients with idiopathic VTE and healthy controls and to assess the contribution of these parameters to the risk for VTE.

Methods:

Haptoglobin phenotypes were detected by immunoblotting using a rabbit anti-human haptoglobin IgG antibody (Dako A/S, Denmark). Haptoglobin plasma levels were measured immunonephelometrically (Dade Behring®, Germany) on a Behring Nephelometer II. Group1 comprised 128 patients (70 women (w), 58 men (m)) with a history of deep venous thrombosis (DVT), group2 105 (58 w, 47 m) with pulmonary embolism (PE) and group3 122 healthy individuals (60 w, 62 m) without a history of arterial or venous thrombosis. Mean age and standard deviation were 46 (16) years in group1, 48 (16) years in group2 and 43 (14) years in group3.

Results:

The Hp2-2 phenotype was significantly more frequent in patients (42% in group1 + group2) than in controls (30%, $p = 0.037$). Including all subjects those with Hp2-2 ($n = 134$) had significantly lower haptoglobin plasma levels (median (Md) = 89.7 mg/dL) than those with Hp1-2 ($n = 170$, Md = 123.5, $p < 0.001$) or with Hp1-1 ($n = 51$, Md = 142.8, $p < 0.001$), respectively. Interestingly, median haptoglobin plasma levels were higher in patients than controls (group1: Md = 118.6 mg/dl, interquartile ranges (IQR) = 90.9 - 160.7, group2: Md = 125.0, IQR = 87.7 - 152.9, group3: Md = 104.8, IQR = 78.2 - 133.3). Differences among each patient group and controls were statistically significant ($p = 0.008$, $p = 0.013$, respectively).

Summary / Conclusions:

The Hp2-2 phenotype was more frequent in patients than controls and may thus act as a risk factor for VTE. Unexpectedly, not lower, but significantly higher basal Hp levels were observed in patients with VTE compared to controls, which might indicate sub-clinical inflammation in patients with VTE. It might be suggested that not the absolute concentration of haptoglobin is of importance for the risk of VTE but the different molecular size and shapes of the haptoglobin proteins encoded by the three haptoglobin phenotypes.

311 THE ESCHQOL STUDY: PILOT TESTING RESULTS CONCERNING HEALTH CARE NEEDS IN HAEMOPHILIA TREATMENT

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Background: Haemophilia and its treatment may impact on patients' quality of life and can affect the everyday life of their families. Quality of life is considered as an important outcome criterion in clinical trials and can be used to describe the impact of the health condition on the patient as well as the effects of treatment strategies and comprehensive care structures.

Aims: In the ESCHQoL Study (European Study of Clinical, Health Economic and Quality of Life Outcomes in Haemophilia Treatment) - funded by the EU - clinical, quality of life and health economic outcomes are assessed as well as structural characteristics of haemophilia care in 22 European countries.

Methods: In this prospective cohort study 2040 patients with haemophilia A or B from the age of 4 will be consecutively enrolled. The project consists of three phases: 1) planning & pilot testing phase, 2) field study phase, 3) data analysis phase. Each recruited patient is asked to complete a comprehensive questionnaire at baseline and after a 6 months follow-up period, during the 6 months period they are asked to fill in a diary on a daily basis. The questionnaire comprises information on socio-demographic (e.g. age), psychosocial (e.g. quality of life) and health economic (e.g. health care consumption) information. In parallel, clinical information are obtained from the physicians; structural data describing the haemophilia care organisation and other information relevant for the calculation of treatment costs are assessed as well by the physicians.

Results: In the pilot testing conducted in the steering committee countries (Italy, Germany, Romania, Hungary, Sweden and U.K.) and in two centres in France, 70 patients (41 adults, 29 children) were investigated in order to test the feasibility of the questionnaire and to investigate problems/wishes concerning haemophilia treatment. The pilot testing showed that children liked the questionnaire and found the questions easy to answer. Parents and adults perceived the questionnaire as good, but long with

repetitive questions. Most of them had no difficulties to understand the questions and found the questionnaire relevant for haemophilia. Venous access and availability of factor concentrate were the problems mentioned most frequently concerning haemophilia treatment.

Conclusion: The pilot testing revealed that the questionnaire was accepted by the respondents, but had to be shortened for adults and parents. The results indicated already after the pilot testing that countries were different in terms of reported problems of haemophiliacs and their parents and wishes for the haemophilia treatment. The ESCHQoL Study is the first study evaluating quality of life in children and adults with haemophilia all over Europe. The general aim of this project is to identify models of health care of haemophiliacs on the basis of clinical characteristics, their possible costs and impact on quality of life.

311b THE ESCHQOL STUDY: PILOT TESTING RESULTS CONCERNING CLINICAL OUTCOMES IN HAEMOPHILIA CARE IN EUROPE

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Introduction: The treatment of patients with haemophilia requires a substantial amount of economical and human resources and is an example for the socio-economic impact of biotechnologies. Haemophilia care impacts not only on the patients in terms of quality of life but also medical treatment, health care consumption and availability of treatment are important issues. In an EU-funded project the ESCHQoL Study - clinical outcomes will be assessed together with quality of life, health economic aspects as well as structural characteristics of haemophilia care in Europe.

Aims: The objectives of the evaluation of clinical outcomes are 1) to provide knowledge about the clinical status and the prevalent treatment modalities in Europe 2) to compare clinical situations and types of treatment among countries and/or regions of Europe (North, South, East) 3) to compare clinical status among groups of patients with different age groups (children, adults), different treatment (prophylaxis, on demand), site of treatment (at home, at the hospital) and complications (inhibitors). **Methods:** In this retrospective/prospective, prevalence-based study on haemophilia care, quality of life and costs 2040 haemophilic patients will be enrolled consecutively from 1 to 3 centres. Patients with haemophilia A and B from the age of 4 will be recruited in 22 European Countries. Clinical information concerning bleeding history, presence of inhibitors, concomitant diseases, surgery, orthopaedic status, treatment modality, type of product and other drugs, medical visits and hospitalisations will be recorded. These data will be obtained both from physicians and patients/parents at baseline and after a follow-up of 6 months. Structural data describing the haemophilia care organisation and other information relevant for the calculation of treatment costs will be collected as well.

Results: The pilot testing results from 70 patients of seven European countries (Italy, Germany, Romania, Hungary, Sweden, U.K., France) will be presented concerning clinical outcomes: Of the patients 41 adults and 29 children (4-16 years), 90.3 % were haemophilia A, 9.7 % haemophilia B, 82.3 % had severe haemophilia. Only adults had inhibitors (10 %), 41.2 % had HCV infection and 11.8 % had HIV infection (none of the children). Prophylactic treatment was given to 33.4 % of enrolled patients: 57 % of the children and only 10 % of the adults.

Conclusion: The pilot testing already showed differences between countries in Europe. In order to uniform treatment care all over Europe the ESCHQoL Study is going to provide detailed and representative data by combining the assessment of clinical, health economic and quality of life outcomes in haemophiliacs (children, adults). With the final results of the ESCHQoL project will allow informed decision making for optimal care of haemophilia patients all over Europe.

AML: FLT3 & MEGAKARYOBLASTIC LEUKEMIA

312 SCINDERIN EXPRESSION IN MEGAKARYOBLASTIC LEUKEMIA CELLS INDUCES DIFFERENTIATION, RELEASE OF PLATELET-LIKE PARTICLES AND INHIBITS PROLIFERATION AND TUMORIGENESIS

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Megakaryoblastic leukemia cells show presence of gelsolin but absence of scinderin (Sc) expression, two filamentous actin-severing proteins present in normal megakaryocytes (Mk) and platelets (Ptl). To test the possibility that Sc was important for Mk maturation and differentiation, experiments with vector-mediated Sc expression in the megakaryoblastic MEG-01 cell line were performed. Sc expression in MEG-01 cells decreased both F-actin (FA) and gelsolin levels, increased Rac2 and activated the PAK/MEKK/SEK/JNK/c-jun, c-fos transduction pathway which was followed by cell differentiation, polyploidization, maturation and apoptosis with release of Ptl-like particles. These particles expressed surface CD41a antigen (glycoprotein IIb/IIIa or fibrinogen receptor), had dense bodies, high affinity serotonin transport and circular array of microtubules. Thrombin induced serotonin release and aggregation of Ptl-like particles, effects blocked by CD41a antibodies. PAC-1 antibody also blocked aggregation. PD98059, blocker of MEK, inhibited CD41a expression, increases in cell volume and protoplasmic extensions in MEG-01 cells over-expressing Sc. Cell proliferation and the ability to form tumours in nude mice were also inhibited by Sc expression. Cells expressing Sc followed in vivo the same fate that in vitro (apoptosis and release of Ptl-like particles). Sc promoter (Sc-Pro) has recently been characterized in our laboratory. Sc-Pro has several AP2 sites and four Dioxin Responsive Element (DRE) sites that recognize transcription factor Aryl Hydrocarbon Receptor (AhR). Our experiments demonstrate: a) that in the Luciferase-Sc-Pro construct assay, Sc-Pro is stimulated by either ATRA (All-trans retinoic acid) or TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin; a ligand for the AhR), b) the presence of AhR in MEG-01 cells, and c) that stimulation of wild type MEG-01 cells by either ATRA or TCDD increased transcription and expression of Sc followed by polyploidization and maturation. It is concluded that the lack of Sc expression in Mk leukemia cells seems to be responsible for their inability to enter into differentiation and maturation pathways characteristic of their normal counterparts. Supported by Canadian Institutes of Health Research (CIHR) and CIHR-CONICET exchange programme.

313 MUTATIONS OF CEBPA, FLT3 AND RAS IN PATIENTS WITH DE NOVO ADULT LEUKEMIA: A STUDY FROM THE ACUTE LEUKEMIA FRENCH ASSOCIATION (ALFA)

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Background: Characterization of chromosomal alterations in acute myeloid leukaemia (AML) has provided to identify different prognostic subgroups. Better understanding of pathogenesis of AML leads to identification of new molecular markers, but their prognostic relevance remains unclear.

Aims: In the order to evaluate the incidence and prognostic significant of CEBPA mutations, FLT3 internal tandem duplications (FLT3-ITDs), FLT3 Asp835 mutation and N- and K-RAS mutations, we performed a retrospective analysis in AML patients (French-American-British [FAB]-M3 excluded). **Patients and methods:** A total of 224 peripheral blood or bone marrow samples from 224 de novo AML adult patients were retrospectively analyzed. All patients (aged 15-65 years, median age : 42 years, median follow-up 5.1 years) were prospectively enrolled in the Acute Leukemia

French Association (ALFA)-9000 prospective trial. Cytogenetics features according to the British Medical Research Council (MRC) classification were favourable, intermediate or poor in 15% (31/207), 67% (139/207) and 18% (37/207), respectively. Complete Remission (CR) was obtained in 79% (167/212) of patients and median overall survival (OS) was 24 months. CEBPA, N- and K-RAS mutations detections were performed by direct sequencing. **Results:** We found 35 CEBPA mutations in 24/224 patients (11%), mostly belonged to the intermediate cytogenetic risk subgroup (81%), and associated with the FAB-M1 risk subgroup (54%). CR rate was not significantly different between mutated and non-mutated CEBPA patients (83% vs 76%), but OS was improved in CEBPA mutated patients (p=.03). FLT3-ITD and FLT3 Asp835 mutations were observed in 23% (52/224) and 8% (18/224) of patients, respectively. Cytogenetic subgroups were intermediate (91%) and poor (9%) for FLT3-ITD, and favourable (20%) and intermediate (80%) for FLT3 Asp835 mutations. CR was not influenced by FLT3-ITD or Asp835 mutations (p=NS), while OS was significantly worse for FLT3 Asp835 mutated patients who belonged to the favourable prognostic subgroup (p=.03). In our series, FLT3-ITD status do not influence OS, but may be the consequence of intensive protocol of treatment. Frequencies of N- and K-RAS mutations were 10% (22/224) and 2% (4/224), respectively. RAS-mutated patients belonged to favourable (43%), intermediate (43%), and poor (14%) cytogenetic subgroups. CR was not influenced by RAS mutations. In intermediate cytogenetic subgroup, RAS-mutated patients had a shorter OS when they had no FLT3 mutation (p=.01). **Conclusion:** We thus propose a risk classification that includes karyotype and analysis of some molecular markers and which defined 3 different prognostic subgroups: (1) Patients with MRC favourable karyotype and/or CEBPA mutation, and no FLT3 mutation (FLT3-ITD or FLT3 Asp835), (2) Patients with MRC intermediate karyotype, or MRC favourable karyotype with FLT3 mutation, and no RAS mutation, (3) Patients with MRC poor karyotype, or MRC intermediate karyotype with RAS mutation. This classification better identify distinct prognostic subgroups, which guides clinicians in the choice of the treatment.

314 IN VITRO ACTIVITY OF THE FLT3-INHIBITOR SU5614 AND STANDARD CYTOTOXIC AGENTS IN TUMOUR CELLS FROM PATIENTS WITH WILD TYPE AND MUTATED FLT3 ACUTE MYELOID LEUKEMIA

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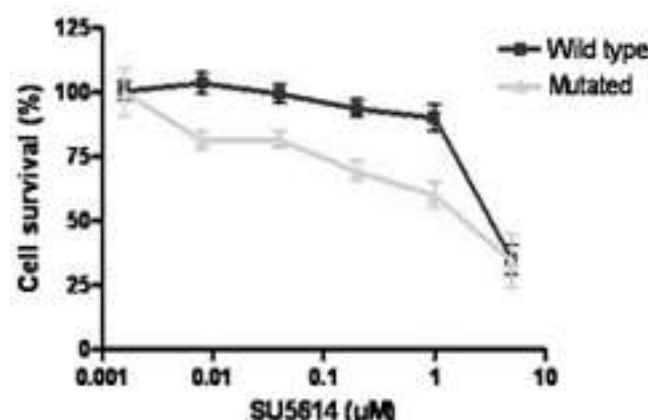
Background: Mutations in the tyrosine kinase receptor gene, FLT3, causing ligand-independent activation of the tyrosine kinase receptor, is the single most common genetic lesion in acute myeloid leukaemia (AML). Two types of FLT3 mutations have been detected: internal tandem duplication (FLT3/ITD) and point mutations in the intracellular tyrosine-kinase domain (FLT3/TKD). In AML, the presence of FLT3/ITD mutation is associated with increased relapse rates and inferior overall survival. Several small-molecule FLT3 tyrosine kinase inhibitors are currently in development and some, including SU5614 (SUGEN), have entered early clinical trials. SU5614 induces growth arrest and apoptosis in AML cell lines expressing constitutively activated FLT3 and inhibit phosphorylation of FLT3 mutation as well as downstream signalling pathways.

Aims: The aim of this study was to evaluate the impact of FLT3 mutation status on in vitro sensitivity of primary AML cells to the FLT3-inhibitor SU5614 and to standard cytotoxic agents. Combinations between SU5614 and standard agents were also investigated.

Methods: Cryopreserved tumour cells isolated from blood or bone marrow from patients with previously untreated AML were investigated. FLT3/ITD was analysed by PCR technique and fragment analysis. FLT3/TKD mutations (codon D835) was analysed by PCR technique and restriction enzyme cleavage. In vitro cell survival after 72h exposure to the FLT3-inhibitor SU5614 and the standard cytotoxic agents amasacrine, cytarabine, etoposide, fludarabine, idarubicin, mitoxantrone and 6-thioguanin was assessed in 96-well microtiterplates using the non-clonogenic in vitro assay, fluorometric microculture cytotoxicity assay (FMCA).

Results: The FLT3 receptor gene mutation status and in vitro drug resistance were successfully analyzed in 17 cases, revealing that 7 cases harbored FLT3/ITD mutations whereas no FLT3/TKD mutation was detected. Tumour cells with FLT3/ITD (n=7) were more sensitive

($p < 0.01$) to the FLT3 inhibitor SU5614 than tumour cells with wild type FLT3 ($n=10$) (see figure below). There was no difference in chemosensitivity to standard cytotoxic agents between the two groups. Combinations of SU5614 with etoposide and amsacrine showed superior effect ($p < 0.05$) compared with the respective single drugs. Conclusions: The FLT3 inhibitor SU5614 may have a therapeutic potential, especially in combination with other cytotoxic agents, in AML patients with ITD in the FLT3 receptor gene. The data also suggest that the poorer prognosis of patients with mutated FLT3 is not due to cellular drug resistance to standard cytotoxic agents.



Caption 1:

315 MOLECULAR THERAPY OF AML: A NEW AND RAPID METHOD TO CHARACTERIZE NOVEL GENETIC VARIANTS IN FLT3 GENE

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FLT3 is the most commonly mutated gene in human acute myeloid leukemia (AML), and has been implicated in its pathogenesis. Since screening of FLT3 in AML patients by PCR followed by gel electrophoresis is time-consuming and fails to detect some very small ITDs, we set up a method for screening of FLT3 receptor mutations with PCR plus denaturing high-performance liquid chromatography (D-HPLC). More importantly, this technique has been used for further identification of new point mutations occurring in the regulatory and catalytic domains of FLT3 receptor. Taking advantage of our knowledge of the FLT3 gene and its aberrations, we determined both the set of primers to use during PCR reaction and the melting temperatures to be used during D-HPLC mutational analysis. WAVEmaker software version 4.1.40 enabled us to find the optimal temperatures to maximize the resolution of heteroduplex and homoduplex peaks, and visualize the position of melting subdomains. Moreover, since D-HPLC peak profile allows accurate measurements of the peak height and area, which are indicative of the dosage, we were able to estimate the ITD/wild type ratio for each analyzed patient; this software analysis tool enable us to replace gel electrophoresis quantification of fluorescent labeled products.

Thirty-four patients were analyzed by PCR amplification, restriction enzyme digestion and agarose-gel electrophoresis (PCR-RE-AGE): nine were found positive for known FLT3 mutations (9/34 corresponding to 26.5% -length mutations plus point mutations). The same patients panel was analyzed by denaturing high-performance liquid chromatography (D-HPLC) based procedure; we could identify 14 sequence variations (7/34 20.6% for ITD, 3/34 8.8% for TKDs mutations, 4/34 11.8% for polymorphism). Additional nucleotide changes were discovered; direct sequencing analysis identified nucleotide alterations in each of the DHPLC-positives but not in any of the DHPLC-negatives, yielding a specificity and sensitivity of 100% for DHPLC based screening. Notable, for one patient we detected a nucleotide change (C > T - nt 2096 - accession number NM_004119), in exon 16 of FLT3 gene, corresponding to the first catalytic domain of FLT3 receptor protein, resulting in the

aminoacid change Ala680Val. Molecular characterization of this new point mutation and studies to determine its functional consequences are ongoing.

In recent years, a series of studies supported the idea that FLT3 is potentially a good pharmacological target in AML, and that therapeutic efficacy and tolerability could strongly depend on the presence of a specific genetic variation in the FLT3 gene. Leukaemic cells from AML patients could harbor additional activating mutations within FLT3 protein that are unknown up to the present. Mutational D-HPLC profiles provide a fingerprint to reveal the presence of sequence alterations. Future therapeutic approaches may involve the design of novel molecules targeting the underlying molecular defects of the FLT3 receptor protein: precise genotype determination would be critical for the selection of such therapies. The D-HPLC method described herein should be of use for such studies.

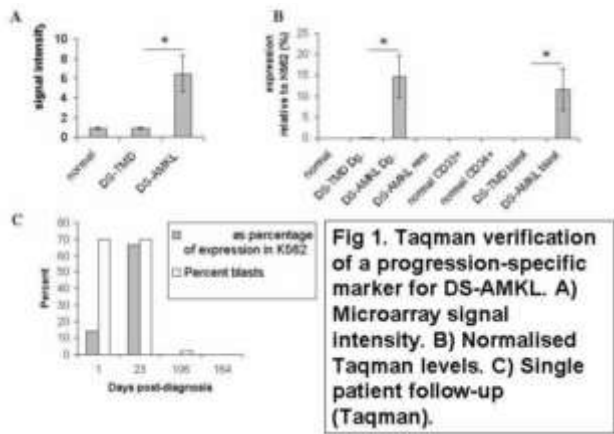
Acknowledgements: COFIN 2003, AIRC, FIRB 2001, ATENEO 60%, 'Hairshow A.I.L.', Fondazione del Monte di Bologna e Ravenna.

316 MICROARRAY TRANSCRIPT PROFILING DISTINGUISHES THE TRANSIENT FROM THE ACUTE TYPE OF MEGAKARYOBLASTIC LEUKAEMIA (M7) IN DOWN SYNDROME, REVEALING A PROGRESSION-SPECIFIC DISCRIMINATING MARKER

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BACKGROUND: Transient myeloproliferative disorder (TMD) is a unique spontaneously regressing neoplasia specific to Down syndrome (DS), affecting up to 10% of DS neonates. In 20-30% of cases, it re-occurs as progressive acute megakaryoblastic leukaemia (AMKL) at ages 2-4. The TMD and AMKL blasts are morphologically and immuno-phenotypically identical, and have the same acquired mutations in GATA1. We and others have recently discovered in utero acquired mutations in GATA1 in DS newborns in TMD and found that they did not predict a development of a future progressive AMKL (Groet et al. Lancet 361:1617-1620, 2003, Rainis et al. Blood, 102, 981-986, 2003). These findings clearly implicate mutation in GATA1 as an early permissive event which is necessary, but not sufficient, for the development of AMKL in DS. The mechanisms causing proliferation in TMD, its obligatory spontaneous regression, and the nature of the additional changes ('second hits') required for the development of the progressive AMKL remain unknown. AIMS: To investigate these questions, we used microarray expression profiling with the aim of identifying genes with significantly differing levels between TMD and AMKL in DS. METHODS: Our study, approved by the North East London Health Authority's ethical committee, was performed entirely on RNA samples of DS-TMD and DS-AMKL cases (for patient sample collection details, see Table1 in Groet et al. Lancet 361:1617-1620, 2003). We performed transcript profiling of 9 TMD patients and 7 AMKL patients using Affymetrix HG-U133A microarrays. Hierarchical clustering was performed using Genespring, and TMD and AMKL groups were compared to each other by parametric test with cross-gene error model, Affymetrix Microarray Suite comparison based on change calls, and paired t-test. Taqman analysis on 11 TMD and 11 AMKL RNA samples verified the expression of selected differing genes, with statistical significance ($p < 0.05$) by Student T-test. The Taqman differences were also reproduced on TMD and AMKL blasts sorted by FACS. RESULTS: Similar overall transcript profiles were observed between the two conditions, which were only separable by supervised clustering on a subset of genes. Among significant differences, we found the downstream targets of GATA1-mediated cell cycle arrest, and overexpression of an oncogene never previously associated with any form of leukaemia. These differing genes had contrasting and directly opposite patterns of expression between the two conditions, were functionally inter-related, and could provide a hypothetical explanation for the cause of the TMD proliferation in utero, and its spontaneous regression during neonatal period. Finally, one gene was identified as a completely specific marker with high expression specific only to AMKL blasts, with no expression in

TMD or normal controls, including non-leukaemic DS (Fig 1). The nature of the specific marker found offers the possibility of novel therapeutic approaches in the treatment of DS-AMKL. CONCLUSIONS: This study provides markers discriminating TMD from AMKL(M7) in Down syndrome. These markers have potential as predictive, diagnostic and therapeutic targets. In addition, the study provides further clues into pathomechanisms discerning the self-regressive from the progressive phenotype.



Caption 1:

Presidential Symposium (6 Best abstracts)

317 AML1-EVI-1 KNOCK-IN MICE SHOW MORE DYSPLASTIC FETAL HEMATOPOIESIS THAN AML1-ETO KNOCK-IN MICE

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Background: The t(3;21)(q26;q22) chromosomal translocation associated with leukemic transformation of myelodysplastic syndrome or chronic myelogenous leukemia results in the formation of AML1-Evi-1 chimeric gene.

Aims & Methods: To investigate its functions in vivo, we generated and analyzed AML1-Evi-1 knock-in mice. We also compared hematopoiesis in AML1-Evi-1 knock-in mice to that in AML1-ETO knock-in mice reported previously. [Results] (1) AML1-Evi-1/+ embryos died around E13.5 due to hemorrhage in the CNS. (2) AML1-Evi-1/+ embryos were histologically found to lack fetal liver hematopoiesis. On electron microscopic examination, the fetal liver from E13.5 embryos showed a marked decrease in erythroid and peroxidase-positive myeloid cells and the presence of dysplastic megakaryocytes with defective demarcation membrane formation. Nucleated erythroblasts were observed in the peripheral blood, but enucleated erythrocytes were not. (3) On in vitro hematopoietic colony forming assays, the fetal liver from E13.5 embryos contained multi-lineage hematopoietic progenitors (CFU-GEMM), while that from E12.5 embryos had only progenitors committed to monocyte/macrophage lineage (CFU-M). No erythroid colonies were seen in the livers at both E12.5 and E13.5. The CFU-GEMM-derived colonies from E13.5 AML1-Evi-1/+ embryos included few erythroblasts, numerous agranular granulocytes with a delayed differentiation, and dysplastic megakaryocytes, although those from E13.5 wild-type litters consisted of numerous erythroblasts and hyper-granular granulocytes, and normal megakaryocytes. Moreover, serial in vitro replating assays unveiled higher self-renewal capacity of hematopoietic progenitors in E13.5 AML1-Evi-1/+ fetal livers than that in wild-type fetal livers. (4) On semiquantitative RT-PCR method with fetal livers, up-regulation of C/EBP-alpha as well as down-regulation of LMO2 were detected. **Summary:** The macroscopic phenotype of AML1-Evi-1 knock-in mice was similar to that of AML1-ETO knock-in and AML1 knock-out mice. Therefore, this is the first report to demonstrate in vivo dominant inhibitory effects of AML1-Evi-1 over AML1. Embryonic lethality at E13.5, severely impaired fetal liver hematopoiesis and high self-renewal capacity of multi-lineage hematopoietic progenitors in the fetal liver are common features in both AML1-Evi-1 and AML1-ETO knock-in mice. However, they differ in lineage-specific fetal hematopoiesis. First, as shown in hematopoietic colony assays, erythropoiesis is severely prevented in AML1-Evi-1 knock-in embryos, but not in AML1-ETO knock-in embryos. Second, myelopoiesis in AML1-Evi-1 knock-in mice shows an abnormal differentiation producing hypo-granular granulocytes, while that in AML1-ETO knock-in mice undergoes normal terminal differentiation into hyper-granular granulocytes. Differences in megakaryopoiesis currently remain unclear. Moreover, expression of C/EBP-alpha that was reported to be decreased in AML1-ETO-expressing cells is increased in AML1-Evi-1 fetal livers. All these data suggest that in vivo molecular and biological functions are different in AML-Evi-1 and AML-ETO. It is important to note that AML1-Evi-1 and AML1/MTG8 develop different types of leukemias, namely stem cell tumor-derived leukemia and de novo acute myelogenous leukemia. More dysplastic fetal hematopoiesis observed in AML1-Evi-1 knock-in mice could at least partly reflect biological natures of AML1-Evi-1-causing leukemia.

318 RE-IIBP IS THE ONLY UNIQUELY DYSREGULATED AND POTENTIALLY FUNCTIONAL TARGET GENE OF T(4;14)(P16;Q32) MYELOMA

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Background: One of the most frequently observed translocations in multiple myeloma (MM) is t(4;14)(p16;q32). Multiple groups, including our own, have identified t(4;14) as a poor prognostic marker, however, the mechanism underlying the aggressiveness of t(4;14) myeloma remains elusive. The initially proposed target gene, FGFR3, is not

expressed in 25-30% of newly diagnosed t(4;14) positive patients and this group continues to do poorly. Interestingly, the der(4) chromosome is detectable in all t(4;14) positive patients, suggesting that a relevant target gene exists on this derivative. MMSET, located on der(4) has been suggested as a potential target gene. It produces four protein products; MMSET type I, II, RE-IIBP, and Exon 4a, however, in some patients the proper forms of MMSET type I, II, and Exon 4a are not produced due to the genomic breakpoint, which separates the proper translation initiation site from the rest of the gene.

Aims: To identify uniquely dysregulated genes in t(4;14) positive myeloma.

Methods: Patients were screened for t(4;14) and FGFR3 expression by RT-PCR. Quantitative RT-PCR on a panel of MM cell lines and CD138 purified patient plasma cells was used to identify target genes. Localization and functional kinetics of MMSET variants was determined by GFP localization and fluorescence recovery after photobleaching (FRAP) experiments.

Results: Within our cohort, 43/312 (13.8%) myeloma and 2/103 (1.94%) MGUS patients are t(4;14) positive and 70% have the MB4-1 breakpoint type and thus the entire translated portion of MMSET remains on the der(4). Furthermore, only 31/43 t(4;14) positive MM patients expressed detectable levels of FGFR3. The expression level of all known and novel genes mapped to the 500 Kb region flanking the breakpoint region were assayed to identify potential target genes. Using this method we identified both MMSET (Type I, II, Exon 4a combined) and RE-IIBP as uniquely dysregulated genes. The median expression level of RE-IIBP was 99.7 (range 27-169) versus 1.1 (range 0.33-11.7), in t(4;14) positive and negative patients respectively. MMSET I, II, and Exon 4a localize to the nucleus and are excluded from the nucleolus. The breakpoint variants all produce proteins and localize to the nucleus, however, they are not excluded from the nucleolus. RE-IIBP localizes to the nucleolus and in cytoplasmic foci. FRAP experiments showed that the live cell kinetics, t1/2 times, were significantly different for the breakpoint variants, 2 sec versus 9 sec (type I) and 39 sec versus >500 sec (type II) for the wildtype proteins.

Conclusion: The only gene that is uniquely dysregulated and potentially functional in all t(4;14) patients is RE-IIBP. MMSET is not a good candidate gene as 30 % of patients produce N-terminal truncations, which do not localize or function like the wildtype proteins. Therefore, the only relevant target gene appears to be RE-IIBP, which is dysregulated in all t(4;14) positive samples at diagnosis and relapse irrespective of breakpoint type or FGFR3 expression status.

319 MUTATIONS IN THE ATP-BINDING POCKET OF FLT3 CONFERRING RESISTANCE TO TYROSINE KINASE INHIBITORS: IMPLICATIONS FOR MOLECULARLY TARGETED THERAPY FOR ACUTE MYELOID LEUKEMIA

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Background:

The success of the small molecule tyrosine kinase inhibitor imatinib (Imatinib mesylate, Glivec, Novartis) for the treatment of chronic myeloid leukemia opened the door to molecularly targeted therapy for other hematological malignancies and solid tumors. Despite good responses to imatinib treatment in the chronic phase, resistance to imatinib develops quickly in patients in blast crisis. Molecularly targeted therapy was developed for acute myeloid leukemia harboring mutations in the FLT3 receptor tyrosine kinase. Mutated FLT3 transforms hematopoietic cells in vitro and in vivo, and kinase activity is required for the proliferation and survival of the leukemic cells. Four structurally different FLT3 kinase inhibitors (PKC412, SU11248, CEP701 and MLN518) are currently under investigation in clinical trials as molecularly targeted therapy for AML.

Aims:

Given the availability of several FLT3 inhibitors with different chemical structure, we developed a strategy to identify mutations that confer resistance to PKC412, and tested the ability of other FLT3 inhibitors to overcome resistance to PKC412.

Methods:

Random mutations were introduced into the ATP-binding region of FLT3, these mutants were expressed in Ba/F3 cells, and were selected for their ability to grow in the presence of PKC412. Resistance was confirmed using western blot analysis of phospho-FLT3 and dose response curves.

Results:

Mutations in FLT3 that conferred resistance to PKC412 were identified at 4 different positions (A627, N659, F691 and G697). The G697R mutation conferred the highest degree of resistance to PKC412 and also conferred high level resistance to SU5416 and K-252a (similar to CEP701). Other mutants showed a clear shift in sensitivity towards the different inhibitors, but remained sensitive to higher concentrations of inhibitor.

Conclusion:

These results indicate that resistance to PKC412 may develop due to point mutations in the ATP-binding domain of FLT3. Our results identify the G697R mutation as a critical mutation in FLT3 conferring resistance to conferred FLT3 inhibitors, and indicate that screens to identify new small molecules to overcome resistance to PKC412 and other inhibitors should focus on this resistance mutation.

320 ECULIZUMAB, A C5 COMPLEMENT-BLOCKING ANTIBODY, CONTROLS HAEMOLYSIS IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA (PNH) WITH RESPONSES MAINTAINED OVER A PROLONGED PERIOD OF 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 is the principle cause of the complement-mediated hemolysis. Eculizumab is a humanized monoclonal antibody that binds to C5 inhibiting terminal complement activation. We have recently demonstrated that eculizumab is extremely effective at controlling intravascular hemolysis in PNH.

Aims: To present 12 months follow-up of eculizumab therapy in 11 transfusion-dependent PNH patients. To assess whether eculizumab continues to be effective at controlling hemolysis and reducing transfusion requirements over an extended treatment period.

Methods: All patients initially received 600mg eculizumab by intravenous infusion weekly for 4 weeks followed by 900mg every other week. Clinical and laboratory measures of hemolysis as well as transfusion requirements were recorded during therapy.

Results: Eculizumab is well tolerated with no dose limiting toxicity for in excess of 18 months of therapy. The dramatic improvement in the biochemical parameters of hemolysis occurring in all patients within a week of starting therapy persists. Mean LDH decreased from 3111U/L over the 12 months prior to treatment to 627U/L over 12 months on treatment ($p=0.002$). Hemoglobinuria (daily urine colour scale) decreased from 3.0 to 0.2 paroxysms/patient-month ($p<0.001$) since starting eculizumab therapy with a significant improvement in quality of life measures related to hemolysis. In addition, PNH type III red cells progressively increased during the treatment period from a mean of 36.7% to 67.1% at 12 months ($p<0.001$). There was also a continued reduction in transfusion requirement from a median 1.8 units/patient/month in the 12 months prior to treatment to 0.2 units/patient/month since starting treatment ($p=0.001$). 5 patients remain transfusion independent after a year of therapy. Patients with purely hemolytic disease (macroscopic hemoglobinuria with normal platelet count; $n=6$) had the best response with cessation of hemolysis, an increase in type III red cells from 38.8% to 77.8% and a decrease in transfusions from a median of 2.0 units/patient-month to 0 units/patient-month. Four patients who did not become transfusion independent initially were treated with subcutaneous erythropoietin in addition to eculizumab. Two of the four became transfusion independent, one has required transfusions at 4 monthly intervals and one remains transfusion dependent and has discontinued erythropoietin. The latter patient has significant marrow failure also with a platelet count below $32 \times 10^9/l$. Interestingly, in most patients LDH levels remain just above the upper limit, the bilirubin remains slightly to moderately elevated (under $50 \mu mol/l$), the haptoglobins remain undetectable and the reticulocytosis persists indicating a low level of

well-compensated, presumably extravascular, hemolysis. Two patients experienced transient breakthroughs in complement activity associated with increased LDH, hemoglobinuria and mild symptoms; these resolved immediately upon next dose of eculizumab and further breakthroughs were prevented by a slight decrease in the dosing interval.

Summary: This study demonstrates that eculizumab is well tolerated, dramatically decreases hemolysis and transfusion requirement and also improves the symptoms of PNH. The effectiveness of eculizumab has been maintained for over 12 months of therapy and is ongoing in all patients.

321 THE HIGH PREVALENCE OF CHLAMYDIA PSITTACI INFECTION IN OCULAR ADNEXAL LYMPHOMAS (OAL) PROVIDES THE RATIONALE FOR THE USE OF ANTIBIOTICS AS A POTENTIAL THERAPEUTIC STRATEGY IN THESE PATIENTS

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Background: Some infectious agents have been variously associated with lymphomagenesis and, in some cases, regarded as novel therapeutic targets. OAL may be antigen-driven disorders; however, the source of this putative antigen(s) is still unknown. Chlamydiae infection is responsible for inflammatory ocular diseases, which could provide persistent antigenic stimulation such as chronic follicular conjunctivitis.

Aims: The present study was carried out to assess whether Chlamydiae infection is associated with the development of OAL. Chlamydial eradication rate and anti-lymphoma activity of antibiotic therapy was also investigated in 8 patients with OAL.

Methods: The presence of Chlamydia psittaci, trachomatis, and pneumoniae DNA was investigated by multiplex touchdown PCR in 40 OAL samples and in 20 non-neoplastic orbital biopsies and 26 reactive lymphadenopathy samples used as controls. The same analysis was performed in peripheral blood mononuclear cells (PBMCs) from 21 OAL patients and 38 healthy individuals. PCR products were sequenced to confirm the specificity of the fragment amplified. The location of the microorganisms within lymphomatous lesions was carried out by immunohistochemical detection of Chlamydia lipopolysaccharide.

Eight patients with chlamydia-positive OAL were treated with the antibiotic doxycycline (100 mg PO, twice a day, for three weeks): six of them had concomitant chlamydia-positive PBMCs and were assessable for Chlamydia-eradication rate, while five of these patients had measurable lymphoma lesions and were evaluable for objective response.

Results: Thirty-two (80%) of 40 OAL samples carried Chlamydia psittaci-DNA, whereas all of the lymphoma samples were negative for Chlamydia trachomatis and pneumoniae. In contrast, none (0%) of the non-neoplastic orbital biopsies carried Chlamydia psittaci DNA (0% vs. 80%; Fisher exact test, $p<0.00001$) and only three of 26 (12%) reactive lymphadenopathy samples (12% vs. 80%; $p<0.00001$) carried the Chlamydia psittaci DNA. Nine (43%) of 21 patients with Chlamydia-positive OAL carried Chlamydia psittaci DNA also in their PBMCs, whereas none (0%) of the PBMCs from healthy donors was positive for Chlamydia psittaci DNA (43% vs. 0%; $p<0.00001$). Sequencing analysis showed a variable heterogeneity degree, indicating that OAL carry unrelated variants of Chlamydia psittaci. Immunocytochemical analysis localized the immunoreactivity for Chlamydia lipopolysaccharide in tumor-infiltrating cells, which were characterized by macrophage-like morphology.

All patients completed doxycycline treatment with excellent tolerance; one month after antibiotic assumption, chlamydial DNA was no longer detectable in the PBMCs of all 6 assessable patients. Objective response was observed in three of the five evaluable patients: one complete remission (22+ months) and two partial responses (7+ and 24+ months). **Conclusions:** Patients with OAL have a high prevalence of Chlamydia psittaci infection both in tumor tissue and in PBMCs. This association appears highly specific and does not reflect the occurrence of a subclinical infection widespread among general population. Macrophages and PBMC may constitute a reservoir for Chlamydia psittaci infection potentially able to provide local growth-promoting stimuli for lymphoma cells. Chlamydia psittaci-eradicating therapy, with doxycycline, is able to induce durable lymphoma regression in OAL patients. A larger prospective trial assessing eradication rate and activity of antibiotic therapy in patients with OAL is warranted.

322 A RANDOMIZED PHASE II STUDY COMPARING IMATINIB WITH CHEMOTHERAPY AS INDUCTION THERAPY IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED PHILADELPHIA-POSITIVE ACUTE LYMPHOID LEUKEMIAS (PH+ALL)

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Background: Elderly pts. with ALL have a very poor prognosis due to a low CR rate, short remission duration and high induction mortality. Results are even inferior in elderly patients with Ph+ALL. Because of the substantial comorbidity of these patients, single-agent Imatinib may be an attractive alternative to induction chemotherapy as it has shown rapid anti-leukemic activity and a favorable toxicity profile in advanced Ph+ALL. Subsequent postremission therapy with combined Imatinib and cytotoxic chemotherapy may delay or overcome the acquired resistance encountered with imatinib monotherapy.

Study design: To establish whether up-front Imatinib monotherapy is superior to age-adapted induction chemotherapy, patients with newly diagnosed Ph+ALL were randomly assigned to receive a 4 week

course of Imatinib at 600 mg/d (IndImatinib) or multiagent induction chemotherapy according to the GMALL elderly protocol (IndCTX). Crossover to the other treatment arm was possible in patients failing to achieve at least a minor response after 2 weeks of assigned therapy. Pts. completing induction with a CR or PR continued treatment with consolidation and reinduction cycles given in conjunction with Imatinib to determine the impact of this strategy on hemtologic and non-hematologic toxicity and on disease-free and overall survival (DFS; OS).

Results: To date, 32 pts. with newly diagnosed Ph+ALL (n=31) or lyBC (n=1) and a median age of 67 (60-79) yrs. have been randomly assigned to receive IndImatinib (n=15) or Indchemo (n=17). Of 12 pts. who have already completed IndImatinib, 11 (92%) achieved a CR and one a PR (8%), for an overall response rate of 100%. In comparison, 8 of 15 evaluable pts. allocated to Indchemo achieved a CR (53%) and 1 pt. a PR (7%), while 6 pts. failed treatment (40%). Four of these 6 non-responders were crossed over to receive Imatinib and 3 achieved a CR. There were 2 deaths during Indchemo (non-response, sepsis) and none during Indimatinib. Severe infectious complications during induction occurred in 8 pts. assigned to Indchemo (pneumonia n=5, sepsis n=3) and in no pt. receiving IndImatinib. Three pts. achieved a molecular remission after the 1st (n=1) or 2nd (n=2) consolidation cycle. After a median follow-up of 4.8 (0.5-18.3+) mo., 15 pts. are in ongoing CR and four pts. have relapsed. Four pts. died in CR a median of 4.1 (2-5.1) mo. after starting treatment and 1 pt. died of transplant-related causes after allogeneic SCT. Estimated DFS and OS in 27 evaluable pts. at 18 mos. is 34.614% and 47.14%, respectively.

Conclusion: Imatinib is highly effective as induction therapy in elderly pts. with Ph+ALL and appears to be superior to standard chemotherapy induction with respect to tolerability, induction mortality and CR-rate. Extended administration of Imatinib and concurrent consolidation chemotherapy is feasible and promising with respect to DFS and OS, although longer follow-up is needed.

Poster Session II

LYMPHOMA: CLINICAL ASPECTS

323 THE HEPATITIS C VIRUS INCIDENCE IN NON-HODGKIN'S LYMPHOMAS PATIENTS

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Since many clinical studies have suggested an etiopathogenetic role of hepatitis C virus (HCV) in non-Hodgkin's B lymphomas (B-NHL), we consecutively evaluated 102 patients affected by B-NHL, in order to assess HCV prevalence. We looked for histological and clinical differences between HCV positive and HCV negative patients.

From 102 patients with NHL who were registered in our Clinic (age between 55-81 years, ratio males/females=1/1) in July 1997-February 2004 period, a number of 61 (59,8%) have antibodies anti hepatitis C virus. From these, 14 (22,9%) have hepatocytolysis syndrome and 4 (6,5%) have colestatic syndrome. The ethanolic etiology was presented in 12% from patients with hepatocytolysis syndrome (the increases of gammaglutamyl transpeptidase in these cases was considerable).

The patients with NHL and antibodies anti HCV positive have the following types of lymphomas: gastro-intestinal type MALT lymphomas (11%), plasmacytoid lymphomas (9,2%), centrocytic/centroblastic lymphomas (10,2%), angiofollicular hyperplasia (AFH), pseudo Castleman's disease multicentric type (5,2%), other types (64,4%).

Conclusion

1. It was noted an increased prevalence of NHL HCV positive to the older patients (average 69 years), female sex (66%).
2. The complete response rate was lower in NHL HCV positive patients subgroup comparatively with NHL HCV negative patients subgroup.
3. The evolution after the treatment in the patients with NHL type MALT and HCV positive was unfavorable comparatively with the patients with HCV negative (the complete and partial response rates was 39% for HCV positive patients comparatively with 82% for HCV negative patients).
4. The use of an aggressive regimens for NHL HCV positive patients was followed by a significantly hepatotoxicity ($p < 0.001$).

The role of HCV in the appearance of NHL is still uncertain, the significance of MALT lymphomas and plasmacytoid lymphomas prevalence so the increased percentage of pseudo Castleman's disease to the patients HCV positive is still unknown and it is worth to be study in the future

324 THE USE OF 18FDG-PET/CT FOR EVALUATION OF NEUROLYMPHOMATOSIS

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Background: NL represents a unique subtype of extranodal non-Hodgkin's lymphoma (NHL) with localized invasion of cranial or peripheral nerves or nerve roots. Less than 100 cases of pathologically proven NL were reported in the literature whereas in approximately half of the patients the diagnosis was established on autopsy. MRI detecting enlarged peripheral nerves was reported to be of great diagnostic utility, although it does not always achieve the optimal visualization of the peripheral nervous system. Most antemortem diagnosed patients were treated with systemic chemotherapy, generally with poor prognosis.

Aims: To report a biopsy-proven case of neurolymphomatosis (NL) successfully evaluated by 18FDG PET/CT.

Methods and Results: A 38-year old woman with previous history of non-Hodgkin's lymphoma of the cervix 5 years ago presented with a painful brachial plexopathy and developed multiple cranial and spinal nerve palsies. The diagnosis of NL was achieved by an open brachial plexus biopsy. The 18FTG PET/CT was successfully used for the evaluation of the extent of disease and demonstrated an extensive involvement of the peripheral nervous system with prominent enhancement of the left facial nerve, right vagus nerve, left brachial plexus and multiple spinal roots (figure). A complete clinical and radiological response and almost complete neurological recovery were achieved by means of systemic high dose (8 g/m², every 14 days) Methotrexate treatment combined with Rituximab 375 mg/m² and intra-CSF injections of Cytarabine.

Conclusion: 18FDG PET can be useful for diagnosis, evaluation of extent, and monitoring of response to therapy in NL.



Caption 1: Image 1: Before



Caption 2: Image 2: After

325 CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT IN MANTLE CELL LYMPHOMA (MCL)

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Background: Patients with MCL usually present with extranodal involvement, including bone marrow, gastrointestinal tract, and Waldeyers ring. However, the real incidence of CNS infiltration in those patients has not been completely established, ranging from 5% to 23% in different studies. In addition, variables predicting CNS involvement in MCL, as well as the clinical outcome of this complication have not been fully assessed.

Aim: To ascertain the incidence and prognostic significance of CNS infiltration in patients with MCL from a single institution.

Patients and methods: 84 patients (62 M/22 F; median age: 61 years) diagnosed of MCL between 1988 and 2002 were studied. The main clinical, biological, and evolutive features were recorded and correlated with CNS involvement.

Results: The histologic subtype of MCL was blastoid in 21/84 cases (25%) and typical in 63/84 (75%). Most of patients presented with advanced clinical stage (93%) including bone marrow involvement in 75 cases (89%). 85% of patients presented with intermediate and high risk IPI. Treatment varied over the time but 28 patients were treated with high-dose methotrexate (MTX) (HyperC-VAD regimen). Eleven of 84 patients (13%; 0.95 CI: 5-20%) developed CNS involvement. In one asymptomatic patient, cerebro-spinal fluid (CSF) infiltration was detected during staging maneuvers. The remaining 10 patients developed CNS involvement during the course of the disease (median time from diagnosis, 25 months; range 5-130), as a part of a resistant disorder or generalized relapse. Those patients presented with neurological symptoms (facial palsy in 4 cases, sciatalgia in 3, diplopia in 2, paraparesis in 2, and confusional syndrome in 1) and displayed atypical lymphocytes in the CSF, consistent with the diagnosis of MCL. All patients were treated with intrathecal chemotherapy, with a minor improvement. Median survival time after diagnosis of CNS infiltration was 3 months (range, 5 days-19 months). The risk of CNS involvement at 5 years was 26% (0.95 CI: 10-42%). Patients with CNS infiltration more frequently had blastoid histology ($p=0.02$), 2 or more extranodal sites involved ($p=0.05$), high LDH serum level ($p=0.009$), and intermediate and high risk IPI ($p=0.02$). In the multivariate analysis, the histologic subtype (blastoid vs. non-blastoid, $p<0.01$; RR: 6) and the IPI (L vs. H, $p=0.01$; RR: 11) retained their prognostic significance.

Conclusions: CNS involvement might be more frequent than previously recognized in patients with MCL. In our study, CNS infiltration was associated with poor histologic variants, multiple extranodal involvement, increased LDH serum level, and unfavorable IPI. Finally, the prophylaxis and treatment of such a complication warrant further studies.

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326 RESULT OF A MULTICENTRE CASE-CONTROL STUDY ON HEPATITIS B VIRUS (HBV) AND LYMPHOMA

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Background/aims A high prevalence of hepatitis C virus infection in lymphomas is now well demonstrated by several observational studies and a large case-control study. There is also evidence of regression of lymphoma in some patients with HCV infection and splenic lymphoma after antiviral treatment. Conversely few data are currently available concerning the role of hepatitis B virus infection in lymphomas. The present study is a multicenter case-control study conducted from January 1998 until February 2001.

Methods All cases received a new diagnosis of not pre-treated B-cell Non Hodgkins Lymphoma (NHL) and the controls were enrolled during the study period from department of traumatology, ortopedic, ophthalmology, surgery, gynaecology, otorhinolaryngology, medicine, dermatology and dentistry in the same hospitals where cases were identified. HbsAg was determined by commercial methods. HBV-DNA was performed by Cobas-Ampliscreen HBV test in all HbsAg-positive

patients and Anti-HBc positive cases and controls. Viral genotype was also determined by INNO-LIPA HBV Genotyping. Adjusted odds ratios and their 95% confidence intervals were computed using multivariate logistic regression model.

Results The HbsAg prevalences and adjusted odds ratios are presented in table 1. A significantly higher HBV infection prevalence was demonstrated in lymphoma patients as compared to the controls. Analysis of HBV infection prevalence in the single NHL histotypes will be presented.

Conclusions Our study, conducted in different geographical areas of Italy using incident cases, demonstrates an association between HBV infection and B-NHL.

Table 1. Prevalence of HCV infection and Adjusted O.R. in B-cell NHL and controls

HbsAg	cases	controls	O.R. (95% CI)	OR adjusted (95% CI)*
Pos	35	12	3.04 (1.5-5.9)	3.65 (1.82-7.4)
Neg	365	381		
tot	400	393		

*adjusted for area of residence, age and sex.

Caption 1:

327 SERIAL PRE-TRANSPLANTATION FDG-PET SCANNING TO PREDICT OUTCOME IN CHEMOSENSITIVE RELAPSED LYMPHOMA

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Background

In a retrospective study of 46 relapsed lymphoma patients (BJH 2003;123:282), we have shown that FDG-PET appears to be an excellent tool to evaluate early chemotherapy response. PET may predict outcome after intensive treatment even better than clinical parameters based on conventional diagnostic methods. Eventually, pre-transplantation PET might enable us to select a group of patients for whom current autologous stem cell transplantation (ASCT) is not beneficial.

Aims

To prospectively evaluate optimal timing and parameters to use in pre-transplantation PET evaluation of patients with relapsed lymphoma.

Methods

36 consecutive chemosensitive (more than 50% tumour reduction on CT scan after DHAP-VIM) patients with relapsed lymphoma (24 aggressive non-Hodgkin lymphoma (NHL) and 12 Hodgkins disease (HD)) were treated with DHAP-VIM-DHAP induction chemotherapy, followed by BEAM and ASCT. Three serial whole-body attenuation-corrected FDG-PET scans were performed: PET1 before treatment, PET2 after DHAP-VIM and PET3 before BEAM and ASCT. Visual analysis and SUV parameters were obtained in each scan. Minimal follow-up was 6 months after ASCT.

Results

PET2 normalised in 16/36 patients, PET3 in an additional 6 patients. 14/36 patients showed persistent abnormal FDG-uptake. 53% of the patients relapsed after ASCT in median 8 (0-27) month. Median follow-up for the non-relapsing patients was 21 (6-44) months. Median progression free survival (PFS) was significantly worse for PET2 positive patients (13 [3-23] versus 23 [21-25] months, RR=5.52, $p=0.018$). Also, PET3 positive patients had a significantly worse PFS (5 [0-16] versus 23 [21-25] months, RR=6.42, $p=0.011$). The positive predictive value (PPV) of abnormal PET3 before transplantation was slightly better than that of PET2: visual (abnormal PET=positive): PPV(PET3) 0.86 versus PPV(PET2) 0.70; SUV (SUVbw >2=positive): PPV(PET3) 0.83 versus PPV(PET2) 0.75. In five patients an increase in SUV between PET 2 and PET3 was found, all these patients relapsed. SUV-parameters did not provide more discriminating parameters than visual assessment.

Conclusion

Pre-transplantation PET predicts outcome after ASCT in patients with relapsed aggressive lymphoma. Neither PET2, nor PET3 alone could

predict outcome during therapy precisely enough to be used for treatment decisions. However, in patients with at least partial response according to conventional CT criteria after induction treatment, increasing FDG uptake on serial PET scanning is highly predictable for treatment failure after ASCT.

328 DIFFUSE SKELETAL UPTAKE OF GA-67 IN HODGKIN'S

DISEASE WITHOUT BONE MARROW INVOLVEMENT

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Background: Bone marrow involvement (BMI) occurs in less than 10% of untreated patients with Hodgkins disease (HD). In most cases of HD without BMI, biopsy specimens reveal nonspecific and reactive changes, such as increased marrow cellularity, inflammatory infiltration by lymphocytes, plasma and mast cells, and stromal changes. Only 20% have normal marrow histology. Ga-67 citrate scintigraphy (GS) has been proved a useful tool in the initial evaluation and management of patients with HD. The uptake of Ga-67 in lesions of HD is well documented, the sensitivity and specificity of GS in HD range from 85-97% and from 90-100%, respectively. However, diffuse increased skeletal Ga-67 uptake is frequently observed on GS in HD, even in the absence of bone marrow (BM) or skeletal involvement.

Aims: The objective of this study was to evaluate the association between Ga-67 skeletal uptake, BM histopathology and laboratory findings, in patients with HD without BMI.

Methods: BM trephine biopsy (BMB) and GS were performed routinely as parts of the initial staging in all patients with HD. Patients with BMI were excluded from the study. Fifty-four patients with HD without BMI were investigated (27 males, mean age 38.917.8 years, range 18-82). Thirty-six patients had nodular sclerosis type and 13 had mixed cellularity type of HD. Seven patients had Ann Arbor stage 1, 33 had stage 2, and 14 had stages 3-4. Twenty-one patients had B symptoms and 24 had bulky disease. Ga-67 uptake in the humerus, femur and vertebrae was assessed by qualitative visual scoring (grades 0, 1 and 2 for normal, mildly increased and markedly increased uptake, respectively). The sum of these grades was defined as skeletal uptake index (SUI). BMB were evaluated histologically and scored semi-quantitatively for marrow cellularity (age adjusted), erythroid, myeloid and megakaryocytic activity, and eosinophils, lymphocytes, plasma and mast cells hyperplasia (scores 0, 1 and 2 for normal, mildly increased and markedly increased, respectively). Laboratory tests included peripheral blood counts, lactic dehydrogenase (LDH), C-reactive protein (CRP), copper and ceruloplasmin serum levels, and erythrocytes sedimentation rate (ESR). Statistical analysis was performed using Spearman's rank correlation coefficient analysis.

Results: Significant correlation was observed between Ga-67 SUI and BM hypercellularity ($r=0.42$, $p=0.002$), BM megakaryocyte hyperplasia ($r=0.44$, $p<0.001$), peripheral blood platelet count ($r=0.38$, $p=0.005$) and peripheral white blood cells count ($r=0.50$, $p<0.001$). Other laboratory parameters, typically reflecting severity of disease or BMI, including LDH, ESR and CRP, were not correlated with Ga-67 SUI.

Conclusions: The results of this study show that skeletal uptake of Ga-67 in patients with HD without BMI is associated with increased bone marrow reactivity. starkp@post.tau.ac.il

329 CLINICAL FEATURES AND INCIDENCE OF HCV INFECTION AND SOLID TUMORS IN 58 CASES OF NON-GASTRIC MARGINAL ZONE B-CELL LYMPHOMA OF MALT

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Aim

Aim of this study was to define the clinical features and outcome and to clarify the role of HCV and the association with solid tumors in a series of 58 patients with non-gastric MALT lymphoma observed in a single Institution.

Methods

We studied 58 patients with a confirmed histology of marginal zone B-cell lymphoma of MALT according to the WHO classification presenting with a clinically prevalent extranodal non-gastric site of disease. The primary sites of lymphoma were: orbit (15), Waldeyers ring (9), parotid gland (8),

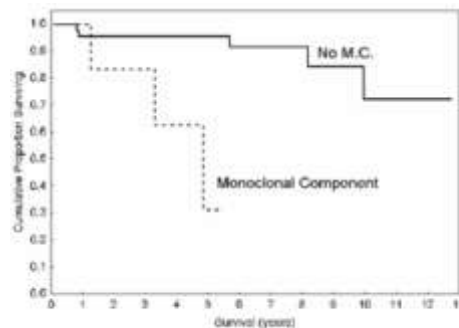
minor salivary glands (6), skin and subcutaneous tissue (7), lung (5), breast (4), female genital tract (2), bowel (1), multiple mucosal sites (1). Results

The majority of pts were from Northern Italy. Median age was 64 years (36-86), (42 F, 16 M). Thirty-two (55%) had stage I disease, 8 (14%) stage II, 3 (5%) stage III, and 15 (26%) stage IV with bone marrow involvement. Peripheral blood was involved in one patient and one had bulky disease. No patient had B symptoms, 3 had ECOG score >1. Eighteen pts (31%) showed nodal involvement and 4 (7%) spleen enlargement. Six pts (10%) had hemoglobin < 11 g/dL. Nine (16%) had a small monoclonal component (8 IgM, 1 IgG). Two were affected with Sjögrens syndrome before diagnosis of parotid MALToma. HCV-serology was positive in 22 of 50 evaluable pts (44%) (6 salivary gland, 5 skin and subcutaneous tissue, 4 orbit, 3 breast, 2 lung, 1 Waldeyers ring, 1 bowel). Eleven pts showed signs of chronic liver disease. Circulating HCV-RNA was detected in all the 8 pts tested. Five pts were HbSag-positive (1 coinfection with HCV). An history of solid neoplasia was present in 10 pts (17%) (4 breast, 2 endometrium, 1 female genital tract, 1 thyroid, 1 lung, 1 parotid carcinoma). Solid neoplasia was previous in 6, concurrent in 2, and subsequent in 2. In one patient the site of cancer and of lymphoma was the same (breast cancer developing 12 months after the diagnosis of breast MALToma). First-line therapy consisted of: CHOP-like regimens in 12 pts, alkylating agents in 15, CVP in 2, radiotherapy in 10, chemo-radiotherapy in 5, surgical resection in 4. CR was obtained in 31, PR in 7. Ten pts were followed without initial treatment. Median follow-up was 2.8 years (range 1-12). Median EFS was 2.8 years. Median OS was not reached. Pts with M component (Fig. 1), presence of nodal disease, >1 extranodal site, and with advanced disease showed a significantly worse OS. There was not difference in survivals according to extranodal site and between HCV+ and HCV- cases.

Conclusion

This study on a single-centre series of 58 pts with non-gastric MALT lymphoma shows frequent dissemination of disease at presentation and an evident association with HCV infection and solid cancers. Despite the indolent nature of these MALT lymphomas, some features such as the presence of M component and nodal dissemination characterize a subset of patients with a worse prognosis.

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Caption 1: OS according to the presence of M component

330 HODGKINS DISEASE IN CHILDHOOD: A 20-YEAR EXPERIENCE AT A SINGLE CENTER

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During the last 20 yrs, 63 children with Hodgkins Lymphoma were treated at our center with treatment strategies, which focused on increasing cure rates and reducing long-term toxicities. The study was retrospective and analyzed the following data: age, sex, follow-up period, stage, histology, therapy, outcome and complications. Age at diagnosis (dx) ranged from 3.5 to 15 yrs (median 12.4). There were 38 male pts. Only ten pts (16%) had stage IV, 16 (25%) III, 21 (33%) II, and 16 (25%) I. Prevailing among histologic subtypes were nodular sclerosis (37/63, 59%) and mixed cellularity (18/63, 29%). Combined, chemotherapy (CT) plus radiation (RT), therapy was used in 49/63 (77%), only RT 9/63 (14%, all stage I), and only CT 5/63 (9%, 1 stage II, 3 stage III, 1 stage IV). With a median follow-up time of 11 yrs the overall survival was 98.3 %. 57/63 children (90 %) are in first complete remission (CR) from 4 to 288 months (median 119 mo). Relapses occurred in 6 of 63 children (9%) 24 to 40 mo (median 30) off therapy despite RT for 1 with stage

	ALL PT N=141	GF A N=75	GF D N=47	GF C N=20
Median [S.E.] time to onset of chemotherapy [95% CI]	3.4 (1.2)	3.1 (1.0)	3.3 (1.0)	3.2 (1.3)
% of PT with planned chemotherapy dose or 80% of PT with cycles delayed	56.8 12.2	72.0 2.0	53.0 0.6	52.8 5.4
% of patients with neutropenia	29.7	17.3	29.8	42.9
Median [S.E.] time to MVL; % evaluable pts				
CR (%)	86	91	95	72
PR (%)	9	0	0	20
NR (%)	4	0	5	8
Not evaluable (%)	(1)	(1)	(1)	(1)
Adverse chemotherapy	5%	1%	4%	55%
IFRT omitted	2%	0%	2%	0%
Progressed while on treatment	4%	0%	4%	0%

Background: Remarkable changes in the incidence and mortality of various cancers have occurred during the past decades. While for most cancers incidence and mortality are decreasing, NHLs have steadily increased, now representing the fifth most commonly diagnosed cancer in the US. Research in defining reasons for this increase have been vivid but yet unsettled. **Aims:** We conducted a literature analysis on trends regarding changes in the incidence, geographic distribution and etiologic factors of NHL. **Methods:** A systematic review was conducted to analyse all available literature on NHLs (excluding CLL, MM and Hodgkins disease). Various cancer web pages (SEER, IARC) were used as supplemental resources for statistical data support. **Results:** From our and previous analyses, an increased NHL incidence at a rate of 3-4% /year can be observed in the 1970s and 1980s. This increase stabilized in the 1990s, with still an annual rise of 1-2%, resulting in an almost duplication of the

NHL incidence. This rise has been noted worldwide. In elderly persons, particularly those >55 years (y), this increase is most prominent, although of note for all age groups, except for the young (<30y.). Concerning race and gender subgroups, this increase is observed in all subgroups worldwide, albeit a male predominance throughout all age groups is seen. Although the NHL incidence has historically been higher in whites than blacks, disproportional increases have recently been observed in the latter. Increases in high-grade NHL, particularly in the diffuse subtype (diffuse-large cell and immunoblastic NHL) and of extranodal disease (END), with most striking proportional increases in the central nervous system (244%) are apparent. With minor geographic variations, END is most frequently found in the stomach, intestine and skin. Differences in geographic distribution are particularly striking for follicular lymphoma, most common in western countries, but relatively rare in developing and far eastern countries. Asians have higher rates of aggressive NHLs, T-cell lymphomas and END. In the Middle east, particularly high rates of gut END are observed, whereas on the African continent endemic Burkitts lymphoma comprises a substantial proportion. An association of HIV and NHL in African countries is weak, most likely due to the early death from infectious causes in AIDS patients. Risk for developing NHL comprises immunosuppression, such as immunodeficiency-syndromes, AIDS or organ transplantations. Certain autoimmune disorders appear to be also associated with NHL. A causal link between infectious agents and lymphomagenesis has been determined, particularly for HTLV-1, EBV and *Helicobacter pylori*. Exposure to environmental agents and occupational risks, as pesticides, radiation, hair dyes, nutrition and others have extensively been studied, however, conclusive data are missing. Conclusion: The NHL incidence has been strikingly rising in the past decades. Changes in the NHL classification and diagnostic tools may have contributed to this phenomenon. Infectious agents are pathogenetically involved in lymphomagenesis, however, their and other environmental agents' and occupational risks' influence on changing the NHL incidence are as yet unresolved.

334 GA-67 UPTAKE: A PREDICTOR OF ACTIVE MINIMALY RESIDUAL DISEASE AND CLINICAL OUTCOME IN PATIENTS WITH DIFFUSE LARGE CELL LYMPHOMA

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Background: Most of the patients with diffuse large cell lymphoma (DLCL) can achieve complete remission (CR) after multidrug therapy, but evaluation of the presence or absence of a MRD following treatment still represents a difficult diagnostic problem. So, 67Ga scan has been considered a good technique for monitoring response after treatment of patient with lymphoma.

Aim: The purpose of this study was to investigate 67Ga scan performed after treatment as a means to predict active minimally residual disease and outcome.

Methods: Sixty-three patients with DLCL were studied with CT and 67Ga scan at the end of intensive chemotherapy.

Results: After treatment, 29 patients had a negative restaging CT. Of these patients, 15 had a positive 67Ga scan, 9 of whom (60%) relapsed; 14 had negative 67Ga scan of whom 1 (7%) relapsed. The 5-year relapse-free survival rate was 92% for those with negative scans compared with 33% for gallium-positive patients ($p < 0.001$). At the same time, 34 patients had a positive restaging CT. Of these patients, 17 had a positive 67Ga scan, 13 of whom (76%) relapsed; 17 had negative 67Ga scan of whom 5 (29%) relapsed. The 5-year relapse-free survival rate was 70% for those with negative scans compared with 23% for gallium-positive patients ($p < 0.004$).

Conclusion: 67Ga scan after intensive chemotherapy is a good predictor of active minimally residual disease and clinical outcome of patients with DLCL.

335 DEVELOPMENT AND VALIDATION OF A CLINICAL PREDICTION RULE FOR BONE MARROW INVOLVEMENT (BMI) IN PATIENTS WITH HODGKINS LYMPHOMA (HL)

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Background: BMI is rare in patients with HL, with an incidence varying between 4% and 14% in reported series. The indications for bone marrow (BM) biopsy in HL are not well established. Many centers perform BM biopsy in all patients, while others use various selection criteria. The use of bilateral BM biopsy may increase the yield of positive results by 16-33%. Aims: To develop a clinical prediction rule for BMI and validate it in an independent patient population. Patients and Methods: We developed a clinical prediction rule for BMI based on 826 HL patients from a single center, who were evaluated with respect to their baseline clinical, histological, and laboratory features. Univariate analysis was based on Fishers exact test. Logistic regression analysis was used to identify independent predictors for BMI and construct the prediction rule. The rule was subsequently validated in 654 patients from 4 other centers. An 25% increase in the yield of positive results by using bilateral biopsies was assumed. Results: Independent prognostic factors for BMI were: x1: B-symptoms, x2: Stage III/IV prior to biopsy, x3: anemia (hemoglobin levels <13 and <11.5 g/dl in males and females), x4: leukocytes <6x10⁹/l, x5: age >=35 years, and x6: iliac/inguinal involvement. Each factor was graded as xi=1, if present, or xi=0, if absent. A simplified score Zs=8x1+6x2+5x3+5x4+3x5+3x6-8 was assigned to each patient, using logistic regression analysis. If patients with Zs >= 0 were considered to have BMI, the sensitivity, specificity, positive and negative predictive value of this prediction rule was 97.8%, 51.5%, 10.6%, and 99.8% respectively. In the validation group they were 98.1%, 40.3 %, 12.7%, and 99.6%. Thus, among 403 and 243 patients who were predicted to be BM negative (e.g. had Zs <0) in the original and the validation group respectively, only one in each group was actually positive. According to the risk of BMI the patients were classified into 3 groups: Low risk (Zs <0, 44% of patients, 0.3% risk of BMI), standard risk (Zs 0-9, 37% of patients, 4.2% risk of BMI), and high risk (Zs >= 10, 20% of patients, 25.5% risk of BMI). If 1000 bilateral biopsies were performed, <1, 11 and 64 additional cases of BMI would be expected to be demonstrated (as compared to unilateral BM biopsy) in low, standard and high risk patients respectively. Conclusions: Based on these data, bone marrow biopsy could be omitted in 44% of HL patients, who are considered as low risk, with a misclassification rate of only 0.3%. Standard risk patients should be staged with unilateral biopsy, but high risk patients may benefit from bilateral biopsy.

336 LYMPHOMA OF THE ORAL CAVITY. TEN-YEAR EXPERIENCE OF A SINGLE HAEMATOLOGY UNIT

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Background: Lymphomas of the oral cavity consist <5% of the malignancies of the region. B-cell lymphoma of the diffuse large cell histology is the predominant type (B-DLCL). Aims: To describe the clinicopathologic characteristics of a group of patients with oral cavity lymphomas and their treatment outcome. Methods: We reviewed the records of 46 patients (21 males and 25 females) with oral lymphomas who were diagnosed and treated in our Service, between 1993 and 2003. Results: The mean age was 57 years and the most prevalent site of involvement was the tonsil (41.3%) followed by the tongue (15.2%), while 8.7% had >1 involved sites. Thirty six patients (pts) (78.3%) had clinical stage I or II and 10 (21.7%) advanced stage disease. A monoclonal lymphocytic population was detected in the blood of 7 pts (15.2%) by PCR (IgH or TCR rearrangement), 4 of them with early stage lymphoma. 56.5% of the lesions were diagnosed as B-DLCL and only 10.8% as peripheral T-cell lymphomas.

Nineteen pts with B-DLCL received CHOP or equivalent chemotherapy regimens and 11 CHOP combined with anti-CD20. Chlorambucil was administered in 4 pts with MALT lymphoma and local radiotherapy as the sole treatment in 1. The pts with T-cell lymphoma received CHOP (3) or interferon alfa-2b (2). Thirty eight pts are in complete remission. There were 3 deaths due to the disease (1 MALT, 1 predominantly small cleaved cell and 1 B-DLCL). Three additional pts relapsed and achieved

complete response with a second chemotherapy course and 2 pts developed a lymphoma of different histology.

Conclusion: We conclude from the above study that oral lymphoma is a rare disease of mainly a diffuse large B-cell histology and a prolonged survival when treated with chemotherapy (10-year Event Free Survival 82.5%).

337 RISK OF NON-HODGKINS LYMPHOMA AND HEPATITIS C VIRUS INFECTION

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Background. Several recent studies have reported a high rate of hepatitis C virus (HCV) infection in non-Hodgkins lymphoma (NHL). HCV prevalence has been reported to be higher (15%) in patients with B-NHL than both in the general population and in other hematological malignancies.

Aim of the present study was to evaluate B-NHL risk associated with HCV infection in Sardinia, a geographical area where HCV infection is endemic. Design and methods. We conducted a case-control study on 153 newly diagnosed B-NHL cases, aged 25-74 years, consecutively admitted to the participating haematological departments from 2000-2003, and 125 population controls, randomly selected from population registers, and frequency matched to cases by sex, age, and residence. Type and stage of B-NHL were defined according to the REAL/WHO classification. Both cases and controls were interviewed using a questionnaire exploring medical and behavioural history, social and demographic characteristics, and environmental and occupational exposures. Testing for HCV antibodies was performed in serum samples using enzyme-linked immunosorbent assay (AXSYM System, ABBOTT, Germany) in duplicate.

Results. Anti-HCV antibodies were detected in 14/153 (9.1%) B-NHL patients. The highest anti-HCV antibodies prevalence was observed among diffuse large B-cell lymphoma (DLCL; 8/53, 15.1%) patients, and follicular lymphoma (2/16, 12.5%) patients. HCV positive cases with small lymphocytic lymphoma/LLC were 3/55 (5.4%), a prevalence comparable with that observed among population healthy controls (6.4%). Among the other 29 cases, including 6 Marginal Zone Lymphoma, 4 MALT, 4 Mantle cell lymphoma, 1 Burkitts Lymphoma, and 14 unspecified B-cell Lymphoma, we detected HCV antibodies only in the Burkitts Lymphoma patient. After adjusting by sex, age and education, the Odds Ratio (OR) for B-NHL associated with positive HCV antibodies was not significantly elevated (OR = 1.5; 95% C.I. = 0.4, 5.0). However, when limiting the analysis to DLCL cases, we observed a 2.6 fold excess risk (OR = 2.6; 95% C.I. = 1.5 4.5).

Conclusion. We found a positive association of HCV infection with B-NHL, which was mostly accounted for by a 2.6 fold excess risk among DLCL patients. The prevalence of HCV positive subjects among cases affected by other B-cell lymphomas was comparable to that observed among population controls. Our results extend those of previous epidemiological studies suggesting a role for HCV infection in lymphomagenesis, and represents the first report of a HCVNHL positive association in a geographic area with similar dietary habits and homogenous genetic background.

Funding: MIUR, CEE V Framework, Compagnia San Paolo di Torino

338 CORRELATION BETWEEN CLINICAL, HISTOLOGICAL, SKIN PCR DATA AND CLINICAL OUTCOME IN PATIENTS WITH EARLY STAGE MYCOSIS FUNGOIDES TREATED WITH LOW DOSE INTERFERON COMBINED WITH PUVA.

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Background: The combination of interferon alpha (IFN-alpha) and PUVA therapy has been reported to be effective in the treatment of early mycosis fungoides (MF).

Aims: Up to now controlled prospective studies exploring the activity and tolerability of the combination with low dosages IFN-alpha and evaluating further clinical outcome are lacking

Methods: We herein present a multicentric prospective phase II clinical study enrolling 89 early MF patients (22 patients in stage IA, 55 IB, 11 IIA and 1 patient in stage IIB), who were treated for 14 months with low dose IFN-alpha (6-18 MU weekly) and PUVA.

Results: A 84% complete remission (CR) and a 98% overall response rate were obtained; the response was reached rapidly with a median time of 4 months and without significant toxic effects. CR was associated at 6 months with a histologically non confluent skin infiltrate (p=0.044), and at 14 months with high epidermal CD1a+ dendritic cell density (p=0.030). Local recurrences with minimal skin disease were observed in 28 cases and responded well to further therapy with low-dose IFN-alpha. A high CD8+ lymphoid T cell density was the only factor associated with a lower relapse rate (p=0.002). All the other factors analysed (age, sex, stage, serum LDH, TCR- gamma gene rearrangement) did not affect clinical response or relapse.

Relapse-free survival curves showed that half of the patients would probably suffer of relapses within 5 years and 80% of the patients within 9 years. The median RFS was significantly shorter in patients with low CD8+ skin density compared with patients with high CD8+ skin density (36 vs 49 months; p=0.04).

Conclusions: The combination of IFN-alpha and PUVA seems to be an effective and well tolerated alternative approach to the more commonly used treatments, i.e. topical chemotherapy, PUVA and total electron beam radiation; a high density of epidermal DCs and CD8+ lymphocytes seem to be an optimal basis to suppress neoplastic cell proliferation and prevent relapses. However, the curative potential of the combination is questionable and probably limited only to 20% patients, who show long standing remissions. Further studies are needed to evaluate the advantage of a prolonged maintenance therapy with low dose IFN-alpha.

339 BCL-2/IgH REARRANGED CELLS IN THE BLOOD AND BONE MARROW OF PATIENTS WITH STAGE I/II FOLLICULAR LYMPHOMA. POSSIBLE CLEARANCE AFTER LOCAL RADIOTHERAPY

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Background: Localized forms of follicular lymphoma (FL) have a better prognosis than the more frequent advanced-stage forms, being curable in 40-50% of cases with local radiotherapy. The accurate definition of truly localized forms is therefore crucial in order to offer an appropriate treatment to patients. Technical devices employed for non-Hodgkins lymphoma (NHL) staging have evolved during the last years increasing the accuracy of disease extension definition. Polymerase chain reaction (PCR) of Bcl-2/IgH rearranged cells provides for the majority of FL patients a sensitive measure of minimal NHL cell contamination in the peripheral blood (PB) and bone marrow (BM).

Aims: To evaluate the role of PCR detection of Bcl-2 positive cells in the PB and BM of patients otherwise considered stage I/II FL and the impact of treatment.

Methods: Between April 2000 and December 2003, 23 consecutive patients with follicular NHL, stage I/IIA according to the Ann-Arbor classification, entered the study. Median age was 56 years, ranging from 27 to 74; 11 were males and 12 females. Histology was centrally revised, confirming in all patients the diagnosis of FL according to the REAL/WHO classification. In 7 of the 23 patients, the diagnosis was FL grade I, in 15 grade II and in 1 grade III. Involved field radiotherapy was performed in all patients (total dose ranging from 30 to 40 Gy). In 2 of them, radiotherapy was preceded by chemotherapy according to the CHOP regimen for three courses. In 1 patient, the anti-CD20 monoclonal antibody (Mabthera) was administered, at the conventional dose of 375mg/m² x 4 doses after radiotherapy. PCR was evaluated in the BM and PB of all patients at diagnosis, re-evaluated 3 months after treatment and, thereafter, every 6 months.

Results: At diagnosis, Bcl-2+ cells were found in the BM and/or PB in 16 of the 23 patients studied; of these, 15 had a major break-point region (MBR) rearrangement and 1 a minor cluster region (mcr) rearrangement. After treatment, in 8 out of 14 Bcl-2+ cases studied, a disappearance of PCR positive cells was observed in the BM and PB, which still persists after a median follow-up of 27 months (range 1-47), while 6 are still persistently positive.

Conclusions: The results of this study indicate that in the majority of stage I FL patients at presentation, PCR analysis can document the presence

of viable Bcl-2+ cells in the BM and PB. A durable disappearance of Bcl-2+ cells from the BM and/or PB samples can be obtained in a high proportion of patients treated with local radiotherapy alone.

340 ROLE OF WHOLE-BODY POSITRON EMISSION TOMOGRAPHY USING FLUORODEOXYGLUCOSE (FDG-PET) IN THE EVALUATION OF RESIDUAL MASS IN HODGKINS DISEASE: A MONOCENTRIC EXPERIENCE

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Background: A common problem in response evaluation of Hodgkin's disease (HD) is the presence of a residual mass after completion of therapy. In fact, in approximately two-thirds of patients a residual mass is present, whereas only about 20% of these will relapse. FDG-PET has emerged as an important tool to monitor response to treatment in lymphomas.

Aims: To evaluate the sensitivity, specificity, positive and negative predictive value of FDG-PET performed at restaging in HD patients with a residual mass following treatment.

Methods: We retrospectively analysed the clinical data from 31 patients with HD in whom a residual mass was documented by CT-scan after the completion of treatment. At least one FDG-PET was performed. M/F ratio was 14/17; median age was 29 years (range 19-61); 2 patients (6%) were stage I, 12 (39%) stage II, 8 (26%) stage III and 9 (29%) stage IV. 21 patients (68%) presented B symptoms. Chemotherapy consisted of ABVD or ABVD/OPP x 6 cycles in 16 patients (52%), ABVD x 4 cycles in 11 patients (36%), ABVD x 2 cycles in 1 patient (3%), BEACOPP x 8 cycles in 1 patient (3%) and ABVD x 8 cycles in 2 patients (6%). 26 patients (84%) received consolidation radiotherapy.

Results: In 19 out of 31 patients (61%), PET proved negative: of these, 18 patients (95%) are in continuous complete remission (CCR) at a median time of 4 months from completion of treatment and 1 patient (5%) has relapsed 3 months later. The remaining 12 patients (39%) resulted PET positive: 2 of them (16%) are in CCR 7 months after therapy, 5 patients (42%) underwent a biopsy that confirmed HD persistence of disease in 4 and fibrosis in 1; 5 patients (42%) underwent treatment because of progression confirmed by CT-scan. Sensitivity, specificity, positive and negative predictive value were 90%, 86%, 75% and 95%, respectively.

Conclusions: Our data confirm the experience of other groups that FDG-PET has a high sensitivity, specificity and negative predictive value, while the positive predictive value is lower (75%). Thus, a negative FDG-PET identifies patients who have an excellent prognosis, while FDG-PET positivity for a residual mass needs to be confirmed by further evidence of disease persistence. By combining FDG-PET with morphological studies (CT-scan) it is possible to achieve better results. To date, FDG-PET should not substitute other investigations, but rather represent a complementary study capable of offering additional information. It is a promising modality, but prospective studies with a long follow-up are required to confirm and possibly improve our present restaging work-up.

341 THE COMBINED PET/CT SCANNER - AN INVALUABLE AID IN THE MANAGEMENT OF COMPLEX LYMPHOMA PATIENTS FROM NORTHERN IRELAND.

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Background: The availability of accurately aligned, whole body anatomical (CT) and functional (PET) images are having a significant impact on diagnosing and staging both Hodgkin and non-Hodgkin's lymphoma.

Aim: We set out to review the first cohort of haematology patients scanned from July 2002 to July 2003.

Methods: Using the haematology consultant radiology codes, all haematology patients who received the combined scan had their data collated retrospectively.

Results: A total of 44 scans; 15 patients with Hodgkin's (34%) and 29 patients with non-Hodgkin's lymphoma (66%). The indications for scanning were prechemotherapy (4.5%); mid chemotherapy (6.8%) if conventional CT suggests a sub optimal response; post chemotherapy (77.2%) seven of whom had minimal residual disease on CT alone; and

post autologous transplant (14.7%) assessment. A total of 38.6% was PET positive, while 61.4% had no uptake at all. A total of 28 patients were confirmed in remission. Of those PET positive, 3 patients had a repeat scan following BEAM autograft which was negative, while a further 2 patients whose mid chemotherapy scan showed poor response from first line therapy, had their treatment switched to salvage chemotherapy and gain had repeat PET/CT scans negative. Six of the seven patients with residual disease on CT had negative PET uptake. The seventh had PET positive residual disease and was transplanted with her subsequent scan PET negative.

Summary/Conclusion: PET/CT has been shown to predict at an early stage good prognosis patients who are likely to be 'cured' with standard chemotherapy from those patients with poorer prognosis who require alternative treatment. The future may necessitate it as the gold standard investigation at all stages of therapy for most types of lymphoma.

342 LYMPHOMAS IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY: THE SINGLE CENTER EXPERIENCE

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The Common Variable Immunodeficiency (CVID) is one of the most frequent primary immunodeficiencies. The usual age of presentation is the second and third decade of life. CVID is characterized by defective antibody formation which causes recurrent bacterial sinopulmonary and intestinal infections. The patients with CVID are prone to autoimmune disorders and malignancies.

In this study we describe the cases of four patients with CVID, who developed lymphoproliferative diseases. All the patients were males between the ages of 16 and 55 years. They developed two Hodgkin's diseases HD and two Non Hodgkin's Lymphomas NHL/Diffuse Large B Cell Lymphomas respectively. Among them, the first two had CVID diagnosed 10 and 12 years ago while the patients with NHL identified the immunodeficiency almost at the same time with the NHL. In all cases the malignancy was extensive, presenting multiple extranodal infiltrations. Two patients with HD and NHL achieved a complete response to the treatment. Between them, one patient with HD remains disease free six years after the end of treatment. The other two with HD and NHL had not responded to the treatment and they died 1 and 3 years later respectively.

LYMPHOMA: TREATMENT WITH CHEMOTHERAPY

343 AVERAGE RELATIVE DOSE AND DOSE-INTENSITY IN PATIENTS TREATED FOR DIFFUSE LARGE B-CELL LYMPHOMA : A RETROSPECTIVE STUDY IN BELGIUM (1995-2000)

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Aims: To describe the applied chemotherapy (CT) regimen and the average relative dose (ARD) and dose-intensity (ARDI) for patients (pts) treated first line for diffuse large B-cell lymphoma (DLBCL).

Methods : Charts of 373 adult patients from 29 centers receiving at least 3 cycles of first line treatment for DLBCL, in or out of a protocol setting between 1995 and 2000, were retrospectively reviewed. ARD and ARDI analysis (delivered vs planned) was only performed for cyclophosphamide (C) and anthracyclines (A) as they are considered to be the most important agents. An overall analysis and stratifications for age, International Prognostic Index (IPI) score and treatment in or out of a study protocol were performed.

Results : Out of 373 pts, an analysis population of 348 pts treated with CHOP-like, ACVBP-like or CHVmP-BV as the most common regimens was identified. CHOP-like was the most used CT-regimen (273 pts, 79%) of which CHOP-21 (210 pts) was the most frequently used; followed by ACVBP (57 pts, 16%) (with ACVBP-14 (46 pts) as the most important type) and CHVmP-BV (18 pts, 5%). For all regimens the relative total dose (RTD) effectively received was 96% for C and 94% for A, respectively (CHOP: C (96%), A (93%); ACVBP: C (100%), A (98%) and CHVmP-BV: C (100%), A (91%)). Overall, the relative total dose intensity (RTDI)

scored 92% for C and 90% for A (CHOP: C (93%), A (91%); ACVBP: C (88%), A (87%) and CHVP-BV: C (95%), A (87%)). This results in an ARD of 94% (SD 14%) for CHOP, 99% (SD 4%) for ACVBP and 95% (SD 16%) for CHVP-BV and an ARDI of 92% (SD 13%) for CHOP, 88% (SD 13%) for ACVBP and 91% (SD 16%) for CHVP-BV, respectively. 10% of the pts did not receive an ARD > 80% (CHOP: 12%, ACVBP: 2% and CHVP-BV: 6%) and 16.5% of pts had an ARDI below 80% (CHOP: 15%, ACVBP: 26% and CHVP-BV: 11%). Overall, 45% of the pts had dose modifications (dose delays (DD), dose reductions (DR) or both): 39% DD (CHOP: 39%, ACVBP: 39% and CHVP-BV: 44%) and 15% DR (CHOP: 16%, ACVBP: 3.5% and CHVP-BV: 22%). Hematological toxicity was the most important reason for CT-adjustment: 44% (CHOP: 43%, ACVBP: 56%, CHVP-BV: 25%), followed by non-hematological toxicity (35%) and other reasons (11%). In total, 56% of pts received granulocyte colony-stimulating factor (G-CSF) treatment (CHOP: 47%, ACVBP: 100%, CHVP-BV: 61%). The difference in survival between pts treated with greater or less than an 80% ARDI was not statistically significant ($P=0.357$). Whether pts were treated in or out of a protocol setting made no difference in relation to ARD and ARDI. Conclusions: Lymphoma pts treated in academic and non-academic hematological departments in Belgium benefit from optimal treatment with respect to dose-intensity. CHOP-like is the most frequently used CT regimen, followed by ACVBP and CHVP-BV. The RTDI effectively received was 92% for C and 90% for A. 56% of all pts and 100% of pts treated with ACVBP are treated with G-CSF.

344 AN OUTPATIENT SALVAGE APPROACH FOR NON-HODGKIN'S LYMPHOMA (NHL) AND HODGKIN'S LYMPHOMA (HL) INCORPORATING RISK-FACTOR STRATIFICATION AND TREATMENT ESCALATION

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Background: We have previously demonstrated significant anti-lymphoma activity using an outpatient-based salvage approach comprising vinorelbine and gemcitabine with an overall response (CR + PR) rate of 55%.

Aims: We now report interim results of a similar outpatient approach for both NHL and HL but incorporating patient stratification and treatment escalation.

Methods: Patients were stratified into Group 1 (G1 - good risk - first relapse following durable first remission - follicular NHL>12 months, all other NHL sub-types and HL>6 months); Group 2 (G2 - poor risk primary refractory, second or subsequent relapse, or non-durable first remission); or Group 3 (G3 - post-transplant relapse following any form of stem cell transplant therapy). Two chemotherapy regimens were evaluated. VGF (vinorelbine 25mg/sqm days 1 and 8, gemcitabine 1000mg/sqm days 1 and 8, pegfilgrastim 6mg SC day 9); F-GIV (same as VGF but with ifosfamide 3000mg/sqm day 1 with mesna uroepithelial protection). G1 and G3 commenced therapy with VGF and G2 with F-GIV. Following 2 cycles of therapy all patients were planned for interim re-staging. Any patients demonstrating disease progression were removed from trial. Responsive patients (>50% reduction in all previous sites of disease and gallium or FDG-PET negativity where baseline positivity was demonstrated) received 2 further cycles of the same therapy, the remainder escalated therapy with F-GIV (G1 and G3) or IVAC (G2) (inpatient treatment with ifosfamide, VP-16 and Ara-C).

Results: To date 45 of a planned 90 patients have been accrued (G1 = 16, G2 = 23, G3 = 6) with a median age of 56 years (range, 18-75). Diagnoses at entry were HL, n = 9 (nodular sclerosing = 8, mixed cellularity = 1) and NHL, n = 36 (diffuse large cell = 24, follicular = 6, others = 6). So far G1 and G2 have received 44 and 72 cycles of VGF and F-GIV, respectively, with cycle 1 grades 3/4 neutropenia or thrombocytopenia occurring in 31% and 17% (VGF) and 74% and 78% (F-GIV) of patients, respectively. Significant non-haematological toxicities were uncommon. Febrile neutropenia, hospital admission, treatment delay or dose-reductions occurred with 7%, 20%, 7%, 0% and 17%, 29%, 6%, 14% of VGF and F-GIV cycles, respectively. There has been 1 treatment-related death. Fourteen of 16 G1 (2 data not yet available) and 22 of 23 G2 (1 not yet evaluated) patients were evaluable for the mandatory interim response analysis with 57% and

59%, respectively, achieving response. Six post-transplant patients (G3) have been treated on protocol and have received 13 and 5 cycles of VGF and F-GIV, respectively, with no unexpected toxicities. Based on published standardised response criteria overall response (CR + CRu + PR) after 2 to 4 cycles of treatment is 54% (n = 41).

Conclusion: Based on this interim analysis we conclude that both VGF and F-GIV can be safely administered on an outpatient basis and show activity against advanced lymphoma. The impact of stratification and escalation remains to be determined.

345 AUTOLOGOUS TRANSPLANTATION FOR AGGRESSIVE NON-HODGKIN'S LYMPHOMA: 13 YEAR EXPERIENCE AT A SINGLE INSTITUTION

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High-dose chemotherapy (HDT) and hematopoietic stem cell transplantation (HSCT) is considered standard of care for patients with relapsed chemotherapy sensitive aggressive non-Hodgkin's lymphoma (NHL). We analyzed the results of 268 patients with relapsed aggressive NHL who underwent HDT and HSCT at our institution between 1/1990 and 5/2003. RESULTS: Fifty seven percent of the patients were males. The immunophenotype was B-cell in 72% of patients and T-cell in 13% (unknown 15%). Nineteen percent (52 patients) had transformed histology. Median age at transplantation was 48 years (range, 16-75 years), and median time from diagnosis to transplant was 1.6 years (range, 0.3-32 years). Fifty-six percent of patients had an IPI score of ≥ 1 at the time of transplantation and 32% had elevated serum lactate dehydrogenase levels (LDH). The median number chemotherapy regimens administered prior to transplantation was 3 (range, 1-9). The disease was sensitive to the chemotherapy administered immediately prior to transplantation in 185 patients (69%) and only 25% of patients were in CR/CRu (PR, 44%; SD, 10%, PD, 19%) at the time of transplantation. The conditioning regimen was BEAM/BEAC in 175 patients (65%) and Carmustine/cyclophosphamide/thiotepa in 62 patients (23%). Fifty-nine percent of patients received PBPC the source of stem cells. The median follow-up of patients who are still alive at the time of this analysis is 50 months (range, 0.4-148). At 10 years the overall survival (OS) and progression-free survival (PFS) for the entire cohort were 37% (95% CI, 30-46) and 24% (95% CI, 17-31) respectively. The median survival for patients who had an IPI score of 0 at the time of transplantation had not been reached at the time of this analysis, whereas patients who had an IPI score of 0-4 had a median survival of 21.5 months. We analyzed the following prognostic factors for OS and PFS; T or B cell histology, gender, age at transplantation, LDH, IPI score, transformation, number of prior chemoregimens, disease stage at transplant, chemosensitivity, response prior to transplant, conditioning regimen, response to transplant procedure, cell type, and period of transplantation (before or after 1996). Patients who did not achieve a CR (or CRu) with HSCT had a median OS of 7.6 months, whereas the median OS for patients who achieved a CR/CRu has not been reached. On multivariate analysis LDH (> normal) at the time of transplantation was associated with a worse PFS and IPI ≥ 1 and BM as source of stem cells was associated with a worse OS. CONCLUSIONS: In the present study IPI score and source of stem cells impacted on overall survival. No differences were observed in patients with T-cell versus B-cell immunophenotype, or with transformed versus de novo aggressive lymphomas. Disease relapse continues to be a major cause for failure after autologous transplantation and continued efforts should be made to improve the transplant procedure. Integration of monoclonal antibodies and radioimmunoconjugates into the pretransplant preparative regimen and allogeneic transplantation may improve the long-term outcomes and are being explored.

346 HIGH EFFICACY OF BRIEF DURATION HIGH INTENSITY MULTI AGENT CHEMOTHERAPY FOR TREATMENT OF IMMUNOCOMPETENT ADULT PATIENTS WITH BURKITT'S AND BURKITT-LIKE NON-HODGKINS LYMPHOMAS

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Background :Burkitt's and Burkitt-like NHL are aggressive lymphomas that are rare in immunocompetent adults . Treatment of adult patients with these lymphomas using intermediate grade regimens usually results

in sub-optimal outcome. **METHODS:** Between 1/1995 and 12/2002, 26 consecutive, previously untreated patients with the diagnosis of Burkitt or Burkitt-like NHL were treated at our institution using a brief, high intensity multi agent chemotherapy program described below. **RESULT:** 19 (73%) were male and 7 (27%) were female. Median age was 25.5 (15-66) years. Staging by Murphys system was stage I in 7 pts, II in 7 pts, III in 10 pts, and IV in 2 pts. 13 (50%) pts presented with extra nodal disease (31% intestinal). The median LDH value was 502 (range 251-4114).

Chemotherapy Schedule: see table 1

CNS prophylaxis was given to all patients. G-CSF was used in Block A or B, or both in 46%. The main toxicity was myelosuppression, other toxicities were minor. One patient died shortly after starting treatment secondary to sepsis. Out of 25 evaluable pts, 22 (88%) achieved CR, 2(8%) had PR, and 1 had progressive disease. At 5 years, 19 patients (73%) are alive and 17 (65%) are disease free.

Patients with stage I and stage II had OS, and DFS of 100%, while patients with stage III had OS of 50% and DFS of 40%. One patient with stage IV, died in CR after Block B secondary to infection and the other died secondary to progressive disease.

Univariate analysis of relevant clinical factors identified the following variables as statistically significant prognostic factors for DFS: LDH at presentation ($p=0.013$), age at diagnosis ($p=0.034$), and stage of the disease ($p=0.043$). Due to the relatively limited sample size, multivariate analysis was not attempted.

CONCLUSION:

The result of this program compares favorably with other reported treatment programs particularly for a better outcome for stage I and II disease patients.

Patients with stage III disease with high risk features and stage IV disease should be considered for more aggressive post remission treatment and SCT.

	ALL PT n=26	GF A n=15	GF B n=11
Median (SD) number of cycles of chemotherapy	1.4 (1.2)	2.1 (1.3)	1.1 (1.0)
% of patients planned chemotherapy dose of 0.15	35.0	73.3	36.4
% of patients cycles delayed	2.3	2.0	0.0
% of patients cycles reduced	26.7	13.3	27.3
Hospitalisation due to neutropenia (% evaluable pts)			
CR (%)	0.0	0.0	0.0
PR (%)	0.0	0.0	0.0
Progressive disease (%)	0.0	0.0	0.0
Death (%)	0.0	0.0	0.0
Survival at 5 years (%)	73.0	86.7	63.6
Survival at 10 years (%)	65.4	73.3	54.5

Caption 1: table 1

347 LOW DOSE SINGLE SHOT 2-CHLORODEOXYADENOSINE (2-CDA) IN PATIENTS WITH HAIRY CELL LEUKEMIA

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Hairy-cell leukemia (HCL) is an uncommon B-cell chronic lymphoproliferative disorder that accounts for about 2% of all leukemias. Typically, the disease follows an indolent, but progressive course and the majority of patients (pts.) require treatment for life-threatening infections due to pancytopenia or symptomatic splenomegaly. 2-CDA, a purine analogue, is considered the treatment of choice for HCL. We previously performed a phase II trial (SAKK 32/93) with one cycle of 2-CDA at a dose of 0.14 mg/kg/day by subcutaneous (s.c.) bolus injection for five consecutive days in 62 pts. with HCL. Complete (CR) and partial remissions (PR) were seen in 47(76%) and 13(21%) patients, respectively, for a response rate (RR) of 97%. However, the administration of 2-CDA to pts. with HCL results in a high frequency of drug-induced severe neutropenia and neutropenic fever. We present here the results of a phase II trial with a low dose single shot 2-CDA (SAKK 32/95).

Patients and methods: A total of 74 pat. were recruited to the pilot trial (n=15) and the subsequent phase II trial (n=59). 68 pts. had a classical HCL, 6 pts. the polymorphocytic subtype. There were 54 men and 20 women with a median age of 58 years (range 34-96 y.). 24 pts. were pretreated

(splenectomy n=10, radiotherapy n=1, interferon alpha n=17, chemotherapy n=9). Prior treatment with purine analogues was not allowed. The median duration from diagnosis of HCL to the first 2-CDA course was 0.25 y. (range 0-18.5 y). The median baseline level of WBC was 3.15 G/l (1.2-20.7 G/l), platelets 88.5 G/l (16-299 G/l), hemoglobin 116 g/l (57-163 g/l). Pts. received 2-CDA as a single shot at a dose of 0.25 mg/kg as a s.c. bolus injection. Response was assessed at days 29 and 71. In case of no or minor response at day 71, pat. received one or two standard courses of 2-CDA.

Results: The RR at day 29 or day 71 after the single shot 2-CDA was 23% (95% CI 14-34%). The maximum number of cycles given to pts. was: 1 cycle in 29 pts., 2 cycles in 40 pts. and 3 cycles in 5 pts (cycle 1: single shot, cycle 2 or 3: standard dose). The total overall RR over all cycles was 66%. Grade 3/4 toxicities occurring in cycle 1 included: Leucocytopenia 52%/12%, neutropenia 32%/41%, thrombocytopenia 10%/7%, anemia 11%/7%, lymphocytopenia 44%/33%, infections 8%/4%.

Conclusion: The infection rate with a low dose of 2-CDA is lower compared with reported data from studies with standard dose 2-CDA. A single shot of low dose CDA induces a RR of only 23% and cannot be recommended for the treatment of HCL.

348 CHEMOTHERAPY-RELATED NEUTROPENIC EVENTS IN PATIENTS WITH NON HODGKIN'S LYMPHOMA - A COMPARISON OF TWO EUROPEAN AUDITS

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Background: European data on chemotherapy (CT) related neutropenic events (NE) and their consequences in patients with non Hodgkin's lymphoma remain sparse. Retrospective audits were conducted in Belgium (Lymphodose 02) and the UK (Audit of Lymphoma Patients).

Aims: To assess the incidence and predictors of CT-induced NE, the occurrence of low average relative dose intensity (ARDI $\leq 85\%$), and the association of NE with low ARDI.

Methods: Variables available in both datasets were merged into a dataset of individual observations to allow for combined analyses. Definitions were harmonised. Cut-off points used were $\geq 15\%$ for CT dose reductions and ≥ 7 days for CT dose delays. NE were defined as neutropenia-related dose delay; dose reduction; or hospitalisation. Multivariate adjusted odds ratios (ORs) were calculated by robust multiple logistic regression allowing for clustering by audit.

Results: The Belgian study included 373 patients with a mean age SD of 61.7 15.3 years and a diagnostic spread of stage I 22%, II 29%, III 18%, IV 31%. The UK audit contributed 125 patients with a mean age SD of 58.0 14.9 years and a diagnostic spread of stage I 15%, II 31%, III 24%, IV 30%. In the Belgian study, CT regimens used were CHOP-like in 73%, ACVBP-like in 16%, CHVMP-BV in 5%, and other regimens in 6%. In the UK audit, CHOP-like regimens were used in 77%, PACEBO/PACEBOM/PMitCEBO in 21%, and other regimens in 2%. Concomitant radiotherapy was recorded in 2% of the Belgian patients vs 11% of the UK patients. CT dose reductions were seen in 24% (Belgium) vs 35% (UK), and neutropenia-related hospitalisations in 29% (Belgium) vs 10% (UK). NE and repeated NE occurred in 39% and 20% of the Belgian patients. The respective figures for the UK audit were 30% and 8%.

NE occurrence was independently associated with age; reduced cycle length; the number of CT cycles planned; and CT regimen type. Details are shown in Table 1.

Mean ARDI SD was 90.0% 16.4% in the Belgian study vs 94.4% 15.0% in the UK audit ($p = 0.010$). Low ARDI occurred in 26% vs 18% of patients ($p = 0.067$). NE and ARDI were associated. ORs were 4.4 (Belgium, CI 2.6-7.5) and 2.9 (UK, CI 1.0-8.3). Multivariate adjustment at the combined dataset level resulted in an overall OR of 4.3 (CI 3.3-5.5).

Summary/conclusions: In both audits, NE and low ARDI occurred in a relevant proportion of patients. Increased occurrence of NE in the Belgian study may in part be attributable to more aggressive CT regimens being used and increased dose reductions in the UK. NE impair chemotherapy delivery in up to a quarter of patients. NE occurrence may be ameliorated by targeting preventive CSF at patients being at increased risk.

On behalf of the Impact of Neutropenia in Chemotherapy European (INC-EU) Study Group

Robust logistic regression allowing for clustering by audit			N = 471
Pseudo R squared of the model = 0.09			
Independent influence	OR (95% CI)	p-value	
Age	1.03 (1.02-1.03)*	< 0.0005	
Cycle length	0.90 (0.88-0.92)**	< 0.0005	
No. of CT cycles planned	1.19 (1.13-1.27)***	< 0.0005	
PACEBO/PACEBOM/PMICEBO regimen	0.99 (0.74-1.32) ****	0.942	
ACVBP-like regimen	6.96 (4.53-10.72)*****	< 0.0005	
CHVMP-BV regimen	2.46 (2.18-2.78) *****	< 0.0005	
* Per additional year of age			
** Per additional cycle day			
*** Per additional cycle planned			
**** Compared to CHOP-like			

Caption 1: Table 1. Independent influences on NE occurrence

349 PEDIATRIC BURKITT LYMPHOMA TREATED WITH LMB PROTOCOLS

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BACKGROUND: Burkitt lymphoma (BL) is one of the fastest growing malignancies in humans. BL and L3 acute lymphoblastic leukemia (ALL) are different forms of the same disease (B-cell disease). Prognosis of stage III/IV BL according to Murphy's classification and FAB L3 ALL was dramatically improved during the two last decades through a very intensive and short duration chemotherapy.

AIMS: The aim of our study was to characterize the clinical features, outcome and complications associated to LMB protocols, in children with BL or L3 ALL, in our Center.

PATIENTS AND METHODS: Between January 1993 and February 2004, sixteen white previously healthy children (15M, 1F) with a median age of 4.7 years (range 2.6-10) were diagnosed and treated with LMB protocols (3 LMB84, 4 LMB89 and 9 LMB96) at the Pediatric Hospital of Coimbra, Portugal. Most children (13/16) presented with advanced BL (7/16 with stage III; 6/16 with stage IV) and two others with L3-LLA. Presentation features included abdominal pain, weakness and anorexia (44%); vomiting and abdominal enlargement (31%); facial edema (25%); bone pain (19%). The primary sites of involvement were abdomen in 9/16, head and neck in 5/16. Hepatomegaly and splenomegaly were observed in 25 and 19% of patients, respectively; pleural effusion in 31%, ascitis in 25%. Bone marrow was involved in 3 patients with BL at diagnosis. CNS involvement was documented in 1 case. Only one child presented with renal impairment. Elevated lactic dehydrogenase was present in 81% and hiperurycemia in 31%.

RESULTS: Induction therapy was performed in our Intensive Care Unit in 12 children, with a high risk of tumour lysis syndrome, which occurred in 5, with acute renal failure in 4. Neutropenia WHO grade IV occurred in all children, thrombocytopenia WHO grade IV in 8/16 and gastrointestinal toxicity WHO grade III/IV in 5/16. All children received erythrocyte transfusions and platelet transfusions were reported in 7/16. All children required growth factor support. One child died with sepsis, due to multiresistant *Pseudomonas aeruginosa* infection, during induction chemotherapy of LMB89 protocol. All the others 15 children achieved complete remission. One patient had CNS relapse 2 months post-LMB96 protocol, underwent autologous stem cell transplant and is now in complete remission. Overall survival is 94% and disease-free survival is 87.5% after a median follow up of 60 months (range 2-132).

CONCLUSIONS: Intensive chemotherapy with LMB protocols and modern supportive care result in a high cure rate of childhood Burkitt lymphoma, in patients with advanced disease.

350 TREATMENT OF HIGH-RISK MEDIASTINAL DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) WITH MEGACHOP AND AUTOLOGOUS TRANSPLANT (ASCT)

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This work is sponsored by a grant from the Ministry of Public Health of the Czech Republic No. XC-8231/3

Background. Treatment of mediastinal DLBCL with age-adjusted IPI 2 or 3 with a standard CHOP-like regimen is unsatisfactory, with only 25-50% long-term survivors. Most of the relapses occur up to 2 years after diagnosis.

Aim. To increase long-term survival of mediastinal DLBCL with age-adjusted IPI 2 or 3 by treatment including high-dose CHOP induction and consolidation with autologous transplant.

Methods. 24 patients with high-risk mediastinal DLBCL diagnosed on the basis of histology and clinical presentation (7 men, 17 women, median age 28 years, range 21-58) were scheduled to one of three following regimens: First regimen (4 pts) included 3 cycles of MegaCHOP (Cyclophosphamide, 3 g/m² d 1, Vincristin 2 mg d 1, Doxorubicin 75 mg/m² d 1, Prednisolone 60 mg/m² d 1-5, G-CSF 5 ug/kg from day 6) and BEAM (BCNU, 300 mg/m², AraC, 1600 mg/m², Etoposid, 800 mg/m², Melphalan, 140 mg/m²) with autologous transplant. Second regimen (10 pts) included 3 cycles of MegaCHOP, followed by 3 cycles of ESHAP (Etoposide, 60 mg/m² d 1-4, Methylprednisolone 500 mg d 1-4, Cisplatin, 25 mg/m² d 1-4, AraC 2 g/m² d 4) and BEAM with ASCT and third regimen (10 pts) was the same as second, only with four doses of Rituximab 375 mg/m² added to chemotherapy. Radiation treatment was added if residual mediastinal mass persisted after autologous transplant.

Results. 16 pts (66%) had IPI 2 and 8 pts (33%) IPI 3. 18 pts (75%) finished the planned schedule. The reason for treatment modification was progression on treatment, toxicity or failure to mobilize stem cells (2 patients each). 20 pts (83%) received radiation therapy. There were two progressions on-treatment; one of the patients was successfully salvaged with double autologous transplant. Two progressions occurred off-treatment: one patient was salvaged with second transplant and one patient who did not received up-front ASCT was salvaged with rituximab-containing regimen (R-ICE) and second-line ASCT. So far, there was no progression in patients treated with Rituximab upfront. The actuarial 2-year survival is 96% and actuarial progression-free survival is 83% after median follow-up of 22 months for living patients (range, 4-66).

Conclusion. High-dose CHOP induction and ASCT consolidation seems to be beneficial to high-risk patients with mediastinal DLBCL. The role of radiation therapy and Rituximab is to be further explored.

351 A(E)BVD CHEMOTHERAPY (CT) PLUS LOW DOSE INVOLVED FIELD RADIATION THERAPY (RT) FOR EARLY CLINICAL STAGE HODGKINS LYMPHOMA (HL): THE EFFECT OF RADIATION DOSE ON RELAPSE FREE SURVIVAL (RFS)

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Background: Early stage HL is highly curable by ABVD-based combined modality therapy (CMT). However the optimal amount of CT and more importantly- the optimal field and dose of radiotherapy (RT) are not well defined yet. **Aims:** To present the long-term experience of our Unit with the use of ABVD or EBVD (E=epirubicin) plus low dose involved field RT (LD-IF RT) in the treatment of clinical stage (CS) IA/IIA HL. **Patients and Methods:** We analyzed 357 patients with a median age of 30 years (14-82). Among them 55% were males, 65% had nodular sclerosis, 62% CS IIA, 17% bulky mediastinal mass (32% bulky disease overall), 5% E-disease, 10% pure infradiaphragmatic disease, and 23% ESR 50. According to the German Hodgkins Lymphoma Study Group classification 54% of the patients had intermediate stage HL (51% unfavorable according to EORTC). The mean SD follow-up of surviving patients was 75 48 months. Complete responders (CRs), CRs unconfirmed (CRus) or very good partial responders (VGPRs, defined as >90% reduction of tumor load) were scheduled for LD-IF RT (3200 cGy). **Results:** RT was not delivered in 6% of the patients and a median of 2800 cGy was given to 94% of them. Among the latter 82% (n=276) actually received LD-RT after CR/CRu/VGPR and formed the basis of this study. There was no difference with respect to baseline characteristics among patients who received <2800 cGy and 2800-3200 cGy. Patients who received <2800 cGy had inferior RFS compared to those who received 2800-3200 cGy (10-year rates 84% vs. 97%, p=0.002). This difference was at least in part attributed to inferior IF control rate in the lower

dose group (10-year IF relapse rate 0% vs. 5%, $p=0.01$). There was no difference in relapse rates for patients treated with 2800-3000 cGy and 3001-3200 cGy. In multivariate analysis, both the delivery of <2800 cGy [relative risk (RR) 7.2, $p=0.009$] and E-disease (RR 8.0, $p<0.001$) were independent prognostic factors for relapse. However OS did not differ between CR/CRu/VGPRs who received <2800 cGy or 2800-3200 cGy (10-year rates 98% vs. 95%, $p=0.22$). Only 2/19 relapses developed exclusively in previously involved and irradiated areas. Two cases of secondary acute leukemia developed after ABVD plus salvage therapy. None of the 3 secondary solid tumors developed within the RT fields. Conclusions: IF-RT at a dose of 2800-3000 cGy is highly effective in CS IA/IIA HL patients, who achieve a CR/CRu/VGPR with A(E)BVD. These RT doses are probably superior than doses < 2800 cGy, partly due to better involved field control rate. The long-term toxicity with respect to secondary malignancies appears to be acceptable, but needs further follow-up to be accurately assessed.

352 MANAGEMENT OF PRIMARY CUTANEOUS T- AND B-CELL LYMPHOMA WITH INTERFERON ALPHA-2B (IFN-A). A SINGLE CENTER EXPERIENCE

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Background: Primary cutaneous lymphoma comprise a wide range of rare diseases of which the commonest T- cell subtypes include mycosis fungoides (MF), Sezary syndrome (SS) and peripheral T-cell lymphoma (PTCL) while follicle center cell lymphoma (FCL), diffuse large B-cell lymphoma (DLBL) and extranodal marginal zone B-cell lymphoma (MZL) represent the B-cell subtypes. The behavior of these diseases is different from nodal NHL and it has been shown that IFN- α can produce long-lasting remissions in early stages MF. On the contrary, there are no reports on the efficacy of IFN- α for primary cutaneous B-cell lymphomas (CBCL). Aims: To present our experience on IFN- α treatment for patients with both primary T-cell and B-cell cutaneous NHL. Patients and Methods: Forty-one patients, 25 with primary cutaneous T-cell NHL (CTCL) (16 MF, 4 SS, 5 PTCL) and 16 with CBCL (4 MZL, 3 FCL, 8 DLBL, 1 lymphoblastic NHL) were included in this study. Of the 25 CTCL patients, 20 were males (median age 48y); 16 received IFN- α as first line treatment, 5 after PUVA and 4 after failure of other treatment modalities. Of the 16 CBCL patients, 8 were males (median age 57y); 14 received IFN- α frontline and 2 after treatment failure. Three to 5 MU IFN- α were administered subcutaneously daily with paracetamol prophylactically 1 hour before injection and two hours after. Results: 17 out of 24 CTCL patients responded (overall response rate 71%, 42% CR and 29% PR) (12/16 MF, 1/4 SS and 3/5 PTCL). Median time to response was 4 months (1-17). Median duration of response was 15 months (8-38). 11 patients relapsed, 1 responder rapidly discontinued treatment because of side effects (cataract), the others are still under treatment with IFN- α . Among patients with CBCL 12/14 responded (overall response rate 86%, 64% CR and 22% PR). Median time to response was 2 months (1-4) and median response duration 23 months (13-30); 4 patients relapsed, 1 stopped treatment because of side effects (depression), another continued with Peg- IFN because of chronic fatigue and the others are still under IFN- α treatment. With respect to histologic subtype, all 4 MZL patients responded; 2/3 FCL and 4/8 DLBL also responded. Flu-like syndrome was observed in the majority of patients at treatment initiation and 60% of patients presented mild leukopenia. Conclusions: IFN- α is an effective treatment in primary cutaneous NHL and is usually well tolerated. As already reported response rate is satisfactory in MF. The response observed in CBCL, especially in low-grade subtypes but also in half of DLBL patients has not been reported previously.

353 HIGH CLINICAL AND MOLECULAR RESPONSE RATES WITH FLUDARABINE, CYCLOPHOSPHAMIDE AND MITOXANTRONE (FCM) IN PREVIOUSLY UNTREATED PATIENTS WITH ADVANCED STAGE FOLLICULAR LYMPHOMA (FL)

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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 analyze in a phase II trial the efficacy of the FCM regimen in previously untreated younger pts with advanced stage FL.

Methods: Between July 1999 and December 2003, 122 pts <65 yrs (56M/58F; median age: 51 yrs), with FL (grade I, 52 cases; grade II, 42; in review, 20) 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). Cotrimoxazole prophylaxis was used until CD4 recovery >300 per mL. In addition to clinical studies, minimal residual disease (MRD) was assessed in peripheral blood (PB) and bone marrow (BM) by real-time PCR technique detecting bcl-2/JH rearrangement at MBR and mcr breakpoints.

Results: At the last update (September 2003), 75 pts had completed the 6 cycles of FCM, with 66 of them (88%) having received >90% of the scheduled doses. Overall, 497 courses of FCM have been administered. Grade III/IV neutropenia and thrombocytopenia was observed in 6% and 0.4% of the courses, respectively. Five episodes of grade III/IV infection were observed, including neutropenic fever (3 cases), pneumonia (1) and progressive multifocal leukoencephalopathy (PML) after the 2nd FCM cycle (1). Three pts died during therapy due to causes most likely not related to FCM (PML, meningioma and bronchoaspiration). Up to now, no late toxicity has been observed. After 3 cycles of FCM, 40/75 pts (53%) achieved CR. At the end of the treatment, 60/75 pts were in CR (80%), 13 in PR (17%) and 2 (3%) were refractory to therapy. Bcl-2/JH analysis was possible in 103 patients, with 64 of them being bcl-2/JH+ (58 at MBR, 8 at mcr). An excellent correlation was observed between BM and PB MRD assessment ($R=0.84$; $p<0.0001$). The amount of cells carrying the bcl-2/JH at diagnosis dramatically decreased after 3 and 6 courses of FCM (at BM: 3.9% 8.9, 0.0097% 0.04, and 0.0078% 0.03, respectively; $p<0.0001$). At the end of therapy, 22 of 30 (73%) patients with available samples had cleared bcl-2/JH+ cells from BM and PB. Molecular follow-up after treatment was available in 23 pts (median follow-up: 12 months). Out of 15 pts t(14;18)-MBR negative after FCM, 9 maintained the PCR negativity, whereas 6 reverted back to MBR positive with low PCR levels (10⁻³ to 10⁻⁴). Two pts t(14;18)-MBR positive after FCM were persistently PCR positive until clinical relapse at 6 and 8 months after treatment. With a median follow-up of 16 months, 11 pts have progressed, with a 24-month failure-free survival of 76% (95%CI: 64-88).

Conclusions: FCM is highly effective in patients with advanced FL. This regimen should be compared to other, more conventional regimens (e.g., CVP, CHOP). Also, whether anti-B cell monoclonal antibodies (e.g., rituximab) add efficacy to FCM should be investigated.

MULTIPLE MYELOMA: CONVENTIONAL TREATMENT

354 OUTCOME OF PATIENTS WITH MULTIPLE MYELOMA AND HYPERTENSION TREATED WITH ANGIOTENSIN-I CONVERTING ENZYME INHIBITORS AT THE TIME OF AUTOLOGOUS STEM CELL TRANSPLANTATION

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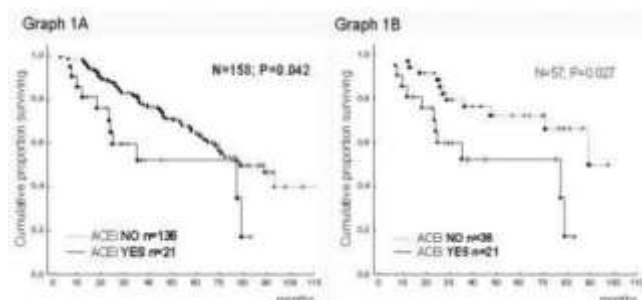
Multiple myeloma (MM) is an incurable disease with an incidence of approximately 4 in 100,000. Angiotensin-I converting enzyme inhibitors (ACEI) belong among the most commonly used antihypertensives. In a retrospective study, we analysed the impact of angiotensin-I converting enzyme inhibitors (ACEI) taken at the time of autologous stem cell transplantation (ASCT) on the outcome of patients with multiple myeloma.

Patients with multiple myeloma who underwent ASCT at the Brno University Hospital from 1995 to 2002 (n=158) were studied retrospectively. Of those, 57 had hypertension as evidenced by hospital discharge notes from chemotherapy or ASCT. Patients taking ACEI at the time of ASCT (i.e. immediately before ASCT and/or immediately after ASCT) were allocated to the ACEI group (n=21). All patients had the hypertension well controlled and had a normal ejection fraction. The induction chemotherapy consisted of 4 cycles of VAD (vincristine, doxorubicin, and dexamethasone). For stimulation, cyclophosphamide was given in a single dose and was followed by G-CSF until harvest. High-dose melphalan was given prior to the infusion of autologous graft. Ninety days after ASCT, maintenance therapy was instituted with interferon-alpha with or without interposed cycles of dexamethasone.

The groups were similar in terms of age, myeloma stage and type, maintenance treatment, diabetes mellitus occurrence, and renal failure occurrence (A and B stage). The ACEI taken by the patients in our study were enalapril in doses 5-20mg/day (n=13), captopril 50 mg/day (n=1), quinapril 20 mg/day (n=1), perindopril 4 mg/day (n=1), lisinopril 10 mg/day (n=1), trandolapril 2 mg/day (n=1), and ramipril 1.25-5 mg/day (n=3). All patients were taking the ACEI for a period of more than 4 months, except one patient who was taking it for two months prior to ASCT. For most patients, ACEI were a part of permanent medication. The overall survival (OS) and progression-free survival (PFS) for all transplanted patients (n=158) was 78.0 and 32.6 months, respectively.

Patients taking ACEI (n=21) had worse OS (40.4 months) compared to patients not taking ACEI (n=137; OS 78.6 months; p=0.042; Graph 1A). Among hypertensive patients, OS was significantly shorter in the ACEI group than in the non-ACEI group (n=36; OS 89 months; p=0.027; Graph 1B). The OS of all patients with hypertension (n=57) was not statistically different to that of all patients without hypertension (n=101; p=0.847). Only one cardiovascular death was reported in the hypertensive patients during the follow-up.

In conclusion, we are the first to report on the adverse effect of ACEI in patients with MM undergoing ASCT. We have found that patients with MM and hypertension treated with ACEI at the time of ASCT have significantly worse overall survival compared to patients with MM receiving other or none antihypertensive drugs. The mechanism of action of ACEI in MM is unclear. Our findings might lead to the reevaluation of the role of ACEI in the pathophysiology of MM, and to the reconsideration of the role of ACEI as a part of antihypertensive medication in MM.



Caption 1: Overall survival of patients taking ACEI

355 A PHASE I/II STUDY OF XCELLERATED T CELLS AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Previous studies have demonstrated an association between survival & lymphocyte recovery following autologous transplantation (Porrata et al., Blood 2001). Furthermore, in a murine model of myeloma, adoptive transfer of activated T cells following bone marrow transplantation has demonstrated a marked anti-tumor effect in vivo (Hou Fowler, BBMT 2003).

Aims: We are conducting a trial in the transplant setting to evaluate the activity of T cells activated and expanded ex vivo with the Xcellerate(TM) Process, which uses anti-CD3 and anti-CD28 antibody coated magnetic beads.

Methods: Following induction therapy, patients undergo leukapheresis to collect peripheral blood mononuclear cells for the Xcellerate Process. Patients then undergo stem cell mobilization and collection, followed by high dose melphalan (200 mg/m²). Three days following peripheral blood stem cell infusion, patients receive 50-100 x 10⁶ Xcellerated T Cells. Results: 36 patients have been treated with median f/u of 205 days post transplant (range 72-415). Using a new bioreactor process instituted during the trial, T cells expanded 268 + 101 fold (mean + SD), with 93.6 + 0.8 x 10⁶ cells infused, & final product 98.5 + 2.3% T cells (n = 19).

There have been no Grade 3 or 4 acute infusional toxicities. Days of neutropenia and thrombocytopenia have been 5 (3-43) and 4 (0-88+) respectively [median (range)]. Lymphocyte recovery has been rapid, with counts reaching > 500/mm³ generally within 1-2 days following T cell infusion. Historically, lymphocyte recovery to > 500/mm³ usually does not occur for 3 or more weeks post-transplant. The T cell repertoire measured 25 days following T cell infusion demonstrates a more normal pattern than prior to T cell infusion (n = 5). This is in contrast to the severe skewing of T cell receptor diversity normally seen in myeloma patients following autologous stem cell transplantation (Mariani et al, BJH 2001). In 25 patients with median f/u of 180 (14-360) days, preliminary best responses to date are: 16% CRs, 40% VGPRs, 36% PRs, 4% SD and 4% with PD, using the pre-induction M-protein as reference.

Conclusions: Xcellerated T Cell therapy leads to rapid lymphocyte recovery and appears to restore a normal T cell receptor repertoire. Additional follow-up is needed to determine if this will translate to improved clinical outcomes.

356 RECOMBINANT HUMAN ERYTHROPOIETIN AND THE RISK OF THROMBOSIS OF MULTIPLE MYELOMA PATIENTS RECEIVING THALIDOMIDE

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Background: Thalidomide (Thal) is widely used in patients with multiple myeloma (MM). Deep vein thrombosis (DVT) is one of its most serious side effects, whose prevalence ranges from 5%, when Thal is used alone, to approximately 25% when the drug is administered together with adriamycin-containing regimens. Recently, three cases of DVT have been reported among seven patients with myelodysplasia treated with Thal and Erythropoietin (Epo).

Aim: Since recombinant human Epo (rhEpo) is commonly used in MM patients with anemia, we retrospectively analysed a series of 193 consecutive patients with MM who received Thal for at least one month, to evaluate whether rhEpo administration increased their rate of thrombosis. Patients and Methods: Patients were 106 males and 87 females, aged 38-88 years (median, 66) at the beginning of Thal treatment. Hundred and forty-four patients (75%) received Thal for relapsing/resistant MM,

the others as maintenance (18%), or as first line therapy (7%), alone or in combination with melphalan/prednisone or vincristine/adriamycin/desamethasone treatments. Median duration of Thal treatment was 6.8 months (range, 1-41). Thal was given as single agent in 81 cases (42%) and in combination with steroids in the other 112 (58%). Forty-seven patients (24%) received rhEpo, 10,000 units thrice weekly, at some time during Thal treatment.

Results: Sixteen of 193 patients with multiple myeloma experienced objectively documented thrombotic complications, none fatal. There were 13 DVT of the lower limbs, and 3 arterial thrombosis (two intestinal ischemic events, and one myocardial infarction). The overall prevalence was 8.3 percent, and the incidence rate 9.46 percent patient-year. The events occurred 1.7-14.7 months from Thal initiation. All but one thrombosis occurred in refractory/resistant patients. Five patients stopped definitively Thal treatment, and other 3 transiently after thrombosis.

The daily Thal dosage of the 16 patients with thrombosis ranged from 50 to 300 mg (median, 125 mg). Three thromboses occurred to patients during rhEpo therapy (6%), and the other 13 were recorded among patients not receiving rhEpo (9%) ($p =$ not significant; Odds Ratio, 95% CI 1.43, 0.39-5.25). Twelve thrombosis occurred to patients on steroid treatment (11%), and the other 4 to patients not receiving steroids (5%) ($p =$ not significant; Odds Ratio, 95% CI 2.31, 0.72-7.44). One event was recorded among the 11 patients who were treated with Thal and chemotherapy ($p =$ not significant; Odds Ratio 1.11, 95% CI 0.36-25.43). One patient was heterozygous for the G1691A mutation of the factor V gene, and in another case DVT was a recurrence in spite of oral anticoagulation in the appropriate PT INR range 2.0-3.0.

Conclusion: rhEpo does not increase the risk of thrombosis of patients receiving Thal for relapsed/refractory or de novo MM.

357 REMISSION STATUS AT FIRST AUTOTRANSPLANT IS A PROGNOSTIC FACTOR OF BETTER SURVIVAL IN MULTIPLE MYELOMA

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Background: High dose chemotherapy with peripheral stem cell support is the best treatment for patients with newly diagnosed multiple myeloma (MM). Compared to conventional chemotherapy, it prolongs the complete remission (CR) rate up to 50%, and the overall survival (OS) up to about a median of 5 years.

Aim: We evaluated the event-free survival (EFS), OS, and prognostic factors of newly diagnosed MM patients treated with Total Therapy I (TT-I) (Barlogie et al., 1997).

Patients characteristics: 422 new cases of MM were diagnosed at our Institution from 1993 until June 2002. Hundred twenty were enrolled in the TT-I program and received at least one autoTx. They were 60 males and 60 females, median age 57 years (range 35-67), followed-up for a median time of 44 months (range 5-117). Clinical staging: IIA (n 15), IIB (n 2), IIIA (n 94), IIIB (n 9). Type of myeloma: IgG (n 69), IgA (n 23), light chain (n 19), non secretory (n 8), plasma cell leukemia (n 1). Laboratory details at diagnosis, median values: serum creatinine, 1 mg/dl (0.46-10.6); Beta2-M, 2.9 mg/dl (0.13-16.8); CRP, 0.4 mg/dl (0.1-14); LDH, 325 U/L (175-631); serum calcium, 9.3 mg/dl (7-14.7). Conventional cytogenetic (or FISH for chromosome 13 deletion) analysis was performed in 60 patients: 36 had a normal karyotype, 3 cases had del 13q-, 1 case had del 11q-, 3 others had a complex karyotype, and in the remaining 17 the assay was not informative. After endoxan (7 gr/sm), all patients mobilised sufficient peripheral hematopoietic stem cells for 2 autoTx.

Results: one hundred and one patients (84%) completed the program. The other 19 received only one autoTx for the following reasons: HBV hepatitis (n 10), severe polyneuropathy (n 1), cardiomyopathy (n 1), progression and death (n 3), allotransplant (n 3), bad performance status after first transplant (n 1). Response to treatment, i.e., CR, very good partial remission (VGPR), partial remission (PR), no response (NR), (Blad et al, 1998), was evaluated after:

See table 1

Median OS was 82 months (43% at 118 m.), median EFS was 42 months (21% at 118 m.).

By univariate and multivariate analysis, patients status after induction therapy was the major determinant of both EFS and OS:

See table 2

Conclusion: The majority of patients achieved CR/VGPR after the 1st and the 2nd autoTx with an acceptable toxicity. However, only patients in CR/VGPR before the 1st autoTx have a significantly prolonged EFS and OS. This suggests the need to improve the induction therapy in order to increase the rate of early response.

	CR/VGPR	PR	NR
Induction	26%	52%	22%
1 st autoTx	69%	25%	6%
2 nd autoTx	84%	15%	1%

Caption 1:

	CR/VGPR	PR/PR	P
EFS	41% at 102 m.	15% at 118 m.	0.018
OS	77% at 102 m.	34% at 118 m.	0.015

Caption 2:

358 THE COMBINED IN VITRO ACTIVITY OF THALIDOMIDE AND ZOLEDRONIC ACID INDUCES NFKAPPAB-MEDIATED ANTIPROLIFERATIVE EFFECTS ON MULTIPLE MYELOMA CELLS

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Background

Thalidomide is an established treatment for relapsed and refractory multiple myeloma (MM) and possesses significant antiangiogenic and immunomodulatory activities. Likewise, the direct antitumor effect of zoledronic acid has been demonstrated, not only in MM cells, but also in breast, prostate and pancreatic cell lines. Zoledronic acid is an inhibitor of the mevalonate pathway.

Aims

To examine the in vitro activities of zoledronic, thalidomide and combinations thereof, on the survival and proliferation of a myeloma cell line, the HS-Sultan. To test whether putative drug effects are mediated by possible alterations on the activity state of the leukemogenic transcription factor NF-kB.

Methods

MM-derived Cell line and Culture conditions: The MM cell line HS-Sultan was purchased from the European Collection of Cell Cultures (ECACC, UK). HS-Sultan cells were cultured in RPMI-1640 medium (GIBCO BRL) supplemented with 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C in a 5% CO₂ humidified atmosphere.

Drugs: Thalidomide (Sigma-Aldrich Corp.) and Zoledronic acid (Novartis Pharmaceuticals Corp.) were dissolved in dimethylsulfoxide and distilled water, respectively.

Measurement of cell-line proliferation rate: The HS-Sultan cell line was cultured at an initial concentration of 10⁴ cells/ml/well. Drugs (thalidomide, zoledronic acid) were added to the wells at various concentrations. After 6 days of incubation, viable cell number was estimated using a Sysmex K-1000 counter (Japan). All experiments were done in triplicate.

Electrophoretic Mobility Shift Assays: Cells (10⁶/ml) were incubated for 20min with drugs, pelleted and washed (x3) with cold PBS. Subsequently, cells were resuspended in 0.2ml of a hypotonic lysis buffer and subjected to sonication for 4sec. NaCl was added to a final concentration of 300mM and samples were incubated on ice for 30min. After centrifugation at 100,000g, protein extracts were recovered in the supernatant. Binding reactions containing 200ng of oligonucleotide probe (end-labeled with γ-P-ATP) and 3µg of total protein extract were conducted at room temperature (30 min) in a total volume of 12µl of binding buffer and 0.6µg of polydIdC. The samples were run in a 4% native polyacrylamide gel, transferred to Whatman paper and visualized by autoradiography.

Results

As can be seen in Fig.1 (above), the antiproliferative effect of either thalidomide (Th) or zoledronic acid (Z) on the proliferation of the HS-Sultan cell line, is only moderate even at high drug concentrations. In contrast, combinations of 10µM thalidomide with 50µM or 100µM zoledronic acid resulted in an inhibition of cellular proliferation by 74% and 93%, respectively.

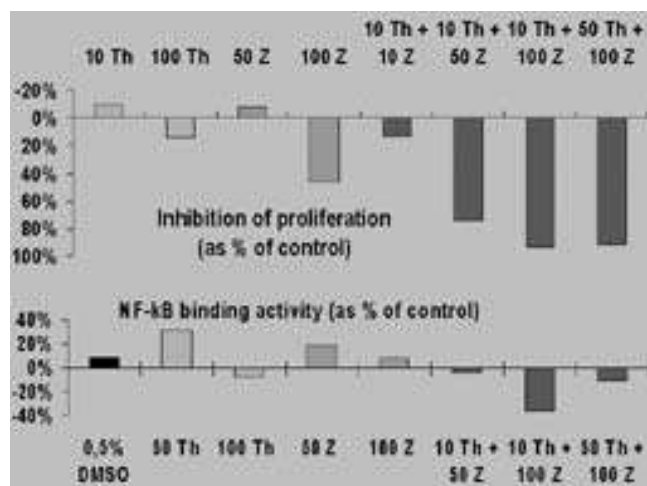
Similarly, the binding activity of the NF-kB transcription factor is significantly reduced when the two drugs are combined (Fig.1, below),

causing a reduction of the NF- κ B band intensity of 39% at 10 μ M thalidomide plus 100 μ M zoledronic compared to control value.

Conclusions

Our experimental data suggest that zoledronic acid enhances the direct antitumor effect of thalidomide. Interestingly, the two drugs showed synergism at concentrations near to those measured in vivo in plasma and bone. Thalidomide has been previously shown to interfere with NF- κ B binding activity. Zoledronic acid enhances this effect.

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Caption 1: Image 1 (drug concentrations in micromoles)

359 AUTOLOGOUS STEM CELL TRANSPLANTATION AS FIRST LINE AND SALVAGE TREATMENT FOR MULTIPLE MYELOMA: A SINGLE CENTRE EXPERIENCE

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Background: High dose chemotherapy (HD-CT) followed by stem cell transplantation (SCT) has become the standard treatment for multiple myeloma (MM) patients aged less than 65 years. The French IFM-group has shown that double tandem transplantation is superior to single SCT but it is still not proven if a second transplant at relapse could maintain or improve these results.

Aims: We have retrospectively analyzed our centre experience of a therapy program including a second on demand transplant for patients relapsed after a first SCT.

Methods: Between August 1993 and March 2003, 139 consecutive patients with symptomatic or progressive MM were eligible for the high-dose program: median age 58 (36-70), M: 69, F: 70; M-component: 53% IgG, 19% IgA, 4% IgD, 20% light chain, 4% non secretory; stage I: 9%, II: 40%, III: 49%, plasma cell leukaemia 2%; stage B: 9%. After debulking with 2-4 VAD courses, peripheral blood stem cells (PBSC) were collected by using cyclophosphamide 4-7 g/m² + G-CSF (in 95 pts.) or two DCEP courses (in 44 pts.). The conditioning regimen was melphalan 200mg/m². 9 patients were treated with a second tandem transplant and they were excluded from the survival analysis. At relapse, a second salvage HD-CT was administered to patients in good performance status and with sufficient PBSC. The response was assessed according to EBMT criteria; overall survival (OS) and event free survival (EFS) were calculated from the beginning of VAD therapy.

Results: 116/139 (83%) patients in permitting clinical conditions and with sufficient number of PBSC actually underwent auto-SCT. Median time from initiation of VAD and transplantation was 6.8 months. Median PBSC infused was 5.0 10⁶/kg. 68/107 patients have relapsed after the first single SCT: a salvage autologous SCT was performed in 24 cases, an allogeneic SCT was performed in 1 case and 2 patients underwent a second autologous SCT followed by a non-myeloablative allotransplant. With a median follow-up of 35.4 months, median OS and EFS were 65.4 and 27.7 months respectively. Median OS and EFS from the second salvage transplantation were 42.6 and 12.7 months. The best response attained after the first SCT was: complete remission (CR) 22%, partial remission (PR) 64% with 24% very good PR, minimal response (MR) 6%;

after salvage auto-SCT the response was: CR 4%, PR 58%, MR 20.8%. The early transplant related mortality (within 60 days) after the first SCT was 2.8% and no early deaths occurred after the second auto-SCT.

Conclusions: A second salvage transplantation is safe and effective in prolonging the survival of myeloma patients. Comparing our findings with the data obtained with tandem transplantation (Barlogie, 1999; Attal, 2003) we observed that OS was comparable, although CR rate and EFS were inferior. These results raise the question of the most appropriate timing for a second transplantation; further studies should be performed to define a subgroup of patients that could benefit from this more conservative approach.

360 EARLY RESPONSE PREDICTS THALIDOMIDE EFFICIENCY IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA

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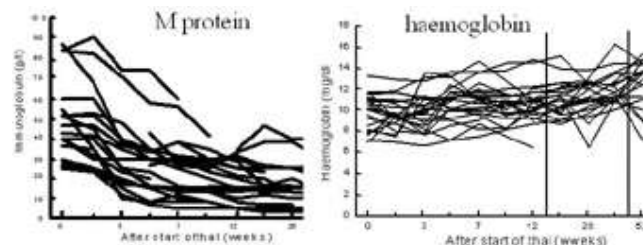
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We studied the initial response of the M protein and haemoglobin after treatment with thalidomide to sixty-five patients who were primary or secondary refractory to melphalan/prednisone or other type of chemotherapy, or relapsed within 6 months after high dose chemotherapy with stem cell support. Thalidomide was given at a dose of 200 mg/day escalating to 800 mg. Response was defined as >25% reduction of M protein combined with a clinical response. Analyses of M protein and haemoglobin was done after 1,3,5,7,9,12,16,20,24 and 52 weeks. Decline of M-protein started early and at least a 25% reduction of M-protein could be detected in 14 of 20 responders (70 %) after 3 weeks. Although 67% of non responders also had a decline of M protein after 3 weeks, there was a marked quantifiable difference between responders and non-responders. After 5 weeks, 20 of 22 responders (91%) had achieved a 25% reduction. Reduction of M-protein continued for 3 months and further decline was seen in only 4 patients. On the other hand, haemoglobin showed a different pattern as any major increase did not occur until 3 months and continued to increase after 6 months. These observations will enable the identification of responders and non-responders at an early stage. The combination thalidomide/dexamethasone may increase response rates over thalidomide alone, but may also produce higher incidence of side-effects. To optimise the effect and minimise the side-effects our observations provide a rationale for starting with thalidomide alone, adding dexamethasone if there is no decline in the M protein after 3 weeks.



Caption 1:

362 RESULTS OF AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA IN AUSTRIA. AN ANALYSIS BASED ON DATA FROM THE AUSTRIAN STEM CELL (ASCT) REGISTRY

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Background:

Median survival with conventional therapy in multiple myeloma is usually less than 40 months. Two prospective randomised trials (IFM 90 and MRC trial) showed superiority of high-dose therapy with autologous stem-cell transplantation over conventional treatment, although other trials were unable to confirm a statistical significant benefit. Here we report the results of high dose chemotherapy in all patients (> 99.5%) with multiple myeloma treated in Austria.

Patient and Methods:

Data on 324 patients treated with autologous stem cell transplantation from 1989 onwards reported to the Austrian Stem Cell Transplantation Registry (ASCT) have retrospectively been analysed. Median age of patients was 55.6 years (range 24.6 - 70.7 years). Source of stem cells was peripheral blood in 317 patients, while only 7 patients received bone-marrow derived stem cells. Hundred-and-seventy patients were treated with single and 98 with double transplantation, while all other patients received >2 autografts, during the whole course of their disease. The influence of total body irradiation (TBI) and interferon maintenance was also analysed. All but one patient were evaluable for survival, and 265 patients were evaluable for response.

Results

A complete response by conventional criteria (without proof of negative immunofixation) was achieved in 37% and a PR in 53% of patients, yielding an OR rate of 90%. Transplant related mortality was 2.5% at 3 months, and median survival 66.5 months at a median follow-up of 14.2 months. Double transplantation yielded a significantly longer overall survival as compared to single transplantation ($p < 0.05$; survival at 1 year 86% vs. 71%, respectively). There was a tendency for a shorter survival in patients receiving total body irradiation as compared to patients receiving conditioning without TBI (survival 51 months vs. not reached, $p = n.s.$). Preliminary analysis shows significant prolongation of maintenance duration and survival with interferon maintenance treatment, but final analysis of a matched pair analysis will be presented.

Conclusion:

High dose chemotherapy with autologous stem cell transplantation revealed a favourable survival (66.5 months) in patients with multiple myeloma of which only a minority have been treated in clinical trials in Austria. Double transplantation was associated with a significantly longer survival, while use of TBI was associated with a tendency towards shorter survival. Preliminary data on interferon maintenance treatment suggest beneficial effects on maintenance duration and on survival. Results of matched pair analysis will be presented.

363 THE COMBINATION OF MELPHALAN, CYCLOPHOSPHAMIDE, PREDNISONE (MCP) FOR TREATING PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINEMIA

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INTRODUCTION: Waldenström's macroglobulinemia (WM) is a rare lymphoproliferative disorder characterized by high levels of IgM immunoglobulin, lymphoplasmocytic bone marrow infiltration, lymphadenopathy and splenomegaly. Despite standard therapy for patients with symptomatic macroglobulinemia is considered the oral administration of alkylating agents, reports about treatment are scanty, and due to the low prevalence of WM, most of the series are based on a low numbers of patients. We report here the results obtained in newly diagnosed WM patients using an oral chemotherapeutic regimen combining melphalan, cyclophosphamide and prednisone (MCP). **PATIENTS AND METHODS:** From July 1973 to April 2002, 100 newly diagnosed consecutive WM patients were observed at the Hematology of the University 'La Sapienza' of Rome. Of these, 28 were asymptomatic and did not require any treatment. The remaining 72 patients (46 males and 26 females) were treated because of the presence of anemia, hyperviscosity syndrome, hemorrhagic manifestations, significant cytopenias or extensive lymphadenopathy and/or hepatosplenomegaly. The laboratory and clinical characteristics of the treated patients were:

median age 61 years (42-87)

Median Monoclonal IgM (range) 3.8 mg/dl (1.5-6.7)

Hb (range) 12g/dl (6.5-16)

Hb <10 gr/dL 37 %

Lymphadenopathy 10%

Splenomegaly 37%

Hyperviscosity syndrome 20%

Treatment consisted of melphalan (6 mg/ m²) + cyclophosphamide (125 mg/ m²) + prednisone (40 mg/ m²) administered orally from day 1 to 7 of each course, repeated every 4-6 weeks for a total of 12 courses. Maintenance treatment consisted of daily chlorambucil (3 mg/m²) and prednisone (6 mg/m²) until progression. Only patients who completed at least one course of treatment were considered evaluable. Responding and stable patients received the programmed maintenance treatment until progression. **RESULTS:** Among 72 treated patients, only one

patient was lost to follow-up few days after initiation of therapy; therefore, a total of 71 patients were available for response to therapy. A partial response, defined as a reduction > 50 % in the serum IgM initial levels, was achieved in 55/71 (77.4%) evaluable patients and 9 of these (14.6%) had a reduction > 75% in the serum IgM level. Moreover additional 7 (9.8 %) patients had a reduction < 50 % but > 25% of the initial IgM levels with disappearance of all clinical symptoms and a reduction in the size of lymphadenopathy, splenomegaly. The remaining 9 (12%) patients had disease progression, including one patient who developed a histologic transformation in non-Hodgkin aggressive lymphoma. After a median follow up of 71.8 months (range 3-195), median overall survival is 66 months with 13/71 patients still alive and 10 still responding. Median event free survival is 47 months. Overall response and Freedom from progression are 64 and 55 months, respectively. The therapy was well tolerated and acute toxicity was limited to transient nausea, vomiting and mild neutropenia. **CONCLUSION:** This chemotherapeutic oral treatment is efficacious and safe for treating patients with WM and results in overall survival are not worse than those obtained with intravenous more aggressive and expensive protocols based on new drugs.

MULTIPLE MYELOMA: NEW TREATMENTS

364 EFFECT OF BORTEZOMIB ON BONE DISEASE IN MULTIPLE MYELOMA

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Bone resorption is increased in multiple myeloma (MM) due to increased osteoclastic activity and suppressed osteoblast function; thus leading to the development of osteoporosis and osteolytic lesions. Receptor activator of nuclear factor-kappa B ligand (RANKL) and its decoy receptor osteoprotegerin (OPG) are implicated in the differentiation and survival of osteoclasts. Our group has previously reported that the ratio RANKL/OPG is increased and correlates with the extent of bone disease, bone resorption, and survival in MM patients. The effect of RANKL on osteoclasts is through many pathways, including the activation of nuclear factor kappa-B (NF- κ B). Bortezomib (VELCADE) is a proteasome inhibitor, which is effective in patients with refractory MM. Its major effect is thought to be through the inhibition of NF- κ B. The aim of this study was to evaluate, for the first time in humans, the effect of bortezomib on bone disease and on markers of osteoclast function and bone resorption in patients with relapsed/refractory MM. We studied 11 patients (7M/4F; median age 59.5 years) who had received more than 2 lines of previous treatment, including high dose therapy with stem cell support. Eight patients had IgG MM, while one patient had IgA, one non-secretory and one light-chain MM. All patients had more than 3 lytic lesions and/or fractures on plain radiographs at the time of treatment initiation. Bortezomib was given at a dose of 1.3mg/m², iv, in 3-week cycles, on days 1, 4, 8, and 11 of each cycle, for 8 cycles. All patients received zoledronic acid both pre-and post-bortezomib administration. Bone pain was clinically evaluated at baseline and on the first day of each cycle. Skeletal survey was performed at baseline, and after the 4th and 8th cycle of treatment. Markers of osteoclast function and bone resorption, such as soluble RANKL (sRANKL), OPG, and tartrate-resistant acid phosphatase isoform-5b (TRACP-5b), were measured at baseline and on day 1 of each cycle. The above markers were also measured in 12 healthy, gender and age matched, controls. Five out of 11 patients had achieved a partial response, while 3 patients had a minimal response, and 3 patients had not responded to bortezomib, according to EBMT criteria. All patients experienced dramatic improvement in bone-related pain after the 3rd cycle of treatment ($p < .01$). In particular, 3 patients who had grade III bone-related pain, achieved complete pain relief after 3 cycles of treatment. At baseline, OPG serum levels were significantly decreased compared to controls ($p < .001$). On the contrary, there was no difference in serum levels of sRANKL and TRACP-5 between patients and controls, possibly due to the long-term administration of zoledronic acid prior to bortezomib treatment. Administration of bortezomib produced no significant alteration in the levels of the above biochemical parameters. These data are in keeping with the recently published report that NF- κ B expression is not required for RANKL or OPG production by osteoblasts in murine models (Xing et al, J Bone Miner Res 2003) and suggest that bortezomib is very effective in controlling bone-related pain in myeloma patients independent of the RANKL/OPG pathway.

365 A PHASE I/II MULTICENTER, SAFETY AND EFFICACY STUDY OF COMBINATION TREATMENT WITH MELPHALAN, ARSENIC AND VITAMIN C (ASCORBIC ACID) (MAC) IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Multiple myeloma (MM) patients relapse after an initial response to chemotherapy. Thus, there is a need to examine new treatments to overcome chemoresistance in MM patients. ATO disrupts key signal transduction mechanisms thereby leading to MM cell growth inhibition and apoptosis. Recent studies in our laboratory also show marked anti-MM effects in vivo when ATO is used alone in SCID-hu murine models of MM. ATO inhibits NF- κ B activation through inhibition of I κ B kinase. Activation of this transcription factor has been shown to be associated with the development of chemoresistance in MM cells. The state of the intracellular glutathione redox system also affects the efficacy of ATO so that decreasing glutathione leads to increased ATO-induced apoptosis. Addition of ascorbic acid, an oxidizing agent that decreases intracellular glutathione levels, to ATO has been shown to increase apoptosis in MM cells. Recent in vitro studies in our laboratory show that ATO can sensitize chemoresistant MM cells to melphalan-induced cytotoxic effects. In vitro experiments also show the most profound anti-MM effects when ATO, ascorbic acid and melphalan are used in combination compared with the effects observed when the drugs are used alone or combinations of any two of these agents.

Based on these pre-clinical studies, the MAC regimen was initially examined in 10 pts with refractory MM. All patients had failed multiple prior therapeutic regimens. Five of the 10 patients had renal failure at the onset of therapy. ATO was administered 0.25 mg/kg twice weekly followed immediately by ascorbic acid 1g IV and oral melphalan (0.05-0.1 mg/kg qd) for 4 days every 4 to 6 weeks. All pts responded to the combination, administered for 14 to 70+ weeks, with significant reductions in M-protein levels (37-85%) and reversal of renal failure in all five patients with elevated serum creatinine at baseline. The regimen was well tolerated. Based on encouraging results from this pilot study, a larger, multicenter trial was recently started.

100 MM patients who showed relapse after responding to first-line chemotherapy and/or having proved to be refractory to chemotherapy will be enrolled. Pts will receive a loading dose of ATO at 0.25 mg/kg IV followed by ascorbic acid 1 g IV days 1-4 of week 1 of each six-week cycle. ATO/ascorbic acid will be given twice-weekly for the next 4 weeks of each cycle. Low-dose melphalan (0.10 mg/kg) will be administered orally for the first 4 days of each cycle. Pts will receive a maximum of 6 cycles followed by weekly maintenance treatment with weekly ATO followed by Vitamin C. The primary objectives of this study are to determine response rate and safety and tolerability of MAC therapy. Currently, 20 patients have been enrolled in this study. Preliminary evidence of antitumor activity has been reported in this heavily pre-treated population. The overall efficacy and safety data to date will be presented at the meeting.

365a FACTORS PREDICTIVE OF CLINICAL OUTCOME WITH BORTEZOMIB (VELCADE) IN PATIENTS WITH RELAPSED, REFRACTORY MULTIPLE MYELOMA

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Background: Bortezomib (VELCADE®) is a potent, reversible inhibitor of the proteasome, a key regulator of cellular protein homeostasis. SUMMIT, a phase 2 trial of bortezomib in 202 patients with relapsed, refractory multiple myeloma, demonstrated a 35% overall response rate (CR+PR+MR) using EBMT criteria. A median duration of response of

14.3 months was observed among patients enrolled in an extension phase of SUMMIT (Proc Am Soc Clin Oncol 2003;22:581).

Aim: This analysis focused on identifying factors predictive of response rate (CR+PR), time to response (TTR), duration of response (DOR), time to disease progression (TTP), and overall survival (OS) with bortezomib in patients enrolled in SUMMIT.

Methods: Bortezomib 1.3 mg/m² was administered on days 1, 4, 8, and 11 every 21 days for up to 8 cycles. Oral dexamethasone 20 mg on the day of and day after bortezomib was allowed for patients with progressive disease after 2 cycles or stable disease after 4 cycles. Factors that were examined included baseline demographic, disease-related, and laboratory parameters, such as age (< 65 years, ≥ 65 years), gender, race, body surface area, Karnofsky performance score (≤ 70, 80, ≥ 90), number of prior regimens, type of prior treatment, percentage of plasma cells in the bone marrow (≤ 50%, >50%), abnormal cytogenetics (including chromosome 13 deletion), hemoglobin (< 10.5 g/dL, ≥ 10.5 g/dL), and myeloma type; β 2-microglobulin, albumin, platelet count, and C-reactive protein were assessed as continuous variables. TTR, DOR, and TTP were calculated for all patients while they were treated with bortezomib alone before dexamethasone was added; OS was calculated for patients treated with bortezomib dexamethasone. Univariate (Cox proportional hazards model) and multivariate (through a stepwise model selection procedure) statistical tests were performed.

Results: By multivariate analyses, the only factors associated with lower response rate were age ≥ 65 years (P<.05) and > 50% bone marrow plasma cells (P<.05). Longer TTR was associated with older age (P=.009) and > 50% bone marrow plasma cells (P=.021). Shorter DOR was associated with hypoalbuminemia (P=.003) and a low Karnofsky performance score (P=.01) in patients with a complete or partial response (n=53). Shorter TTP was associated with elevated C-reactive protein (P=.011) and abnormal cytogenetics (P=.02). OS was decreased in patients with >50% bone marrow plasma cells (P=.014), thrombocytopenia (P=.015), hypoalbuminemia (P=.001), and low Karnofsky performance score (P=.032). Chromosome 13 deletion, β 2-microglobulin, and type or number of prior therapies were not significant predictors.

Conclusion: In patients with relapsed, refractory myeloma, factors predictive of outcome with bortezomib tended to be associated with high tumor burden. Older age was predictive of a lower response rate and a longer TTR but not of DOR, TTP, or OS, suggesting that older patients who respond to bortezomib may experience clinical benefits similar to younger patients. Response to bortezomib appeared to be independent of chromosome 13 deletion and β 2-microglobulin concentration parameters often considered to be risk factors for poor outcome.

366 PAD COMBINATION THERAPY (PS-341/BORTEZOMIB, ADRIAMYCIN AND DEXAMETHASONE) FOR PREVIOUSLY UNTREATED PATIENTS WITH MULTIPLE MYELOMA

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Background: The most favourable outcomes for patients with MM are seen in those achieving a complete or near complete remission following initial therapy. Therefore, it is logical to explore strategies aimed at improving initial response rates. Bortezomib has shown significant activity in patients with advanced MM and is superior to pulsed dexamethasone in this setting. Additional efficacy is seen when dexamethasone is combined with bortezomib and in vitro synergy is observed with cytotoxic agents such as adriamycin. On this basis, the PAD regimen was evaluated as front line therapy for patients with MM. Aims: The primary objective of this Phase I/II study was to assess the feasibility of harvesting PBSC after PAD, with secondary objectives being assessments of safety, toxicity, response rate (RR), PFS and OS. Methods: Patients with previously untreated MM were eligible. Patients were treated with 4x21 day cycles of PAD comprising bortezomib 1.3 mg/m² on days 1,4,8 & 11 along with 40 mg dexamethasone on days 1-4, 8-11 & 15-18 during cycle 1 and days 1-4 during cycles 2-4. During days 1-4 of each cycle, patients also received 0, 4.5 or 9 mg/m² of adriamycin at levels 1,2 & 3 respectively. Following harvesting, patients received high dose melphalan (MEL200) with PBSC rescue. Gene expression profiling was performed on purified plasma cells from diagnostic samples for pharmacogenomic analysis. Results: Thus far, 19 patients have been enrolled (17 male and 2 female, median age 55 years, range 37-66). 18 and 15 have completed at least one or four cycles respectively. PR/CR

rates are 67% and 93% after one and four cycles respectively. 11/12 patients mobilised PBSC successfully (median 2.4×10^6 CD34+ cells/kg, range 1.6-7.4) after a median of 1 (range 1-4) days of collection. 8 patients have received MEL200 with median neutrophil ($>0.5 \times 10^9/L$) and platelet ($>20 \times 10^9/L$) engraftment of 17 (13-23) and 12 (10-33) days respectively. Of 6 patients who are assessable at 3 months post MEL200, all have achieved at least a PR (CR 2, VGPR 2 & PR 2). Toxicities have generally been acceptable with 12 grade 3 events: 7 infections, 2 nausea and vomiting, 2 neuropathy and 1 postural hypotension. Overall, 39% and 44% of patients have experienced sensory or painful neuropathy. Of note, neuropathic symptoms are improving in all patients after completion of therapy. Conclusions: These preliminary data show that PAD is well tolerated in the majority of patients, is effective and does not prejudice subsequent PBSC collection. PAD warrants further evaluation as first line therapy in prospective randomised trials.

367 THE SAFETY OF PROLONGED THERAPY WITH THE PROTEASOME INHIBITOR BORTEZOMIB (VELCADE) IN RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (MM)

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Background: The efficacy and safety of bortezomib (VELCADE®, formerly PS-341) in patients with relapsed and/or refractory MM were demonstrated in two phase 2 trials: CREST (N=54) and SUMMIT (N=202). In CREST, patients with relapsed or refractory disease received bortezomib 1.0 or 1.3 mg/m² 2x/week x2 q3 weeks for up to 8 cycles. Overall response rates (ORR; CR+PR+MR) using modified EBMT criteria were 33%/50% with bortezomib 1.0/1.3 mg/m², respectively. In SUMMIT, patients with relapsed and refractory disease received bortezomib 1.3 mg/m² on the same schedule, and the ORR was 35%, including 4% CR and 6% near CR (100% reduction in M-protein with positive immunofixation).

Aim: To evaluate long-term safety following extended treatment with bortezomib in CREST/SUMMIT patients.

Methods: Patients from both studies who achieved clinical benefit and, in the investigators opinion, would benefit from continuation or retreatment with bortezomib, were eligible for an open-label extension study. Individual treatment regimens were based on bortezomib dose and schedule and patient tolerance in the original study. Dexamethasone was allowed for patients experiencing PD. Safety evaluations were performed every dosing visit.

Results: Overall, 63 of 256 (25%) of CREST and SUMMIT patients were treated in this study (17 and 46, respectively). The median age was 60 years (4284), and the median number of prior therapeutic regimens was 5 (115). In general, patient and disease characteristics of these patients reflected the CREST/SUMMIT populations, except for a lower incidence of abnormal cytogenetics and somewhat lower beta2-microglobulin and C-reactive protein levels. The total bortezomib administration was a median of 56 doses given over a mean of 49.4 weeks (26.8 weeks in the extension trial). The mean dose intensity of bortezomib (1.5 mg/m² per week) was similar to that observed in CREST/SUMMIT. The adverse event (AE) profile was consistent with that in CREST/SUMMIT; most common toxicities were diarrhea NOS (52%), fatigue (48%), thrombocytopenia (44%), and nausea (43%). AEs not observed in CREST/SUMMIT included seizures (2 patients) and pulmonary hypertension (1 patient). Of the most commonly reported events, the incidence of lower-limb edema, hyperglycemia, and increased serum creatinine was higher compared to the same patients experience in CREST/SUMMIT. The occurrence of infections, hypotension, and cardiac rate/rhythm abnormalities were not increased. The incidence of treatment-emergent peripheral neuropathy was lower.

Conclusion: Bortezomib can be administered dexamethasone for up to 32 cycles with a safety profile in this trial similar to that of the first

6 months of treatment. There is no evidence of prolonged exposure-related cumulative or permanent toxicity. The optimal duration of bortezomib treatment and the role of maintenance therapy continue to be evaluated.

368 CHARACTERIZATION AND REVERSIBILITY OF PERIPHERAL NEUROPATHY IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA TREATED WITH BORTEZOMIB (VELCADE). THE SUMMIT AND CREST STUDY GROUP.

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Background: Proteasome inhibition as an antineoplastic strategy has evolved to a clinical reality with approval of the first-in-class proteasome inhibitor bortezomib (VELCADE®) for the treatment of multiple myeloma (MM) in patients with relapsed, refractory disease who have received 2 prior therapies and progressed on their last therapy. Two phase 2 clinical trials (SUMMIT [N = 202] and CREST [N = 54]) demonstrated that bortezomib is active in heavily pretreated patients with manageable toxicities. Peripheral neuropathy [PN] is a known adverse effect of bortezomib. Grade 3 or 4 PN was observed in 13% of patients evaluated in SUMMIT and CREST.

Aim: To further characterize the frequency, severity, symptomatology, reversibility, and electrophysiology of PN with bortezomib.

Methods: Bortezomib was administered to patients with relapsed and/or refractory MM (SUMMIT, 1.3 mg/m² [N = 202]; CREST, 1.0 mg/m² [n = 28] or 1.3 mg/m² [n = 26]) twice weekly every 21 days. Patients with progressive disease after 2 cycles or stable disease after 4 cycles were permitted dexamethasone 20 mg the day of and day after bortezomib. PN was assessed by patient-completed FACT/GOG-Ntx questionnaire, neurologic examination, and adverse events. A subset of patients underwent neurophysiological testing.

Results: Baseline symptoms of PN were present in 81% (194/239) of patients by FACT-GOG-Ntx questionnaires and in 83% (203/244) following examination by a neurologist. Treatment-emergent PN was reported in 35% (90/256) of patients (grade 1/2 in 22% [57/256], grade 3 in 13% [32/256], and grade 4 in 0.4% [1/256]) and was more common among patients receiving bortezomib 1.3 mg/m² (37%; 84/228) compared with 1.0 mg/m² (21%; 6/28). Among evaluable patients with baseline PN, 16% (31/192) developed worsening PN (grade 3 or 4), whereas only 3% (2/60) of patients without PN at baseline developed grade 3 PN (no grade 4). Symptoms were primarily sensory, characterized by paresthesia, burning or painful dysesthesia, or numbness, and more frequent in the lower than upper extremities. PN-associated dose reduction occurred in 12% and drug discontinuation in 5% of patients. Nerve conduction studies (NCS) and quantitative sensory testing (QST) showed no electrophysiologic change from baseline in the majority of evaluated patients (10/12), progression by NCS in 1, and progression by QST in 1. A length-dependent sensory axonal polyneuropathy with predominant small-fiber involvement was the common abnormality. Of 35 patients with either grade ≥ 3 PN or PN leading to discontinuation, resolution, or improvement occurred during treatment in 13 and after treatment in 11, indicating potential for partial or complete reversibility in 71% (25/35). Among patients with ongoing post-study PN, the median time from last dose to resolution or improvement was 47 days (range 1529).

Conclusion: Treatment-emergent PN with bortezomib is dose dependent, is usually of mild to moderate severity, and has a potential for partial or complete reversibility in the majority of patients. If severe PN occurs, baseline PN was usually present. Further analyses are ongoing to study the pathophysiology and reversibility of PN with bortezomib as monotherapy and in combination with other antineoplastic agents.

369 BORTEZOMIB IN COMBINATION WITH DEXAMETHASONE FOR THE TREATMENT OF PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA

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Background: Proteasome inhibition is a novel antineoplastic strategy that produces cell-cycle arrest and apoptosis of myeloma cells in vitro. Experimental evidence has demonstrated additive activity when bortezomib is combined with dexamethasone and has suggested that bortezomib may overcome resistance to dexamethasone. The proteasome inhibitor bortezomib (VELCADE®, formerly PS-341) has shown potent antitumor activity and produced durable responses in heavily pretreated patients with relapsed and/or refractory multiple myeloma in 2 pivotal clinical trials, SUMMIT and CREST. Using European Group for Blood and Marrow Transplant (EBMT) criteria, treatment with bortezomib 1.3 mg/m² resulted in a rate of response (CR + PR + MR) of 35% in 193 patients in SUMMIT. In CREST, the response rate was 33% among patients treated with bortezomib 1.0 mg/m² (n = 27 evaluable patients) and 50% among patients treated with bortezomib 1.3 mg/m² (n = 26).

Aim: This analysis specifically addressed the clinical value of adding dexamethasone to bortezomib by evaluating the number of additional responses and adverse events among patients enrolled in SUMMIT and CREST receiving this combination.

Methods: Bortezomib 1.0 or 1.3 mg/m² was administered by intravenous bolus on days 1, 4, 8, and 11 of a 3-week cycle for up to 8 cycles to patients who had failed either 2 or more lines of therapy (SUMMIT) or first-line therapy (CREST). Patients with progressive disease after 2 cycles or stable disease after at least 4 cycles were eligible to receive oral dexamethasone 20 mg on the day of and day after each dose of bortezomib. Additional responses in patients receiving combination therapy were assessed using EBMT criteria. In both trials, the response rate with combination bortezomib + dexamethasone was a secondary endpoint.

Results: Combination bortezomib + dexamethasone resulted in additional responses in 13 of 74 evaluable patients (18%) in SUMMIT and in 9 of 27 (33%) in CREST. Of these 22 additional responses, 2 complete, 8 partial, and 12 minimal responses were observed. Responses were observed in 8 patients who were previously refractory to dexamethasone (had progressive disease within 30 days of a steroid-containing regimen). Combination treatment did not appear to alter the profile of adverse events or increase their incidence compared with bortezomib monotherapy. Drug-related adverse events occurring in ≥ 20% of the patients receiving combination bortezomib 1.3 mg/m² plus dexamethasone were fatigue (24%), thrombocytopenia (24%), diarrhea (22%), and nausea (21%).

Conclusion: Among patients with relapsed and/or refractory myeloma, bortezomib in combination with dexamethasone provides additional clinical responses in some patients and manageable adverse events that were not increased in incidence compared with bortezomib alone. Further studies of this combination are warranted.

370 BORTEZOMIB AND MELPHALAN COMBINATION THERAPY IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (MM): A PHASE I/II STUDY

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Background: Bortezomib (VELCADE®), a novel, reversible proteasome inhibitor, has demonstrated durable responses for the treatment of

patients with multiple myeloma who have received at least 2 prior therapies and are progressing on their last therapy. In vitro work has shown that bortezomib can effectively enhance the cytotoxic potential of subtoxic concentrations of other therapies for MM, including melphalan, in chemotherapy-resistant cells (Ma et al, Clin Cancer Res 2003;9:1136), and has suggested that antitumor effects might be achieved with lower doses of bortezomib and other chemotherapeutics when used in combination. Therefore, this approach may minimize toxicity and help overcome chemoresistant multiple myeloma. **Aim:** The objective of this dose-escalation phase I/II study was to determine an optimal dosing regimen and assess the safety, tolerability, and preliminary efficacy of bortezomib + melphalan therapy for patients with refractory or relapsed MM, starting with doses below those usually recommended for each agent.

Methods: Bortezomib (0.7 mg/m²) was given intravenously on days 1, 4, 8, and 11 of a 28-day cycle for up to 8 cycles. Oral melphalan (0.025, 0.05, 0.1, 0.15, 0.25 mg/kg) was administered to five 3-patient cohorts on days 14 every 4 weeks. Because of the lack of significant dose-limiting toxicity, enrollment is proceeding with a 3-patient cohort receiving the next higher dose of bortezomib (1.0 mg/m²) and the lowest dose of oral melphalan (0.025 mg/kg).

Results: Eighteen patients (56% male) with a median age of 55 years (range, 33-77 years) were enrolled. The IgG myeloma subtype occurred in 67% of patients, and the median beta 2-microglobulin level was 5.0 mg/L (2.214 mg/L). This was a heavily pretreated population (27 prior therapies), including melphalan (8 patients), thalidomide (9 patients), VAD (12 patients), and autologous stem cell transplantation (7 patients). Toxicity was manageable in this study. One grade 4 adverse event (anemia) was observed in the 1.0 mg/m² bortezomib dose group. Grade 3 events were predominantly associated with myelosuppression (anemia, neutropenia, and thrombocytopenia) and were observed among patients with baseline cytopenia. The most common grade 1 or 2 toxicities were nausea, fever, fatigue, diarrhea, neutropenia, anemia, and thrombocytopenia. Treatment-related neuropathy (grade 1) developed de novo in 2 patients in this study. In patients with mild-to-moderate (grade 1-2) neuropathy at baseline (n = 8), symptoms remained stable in 75% (6/8), worsened transiently in one patient, and resolved in the remaining patient. Overall, responses were seen in 12/18 patients (67%), with partial responses in 7/18 (39%), minor responses in 5/18 (28%), stable disease in 5/18 (28%), and progressive disease in 1/18 (6%). Two of 3 patients receiving the higher bortezomib dose at 1.0 mg/m² showed an IF + CR. Previous treatment with melphalan did not affect the response rate.

Conclusion: This novel combination provided encouraging evidence of antitumor activity in heavily pretreated patients with MM and toxicities were manageable. The full potential of this regimen is being explored in additional cohorts at the higher fixed dose of bortezomib 1.0 mg/m² with escalating doses of melphalan.

371 EVALUATION OF THE SEVERITY AND RISK OF THROMBOCYTOPENIA WITH BORTEZOMIB THERAPY IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background: The first-in-class proteasome inhibitor bortezomib affects several aspects of cell physiology, including NF-κB inhibition. In two phase 2 trials (SUMMIT and CREST) of relapsed and/or refractory MM, bortezomib demonstrated durable responses and meaningful survival benefits. Thrombocytopenia was the most common grade 3 adverse event in each study (28% and 24% of patients, respectively). Thrombocytopenia secondary to conventional chemotherapy is related to destruction of megakaryocytes and their progenitors within the bone marrow. However, preclinical evidence suggests that bortezomib is not toxic to hematopoietic stem cells. Thus, we hypothesized that

thrombocytopenia associated with bortezomib therapy may be due to reversible inhibition of thrombopoiesis rather than direct marrow toxicity. Aim: To further characterize the frequency, severity, and recovery of thrombocytopenia and assess potential predictive factors for the development of grade 3/4 thrombocytopenia associated with bortezomib therapy.

Methods: Patients receiving bortezomib 1.3 mg/m² on days 1, 4, 8, and 11 every 21 days in SUMMIT (N=202) and in CREST (n=26) were included in this report. Patients with PD after 2 cycles or SD after 4 cycles were eligible to receive oral dexamethasone 20 mg the day of and day after bortezomib administration. Potential predictive factors examined included patient age, baseline platelet count, baseline M-protein levels, number of prior regimens, and baseline percentage of plasma cells in the bone marrow (<50%, >50%). The effect of bortezomib on peripheral blood thrombopoietin (tpo) levels was examined in a subset of patients with MM from a separate study.

Results: Among 228 patients who received bortezomib 1.3 mg/m², the incidence of grade 3/4 thrombocytopenia was inversely associated with baseline platelet concentration, occurring in 13% of patients with baseline levels >200,000/L, 56% with 100,000-200,000/L, and 90% with 70,000-100,000/L. The incidence of grade 4 thrombocytopenia increased significantly when the baseline platelet count was <70,000/L. Interestingly, when the degree of thrombocytopenia was analyzed with respect to the severity of disease as measured by percent plasma cells in the marrow or as quantity of the paraprotein in the blood, the platelet nadirs by percent reduction were similar regardless of disease severity. On average, the platelet count reduction during the treatment period was ~60% of baseline platelet count independent of tumor burden. Only 14% of patients required platelet transfusions during treatment. Recovery of platelet count to baseline was observed during the 10-day rest periods within each cycle among patients with SD or better. Thrombocytopenia associated with bortezomib therapy was accompanied by an appropriate increase in serum tpo levels, thereby eliminating reduced production of tpo as an etiology for thrombocytopenia among these patients.

Conclusion: Although clinically significant thrombocytopenia may be observed with bortezomib, it is more frequent in patients with lower baseline platelet counts. The absolute decrease in platelet counts during therapy is predictable and transient, with a return to baseline levels during rest periods among patients without PD. The rapidity of recovery and appropriate increase in tpo levels with thrombocytopenia suggest that the mechanism is not related to marrow destruction, and thus may not be dose limiting.

372 PHASE I STUDY OF THE PROTEASOME INHIBITOR BORTEZOMIB AND PEGYLATED LIPOSOMAL DOXORUBICIN IN PATIENTS WITH ADVANCED HEMATOLOGIC MALIGNANCIES

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Background: The proteasome is a multicatalytic proteinase complex involved in ubiquitin-dependent and independent intracellular protein degradation. Preclinical and clinical studies have identified this protease as a novel target for therapy of hematologic malignancies, including multiple myeloma (MM) and non-Hodgkins lymphoma (NHL). In addition, modulation of proteasome function may overcome several mechanisms of chemoresistance that can decrease the effectiveness of standard chemotherapeutic agents. These mechanisms include inherited resistance through translocation of anti-apoptotic Bcl-2, acquired resistance through overexpression of the P-glycoprotein efflux pump, and inducible resistance through activation of nuclear factor-kappaB. Proteasome inhibitor/anthracycline regimens have demonstrated synergistic antitumor activity using in vitro models, suggesting these combinations might have enhanced clinical efficacy.

Aims: We sought to evaluate the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of the combination of the proteasome inhibitor bortezomib (VELCADE®; formerly PS-341) and pegylated liposomal doxorubicin (Doxil®/Caelyx®) in patients (pts) with advanced hematologic malignancies.

Methods: Pts received bortezomib as an intravenous bolus on days 1, 4, 8, and 11 of a 3-week cycle at doses ranging from 0.90 to 1.50 mg/m², while pegylated liposomal doxorubicin was administered on day 4 at 30 mg/m². The MTD was defined based on the first cycle, while response evaluations were performed every two cycles.

Patients: 42 pts were enrolled, including 24 with MM, 7 with NHL, and 5 with acute myeloid leukemia (AML).

Results: A mean of 4.7 cycles (range 1-11) has been administered to these pts, 38 of whom were evaluable for toxicity. Grade 3/4 therapy-related DLTs in cycle 1 included one episode of diarrhea, hypotension, confusion, and syncope at 0.90 mg/m², one of hyponatremia at 1.30 mg/m², one of neutropenia at 1.40 mg/m², and one of constipation at 1.50 mg/m². Grade 3/4 therapy-related toxicities in later cycles included fatigue, cytopenias, peripheral neuropathy, constipation, diarrhea, palmar-plantar erythrodysesthesia, and impotence. Other commonly seen grade 1/2 toxicities included nausea, vomiting, rash, mucositis, dehydration, and renal insufficiency. There were no clinically relevant sustained decreases in left ventricular function in this cohort. Among 22 MM pts evaluable for response there were 5 complete responses (CR), 3 near CR, and 8 partial responses (PR), while 5 pts had either minor responses or stable disease. Only 1 pt progressed, but did so by bone marrow immunohistochemistry and has improved clinically off therapy. 13 MM patients had disease that previously didn't respond to, or progressed on anthracycline-based therapy, and of these there were 4 CRs, 1 near CR, and 3 PRs. One pt proceeded on to stem cell transplantation and was mobilized and engrafted successfully. Among pts with other diseases, two with B-cell NHL had PRs, one with a peripheral T-cell NHL had a CR, and two pts with AML had PRs.

Conclusion: Bortezomib and pegylated liposomal doxorubicin is a well tolerated regimen with significant activity against multiple myeloma in this study and possibly in other refractory hematologic malignancies. The regimen recommended for Phase II is 1.30 mg/m² of bortezomib with 30 mg/m² of pegylated liposomal doxorubicin, and studies in myeloma are planned.

ALL: THERAPY & MRD

373 SURVIVAL AND PROGNOSTIC FACTORS OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN AT THE NATIONAL CANCER INSTITUTE, EGYPT

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Background and Aim: With intensive multiagent chemotherapy, 95% of children with acute lymphoblastic leukemia (ALL) achieve complete remission and 70% are long term survivors. The aim of this study was to improve survival of pediatric ALL patients in our institute as well as to define prognostic factors that have significant impact on outcome.

Patients and Methods: This study included 154 newly diagnosed ALL patients below the age of 18 years during the period between 1st of August 1998 and 31st of December 2000. They were all treated according to NCI treatment protocol modified from study XIII for high risk ALL of St. Jude Children Research Hospital.

Results: B-Precursor phenotype was encountered in 73.4% of patients, and T-cell phenotype in 26.6%. According to NCI criteria for risk classification, 58.4% of patients were in the high risk group (90% of T-lineage compared to 47% of B-lineage ALL, p<0.001).

Nine patients (5.8%) died during induction. After induction therapy, 135/145 evaluable patients (93.1%) achieved complete remission, while 10 patients (6.9%) failed to achieve remission. With a median follow up period of 43 months, the 3-year disease-free survival (DFS) and its probability at 5 years (pDFS) were 803.6% and 75.34%, respectively, while the 3-year event-free survival (EFS) and 5-year pEFS were 693.8% and 65.24%, respectively. The adverse events included 9 induction deaths, 4 refractory leukemias, 27 relapses (16 hematological, 8 isolated CNS and 3 combined hematological and CNS), 7 deaths in remission and 3 secondary myeloid leukemia. B-Precursor ALL had 5-year pDFS and pEFS of 80.54% and 71.84.5% compared to 60.28.6% and 46.78% for T-cell, respectively (p<0.01, log rank test).

Prognostic factors that had statistically significant unfavorable impact on survival by univariate analysis were age >10 years, CNS involvement, T-lineage phenotype, high risk group, DNA index <1.16 and slow early response to treatment. On multivariate analysis, CNS involvement, high risk group and slow early response to treatment persisted to have statistically significant unfavorable prognosis. Risk classification demonstrated prognostic significance for B-lineage but not T-lineage ALL.

Conclusion: This study was effective in improving survival rates of pediatric ALL patients in our institute. CNS involvement at diagnosis, risk classification and early response to treatment were defined as important

prognostic factors and would allow the identification of patient groups for appropriate risk-directed therapy in our future studies.

374 ROLE OF LIPOSOMAL DAUNORUBICIN, FLUDARABINE AND CYTARABINE (FLAD) IN THE SALVAGE THERAPY OF ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background and aims. Patients with relapsed or refractory acute lymphoblastic leukaemia (ALL) experience very poor outcome, and prognosis is further worsened by the presence of an unfavourable karyotype. The combination of Fludarabine plus Ara-C (anthracycline) showed clinical activity in patients with relapsed ALL. Compared to the unencapsulated anthracycline, liposomal daunorubicin (DaunoXome, DNX) is characterised by higher tumour cell delivery and reduced toxicity profile. Over the last two years some clinical studies have utilised daunoXome, alone or in association with Ara-C, in patients with poor risk AL. On the basis of these encouraging data, we designed a pilot trial to evaluate the association of fludarabine, Ara-C, and DaunoXome (FLAD) in high risk ALL patients.

Patients and methods. The FLAD regimen consisted of three days of treatment with a 30-minute infusion of Fludarabine 30 mg/sqm, followed 4 hours later by a 4 hour infusion of Ara-C 2 g /sqm and a 60 minute infusion of DNX 80 mg/sqm. Patients in complete remission (CR) after induction received a further identical consolidation course. Allogeneic (BMT) or autologous stem cell transplant (ASCT) followed when possible. Currently, our study includes 17 consecutive patients, enrolled between March 2001 and December 2003. Ten patients (59%) were refractory to induction chemotherapy, and 7 (41%) had relapsed (one of them following BMT). Four patients had t(9;22), one had t(11;15), and the remaining 3 showed complex karyotypes.

Results. Ten out of 17 patients (59%) reached CR after the first course of FLAD. Five patients in CR were transplanted from related donors. Another patient underwent autologous stem cell transplant. After a median follow up of 8 months, 8 patients are alive and 6 of them are still in CR. One patient died in CR of cerebral aspergillosis, while another died of cerebral haemorrhage after reinfusion of autologous stem cells. The other 7 died of disease. The median duration of CR was 7 months (range 2-14), the median survival was 11 months (range 2-15).

FLAD was well tolerated and no deaths during induction were recorded. The only complication we observed was encephalitis caused by HSV. Neutrophil ($>0.5 \times 10^9 / l$) and platelet ($> 50 \times 10^9 / l$) recovery required a median of 15 days (14-23) and 19 days (14-28), respectively.

Conclusions. In our study the FLAD regimen showed relevant antileukaemic activity, not only in relapsed patients, but also in those who were refractory to standard induction therapy (7 CR out of 10 refractory patients). Furthermore, FLAD managed to overcome the negative impact of poor prognosis karyotype, since 5 out of 7 patients with t(9;22) or complex karyotype achieved CR. FLAD regimen may be included into the salvage strategy of ALL patients, since, despite the short duration of response, the high complete remission rate and the low toxicity allow to perform allogeneic transplantation in a significant number of patients.

375 DETECTION OF MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKEMIA USING AN ALLELE SPECIFIC OLIGONUCLEOTIDE PCR ASSAY

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Background: Modern sensitive techniques are routinely used to detect residual leukemic cells in the peripheral blood (PB) of patients who, on clinical examination and conventional morphological criteria, are in complete remission. The majority of patients reach complete remission (CR) after about 4 weeks of chemotherapy. CR does not mean that leukemia cells are totally eradicated from the body, but that their level is beyond the sensitivity level of classical cytomorphologic methods. At this time, up to 1010 malignant cells can still remain in patient. They represent the minimal residual disease (MRD). MRD monitoring has demonstrated high prognostic value for predicting subsequent relapse. Immunoglobulin chains (Ig) and T-cell receptor (TCR) gene rearrangements are excellent patient specific PCR targets for MRD detection.

Aims: To evaluate the kinetics of molecular response achievement 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- δ gene rearrangements were present in leukemia cells, one-sized PCR signal was obtained which was used to monitor MRD with lower sensitivity. 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. A positive signal, as analyzed by polyacrylamide gel electrophoresis and silver staining, corresponded to the presence of residual blasts in the remission sample.

Results: The ASO-PCR method was able to detect residual malignant cells in PB samples from patients who were considered to be in CR (with sensitivity of 10-4 to 10-6). TCR- δ gene was rearranged in most cases of T-ALL and seemed to be a very suitable target to follow up the malignant clone. Monitoring the kinetics of MRD during the first year of therapy permitted identification of two MRD-based risk groups of patients with low and high risk of relapse. After therapy, rising or persistently high level of MRD was detected prior to hematological relapse; these patients were in high risk of relapse. By contrast, patients with low, stable or falling MRD remained in remission. There was also a group of patients that their MRD level was fluctuating during the course of the disease; with periodic and frequent monitoring of MRD level we could anticipate the patients clinical outcome.

Conclusion: The application of the ASO-PCR method provides a sensitive and highly specific detection of MRD in ALL patients. Monitoring the kinetics of MRD with this technique provides 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.

376 SUBTYPE-ORIENTED STRATEGIES IMPROVE OUTCOME OF OLDER ADULTS (>55 YEARS) WITH ACUTE LYMPHOBLASTIC LEUKAEMIA: ANALYSIS OF NORTHERN ITALY LEUKAEMIA GROUP (NILG) DATA

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Background. While the outcome of adult patients with ALL is improving as shown in recent clinical trials, the results achieved in older patients (>55 years) remain largely unsatisfactory. Prospective NILG studies conducted since 1979 enrolled patients up to an age of 74 years.

Aims. The purpose of this report is to compare outcome of two consecutive patient cohorts, i.e. before and after 1996, when new risk-oriented studies were started, aiming to evidence possible therapeutic benefits from the latter strategy in this particular age group.

Methods. The newer risk-oriented trials included different treatment elements for different risk groups, that were identified through clinical (protocol 08/96) and biological (protocol 09/00, with MRD evaluation) approaches. Clinical risk groups were B-lineage (lin) standard-risk (SR, i.e. CD10+, blast count <10.000 and Ph/BCR-ABL-), B-lin high-risk (HR, nonSR Ph/BCR-ABL-), Ph (Ph/BCR-ABL+), and T (T-lin). In study 09, all patients remaining MRD+ through early consolidation were declared HR regardless the clinical risk profile. Risk-specific induction-consolidation elements in these protocols included CY as fifth induction drug plus HD-ARA-C/MTX courses in T-lin, early anthracycline-rich consolidation/HD-MTX/intensive maintenance for SR (08)/MRD- (09) groups, autologous or allogeneic stem cell transplantation (SCT) for HR/MRD+ T-/B-lin subsets, and repeated high-dose cycles with autologous blood stem cell support for HR/MRD+ patients unable to undergo SCT (09).

Results. Ninety-seven total patients were evaluable. Diagnostic characteristics by treatment group were (historical vs. risk-oriented): no. 56 vs. 41, male 22 vs. 14, median age 62 (56-73) vs. 62 (56-74) years, risk class SR B-lin 8 vs. 10, HR B-lin Ph- 23 vs. 14, Ph/BCR-ABL+ 14 vs. 11, T-lin 4 vs. 6, unknown 7 vs. 0. Median follow-up period for the 'risk-oriented' cohort is >4 years. The number of autologous/allogeneic SCT procedures was 0 vs. 5. Treatment outcome is shown in the table.

Conclusion. This retrospective review suggests that older adults may benefit from the application of risk-oriented schedules as those herein employed for the management of younger patients, requiring no or minimal dose reductions. This is probably due to a general trend towards improved physical conditions in older age groups and a better supportive care level. Thus, upper age limits for inclusion into curative-intent, chemotherapy-based regimens could be extended up to 65 years and even further in selected, fit patients.

	Historical	Risk-oriented	p value
N	56	41	
CR (%), NR(%)	39 (70), 15 (27)	31 (76), 5 (12)	ns, 0.07
A/W in CR1 (%)	4 (10)	15 (48)	0.000
Recurrence (%)	28 (72)	13 (42)	0.012
Induction/CR death	2/7	5/3	-
5-year survival (%)	9	28	0.01
5-year DFS (%)	14	37	0.01

Caption 1:

377 SUBTYPE-ORIENTED STRATEGIES IMPROVE SURVIVAL IN ADULT T-ALL: ANALYSIS OF NORTHERN ITALY LEUKEMIA GROUP (NILG) STUDIES SINCE 1991

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Background. Outcome of adult T-ALL is improving as shown by recent clinical trials. Prospective NILG studies conducted between 1991-1995 (Group 1: 'IVAP', '07/93') lacked some effective T-ALL-specific elements such as cyclo (CY) in induction phase and HD-ARAC/HD-MTX for postremission therapy. Since 1996, new studies were initiated (Group 2: '08/96', '09/00'), incorporating these drugs into a risk-adapted strategy. Aims. To evaluate the role of the newer risk-adjusted strategy in improving the outcome of adult patients with T-ALL.

Methods. In the new regimens, for T-ALL, the induction phase included (i) fractionated (f-) CY (600/1200 mg/m² over 4 days) added to idarubicin-VCR-ASP-PDN, while intensified postremission phases featured an early intensified CNS prophylaxis plus (ii) HD-ARAC ('08': 1 cycle; '09': 2-4 cycles), (iii) HD-MTX ('08': 2 cycles), (iv) HD-MTX/ARAC combination ('09': 2-4 cycles) or (v) allogeneic/autologous stem cell transplantation (SCT) in selected high-risk patients ('09': based on MRD-defined risk stratification). Results. Ninety-six total patients were evaluable. Diagnostic characteristics by treatment group were as follows (Group 1 vs. Group 2): male 29 vs. 38, median age 28 vs. 32.8 (range 15-73 vs. 14-66), median blast count (x10e9/l) 31 vs. 9.8 (range 0-675 vs. 0-240), abnormal cytogenetics 3 vs. 14, lymphoblastic lymphoma 0 vs. 9. Median follow-up period for Group 2 is >2 years. The number of SCT procedures was comparable among Group 1 (16 auto, 5 allo) and Group 2 (13 auto, 6 allo). Principal treatment results are given in the table.

Conclusions. The overall results enforce the view that HD-MTX, HD-ARAC and SCT procedures (in high-risk patients) are necessary for the optimal management of adult T-ALL. Furthermore, adding CY to standard induction regimens may diminish the risk of primary refractoriness (5/35 vs. 2/42 with f-CY) and/or make the tumoural burden more susceptible to cyto-reduction from further postremission therapy.

	Group 1	Group 2	p value
N	42	54	
CR (%), NR(%)	35 (83), 5 (14)	49 (91), 2 (4)	-
A/W in CR1 (%)	8 (23)	27 (55)	0.003
Recurrence (%)	25 (71)	18 (37)	0.002
Induction/CR death	4	7	-
3-5-year survival (%)	24-21	49-40	0.1
3-5-year DFS (%)	23-23	47-37	0.1

Caption 1:

378 POLYMORPHISM OF THE THIOPURINE S-METHYLTRANSFERASE GENE AND THE INDIVIDUALIZED TREATMENT FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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BACKGROUND. Thiopurine S-methyltransferase (TPMT) catalyzes 6-mercaptopurine (6-MP) which is a major drug in the continuation therapy for children with ALL. There is inherited, racially different polymorphism in TPMT locus. The aim of the current study was to determine the genotypes of TPMT in Taiwanese population with special emphasis on patients who were intolerant to 6-MP.

METHODS. Frozen bone marrow samples from 225 adults and 105 children (totally 330) including 31 cases of childhood ALL with ANC > 500/L, as well as blood from 51 children with childhood ALL and ANC < 500/L during continuation therapy consisting of 6-MP, were examined. An allele-specific PCR assay was used to detect G238C mutation (TPMT*2) and PCR-RFLP analysis was used to detect the mutations of G460A and A719G. Direct sequencing of each PCR product of mutant was performed.

RESULTS. TPMT*3C (A719G) was the only mutation detected. Excluding childhood ALL, the frequency of TPMT*3C mutation was 2.8% (7/248), which represent the frequency of TPMT polymorphism in Taiwanese populations. In children with ALL and ANC < 500/L, the frequency of TPMT*3C mutation was 4/51 (7.8%) (3 heterozygous and 1 homozygous mutation). None of the 31 children with ALL and ANC > 500/L had TPMT*3C mutation. All the 4 patients with TPMT*3C mutation experienced profound neutropenia with repeated infections during 6-MP therapy. Guided by this study, for those with heterozygous mutation, dosages of 6-MP were then tailored to 1/3 to 1/6, and for the patient with homozygous mutation to 1/10. After the dose modification, they did not encounter severe neutropenic infection during the subsequent 6-MP course chemotherapy, and two of them had completed chemotherapy smoothly.

CONCLUSION. TPMT*3C is the only mutant allele identified in Taiwan with a frequency of 2.8%. Individualized 6-MP treatment for children with ALL based on TPMT polymorphism improves efficacy and safety of chemotherapy.

379 RETROSPECTIVE COMPARISON OF QUALITATIVE AND QUANTITATIVE RT-PCR IN DIAGNOSING AND MONITORING THE ALL1-AF4 FUSION TRANSCRIPT IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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We compared quantitative reverse-transcriptase polymerase chain reaction (Q-RT-PCR) to qualitative RT-PCR in determining response to therapy and predicting clinical outcome in 18 retrospectively selected patients with acute lymphoblastic leukemia (ALL) positive for the chimeric gene ALL1-AF4 and with frozen RNA samples collected at diagnosis and during follow-up (96 samples analysed). The ALL1-AF4 junction was detected by qualitative RT-PCR in 18 patients and by Q-RT-PCR in 17 patients (1 patient harboured the rare e10-e6 ALL1-AF4 junction, which falls outside of the primer and probe location designed for the Q-RT-PCR). At diagnosis, the mean number of ALL1-AF4 copies (i.e., ALL1-AF4/ABL x 104) in leukemic cells was 5,926 (range: 435-22,045). Comparing the mean number of ALL1-AF4 copies at diagnosis with the WBC count at diagnosis, the achievement of complete molecular remission and the occurrence of hematological relapse, we found that the only statistically significant association was the trend towards an inverse association between the WBC count and the mean number of ALL1-AF4 copies at diagnosis (p=0.052). At hematological relapse 3 of the 8 patients who persisted molecularly positive after the induction treatment showed a number of ALL1-AF4 copies slightly lower than that detected at diagnosis and at complete hematological relapse. In three of

the 12 patients negative to qualitative RT-PCR after induction therapy, a small number of ALL1-AF4 copies was detected by Q-RT-PCR. Thus 9 patients were negative and 8 positive. Seven of the 8 positive patients suffered a relapse, including 2 of the 3 patients positive to Q-RT-PCR yet negative to qualitative RT-PCR. Moreover, we retrospectively compared 39 tests collected during the follow-up of the 9 patients who converted to a negative qualitative and quantitative PCR status (mean of 4.3 tests per patient; range: 3-9). During the follow-up of these patients we found 2/39 (5%) discordant results that concerned two different patients who tested negative to the qualitative assay yet positive to the Q-RT-PCR. In one of these patients, at 44 months from diagnosis, the Q-RT-PCR revealed a slight increase in the number of ALL1-AF4 copies, which during the remainder of the 62-month total follow-up returned to an undetectable level. By contrast, in the second patient, the re-appearance of a Q-RT-PCR positivity preceded of 3 months the hematological relapse. The results, together with the fact that Q-RT-PCR is limited to detect only those products generated by the majority of junctions, suggest that qualitative RT-PCR is more appropriate for the routine diagnosis of this genetic alteration. However, Q-RT-PCR is more accurate in assessing the molecular response after induction treatment and could be more useful in clinical decision-making in ALL1-AF4-positive ALL patients.

380 HETEROGENEITY OF CHILDHOOD PH+ALL AS ASSESSED BY MRD MONITORING WITH COMPARATIVE QUALITATIVE AND REAL TIME RT-PCR

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Background: in different therapeutic protocols, the presence of the Ph chromosome is associated with a particularly high risk for treatment failure. Qualitative and quantitative (RQ) RT-PCR determination of the kinetic of clearance of Ph+ leukemic blasts during and after induction chemotherapy might reflect different propensity of Ph+ALL to achieve complete remission. A previous prospective AIEOP RT-PCR study in 27 children with Ph+ALL showed that high sensitive qualitative RT-PCR monitoring at early treatment phases unravels differences in treatment response and further discriminates patients with potentially different risk of relapse (Cazzaniga et al., Br J Haematol, 2002; 119, 445-453). The introduction of Imatinib may further reinforce the need to identifying patients with different kinetic of tumor reduction in order to better address clinical choices.

Aim of the study was to compare the MRD results obtained in Ph+ALL by RT an RQ-PCR to further refine the follow up monitoring.

Methods: a series of 13 childhood Ph+ALL was analyzed, with both qualitative and RQ PCR, in parallel. RT-PCR was performed according to Biomed-1 guidelines. RQ-PCR was performed according to EAC guidelines.

Results: we confirmed by quantitative PCR the results previously obtained by qualitative PCR. Moreover, we found follow up time points positive at low levels by RQ-PCR but negative by qualitative PCR, indicating a higher sensitivity for the RQ-PCR method. In other cases the RQ-PCR clearly showed a progressive increase or decrease of the BCR/ABL expression, not visible by qualitative PCR. The gene expression of two different housekeeping genes was also evaluated.

Conclusion: the RQ-PCR can fully substitute RT-PCR in molecular monitoring of Ph+ childhood ALL. Two are the main advantages of the RQ-PCR monitoring: i) the possibility to amplify the expression of one or more housekeeping genes allows to check for the quality and quantity of cDNA amplified, and then to correct the fusion gene expression values; ii) the possibility to clearly monitor the kinetic of the fusion gene expression, which can potentially have a clinical application. The prospective screening of a larger series of patients enrolled in the AIEOP-BFM ALL 2000 is still ongoing.

381 VINCRISTINE, DEXAMETHASONE AND DAUNOXOME (VDXD) THERAPY IN ELDERLY ALL PATIENTS: AN UPDATE

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Acute lymphoblastic leukemia (ALL) represents a rare malignancy in the elderly patients associated with a poor prognosis. Few authors have

specifically focused on the treatment of ALL in this setting of patients and most available data arise from prospective trials including also younger patients. Particularly, Ellison (Ellison et al, J Clin Oncol 1991), Kantarjian (Kantarjian et al, Br J Haematol 1994) and Larson (Larson et al, Blood 1995) reported CR rates of 33%, 58% and 39%, respectively. Median DFS were 14, 12 and 12 months whereas median OS resulted 2, 10 and 1 month, respectively. We recently published the results of the combination VDXD in the treatment of 15 ALL patients aged 60 years who had a median follow-up of 20 months. Induction therapy consisted of vincristine 2 mg i.v. on days 1, 8, 21, 28; Daunoxome 50 mg/m² i.v. on days 1-3 and 28-30; dexamethasone 40 mg i.v. on days 1-4 and 28-31. Patients in complete remission (CR) received one or two consolidation cycles of cyclophosphamide, cytarabine and topotecan followed by a two-year rotating maintenance course including vincristine, Daunoxome, cyclophosphamide and prednisone. We reported a CR rate of 73% and a median DFS and OS of 21 and 23 months, respectively (Offidani et al, Hematol J 2003). We updated the results after enrolling 17 patients of whom, those still alive, had at least 24 months of follow-up.

Median age was 70 years (range 61-79); 9 (53%) had a performance status grade 2; 7 patients (41%) were bcr-abl positive and 11 (65%) had comorbidities. CR was achieved by 13 patients (76.5%), 3 patients (18%) died during induction therapy and one patient (6%) had resistant disease. Deaths were related to infection in two and to brain hemorrhage in one patient. Major toxicities were neutropenia, thrombocytopenia and infection whereas nonhematological toxicity was negligible.

After a median follow-up of 36 months, 11 patients (85%) had relapsed and 2 patients are still disease free at 24 and 58 months. Median DFS resulted 18 months. Thirteen patients (75.5%) had died whereas 4 are still alive at 24, 36, 36 and 58 months. Median OS was 21 months.

Compared with above mentioned studies, our updated results with VDXD regimen in ALL elderly patients confirm again a low mortality rate, a substantial higher CR rate and a longer DFS and OS.

382 RESULTS OF PETHEMA ALL-96 PROTOCOL FOR STANDARD RISK ADULT ALL PATIENTS

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Background. The results of the therapy in standard-risk (SR) adult ALL are promising, with CR over 90%, and DFS and OS probabilities of 50-60%. While no pretreatment prognostic factors have been identified consistently, the presence of MRD appears to predict a poorer outcome in these patients.

Aim. To report the results of PETHEMA ALL-96 trial, a pediatric-based protocol, in adults with SR ALL.

Patients and therapy. Criteria for SR-ALL: age 15-30 yr., WBC count <25x10⁹/L and absence of t(9;22)(or BCR/ABL), t(1;19) or 11q23 rearrangements. Induction: VCR, DNR, PDN, ASP and CPM over 5 weeks. Patients with slow response to therapy (>10% of BM blast cells on day 14) were allocated to a high-risk ALL protocol. Consolidation-1 (C1): MP, HD-MTX, VM26 and ARA-C. Consolidation-2/reinduction (C2): VCR, DNR, DXM, ASP and CPM. Maintenance-1: MP+MTX with monthly reinduction cycles (VCR, PDN, ASP) up to 1 yr. Maintenance-2: MP+MTX up to 2 yr. in continuous CR. CNS prophylaxis consisted of triple i.t. therapy (MTX+ARA-C+DXM), 14 doses over the 1st yr.

Results. 56 patients included, mean (SD) age 20(5) yr. 35 males, WBC count 8.2(8.6) x10⁹/L. Phenotype: pro-B 5, Common+pre-B: 38, T: 13, ALL with myeloid markers 6/56. Cytogenetics (41 evaluable cases, after review): normal 22, hyperdiploid 10, hypodiploid 1, other 8. Evaluable for response: 52 pts. CR 48 (92%), early death 2, resistance 2. Slow responders 8. Median follow-up 29 mo. 5-yr (95%CI) OS and DFS probabilities 63%(49-78) and 68%(53-83). No pretreatment variable had influence on CR, DFS or OS. The only prognostic factor for survival was the slow response to therapy (5-yr OS probability 25%[0-61] vs. 80%[65-

95], $p = 0.0003$ (OR 7.36, 95%CI 2.1-25.9, $p = 0.002$). Tolerable toxicity, although there were dose modifications or delay of the therapy in 10% of C1 cycles, 7% of C2, 19% of M1 and 10% of M2. Conclusion. Treatment of adult SR ALL patients with the PETHEMA ALL-96 protocol is adequate. No pretreatment prognostic variables have been identified and slow response to therapy emerged as the only prognostic factor for survival.

383 ADULT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: CLINICAL CHARACTERISTICS AND RESULTS OF TREATMENT ACCORDING TO THE IMMUNOLOGIC PHENOTYPE IN PATIENTS TREATED WITH TWO TRIALS OF THE SPANISH PETHEMA GROUP

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Background and aim: To describe the main clinical characteristics and the results of treatment according to immunologic subgroups of T-ALL in 81 adult patients included in two Spanish PETHEMA ALL-trials (ALL-96 and ALL-93).

Patients and methods: Between 1993 and 2003 81 adult patients (age >15 years) with T-ALL were treated in two PETHEMA ALL trials (ALL-96 for standard risk, 18 pts, ALL-93 for high risk, 63 pts). According to the immunologic criteria (EGIL) the subtypes of T-ALL considered were: pro-T/pre-T (CD7, TdT, cytoplasmic CD3, CD5 and CD2 positive, 13 pts), thymic (CD1a, CD4, CD8 positive, 21 pts), mature-T (surface CD3 positive, CD1a-, 21 pts). 26 pts were not classifiable (26 pts). All patients in both trials received 5-drug induction therapy. Consolidation consisted of 2 cycles and maintenance-reinductions (2 yr) in ALL-96, and 3 cycles of consolidation therapy for 3 months followed by allogeneic SCT if sibling donor available or randomisation to autologous SCT or 3 cycles intensification followed by maintenance (2 yr).

Results: There were 54 males and 27 females. The median age of the series was 26 (range 15-77). For the whole series LFS probability was 42% (31-53), for ALL-96 62% (32-92) and for ALL-93 36% (24-48), with a median follow-up of 5 years. The main clinical characteristics and results of treatment according to immunological groups are referred in the table. **Conclusions:** The overall results of therapy in adult T-ALL according to the risk factors stratification are comparable to those previously described. We did not find differences in prognosis among the subsets of T-ALL, although the number of cases in each subgroup is small.

Number (%)	Pro-T/pre-T	Thymic	Mature
Sex (M/F)	13 (100)	21 (100)	21 (100)
Median age (range)	7 (4-14)	14 (7-21)	14 (7-21)
CD5 (immunoblastic) (%)	100	100	100
WHO type (%)	100 (100)	100 (100)	100 (100)
Median survival (months) (%)	5.5	2.1	10.1
Survival at 10 years (%)	30	0	30
CR (%)	100	100	100
LFS (pts) (%)	13 (100)	21 (100)	21 (100)
OS (pts) (%)	13 (100)	21 (100)	21 (100)

Caption 1:

384 PROTEASOME INHIBITORS - A POTENCIAL THERAPEUTIC APPROACH IN ACUTE LYMPHOBLASTIC LEUKEMIA

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The ubiquitin/proteasome pathway is responsible for degrading many critical regulatory proteins, including those involved in cell-cycle and apoptosis regulation. Ubiquitination serves as a signal for specific cellular protein degradation.

Recently, it has been 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 human leukaemia.

The aim of this study was to evaluate the effect of two classes of proteasome inhibitors, a boronic acid derivative, MG262 and a peptidyl aldehyde, PSI, on acute lymphoblastic leukaemia.

For this purpose, human T acute lymphoblastic leukemia cell lines in culture, sensitive and resistant to azaguanine, CEM and CEM/AZAG, respectively, were used. Cell viability was estimated by tripan blue exclusion and cell morphology was evaluated by light microscopy examination of May-Grenwald Giemsa stained cells incubated with increasing concentrations of the compounds. Cell death was evaluated by annexin V/propidium iodide incorporation and detected by flow cytometry. Expression of proteins involved in apoptosis regulation, Bax and p53 were determined by flow cytometry using monoclonal antibodies. Polyubiquitinated proteins were identified by flow cytometry and western blot analysis.

Our results show that proteasome inhibitors suppresses cell growth and induce apoptosis in CEM cells sensitive and resistant to azaguanine. We also observed an increase in Bax expression and accumulation of polyubiquitinated proteins in proteasome inhibitors CEM and CEM/AZAG treated cells.

Our data suggests that proteasome inhibitors may constitute a therapeutic strategie to treat human acute lymphoblastic leukaemia and may also be usefull to overcome cellular resistance to chemotherapeutics agents.

This work was supported by GAI (Gabinete Apoio Investiga o), Faculty of Medicine, University of Coimbra

385 IMATINIB IN ADULT AND ELDERLY PH+ ALL PATIENTS: PRELIMINARY RESULTS OF THE GIMEMA PROSPECTIVE STUDY LAL0201

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1. Background

The BCR/ABL rearrangement worsens the prognosis of acute lymphoblastic leukemia (ALL), both in adults and in children, and represents an independent prognostic factor that significantly influences disease outcome. Recently the tyrosine kinase inhibitor Imatinib has become available; so far, however, only limited experiences in Ph+ ALL patients have been reported. In 2001 the GIMEMA Group started a protocol to evaluate Imatinib in adult (study A, 18-60 years) and elderly (Study B, >61 years) Ph+ ALL patients.

2. Aims

Study A: to evaluate the activity of imatinib, as single therapy, in inducing or maintaining Complete Molecular Remission (CMR) in patients in 1st Complete Hematological Remission (CHR) during the post-consolidation phase. Study B: to evaluate the activity of imatinib, in association to PDN, in inducing CHR (+CMR) during the induction phase.

3. Methods

Study A: Patients received therapy with imatinib p.o. at the dosage of 800 mg/day for 6 months. After completing the 6-months therapy, in the absence of safety concerns, if the investigator considered the patient eligible for treatment, additional therapy with Imatinib was added. Study B: seven days PDN pre-treatment followed by induction treatment with Imatinib 800 mg/day p.o. associated to PDN 40 mg/sqm/day for 30 days.

4. Results

Study A: a total of 22 patients in 1st HCR after the GIMEMA LAL 2000 induction treatment have been enrolled, 7 in CMR and 15 still BCR/ABL+.

All 7 PCR- patients are in CHR (6 still PCR- and 1 becoming PCR+) after a median follow up of 19 months (7.6-24). Five are still on imatinib and 2 underwent an alloSCT at 10 e 11 months after CR.

Eleven out of the 15 PCR+ patients are in CHR after a median follow up of 19 months (1.2-26): 7 are on Imatinib, 2 discontinued treatment and 2 underwent an alloSCT. Of the remaining 4 PCR+ patients, 1 died in CR after an alloSCT and 3 relapsed at 4, 9 and 11 months respectively. Study B: a total of 12 patients have been enrolled. Median age was 67.5 yrs (61-78), 9 were females. Five patients are not evaluable for response

(4 too early, 1 not evaluable); all 7 evaluable patients obtained a CR with the prednisone-Imatinib association induction treatment. Five are still in CCR at 2, 4, 5, 9 and 13 months, 1 relapsed at 4 months and 1 died in CR due to a 2nd neoplasia.

5. Summary

Treatment with Imatinib proved feasible and well tolerated both in adult and elderly patients. A total of 18 out of 22 adult enrolled patients are alive in CCR, with 3 of them submitted to an allogeneic SCT and 12 still on Imatinib. In the elderly patient group, all patients evaluable for response achieved CR, so far lasting for 2 to 13 months, with an induction treatment consisting in Imatinib alone plus prednisone. Further studies should be done to better evaluate the best integration of Imatinib in the entire treatment strategy of this disease.

386 PREDNISONE PRE-TREATMENT IS A PROGNOSTIC FACTOR FOR COMPLETE REMISSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS

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1. Background. Since 1996, the GIMEMA trials for adult acute lymphoblastic leukemia (ALL) included a central handling of peripheral blood and/or bone marrow samples at baseline, thus allowing to accurately define the biological profile and to determine its impact on outcome on a large series of homogeneously treated patients.

2. Aims. Since 2000 a one-week pre-treatment with steroids has been administered to all patients in order to allow a larger series of centers to collect and send biological samples before starting the induction treatment, and also to verify whether it was possible to earlier identify a category of patients with a worse prognosis, according to the response to steroids.

3. Methods. The protocol design provided a 7-day (-6 to 0) pre-treatment with an escalating dose of prednisone up to 60 mg/sqm. On day 1, before starting the induction, the response was assessed according to the absolute blast count in the peripheral blood (< vs >1,000/mm³). The induction included high dose daunorubicin, followed, for patients in complete remission (CR) by a consolidation with high dose ARA-C, chemo and radio prophylaxis of central nervous system involvement and periodical re-inductions over a 3-year maintenance period. Patients with adverse cytogenetic features [t(9;22) t(4;11), t(1;19)] achieving complete remission (CR) were treated according to the HAM protocol that included high dose ARA-C (3g/sqm/12hrs for 4 days) and mitoxantrone (10m/sqm/24hrs for 3 days) followed by either imatinib for Ph+ ALL or allogeneic or autologous hemopoietic stem cell transplantation based on the availability of an HLA-identical sibling for the others.

4. Results. A total of 317 patients were registered and were evaluable for the response to the induction treatment. The median age at diagnosis was 35 years (15-60) and the median WBC count at diagnosis was 15x10⁹/L (0.3-800); 78 (20%) were T-cell ALL and 119 (38%) had cytogenetic high-risk features [96 Ph+ ALL, 17 t(4;11) and 6 t(1;19)]. Of the 313 patients evaluable for the response to steroid pre-treatment, 239 (76%) showed a reduction of blasts to less than 1x10⁹/L. The overall CR rate, including also 4 Ph+ patients achieving response after HAM, was 83% (263 cases), with 8% (25 patients) resistant and 29 patients (9%) who died during induction. The probability of response was significantly higher in the group of responders to steroids, with a CR rate of 87% versus 69% in the non-responders (p=.0005). Also the post-CR outcome was better in the group of steroid responders, with a median disease-free survival (DFS) of 20 months versus 12 months in non-responders (p=.04).

5. Comments One-week pre-treatment with steroids is feasible in adult ALL patients treated according to the present GIMEMA protocol, allowing all participating centers to centralize, on a national basis, all needed biological samples from PB and BM before starting the chemotherapy induction treatment. Moreover, the sensitivity to steroids allows to identify a separate category of patients with a worse prognosis, both in terms of

CR probability and in terms of DFS, in which alternative treatments could be considered.

387 POTENTIAL USE OF CD40L+ BCP-ALL CELLS-LOADED DC TO TRIGGER ANTITUMOR IMMUNITY IN B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA.

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Background: Childhood B-cell precursor Acute Lymphoblastic Leukemia (BCP-ALL) is the most common form of cancer in children. Immunization strategies in cancer frequently have as common denominator the targeting of dendritic cells (DC) with its maturation stage playing a pivotal role in the development of functional CD8+T cells and polarization of CD4+T cells toward IFN- γ production. Aims: The finding that DC can present antigens derived from apoptotic cells prompted us to explore the feasibility of using DC pulsed with apoptotic BCP-ALL cells as adjuvants for stimulating immune responses against leukemic cells. Methods: To promote DC maturation during phagocytosis we induced the expression of CD40L on BCP-ALL cells by bystander transfer from a stable infected human bone marrow stromal cell line (HBMS) highly expressing human CD40L. After 24h of co-culture, more than 30% of BCP-ALL cells expressed CD40L. These cells were then induced to undergo to apoptosis by UV irradiation, and co-cultured with immature monocytes derived-DC at 2:1 CD40L+BCP-ALL cells/DC ratio for 24 hours. At the end of incubation, DC were analyzed for CD80, CD83 and CD86 expression by flow cytometry and IL-12p70 production by ELISA. Results: DC incubated with CD40L+ BCP-ALL cells expressed high level (more than 70%) of the maturation marker CD83, while no CD83 up-regulation was observed if CD40L- cells were used. Moreover, further maturation induced by phagocytosis of CD40L+ BCP-ALL cells resulted in higher APC activity, associated with higher expression of co-stimulatory molecules (CD80 and CD86) and higher ability to stimulate a strong proliferative response of allogeneic T cells in MLR assay. In addition, CD40L+ BCP-ALL cells-loaded DC secreted IL-12p70 (0.3-2 ng/ml). In two selected cases we tested the IFN- γ and IL-4 production by intra-staining analysis of naive lymphocytes co-cultured with CD40L+ BCP-ALL cells-loaded DC. In line with previous finding, these cells able to produce IL-12p70, induced a very high ($\geq 30\%$) Th1 polarization. The capacity of CD40L+BCP-ALL cells-loaded DC to induce anti-leukemia cytotoxicity was detected by 51Cr release assay after co-culture with T-cells in an allogeneic setting. T-cells and monocyte-derived DC were obtained from healthy donors. DC were pulsed with BCP-ALL cells expressing or not CD40L. After co-culture with CD40L+BCP-ALL cells-loaded-DC, T cells showed strong proliferation in response to leukemic cells. Moreover, T-cells cultured with CD40L+BCP-ALL cells pulsed-DC showed higher reactivity against leukemic cells than those cultured with BCP-ALL cells pulsed-DC (mean value 47.7%, range 30%-74.5% and mean value 13.5% range 0%-20%, respectively). In addition, blocking of MHC class I on target cells significantly abrogated leukemic blast lysis. Conclusion: we have demonstrated that, after phagocytosis of CD40L+BCP-ALL cells, DC matured in potent APC able to generate cytotoxic activity against leukemic cells. Based on these preliminary findings we suggest that the DC-based vaccine may be an attractive strategy for BCP-ALL immunotherapy.

CLL: BIOLOGY

388 RECOGNITION OF MIC-A AND ULBP3 BY VD1 T CELLS IN

B-CLL: IMPLICATIONS FOR ANTI-CANCER SURVEILLANCE

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The effectors of mucosal and natural immunity, including gd T lymphocytes, are known to play an important role in host defence against tumors. Two main subsets of gd T cells are known: one, expressing the Vd2 TCR, is found in the peripheral blood, while T cells expressing Vd1 are resident in epithelial and mucosal associated lymphoid tissue. There is increasing evidence that ab and gd T lymphocytes make distinct contributions to anticancer surveillance. Indeed, down-regulation of expression of MHC alleles, as well as tumor-associated antigens, is observed frequently during tumor progression, resulting in an impairment of MHC-restricted, ab-T cell dependent anti-tumor immunity. Due to the recognition of non-peptide antigens and the lack of requirement for classical antigen-presenting molecules, gd T cells might represent a non-redundant system in anti-cancer surveillance. However, little is known on the distinct role possibly played by Vd2 and Vd1 T cells in limiting the onset and spreading of Chronic Lymphocytic Leukemia of B cell type (B-CLL). In this research we have attempted to clarify this point.

Herein we analysed by immunofluorescence 32 patients with B-CLL (23 at low risk stage and 9 at intermediate risk stage, according to the Rai modified classification), chemotherapy nave, and we found that in 15 patients (12 low risk and 3 intermediate) circulating Vd1 T lymphocytes were significantly increased (100-300cells/ml) compared with 15 healthy donors matched for sex and age (50-100 cells/ml). Despite their functional cytolytic machinery, evaluated by 51Cr-release assay, these lymphocytes do not kill the autologous tumor cells, which lack the stress-inducible, MHC-related MIC-A antigen, and express low or undetectable levels of the UL16 binding proteins (ULBPs) usually recognized by Vd1 T cells via NKG2D. Interestingly, the anti-tumor activity could be reconstituted by transfecting B-CLL cell lines with MIC-A or by inducing transcription, analysed by PCR, and expression of MIC-A, and up-regulation of ULBP3, at the cell surface of the autologous B-CLL cells upon in vitro exposure to trans retinoic acid. Moreover, soluble MIC-A was detectable in the supernatant of cultured leukemic B cells and in the sera of 4 patients. Of note, in 6 out of the 18 patients who did not have an increased number of circulating Vd1 T cells the disease progressed in the last year (2 patients from low risk stage to intermediate, and 4 from intermediate to high risk stage). These results point to a role for Vd1 T lymphocyte in the anti-tumor defence against B-CLL and highlight a potential therapeutic use of retinoic acid in enhancing the activation of the gd T cell subset which preferentially respond to B-CLL tumor cells. Finally, we propose to evaluate if ULBP3 expression, at the mRNA or protein level, and Vd1/Vd2 ratio can be considered as possible markers of disease progression in the next future.

389 PRAME ANTIGEN IS FREQUENTLY EXPRESSED IN LYMPHOPROLIFERATIVE DISEASES

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Background: PRAME (preferentially expressed antigen in melanoma) is an antigenic protein with 509 amino acids recognized by T lymphocytes and expressed in trophoblasts, testis and in some neoplasias. We have demonstrated that PRAME mRNA is expressed in about 25% of cases of chronic lymphoproliferative disorders, especially chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). Aims: To produce a monoclonal antibody against PRAME and to define its reactivity in lymphoproliferative diseases. Materials and methods: A 562-nt fragment of the PRAME cDNA that includes the region that codes for the immunogenic 9-peptide was clone in pCR2.1-TOPO vector in DH5 alpha cells and subcloned into a pET24a expression vector, and the 196-amino acid peptide was expressed in BL21(DE3) cells as His-tagged protein. The monoclonal antibody (MoAb) against PRAME was generated from splenocytes from mice immunized with the

purified peptide. The expression of PRAME protein was quantified by flow cytometry in the peripheral blood leukocytes from 26 cases of CLL, seven cases of MCL in leukemic phase and 15 healthy controls (blood donors). Quantitative Fluorescence Cytometry was performed using saturating amounts of the PRAME MoAb. The calibration curve plotting the Mean Fluorescence Intensity versus Antibody Binding Capacity (ABC) was constructed based on the analysis of the DAKO QIFIKIT beads coated with well defined amounts of mouse MoAb. The Specific Antibody Binding Capacity (SABC) is the number of PRAME MoAb per cell after the correction for background. Results: Twenty-five cases of CLL (96%) and 6 cases of MCL (86%) were positive (more than 20% of positive lymphocytes). For CLL, mean positivity was 59% of cells (range 16 to 95%), and for MCL mean positivity was 75% of cells (range 12 to 94%). The mean SABC values were of 10,339 sites per cell (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 the peripheral blood (PB) from healthy controls, the percentage of PRAME positive cells varied from 3 to 18% (mean 7%), in which the SABC values ranged from 1,628 to 1,781 sites per cell. Even in the two cases of CLL and MCL that had less than 20% of positive cells, the PRAME+ cells expressed 3,075 and 12,347 sites per cell respectively, therefore significantly higher than the observed in normal PB lymphocytes. Conclusion: PRAME is strongly expressed in the neoplastic clone in most cases of CLL and MCL. This finding supports the suggestion that this antigen may be further explored as a target for diagnostic and therapeutic approaches.

390 P53 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. The biology and clinical course of malignancies is determined by the altered expression of genes involved in the regulation of cell cycle and apoptosis. The p53 gene is the most frequently mutated gene in human tumors. It has been shown that approximately 10-15% of patients with chronic lymphocytic leukemia (CLL) have mutations in p53. The aim of the study was to determine the expression of p53 in CLL and to investigate whether the expression of p53 by CLL cells is correlated with stage of the disease, with disease progression and response to therapy.

Material and methods. 160 patients (pts) with CLL were evaluated, 81M/79F, average age was 59 years (age range 38 - 75 yrs). According to the Binet classification 39 pts were in stage A, 73 pts were in stage B, 48 pts were in stage C. P53 expression was investigated with immunocytochemistry on peripheral blood samples using monoclonal antibodies 'DAKO' (Denmark).

Results. P53 expression was detected in 48/160 (30%) untreated CLL pts, 19/160 (12%) of them had stage B, 29/160 (18%) had stage C. P53 expression was correlated with stage of the disease: the number of p53-positive cells was 49,4% in pts with stage B and 65,3% in pts with stage C (p=0,001). All pts received chemotherapy (CT) with fludarabine. After CT p53 expression was detected in 3% pts, all pts in this group were found to have an aggressive form of CLL characterized by advanced Binet stage and resistance to CT.

Conclusion. Our data showed that p53 expression was associated with stage of the disease and with response to CT: pts with p53-positive CLL cells after treatment had poor response to CT as compared to pts without p53-positive CLL cells after treatment. Therefore these findings indicate that p53 expression may be used as an additional indicator of disease activity, as an indicator for drug resistance and as a prognostic factor in CLL.

391 TRANSFERRIN RECEPTOR-1 AND -2 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Transferrin receptor-1 (TfR1, CD71) plays a major role in cellular iron uptake in both normal and neoplastic cells. TfR2, another receptor for transferrin involved in an alternative iron transport pathway, was recently cloned and found to exist in two different isoforms (alpha/beta); TfR2beta mRNA is detected by RT-PCR in all tissues, while TfR2alpha has more restricted expression. CD71 is considered as one of the

classical activation markers up-regulated upon B-cell activation; recently, a significantly higher expression of CD71 was reported in the mutated group of B cell chronic lymphocytic leukemia (B-CLL; Blood 2002; 99; 4087). We performed RT-PCR analysis for qualitative and quantitative evaluation of Tfr1/Tfr2 mRNA in B-CLL and cellular immunophenotypic analysis for CD71 expression in peripheral blood samples of 105 patients with CLL (CD5+CD19+CD20+CD23+); we also explored possible associations between Tfr1/Tfr2 mRNA expression, CD71 expression and immunoglobulin heavy chain (IgH) mutation status. Most patients were at early clinical stages (Rai stages 0-II: 92 pts; stages III-IV: 13 pts); the median time from diagnosis was 36 months (0-114) and the median follow up time 43 months. Sixty-four patients (61%) carried mutated IgH genes (less than 98% homology to germline), whereas 41/105 cases carried unmutated Ig genes. There were only 5 cases with less than 20% CD71 expressing cells; in the remainder (100/105 cases), the percentage of CD71-expressing neoplastic B cells was generally high (>90%, range: 23.3-100.0%), without any association with Ig mutation status. Tfr1 cDNA sequences were amplified in all samples; Tfr2alpha mRNA was not detected in 47/98 analyzed cases, of which 32 carried mutated and 15 unmutated IgH genes; Tfr2beta cDNA sequences were amplified in 87/98 analyzed samples. Densitometric analysis of quantitative RT-PCR products corresponding to Tfr1 exons 15-17, was done in order to determine Tfr1 mRNA levels in 98/105 cases. Each sample was tested in at least three independent experiments. Samples were classified into four groups (A-D, each representing a one-log difference in concentration); group A (highest expression), 21 cases: 15/21 IgH mutated, 13/20 Tfr2alpha mRNA (-); group B, 27 cases: 20/27 IgH mutated, 10/23 Tfr2alpha mRNA (-); group C, 25 cases: 15/25 IgH mutated, 12/25 Tfr2alpha mRNA (-); group D, 25 cases: 11/25 IgH mutated; 12/23 Tfr2alpha mRNA (-). A significant association was identified between IgH mutated cases and high Tfr1 mRNA levels (A+B groups). The almost uniformly high CD71 expression in CLL is in keeping with the activated status of neoplastic cells, regardless of IgH mutational load; this is not paradoxical given that CLL cells (both IgH mutated and unmutated) are antigen-experienced cells. High CD71 expression in the context of widely divergent Tfr1 mRNA levels suggest posttranscriptional control of Tfr1 gene expression in CLL; this is further supported by the significant association of IgH mutated cases only with Tfr1 mRNA and not with CD71 levels.

392 IMMUNOGLOBULIN KAPPA GENE LOCUS REARRANGEMENTS TO THE KAPPA DELETING ELEMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA WITH CLONOTYPIC LAMBDA LIGHT CHAIN

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In lambda-light chain expressing B cells, immunoglobulin (Ig) kappa genes are inactivated via site-specific rearrangements of the kappa deleting element (KDE) either to an unrearranged Vk gene segment upstream of a VkJk joint or to the heptamer recombination signal sequence in the Jk-Ck intron (Jkl). We analyzed Vk-KDE and Jkl-KDE rearrangements in a series of 85 B-CLL cases expressing lambda light chain. DNA-PCR amplified VkJk joints in 46/70 analyzed cases (66%). Vk/KDE rearrangements were amplified in 45/74 cases analyzed (61%); sequence information was available for 40 Vk/KDE rearrangements and revealed usage of the following genes: 3-20/A27 (8 cases); 2-30/A17 (4 cases); 1-16/L1, 1-5/L12 (3 cases each); 1-17/A30, 4-1/B3, 2D-26/A5, 1-33-1D-33/O8-O18 (2 cases each); 2D-30/A1, 2D-28/A3, 2D-24/A7, 1-27/A20, 5-2/B2, 1-12/L5, 3-11/L6, 1-8/L9, 1-6/L11, 1D-13/L18, 1D-12/L19, 3D-11/L20, 1D-43/L23, 1-39-1D-39/O2-O12 (1 case each). Analysis of Vk-KDE junctional regions revealed insertion of a median of 2 N nucleotides (range, 1-12) by Tdt activity in 26/40 rearrangements (63%). A median of 2 (range, 1-12) nucleotides were removed by 5-exonuclease activity from the 3-end of the Vk segment in 31/40 rearrangements; 3-exonuclease removed a median of 2 (range, 1-16) from the 5-end of the KDE in 29/40 junctions. Jkl/KDE rearrangements were amplified in 49/75 cases analyzed (65%); sequence information was available for 44 rearrangements. A median of 1 N nucleotides (range, 1-9) were identified in 21/44 Jkl/KDE junctions; a median of 4 nucleotides (range, 1-11) were deleted by 5-exonuclease activity from the Jkl heptamer sequence, while 3-exonuclease had removed a median of 3 (1-9) nucleotides from the

KDE sequence in 38/44 junctions. Taking VkJk, Vk/KDE and Jkl/KDE rearrangements together, 77/85 B-CLL cases under study (90%) had at least one rearranged Igk allele. In conclusion, similar to normal peripheral blood naive IgM+ B cells, rearrangements to the KDE inactivate the Ig kappa locus in a large fraction of B-CLL cases with clonotypic lambda light chain; importantly such rearrangements were detected in CLL cases of both the mutated and unmutated subtypes. Evidence for Tdt, 5-exonuclease and 3-exonuclease activity was obtained in the majority of both Vk-KDE and Jkl-KDE rearrangements resulting in junctional regions with high heterogeneity. The frequent use of certain Vk segments in Vk-KDE rearrangements (eg., 3-20/A27, 1-5/L12) may be related to their overutilization in the rearrangement process (as reported in normal peripheral blood IgM+ B cells). Finally, these results indicate that CLL clonogenic cells had attempted several rearrangements at the Ig kappa loci before finally switching to lambda light chain expression.

393 DEREGULATION OF NOTCH2 SIGNALING IN B-CLL

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We have recently shown that Notch2 signaling is involved in the overexpression of CD23 in B-CLL cells. Notch2 plays a determining role in the development and maintenance of pre-activated CD5 positive B-cells, suggesting a possible oncogenic function for Notch2 in the pathogenesis of B-CLL. Here we present evidence that B-CLL lymphocytes express a constitutively active form of Notch2 (Notch2IC). Although cultivated B-CLL cells lose their nuclear Notch2IC activity within 24 hours as demonstrated by EMSA, Notch2IC could be transiently re-induced by the protein kinase C activator TPA. The activity of Notch2IC was not affected by the gamma-secretase inhibitor DAPT, excluding wild type Notch2 signaling in the leukemic cells. In some B-CLL samples, TPA induced Notch2IC could be down-regulated by the PKC- inhibitor Rottlerin. Accordingly, the physiological PKC- δ stimulator IFN- γ induced Notch2IC in some B-CLL cases. In all experiments, the activity of Notch2IC correlates with the expression of its target gene CD23 and with an anti-apoptotic state of the leukemic cells. Control experiments in lymphocytes from healthy donors demonstrated that wt-Notch2 signaling is also Rottlerin sensitive. Since CD5 positive B-cells are thought to be subjected to positive selection, we next investigated whether Notch2 modulates B-cell receptor (BCR) signaling and found that enforced expression of a constitutively active form of Notch2IC rescues BL41 cells from BCR mediated apoptosis. In summary, our data provide evidence that Notch2 is deregulated in B-CLL cells and might be implicated in the positive selection of the malignant clone.

394 PROTEASOME INHIBITOR INDUCED APOPTOSIS IN B-CLL CELLS IS ASSOCIATED WITH NOTCH2 INACTIVATION AND DOWNREGULATION OF CD23

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Recently, proteasome inhibitors (PI) have attracted interest as novel anticancer agents in B-cell chronic lymphocytic leukemia (B-CLL). Inhibition of the proteasome induces apoptosis in B-CLL cells 10 times more efficiently than in normal cells thus providing the basis for a therapeutic application. A prominent feature of B-CLL cells is the high expression of CD23 which is closely related to Notch2 signaling. Since many components of the Notch signaling cascade are regulated by the 26S proteasome, we studied the effect of PI on Notch2 activity. Upon exposure of B-CLL cells to PI, transcriptionally active nuclear Notch2 (Notch2IC) gradually disappeared as shown by electrophoretic mobility shift assays. This process was accompanied by a down-regulation of the mRNA for the Notch2 target gene CD23a, and loss of CD23 surface expression. We found a direct correlation between the disappearance of CD23 from the surface of the malignant cells and the rate of apoptosis induced by PI. The sensitivity of the leukemic cells from different B-CLL cases to PI-induced apoptosis inversely correlated with the amount of the Notch2 complex in band shift assays. PI-mediated inactivation of Notch2 occurred as a specific process as NF-AT, another transcription factor complex, was only slightly affected and the surface expression of its target gene CD5 remained unchanged. Inhibition of the apoptotic process by caspase inhibitors did not prevent Notch2 inactivation and CD23 downregulation thus demonstrating that the observed effects occur early in the apoptotic cascade. In a Notch2 'gain-of-function' model, BL41 cells stably transfected with constitutively active Notch2 showed increased resistance toward PI-induced apoptosis as compared to non-transfected cells.

Our results indicate that in B-CLL cells proteasome inhibitors interfere with Notch2 signaling and decrease CD23 expression in parallel to the induction of apoptosis. The protective function of Notch2 in a human B-cell line suggests that loss of Notch2 signaling upon PI treatment might be required for the induction of apoptosis.

395 INVOLVEMENT OF NUCLEOSIDE TRANSPORTERS IN NUCLEOSIDE-DERIVED DRUG RESPONSIVENESS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: Nucleoside derivatives are widely employed in the treatment of hematological malignancies. In particular, fludarabine is considered the treatment of choice for most patients with chronic lymphocytic leukemia (CLL). In the last years, the intracellular events involved in the pharmacological action of these compounds have been extensively studied.

Aims: To analyze the role of plasma membrane transporters in nucleoside-derived drug bioavailability and action in leukemia cells.

Methods: mRNA of the five nucleoside transporter isoforms cloned so far (CNT1, CNT2, CNT3, ENT1 and ENT2) were analyzed by real-time PCR in cells from 22 CLL patients. Nucleoside uptake was measured using a rapid filtration method using radionucleosides. We have generated a polyclonal antibody against ENT2 and the expression of ENT2 was analyzed by Western Blot. Cell viability was determined by the MTT assay.

Results: CNT1 mRNA was not detected in CLL cells and high variability in the other nucleoside transporters mRNA levels was observed among CLL patients. No statistical correlations were observed among the five nucleoside transporters analyzed. Although all CLL patients showed expression of CNT2 and CNT3, only half of the samples had concentrative activity, whereas no concentrative uptake of fludarabine was detected in CLL samples. However, a significant correlation between fludarabine uptake via ENT carriers and ex vivo sensitivity of CLL cells to fludarabine was observed. Furthermore, a good correlation was obtained between the expression of ENT2 by Western Blot in CLL cells and fludarabine uptake via ENT carriers and also with ex vivo sensitivity of CLL cells to fludarabine.

Conclusions: No relationship between mRNA levels and ex vivo fludarabine cytotoxicity was observed. Moreover, fludarabine accumulation in CLL cells was mostly, if not exclusively, mediated by ENT-type transporters whose biological activity was clearly correlated with fludarabine cytotoxicity. Finally, a correlation between ENT2 expression and nucleoside transporter related biological activity was detected, which reveals a role of ENT-mediated uptake in drug responsiveness in CLL patients.

396 RAPAMYCIN INHIBITS MITOGEN-ACTIVATION OF B-CLL CELLS

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The majority of circulating leukemic B-cells in chronic lymphocytic leukaemia (B-CLL) are growth arrested in the G0/G1 phase of the cell cycle. However, there is growing evidence for the presence of proliferation centers in lymph nodes and bone marrow. Pharmacological compounds, which target this population, might have additional benefit in therapy of B-CLL. Rapamycin is a potent inhibitor of the proliferation of T and B lymphocytes in response to antigens and cytokines. Therefore, we investigated the effect of rapamycin on the activation and proliferation of B-CLL cells in response phorbol ester 12-O-tetradecanoyl-phorbol 13-acetate (TPA), IL-4 and the redox-active enzyme thioredoxine.

Cells were exposed to stimulation under in vitro co-culture conditions, which prevent spontaneous apoptosis of the leukemic cells (Shehata et al Blood 2002) in absence or presence of Rapamycin for 48 hours. Cultures were subjected to microscopic examination and cell viability was assessed by FACS analysis using annexin V staining and by MTT assays. Cells were also stained with propidium iodide for cell cycle analysis.

Stimulation of B-CLL induced homotypic cell aggregation, increased expression of CD23, release of sCD23 and cell cycle progression (10-20 % of the cells progressed into S+G2/M phases of cell cycle as compared to 1-2% in unstimulated cells). Rapamycin abolished the formation of homotypic cell aggregates, inhibited the expression of CD23 and release of sCD23. In addition, rapamycin significantly inhibited cell cycle progression although it exhibited a moderate effect on cell viability. The inhibitory effect of rapamycin on cell cycle was also observed

in a cell line, which was generated from a patient with B-CLL. Since rapamycin prevents cell cycle progression through inhibition of mTOR-induced activation of ribosomal p70 s6 kinase (p70 s6K), we evaluated the effect of rapamycin on the phosphorylation of p70 s6K in B-CLL cells. Immunofluorescence studies showed the presence of intense immunoreactivity with phosphospecific p70 s6K (Thr389) antibody in the stimulated cells and in the cell line, and that rapamycin reduces the phosphorylation of p70 s6K.

These data indicate that rapamycin inhibits the activation and cell cycle entry of B-CLL cells in response to mitogenic stimuli. They also suggest a potential role for rapamycin in targeting the proliferating cell pool in B-CLL.

397 FISH REVEALS MULTIPLE CELLULAR CLONES MARKED BY DIFFERENT NUMBERS OF D13S319 AND RB1 ALLELES IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Bands 13q12-14 deletions are the most common karyotype defects in B-CLL, suggesting they might contain an as yet unidentified tumor suppressor gene.

Our study was aimed at determining whether B-CLL patients may simultaneously harbour cellular subclones with distinct numbers of D13S319 and Rb1 alleles and whether this finding correlates with any clinical parameter and disease outcome.

Interphase FISH was carried out on sixty-nine patients. LSI 13 and D13S319 probes, commercially obtained from Vysis (Downers Grove, IL, USA), were simultaneously applied according to manufacturers guidelines. Normal cells carried two green and two red signals whereas those with a deletion/nullisomy of either probe showed either three or two signals. A nullisomy involving both the loci was never observed so that any FISH pattern was sufficient to document effective hybridisation and allowed us to fix cut-off values at 6%. A total of five hundred cells were analysed for each patient.

Seven patients (10%) showed a deletion of one D13S319 locus and of one Rb1 allele, two a subclone with the same pattern along with another one with one D13S319 allele deleted, ten (14.5%) the deletion of one D13S319 allele, two the deletion of both D13S319 alleles, five (7.2%) a complex pattern, one the deletion of both D13S319 alleles along with the deletion of one Rb1 allele and one the deletion of one Rb1 allele. Overall an abnormal FISH pattern was detected in 42-500 cells from 28 patients (40.5% of cases). The most interesting FISH findings were observed in patients with a complex pattern. Three of them showed two different subclones, one with the deletion of one D13S319 locus and the other with a nullisomy of the same locus in more than two hundred cells. The remaining two patients showed two subclones, one with the D13S319 locus deletion and the other with a nullisomy of the same locus along with a deletion of the Rb1 locus in more than one hundred nuclei. Most of these patients on clinical diagnosis were in early disease stage. In contrast, patients with a monosomy of both the loci were mostly in advanced disease stage. According to Binet three patients were classified as stage B and three as stage C; according to Rai two patients were classified as stage two, two as stage three and one as stage four. Clinical progression occurred in three patients. A monoclonal protein was present in three patients with the deletion of the D13S319 allele.

Our study shows that: i) subclones marked by different numbers of D13S319 alleles may be simultaneously present in B-CLL patients and this very feature might suggest that 13q- is the product of chromosome evolution within the neoplastic clones. This datum is further strengthened by the fact that two of our patients with the deletion of one D13S319 allele spontaneously converted to a normal FISH pattern on subsequent analyses; ii) patients with band 13q14 monosomy frequently presented in an advanced stage on clinical diagnosis and three experienced disease evolution.

398 INDUCTION OF APOPTOSIS IN B-CLL CELLS BY PI3-K INHIBITORS AND SIRNA

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The expansion of the malignant clone in B-CLL appears to be due to inhibition of apoptosis and prolonged survival of the leukemic cells rather

than due to increased proliferation rate. Paradoxically, these leukemic B-CLL cells die spontaneously by apoptosis in cultures suggesting the lack of survival signals under in vitro conditions. Here, we describe an in vitro model which supports survival of B-CLL cells and investigate the antiapoptotic mechanisms in B-CLL cells.

Co-cultivation of B-CLL cells with human bone marrow fibroblasts (BMFs) under serum free conditions inhibited apoptosis and enhanced viability of the leukemic cells in comparison to suspension cultures. The co-culture also allowed preservation of the malignant phenotype of B-CLL cells (CD5, CD19, CD23) throughout the incubation periods (1 day - 4 weeks). Since phosphatidylinositol 3-kinase (PI3-K)/Akt pathway is emerging as a potent anti-apoptotic pathway which can be activated by ligation of B-cell receptor, cytokines and growth factor receptors and integrins. B-CLL cells were treated in co-cultures with the PI3-K inhibitors wortmannin and Ly294002 or with small interference RNAs (siRNAs) against PI3-K (p110 catalytic subunit) and against Akt1. Cell viability and transfection efficacy were assessed by flowcytometry. Inhibition of PI3-K and Akt1 significantly abolished the supportive effect of the fibroblasts and induced apoptosis in B-CLL cells within 15 hours of incubation. Interestingly, the leukemic cells were more sensitive to PI3-K inhibitors than T cells, monocytes and BMFs. Induction of apoptosis was associated with a significant decrease in the intracellular PIP3, PI3-K, PDK1 and Akt1. There was also significant decrease of downstream targets of PI3-K/Akt pathways including NF-kappa B, IKK and dephosphorylation/activation of the tumour suppressor protein PTEN.

In conclusion, these results demonstrate that human BMFs support B-CLL survival in vitro and may mimic the situation in the lymphoid tissues in vivo. The results also indicate that PI3-K/Akt pathway is involved in the protection of B-CLL cells from apoptosis and that PI3-K inhibitors are selectively targeting the leukemic B-CLL cells.

399 EXPRESSION AND FUNCTION OF THE CXCR5 CHEMOKINE RECEPTOR ON B LYMPHOCYTES FROM PATIENTS WITH B CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND OTHER B CELL NEOPLASIAS.

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Chronic lymphocytic leukemia (B-CLL) is characterized by the relentless accumulation of mature, functionally incompetent B lymphocytes in the blood, secondary lymphoid tissues, and the marrow. The mechanisms that regulate the dissemination of CLL cells within these tissue compartments are largely unknown. Chemotactic gradients established by constitutively expressed chemokines may be critical for regulating the trafficking of CLL cells. Recently, we characterized the chemokine stromal cell-derived factor-1 (SDF-1) as an important mediator for the marrow homing and for stromal cell- or 'nurse-like' cell-dependent survival of CLL cells (JA Burger et al., Blood 94:3658-67, 1999 and Blood 96:2655-63, 2000). Here, we examined the expression and function of the CXCR5 receptor for the chemokine B cell-activating chemokine-1 (BCA-1/CXCL13) in CLL and other B cell neoplasias. Normal, mature B cells and a subset of memory T cells express CXCR5 chemokine receptors and migrate in response to BCA-1. By flow cytometry, we detected high levels of surface expression of CXCR5 on all cases of 40 B-CLL patient samples, as well as on leukemic B cells from patients with follicular lymphomas and other low grade B cell lymphomas. In contrast, CXCR5 was not detectable on neoplastic B cells from patients with multiple myeloma, a B cell malignancy that generally does not involve secondary lymphatic tissues. Actin polymerization in response to stimulation with 200 ng/ml BCA-1 was detected in each of 12 CLL samples and this response was rapid and transient with a peak response within 15s to levels that were comparable to CLL cells stimulated with the chemokine SDF-1a. In contrast to SDF-1a, which induces a bell-shaped dose-response curve in chemotaxis assays with CLL cells, chemotaxis of CLL cells to BCA-1 was characterized by a gradual increase in chemotaxis towards BCA-1 concentrations between 500 and 2500 ng/ml. In contrast to CLL B cells, multiple myeloma B cells did not display these functional responses to stimulation with BCA-1.

Stimulation of CLL B cells with BCA-1 induced a prolonged, transient activation (phosphorylation) of p44/42 MAP kinases (ERK1/2) that was comparable to the response detected after stimulation of CLL cells with SDF-1a.

Because BCA-1 is thought to be predominantly secreted by follicular dendritic cells (FDC) within secondary lymphatic tissues, we examined tissues (lymph nodes, spleen) from patients with B-CLL, follicular lymphomas, and healthy controls for BCA-1 expression

by immunohistochemistry. We detected BCA-1 expression by FDC predominantly in germinal centers in follicular lymphomas and healthy controls, whereas the relative FDC density and BCA-1 expression was lower in pseudofollicles of B-CLL lymph nodes. Disturbances of B cell colonization within specific anatomic compartments of lymph nodes and spleen in CXCR5-deficient mice indicate that chemotactic recruitment by locally produced BCA-1 is important for the recruitment of B cells to distinct areas of secondary lymphoid tissues. Collectively, our observations therefore indicate that B-CLL and other low grade B cell lymphomas (but not multiple myeloma) display functional CXCR5 receptors that may play a key role for trafficking and homing of B-NHL cells to secondary lymphoid tissues.

400 GENE EXPRESSION PROFILING IN CHRONIC LYMPHOCYTIC LEUKEMIA: CORRELATION WITH DISEASE STABILITY AND PROGRESSION, AND P53 MUTATIONS

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Background:

Chronic lymphocytic leukemia (CLL) is a disease with an heterogeneous clinical course, with some patients showing a very indolent behavior and not requiring treatment for a long period, if ever, and others showing an aggressive clinical picture and requiring treatment after a short observation time. Different biologically-based analyses show prognostic relevance: the IgVH mutational status, the presence of cytogenetic abnormalities, p53 mutations and, more recently, ZAP-70 expression. In fact, to date it is well established that the presence of an unmutated status of the IgVH variable genes, as well as the presence of p53 mutations and abnormalities involving chromosomes 11 and 17, as well as an increased expression of ZAP-70 on CLL cells, are associated with a poor outcome.

Aims:

In this study, we aimed at identifying gene expression patterns associated with a different clinical course. Taking advantage of the data available on the p53 mutational status, we also sought to identify gene profiles associated with this poor prognostic marker. In addition, we performed a comparison between mutated and unmutated IgVH cases.

Methods:

We evaluated the gene expression profile in 30 untreated CLL patients with a well-documented clinical course: 14 patients had a highly stable disease (9-18 years without treatment) and 16 had an aggressive clinical picture that required therapy. Gene expression profile was evaluated using the Affymetrix U95Av2 gene chips. The diagnosis of CLL was based on the presence of >5,000 lymphocytes/l expressing a typical CLL phenotype. All samples evaluated in this study had >90% CD5/CD20+ cells; none of the patients had received any treatment at the time of the study.

Results:

Comparison between patients with stable and progressive disease identified 83 genes differentially expressed, the majority having higher expression levels in the progressive cases. A set of genes highly expressed in the progressive samples play a role in cell cycle progression and among the few ones more highly expressed in the stable patients some are known to be associated with growth arrest, suggesting that cell cycle deregulation induces a more aggressive clinical scenario. In agreement with previous findings, comparison between mutated and unmutated IgVH identified a large set of genes that were more highly expressed in the unmutated group. Comparison between p53 mutated and wild-type cases selected a large set of genes and identified a very strong signature, characterized by the down-regulation of several genes in the mutated samples; the majority of genes highlighted by this analysis are involved in apoptosis, DNA repair, growth inhibition, transcription regulation and tumor suppression.

Conclusions:

These results show that gene expression analysis can identify specific patterns associated with disease stability or progression in CLL, as well as with the presence of p53 mutations. Further characterization of genes associated with an aggressive course could provide new therapeutic targets for CLL patients. The presence of a strong signature in p53 mutated CLL samples confirms the importance of the integrity of the p53 pathway.

401 THE BONE MARROW HISTOLOGICAL SIGNATURE OF T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKAEMIA

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Background:

Bone marrow (BM) involvement in T-cell large granular lymphocytic (LGL) leukaemia occurs in 69-100% of cases but there have been few reports focusing on the detailed morphology of the BM in this condition.

Aims:

To describe the histological features of BM involvement in T-cell LGL leukaemia, identify any characteristic pattern of involvement, and explore possible relationships between the pattern or degree of infiltration, and associated cytopenias.

Methods:

BM trephine biopsies from 18 patients with T-cell LGL leukaemia were reviewed. Immunohistochemistry was performed using the following antibodies: CD3, CD 20, CD79a, CD4, CD5, CD8, CD16, CD56 and CD57. Anti-CD68 was included to identify macrophage activity, and anti-CD34 to highlight intra-sinusoidal cells.

Results:

The BM was normo- or hypercellular in 89% of cases. An interstitial lymphocytic infiltration was visible on H+E sections with an additional nodular component seen in 56%. Apoptotic figures were present in 89% of cases and haemosiderin deposition in 61%.

All cases showed trilinear haematopoiesis with normal or increased megakaryopoiesis and erythropoiesis in the majority. Myelopoiesis was normal or reduced in 89% with occasional left shift and myeloid arrest. Reticulin fibrosis was present in all cases assessed (Grade I/II in 64%; Grade III in 36%).

Immunohistochemistry confirmed the interstitial infiltration in all cases and highlighted lymphoid aggregates in 3 additional specimens. Interstitial CD3+ cells were predominantly CD8+ in 94% of cases assessed (one case had a CD4+, CD8- phenotype). A preferential localisation of T-cells was seen in 89% of assessable cases with CD8+ cells predominating in the interstitium and CD4+ cells surrounding central condensations of CD20+ B cells.

Lymphoid nodules (median 9, range 0-18) were visible and ranged in size from 0.1mm 4mm. No correlation was found between either number of nodules ($r = -0.32$), or size of largest nodule ($r = -0.2$), and neutrophil count. Degree of infiltration (CD3+) also did not correlate with neutrophil count ($r = -0.1$).

Other features seen in most cases were: intra-sinusoidal T lymphocytes in 92%; increased macrophages in 89%; and eosinophilia in 94% (this was a striking feature in the majority).

Conclusion:

We confirm an interstitial infiltration of CD3+, CD5+, CD8+, CD57+/- cells in all cases of T-cell LGL leukaemia with an intra-sinusoidal component in 92% of cases. The presence of apoptosis, ineffective erythropoiesis (as reflected by BM iron deposition), increased macrophages and striking eosinophilia have not been previously described. We have identified lymphoid aggregates/nodules in 72% of cases. The presence of nodules with non-clonal B-cell centres surrounded preferentially by CD4+ cells, with a predominance of CD8+ cells in the interstitium, was a characteristic finding and may constitute the histological signature of this condition as well as prove of diagnostic value particularly when DNA analysis is not readily available. Similar patterns of infiltration have not been noted in our institution, on review of BM biopsies from patients with other variants of non-Hodgkins lymphoma with, and without BM involvement.

402 CD43 ANTIGEN EXPRESSION IN B-CELL CHRONIC LYMPHOPROLIFERATIVE DISEASES

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BACKGROUND: Flow cytometry immunophenotyping is the basis for the diagnosis of B-cell lymphoproliferative diseases. A scoring system to discriminate between chronic lymphocytic leukemia (CLL) and other B- cell chronic lymphoproliferative diseases (non-CLL) has been widely used (Moreau et al. Am J Clin Pathol, 1997). There are however some cases with non conclusive immunophenotyping. It has been described that CD43 antigen can help in the differential diagnosis of CLL from other B-cell chronic lymphoproliferative diseases.

AIMS: To describe CD43 antigen expression in lymphoproliferative diseases and its possible use in the differential diagnosis of CLL from other non-CLL diseases.

METHODS: We made a retrospective flow cytometry analysis of 122 patients (67 male) diagnosed of chronic lymphoproliferative diseases from 1995 to 2003. Mean intensity and percentage of positive cells were analyzed for each of the following markers: CD5, CD23, CD79b, FMC7 surface immunoglobulines (slg) and CD43. The classical scoring system described by Moreau was used to diagnose CLL. CD43 antigen was considered positive when expressed in more than 50% of cells. Final diagnosis was reached by the combination of cytomorphology, flow cytometry, histology and molecular biology.

RESULTS: Median age was 69 (range 32-91). Hematologic diagnosis was: 107 CLL (88%), 8 splenic marginal zone lymphoma (6%), 4 hairy cell leukemia (3%), 1 follicular lymphoma (0.8%), 1 lymphoplasmocytic lymphoma (0.8%) and 1 without definitive diagnosis. CD43 antigen was positive in 97 CLL patients (90%) and in 2 non-CLL patients (13%) ($p < 0.001$). 91 CLL patients (84%) and 13 non-CLL patients (12%) were correctly classified using the classical scoring system (≥ 4 points for CLL and < 3 points for non-CLL). In 12 out of 16 CLL cases (75%) and 2 out of 2 non-CLL cases with discordant immunophenotyping (≤ 4 points for CLL and < 3 points in non-CLL) CD43 antigen improved the accuracy of diagnosis.

CONCLUSION: We conclude that CD43 antigen is strongly expressed in most CLL cases. CD43 expression can help in the diagnosis of chronic lymphoproliferative diseases specially those with non-conclusive immunophenotyping.

403 BONE MARROW ASPIRATE VERSUS BONE MARROW BIOPSY IN DETECTING NON HEMATOLOGICAL MALIGNANCIES

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The bone marrow (BM) is commonly involved by metastatic tumours. Metastasis may be present in the BM in absence of any abnormalities and the examination of BM may be the unique available modality for discovering their presence. The value of BM aspirates in detecting metastasis has usually found to be less than that of histological biopsy by the most of the authors (Bearden et al. J Clin Path 27:738, 1974; Roedel: Ann Clin Lab Sci 4:193, 1974). However in the literature there are present also reports in which both procedures are recommended as complementary (Garrett et al. Cancer 38:2401, 1976; Gurmukhi et al. Cancer 40:2317, 1977).

Aim of this retrospective study is to assess the value of the aspirate versus biopsy in detecting the presence of BM metastases in our experience from January 1990 to December 2003.

Number 142 cases (0, 6%), out of about 25,000 BM specimens analysed in our hematological diagnostic laboratory, have been selected: N 126 based on the clinical request of suspect for BM metastases and N 16 based on the presence of metastases at BM evaluation, regardless the clinical suspect. BM aspirate is harvested from the superior posterior iliac crest and the microscope analysis is carried out on at least five May-Grünwald-Giemsa stained BM smears.

Number 112 BM aspirate specimens (79%) are positive for presence of metastases, while 30 are negative (21%).

The primary sites of metastatic malignancies show following distribution: breast 27,6% (#31), lung 17, % (#19), prostate 16% (#18), stomach 6,4% (# 7), others 16% (neuroblastoma # 3, colon # 3, bladder # 3, thyroid # 2, liver # 2, uterus # 2, pancreas # 1, oral cavity # 1, rhabdomyosarcoma # 1), unknown 17% (#19). All these cases have been confirmed at marrow biopsy or at autopsy.

In 16 cases, out of these 112 BM aspirate positive specimens, the clinical suspect is of hematological or infectious disease and the marrow biopsy has been requested as consequence.

Number 2/112 positive bone marrow aspirate were negative at first marrow biopsy, while result positive at second biopsy specimen, performed on the basis of the BM aspirate positive result.

Out of 30 marrow aspirate negative specimens, all with clinical suspect of marrow metastases, 4 cases (13, 3% of all negative marrow aspirate specimens) result positive to marrow biopsy.

Analysis of these data reveals a very strong concordance between marrow aspirate and biopsy, having 4/30 false negative and 2/112 true positive on BM aspirate analysis: these data seems to be similar to those reported in the literature.

Our data confirm the absolute complementarity of BM aspirate and biopsy in the detection of metastases as well its usefulness to fasten the diagnosis of metastases, when unsuspected.

In our experience both aspiration and biopsy appear to be indicated for full evaluation of bone marrow in cancer patients.

404 SPLEEN HISTOLOGY IN LEUKAEMIAS OF LARGE GRANULAR LYMPHOCYTES

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Background: Lymphoproliferative disorders of large granular lymphocytes (LGLs) are rare and characterised by expansions of CD3+ T-cells or CD3 NK-cells. Splenomegaly is present in 20-50% of patients with T-cell LGL leukaemia and is often moderate. Information on the spleen histology in LGL leukaemias is scanty. In the only reported series, Agnarsson et al (Human Pathology 1989, 20: 643-651) described expansion of red pulp with intra-sinusoidal lymphoid infiltration in 5 cases, accompanied by prominent white pulp germinal centres. Immunohistochemistry performed in 2 cases, showed a CD3+, CD8+ population in the red pulp.

Aims: To describe comprehensively, using immunohistochemistry, the histological features of the spleen in leukaemias of LGLs.

Methods: Retrospective review of spleens from 7 patients with LGL leukaemia (5 T-cell, 2 NK-cell) was performed. Spleen sections were examined using anti T-cell and NK-cell antibodies (CD2, CD3, CD4, CD5, CD8, TCRb, CD56, CD57), antibodies against the cytotoxic granule proteins (TIA-1, granzyme B and perforin), CD45RO, CD43, Bcl-2, CD25 and CD20. **Results:** The weight of the spleen was available in 5 cases and ranged from 710g to 1850g (median weight 780g). Mild to moderate lymphocytic infiltration confined to the red pulp was seen in all cases but did not distort the architecture of the red pulp. All cases demonstrated preservation of the white pulp with prominent germinal centre formation noted in 4 cases. Moderate to marked expansion of the germinal centre marginal zones was a feature in 5 cases. Germinal centres were intimately surrounded by the lymphoid infiltrate, but the boundary between the malignant infiltrate and the white pulp was sharply demarcated with no spill over or invasion of cells seen.

Lymphocytes were TIA-1+, perforin+ in all cases and granzyme B + in 6. The abnormal infiltrate was CD2+, CD43+, CD4-, CD5-, CD45RO-, CD25-, Bcl-2-, CD20- (1 case CD2-) in contrast to the normal spleen which showed CD3+, CD5+, CD45RO+ cells in the red pulp. With rare exceptions, the 5 T-cell cases showed a CD3+, CD8+, CD56-, CD57- phenotype (1 case CD8-, 1 case CD57+) and the 2 NK-cell cases a CD8-, CD56+/-, CD57+/- phenotype. CD20 immunostaining highlighted negative tumour cells abutting on, but not infiltrating positive B-cells of the white pulp marginal zone. Non-caseating epithelioid granulomas were noted in 1 case.

Conclusions: The common pattern of spleen involvement in both T-cell and NK-cell leukaemic proliferations was lymphoid infiltration by small cells clearly confined to the red pulp with preservation of the white pulp. Germinal centre hyperplasia with expansion of the marginal zones was common. Infiltrating cells expressed cytotoxic granule proteins in all cases and had a CD45RO-, CD5- phenotype in contrast to normal splenic T-cells which are CD45RO+, CD5+. Typical neoplastic features of spill over and/or invasion of other compartments was distinctly absent and may help to distinguish this disorder from other lymphoproliferative disorders of the red pulp such as hairy cell leukaemia where such behaviour results in the formation of red cell lakes and atrophy of the white pulp.

AML: BIOLOGY

405 THE INFLUENCE OF SHORT CULTURE OF LEUKEMIC BLASTS ON THE EXPRESSION OF CO-STIMULATORY MOLECULES AND THE ABILITY TO INDUCE THE PROLIFERATION OF AUTOLOGOUS T-LYMPHOCYTES.

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Background: Activation of autologous cytotoxic lymphocytes T induced by co-stimulatory molecules present on blast cells plays an important role in immunological response to leukemic antigen.

Objectives The aim of the study was to assess the expression of co-stimulatory molecules: B7-1 (CD80) and B7-2 (CD86) on blast cells of patients with acute leukemia, and the possibility of the induction of this expression during short-term culture. The capability of the cultured leukemic blasts to stimulate the proliferation of autologous lymphocytes T was also analyzed.

Patients and Methods: Forty patients were enrolled in the study: 33 with AML and 7 with ALL. The flow cytometry analysis of the co-stimulatory

molecules expression with monoclonal antibodies CD80 and CD86, and short-term cultures were performed on leukemic blasts. Blast cells of 13 patients were tested for their capability to stimulate the proliferation, as assessed by 3H-T incorporation, of autologous T-lymphocytes. IFN-gamma and IL-4 concentration in supernatants of mixed lymphocyte cultures was tested by ELISA method.

Results: The percentage of CD86-positive cells was significantly higher in patients with acute myeloid leukemia M4 and M5 as compared with other acute leukemia FAB subtypes (p=0.0001). We observed an increase of CD80 expression on blasts after short term culture with and without daunorubicin, and this increase was higher on M4 and M5 blasts than on blasts of other leukemia sub-types. In this group CD86 expression after culture was high, similar to before culture. In six out of 13 mixed lymphocyte cultures the stimulation of the proliferative activity of the lymphocytes was observed (stimulation index ≥ 2). IFN-gamma concentration higher than in non-stimulated cultures was stated in seven out of 13 mixed lymphocyte cultures, and was higher (on the limit of statistical significance) in the patients in whom CD80 expression on blast cells was induced after culture. IL-4 was detectable in the supernatant of no culture.

Conclusions. Our observations confirm the ability of leukemic blast cell to differentiate towards the professional antigen-presenting cells. Those cells are able to stimulate the proliferation of autologous lymphocytes T and thus to drive antileukemic immunological response.

406 UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR AND SOLUBLE MATRIX METALLOPROTEINASE-9 IN ACUTE MYELOID LEUKEMIA PATIENTS: A POSSIBLE RELATION TO DISEASE INVASION

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Extracellular proteolytic enzymes of the urokinase-type plasminogen activator (uPA) and metalloproteinase family play a crucial role in the matrix degradation and tissue remodeling process characteristic of malignant disorders. The receptor for urokinase plasminogen activator (uPAR) serves to localize and intensify the action of UPA and is expressed on the surface of malignant cells. Although the biological significance of MMP-9 and soluble urokinase receptor in growth and progression of lymphoid neoplasm is declared, its clinical significance in AML is not fully elucidated. In this study we determined the levels of soluble urokinase-type plasminogen activator receptor (suPAR), cellular uPAR and sMMP-9 in 43 newly diagnosed AML patient at diagnosis, before chemotherapy and ten normal subjects served as a control group. After chemotherapy suPAR and MMP-9 were determined at remission and relapse. The levels of suPAR, cellular PAR were significantly higher (P=0.001,0.001) and MMP-9 was significantly lower (P=0.001) in AML patients at diagnosis as compared to controls. suPAR and MMP-9 levels were significantly lower in AML patients who achieved complete remission as compared to those who did not (P=0.001 for both). Levels of suPAR and MMP-9 were significantly correlated to peripheral blood blast cells (r=0.88, P=0.001; r=0.65, P=0.001 respectively) and blast cell distribution ratio (r=0.84, P=0.001; r=0.65, P=0.001 respectively). suPAR, cellular PAR and MMP-9 were significantly higher in patients with extramedullary infiltration as compared with those without (P=0.001,0.001, <0.05). The suPAR, cellular uPAR, and MMP-9 levels were uneven in AML FAB subtypes being highest in M5 (P<0.05 for all). MMP-9 and suPAR levels were correlated with the disease status. In AML survivors MMP-9, cellular uPAR and su-PAR were significantly lower as compared to non-survivors (P=0.001 for all).

In conclusion: MMP-9 and su-PAR levels might be used as a marker for disease activity and may contribute to blast cell dissemination. MMP-9 and suPAR may be target molecules in the strategy of treatment of AML.

407 ELEVATED SKP2 PROTEIN EXPRESSION IN ACUTE MYELOGENOUS LEUKEMIA: ITS ASSOCIATION WITH THE CONSTITUTIVE PHOSPHORYLATION OF PTEN PROTEIN AND POOR PROGNOSIS

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Background & Aims: The F-box protein S-phase kinase-associated protein 2 (Skp2) positively regulates the G1-S transition by controlling

the stability of several G1 regulators, such as the cell cycle inhibitor p27Kip1. However, the clinical significance of Skp2 in patients with acute myelogenous leukemia (AML) remains unknown. Aims: To evaluate the Skp2 protein expression in AML and its association with the constitutive phosphorylation of PTEN protein and the prognosis.

Methods: In this study, we examined the clinical and biological significance of Skp2 protein expression in AML and evaluated the relationship between Skp2 and p27Kip1 expression and PTEN phosphorylation.

Results: Western blot analysis showed that a high Skp2 protein expression was identified in 57 (57.6%) cases, and was significantly correlated with unfavorable cytogenetics ($P=0.035$), but not with age, the white blood cell count, serum lactic dehydrogenase levels and the French-American-British subtype. An inverse correlation was not observed between the expression of the Skp2 protein and the p27Kip1 protein. However, the p27Kip1 protein was preferentially localized in the cytoplasm in the high Skp2 expression group. The cytoplasmic to nuclear ratio of p27Kip1 protein expression was significantly correlated with the Skp2 protein expression levels ($P < 0.001$). Survival analysis using the Kaplan-Meier method showed that Skp2 overexpression was significantly associated with shorter disease-free survival (DFS) and overall survival (OS) ($P=0.0386$ and $P=0.0369$, respectively). Skp2 overexpression was highly correlated with the constitutive phosphorylation of PTEN protein. The frequency of PTEN phosphorylation was significantly higher in the group with high Skp2 protein expression than with low Skp2 expression ($P=0.035$). Multivariate analysis using the Cox proportional hazard model showed that Skp2 expression was an independent prognostic factor both in the DFS and OS.

Conclusion: These findings suggest that Skp2 expression is an independent marker for a poor prognosis in AML. The presence of a positive correlation between Skp2 and pPTEN suggests that an aberration in the PTEN/Skp2 signaling pathway might be operating in AML.

408 CYTOPLASMIC MISLOCALIZATION OF P27KIP1 PROTEIN IS ASSOCIATED WITH CONSTITUTIVE PHOSPHORYLATION OF AKT/PKB AND POOR PROGNOSIS IN ACUTE MYELOGENOUS LEUKEMIA

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Background: Cyclin-dependent kinase (CDK) inhibitor p27Kip1 functions at the nuclear level by binding to cyclin/CDK2. Recently it was shown that Akt/PKB-dependent phosphorylation of p27Kip1 leads to cytoplasmic mislocalization of p27Kip1, and abrogates its activity. Until now, cytoplasmic mislocalization of p27Kip1 in relation to Akt/PKB phosphorylation and its clinical significance has not been evaluated in acute myelogenous leukemia (AML).

Aims: To evaluate the localization of p27Kip1 protein in AML and its association with constitutive phosphorylation of Akt/PKB and prognosis.

Methods: We evaluated the expression and localization of p27Kip1 in AML cells in the context of Akt/PKB phosphorylation. Western blot analysis of nuclear and cytoplasmic fraction of AML cells revealed variable localization of p27Kip1 according to the patients.

Results: The cytoplasmic mislocalization of p27Kip1 was significantly associated with the constitutive Ser473 Akt/PKB phosphorylation in AML cells ($P<0.05$). Transfection of U937 cells with the expression construct encoding the constitutively active form of Akt/PKB resulted in a remarkable increase in the levels of cytoplasmic p27Kip1 in vitro. In contrast, transfection of U937 cells with expression construct encoding dominant negative Akt/PKB resulted in a recovery of nuclear localization of p27Kip1. Disease-free survival and overall survival time of the patients showing the high C/N ratio of p27Kip1 localization was significantly shorter than those of the patients showing the low C/N ratio of p27Kip1 ($P=0.0353$, $P=0.0023$, respectively). Multivariate analysis demonstrated the high C/N ratio of p27Kip1 localization as an independent prognostic variable (relative risk=2.399, $P=0.008$).

Conclusion: These findings further extend our understanding of the role of p27Kip1 in AML, and buttress the case of p27Kip1 mislocalization as a prognostic indicator and Akt/PKB/p27Kip1 pathway as a ready target for anti-leukemia therapy.

409 EVOLUTIONARY ALGORITHMS IN LEUKEMOGENESIS. A HYPOTHETICAL MODEL

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BACKGROUND. The leukemic haemopoiesis is defined by the expansion of an abnormally mutated clone associated with the inhibition of the surrounding normal clones. Acute leukemias are associated with a maturation arrest within the leukemic clone, whereas in chronic leukemias the leukemic clone is capable of undergoing terminal differentiation.

AIMS. To imagine a simple mathematical model describing hematopoiesis as an evolutionary tree and to define the theoretical pathways towards a leukemic haemopoiesis as well as the prognostic and therapeutic implications of these pathways. METHOD. We devised an evolutionary tree starting with one cell that undergoes four divisions during the expansion phase, afterwards losing cells randomly during the contraction phase. The system 'dies' after the last cell is lost. We assumed that the offspring cells are never completely identical to parent cells and to each other, acquiring various mutations. Such mutations are analogous to the genetic and epigenetic changes that accompany normal differentiation but also leukemic transformation. If a certain mutation is present in more than 20% of the final cells (as in the diagnostic criteria for acute myeloid leukemia) the situation becomes dangerous - an abnormal monoclonal pattern is present and if the mutation itself is abnormal a leukemic picture is present. RESULTS. Analyzing this evolutionary algorithm the following patterns arise:

A. A monoclonal pattern is obvious if the abnormal mutation occurs early, during the first or second division.

B. If the non-leukemic clones are affected by an intrinsic or extrinsic event leading to impaired proliferation or early death, an abnormal clone which has somehow escaped may gain relative dominance over a depleted environment even though it would not make an impact in a normal environment.

C. If the abnormal mutation confers an exceptional proliferative capacity, undergoing more divisions than normal cells, the cells bearing this mutation may soon become dominant

D. If the abnormal mutation confers 'immortality', without exaggerated proliferation. In that case this mutation has a high chance of representing >20% of the cells, especially during the contraction phase.

E. Combinations of these patterns may further enhance the leukemic risk of the system.

DISCUSSION AND CONCLUSIONS. Such models can be superimposed on actual leukemic entities, for example model C resembles 'de novo' acute leukemias while model B is more appropriate for secondary acute leukemias, acute leukemias with dysplasia and in the elderly, while a combination of models A, C and D could describe chronic myelogenous leukemia. They may also have implications in therapy, for example predicting a good chance of success for aggressive chemotherapy only in model C. The assessment of individual cases according to such patterns, may enable clinicians to make more logical decisions concerning treatment strategy.

410 XIAP AND XAF-1 ARE STRONG PROGNOSTIC MARKERS IN AML

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Aberrant expression of apoptosis-associated genes has been detected in leukaemia. XIAP is a member of the inhibitor of apoptosis proteins (IAP) family, while XIAP-associated factor-1 (XAF-1) is a pro-apoptotic gene shown to be an antagonist of XIAP. Using real-time RT-PCR (RQ-PCR) protocols, we quantified, and assessed the prognostic value of XIAP and XAF-1 in presentation samples of 25 AML patients. All patients had a standard/good prognostic karyotype.

10/25 patients relapse within 2 years of presentation, while the other 15 patients remain in remission. ABL was used as a control and the level of these transcripts expressed as a ratio of their expression in NB4 cell line. XIAP transcript was detected in 17/25 patients, while XAF-1 transcript was detected in 21/25 patients. In relapsed patients, the mean XIAP and XAF-1 transcripts at presentation was 0.012206 and 0.048448 respectively. Presentation samples from patients who achieved and in CR had mean XIAP and XAF-1 transcripts levels of 0.001264 and 0.429343 respectively.

For prognostic value evaluation the level of 0.1 for both genes was considered as a cut-off point to discriminate between good and poor prognosis groups. A difference of a minimum of 1 log is considered

significant. 7/25 patients had XIAP levels >0.1, of those 4 relapsed within 2 years. On the other hand 18/25 patients had levels <0.1 and only 6 patients relapsed within 2 years. 3/14 patients with XAF-1 levels >0.1, and 7/11 patients with levels <0.1 relapsed within 2 years of presentation. Patients with either XIAP levels higher than 0.1 or XAF-1 levels lower than 0.1 had worse overall survival than the other patients in this cohort ($p=0.0036$ and $p=0.01$ respectively). These data show that both genes are widely expressed in AML patients, and that their level at presentation may have a strong prognostic significance in AML patients especially those with standard/good karyotype.

411 FLT3 GENE MUTATIONS IN ACUTE MYELOID LEUKAEMIA : ASSOCIATION WITH NORMAL AND T(15;17) KARYOTYPES

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FLT3 gene alterations, including internal tandem duplications (ITDs) and Aspartate835 (D835) mutations, occur frequently in acute myeloid leukaemia (AML). We examined a total of 180 adults of AML and biphenotypic leukaemia seen at our institution from 1999 to 2003 to evaluate the distribution and frequency of FLT3 mutations among these patients. Genomic DNA Polymerase Chain Reaction assays were performed to detect (a) FLT3/ITDs located from exon 14 to exon 15 and (b) D835 mutations in exon 20. FLT3 gene alterations were detected in 54/180(30%) patients including ITDs in 37 cases (69%), D835 mutations in 14 cases (26%) and both types of alteration in 3 patients (6%). According to FAB subtype, FLT3/ITD mutation positive cases comprised : 20/46(43%) AML-M1, 11/65(17%) M2, 4/20 (20%) M3, 2/10 (20%) M5, 1/9 (11%) unclassified subset and 2/14 (14%) ABL. The majority (31/40 or 78%) of the FLT3/ITD-positive patients had a normal karyotype while 4 (10%) had t(15;17)(q22;q11). In addition, of the 17 patients positive for D835 mutations, 7 (41%) revealed a normal karyotype and 5 (29%) had t(15;17). Of the three cases with both mutations, one was a M1 with normal karyotype, another had a t(15;17) karyotype while the third was biphenotypic leukaemia with t(11;20)(p15;q11). Overall, alterations in FLT3 gene were detected in 36 of 86 (42%) patients with normal karyotype and 8 of 20 (40%) patients with t(15;17). FLT3 mutations are also more common in patients with PML bcr3 or short (S) transcript (3/8 or 37.5% for both mutations). In contrast to other reports, our incidence of D835 mutations appeared to be as high as ITDs in the APL cohort though the study group was small. Nucleotide sequencing found the FLT3 mutations to be all in-frame with the ITDs ranging from 15bp to 120bp and the most frequently encountered D835 mutation being D835Y. Rare D835 mutations, such as a change in 3 amino acid residues from DIM toPLR in one patient and a deletion of I836 in another case were also encountered. In several of the patients with ITDs, insertions of 1 to 9 nucleotides were found in addition to the duplicated sequences. Thus, FLT3 mutations are a frequent occurrence in AML patients and in particular, show high correlation with normal and t(15;17) karyotypes. FLT3/ITD mutation appeared to be most prevalent in patients with M1 morphology in our series. We did not find patients with M3 subtype to be as highly associated with FLT3/ITD mutation as observed in the Western study populations but our mutation frequency was similar to rates reported in the Taiwanese and Japanese study populations. The high frequency of FLT3 mutations in patients without detectable cytogenetic aberrations may provide a means to better understand the pathogenesis of these poorly characterized AML subgroups.

412 USING INTERFERON CAPTURE TO IDENTIFY ANTI-RECIPIENT AML ACTIVITY IN IN-VITRO PRIMED, MATCHED DONOR LYMPHOCYTES

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Allogeneic marrow transplantation for leukaemia is associated with improved outcome due to the Graft versus Leukaemia (GvL) effect. Clearly, there is a role for alloreactive lymphocytes in leukaemia treatment. We have begun to characterize the in-vitro allogenicity of recipient acute myeloid leukaemia (AML) blasts to matched sibling donor lymphocytes. In data presented earlier this year on the first of our matched pairs, we showed that we could separate innate natural killer (NK) activity (cytotoxic to K562) from anti-recipient AML activity in donor lymphocytes that had been primed with recipient AML blasts and then flow-sorted into fractions. In short, in a chromium release assay, the ratios of anti-AML blast kill over anti-K562 kill were 0.77, 2.7, 0.85, and 0.32, respectively, for unfractionated donor lymphocytes, CD4+ cells, CD8+56- and CD8+56+ cells (NKT cells). We have since isolated clones from CD4

and CD8+ fractions although even the CD4+ clones were primarily cytotoxic to K562 rather than to AML targets. Consequently, in a second matched pair, we have researched ways of selecting donor lymphocytes specifically cytotoxic to AML targets. Interferon secreted by primed lymphocytes when they subsequently encounter the priming antigen, can be trapped at the cell surface using Interferon-Capture. Preliminary data suggest that interferon-capture, combined with flow sorting, shows promise in identifying recipient AML-responsive donor lymphocytes and should help to resolve the various activities in alloreactive populations as well as assist in isolating anti-AML clones.

413 CHARACTERIZATION OF BONE MARROW STROMAL CELLS FROM ACUTE MYELOID LEUKEMIA PATIENTS

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The interaction between hematopoietic precursors and bone marrow stromal cells (BMSC) is needed to allow normal blood cell differentiation. It is supposed that abnormalities occurring during such interactions may account, at least in part, for the oncogenesis in acute leukemias, making of interest the definition of the functional role of BMSC in normal hemopoiesis and neoplastic transformation. Herein, we studied 10 patients suffering from acute myeloid leukemia (AML), six M1/2, one M3, one M5 and two post-MDS (according to the FAB classification), 9 out of 10 in post-chemotherapy complete remission. BMSC were obtained from all of these patients, with the exception of the two post-MDS AMLs, and maintained in culture for two months. These cells were analysed for the expression of a panel of surface markers at different culture passages (from 1 to 4). BMSC were CD44+, CD105+, beta1 integrin+, ICAM1+, HLA-I+, HLA-II+, CD45-, CD31-, and they constitutively expressed the stress-inducible MHC-related molecules MIC-A. This molecule is a reported ligand for the NKG2D receptor expressed by natural killer (NK) and CD8+ T lymphocytes, effector cells that are thought to play a role in host defence against tumors. NK cells have also been shown to regulate normal differentiation of hemopoietic precursor into the myeloid or lymphoid cell lineage. Moreover, it has been stated that NK cells are not able to damage autologous cells, as they receive negative signals through inhibitory receptors, including killer Ig-like receptors (KIR) or C-type lectin inhibitory receptors (CLIR), which bind to HLA-I discrete alleles. Surprisingly, we found that autologous IL2-activated, but not freshly isolated, NK cells lysed BMSC, while T lymphocytes did not kill self or non-self BMSC. Binding of ICAM-1 expressed by BMSC to its receptor, the integrin LFA-1, expressed by NK cells is essential for signal transduction and delivery of the lethal hit. Conversely, it appears that HLA-I molecules do not protect BMSC from NK mediated injury. The contribution of MIC-A recognition by NKG2D to this phenomenon is currently under investigation. Taken together, these data suggest that NK cells, when activated as it may occur during the first response to viral infections, are able to eliminate BMSC, thus altering the normal interactions with hemopoietic precursors and possibly affecting their differentiation. This mechanism might also contribute to the development of aberrant precursors as observed in acute leukaemias.

414 ACUTE MYELOID LEUKEMIA WITH MULTILINEAGE DYSPLASIA PRESENTING AS DE NOVO DISEASE: DIVERSE PROGNOSIS ACCORDING TO CYTOGENETICS

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Background: Acute myeloid leukemia with multilineage dysplasia (AML-MD) is recognized in the WHO classification as an individual AML subtype. However, few studies have analyzed the specific characteristics and prognosis of this entity.

Aim: To analyze main clinico-hematological features and the outcome of a series of patients from a single institution diagnosed with AML-MD.

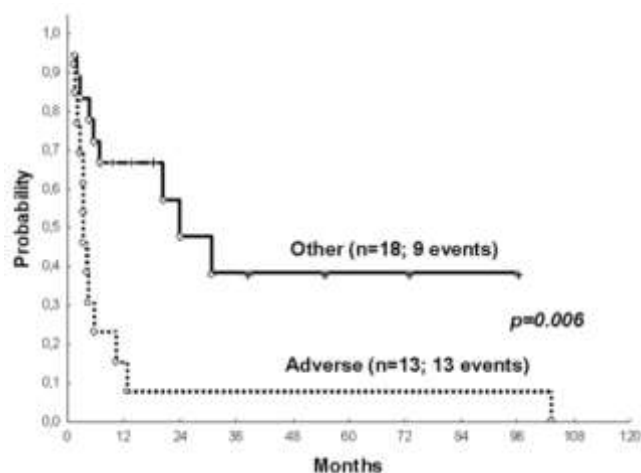
Patients and methods: Forty-two (median age, 59; 22 M/20 F) of 263 patients (16%) consecutively diagnosed with de novo AML during the period 1994-2003 fulfilled the WHO criteria of AML-MD and were included in the analysis.

Results: At diagnosis, median WBC count, Hb level and platelet count were 3.9 x 10⁹/L (0.5-95), 8.4 g/dL (4.2-11), and 54 x 10⁹/L (6-1105). Median percentage of peripheral blood and bone marrow blast cells were

14% (0/90) and 34% (20/83%), respectively. Dysplasia >50% in erythroid, granulocytic and megakaryocytic lineage were observed in 33%, 90%, and 79% of patients. According to MRC definition of cytogenetic risk category, 40% of patients had an adverse karyotype (complex, n=19, abn(7q), n=4, abn(5q), n=3, abn(3q), n=1), 20 (48%) presented with a cytogenetic abnormality belonging to the intermediate-risk category (normal, n=13; +8, n=4; abn 20q, n=2; i(17q), n=1), and cytogenetics was not available in the 4 remaining cases. Thirty-three patients underwent standard induction chemotherapy, with 45% patients achieving complete response (CR), 48% being refractoriness to chemotherapy, and 6% dying due to a treatment-related cause. After a median follow-up of 29 months, 6 patients relapsed, resulting into a median duration of response of 40 months. Median survival for overall series was 6 months, with a 3-year survival of 26% (9) vs. 0 among patients receiving intensive chemotherapy and a palliative approach, respectively (p=0.03). Within the subset of patients receiving intensive chemotherapy, cytogenetics (adverse vs. other) was the only variable at diagnosis with prognostic value for both achievement of CR (15% vs. 61%, p=0.01) and survival (median survival, 3 months. vs. 23 months, p=0.006; figure 1).

Summary: In conclusion, AML-MD constitutes a AML variety with a highly diverse prognosis according to its cytogenetic profile. Nonetheless, a further biological characterization of this category is required to identify subsets of patients with a differentiated clinical course.

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Caption 1:

415 AMPLIFICATION OF THE 11Q23 REGION ON DOUBLE MINUTES AND HOMOGENEOUSLY STAINING REGION ON TWO UNRELATED CLONES IN A PATIENT WITH ACUTE MYELOID LEUKAEMIA M2

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Amplification of MLL is a recurrent but rare genetic change that affects mainly elderly patients with AML or therapy-related MDS, and complex karyotypes. The clinical course is aggressive and the patients have a short survival. MLL amplification can be detected by G-banding as homogeneously staining region (hsr), double minutes (dmin), ring or marker chromosomes. We report the clinical, cytogenetic and molecular data of a patient with AML-M2 who presented MLL amplification as hsr(11)(q23) and dmin in two different clones, and the delimitation of the amplified region. FISH analysis was performed with 6 BACs located between 11q13.4 and 11q25: 699M9 (that covers the CCND1 gene), 241D3 (ATM), 635F12 (ZNF145), 640N11 (GRIK4), 1007G5 (ETS1) and 654C20 (OPCML), and the commercial probe LSI MLL Dual Color (Vysis). Six other BACs located between 2q36 and 2q37 were used. G-banding karyotype at diagnosis of a 81-year-old man with AML-M2 showed 3 different clones: 46,XY,del(5)(q31q35),+8,-20[15]/46,idem,10-17dmin[3]/45,XY,del(5)(q31q35),hsr(11)(q23),-20[12]. The patient died 3 weeks after diagnosis. This karyotype suggests that the clones with dmin and hsr(11) progress independently from the first one, that apparently had not 11q abnormalities. FISH analysis showed MLL amplification in

clones 2 and 3. We delimited the amplified region in 11q. The amplicon extended from MLL (11q23.1) to OPCML (11q25), being all probes amplified in both the hsr and dmin clones (3-17 copies). The copy number of the probes centromeric to MLL were normal. Besides, in all three clones RP11-640N11, that covers the GRIK4 gene, showed one signal in the normal chromosome 11, whereas the other signal split and hybridised to both the der(11) and chromosome 2q36. None of the designed probes for 2q36-q37 were neither deleted nor split, suggesting a cryptic ins(2;11) in all cells, including the clone without 11q amplification. This finding could suggest that GRIK4 might be involved in the leukemogenesis of this patient. Semi-quantitative RT-PCR analysis showed that MLL, but not GRIK4 was overexpressed in the BM of the patient, although both genes were amplified. 3RACE experiments with primers in the GRIK4 gene showed no new sequences on 2q36. In conclusion, we could define the amplified 11q region in a patient who had a very aggressive form of AML. The amplicon containing MLL extended from 11q23 (MLL) to 11q25 (OPCML) in both the hsr(11)(q23) and dmin clones. This is the second time that a patient showing two different clones containing hsr and dmin amplification is reported. Interestingly, a cryptic ins(2;11) has been detected in all clones, suggesting that this 11q rearrangement could be involved in the leukemic progression.

416 HIGH EXPRESSION OF APAF-1LN IN ACUTE MYELOID LEUKEMIA MAY BE ASSOCIATED WITH FAILURE OF REMISSION INDUCTION THERAPY

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Background: Cytochrome c/ Apaf-1/ caspase-9 pathway is the main pathway involved in the apoptosis initiation by anticancer drug treatment. The original Apaf-1 comprises of three functional domains, the N-terminal CARD (caspase recruitment domain) followed by a putative ATPase domain and a C-terminal domain with a 12 WD-40 repeat region. Alternative splicing can create an NH2-terminal 11-amino acid insert between the CARD and ATPase domain or an additional COOH-terminal WDR between the fifth and sixth WDRs. The Apaf-1XL and Apaf-1LN isoforms have the insertion between CARD and ATPase domains (11 amino acids) and Apaf-1XL has also the additional COOH-terminal WDR. In normal human tissues, it seems that the Apaf-1XL isoform is the major form expressed. However, cancer cell lines express significant amounts of Apaf-1LN, which lacks the WDR. The relative expression of Apaf-1 isoforms in AML remains poorly characterised.

Aim: To analyse the mRNA expression of different Apaf-1 isoforms in AML and correlate with response to remission induction therapy.

Methods: Bone marrow samples from 26 patients with acute myeloid leukaemia were submitted to semi-quantitative RT-PCR. Median age was 47 y.o., 2 cases had previously myelodysplasia syndrome and 24 cases were AML de novo (FAB: 5 M1, 6 M2; 4 M3, 6 M4, 2 M5, 1 M6). Remission induction therapy included anthracyclines, cytarabine and 6 thioguanine for patients younger than 60y.o and etoposide, mitoxantrone and 6-thioguanine, for patients 60+ y.o. Fifteen normal controls also participated in the study.

Results: All samples examined, AML or normal controls, contained the 11-amino acid NH2-terminal insertion. Regarding the C-terminal domain, all samples showed both isoforms, with and without the extra WDR, although the relative amounts of the two types varied among the samples. The product containing the extra WDR (Apaf-1 XL) was the most expressed in all normal controls. In contrast, 50% of the AML samples expressed equally or mostly the Apaf-1LN isoform (lacking the extra WDR). Comparing both AML groups, group 1: expressing mostly the Apaf-1XL (Apaf-1XL/Apaf-1LN ratio > 2) and group 2: Apaf-1XL/Apaf-1LN ratio < or = 1, no difference was observed regarding age, AML subtypes, expression of CD34, HLA-DR or lymphoid markers. However, regarding the response to the first cycle of remission induction therapy, 30% had a complete remission (less than 5% marrow blasts and hematological recovery) being only one patient from group 2, 30% did not attain complete remission (all cases from group 2) and 40% of the patients had early death (equally distributed in both groups).

Conclusion: We showed, for the first time, that Apaf-1LN is higher expressed in AML and is associated with a poor response to remission induction therapy. Previously, Benedict et al showed that only the isoforms with the extra WDR activated pro-caspase 9 (JBC 275: 8461, 2000). Thus, we may speculate that in acute myeloid leukemia, a lower pro-caspase 9 activation could also be involved in the deregulation of apoptosis and chemotherapy resistance.

417 ANALYSIS OF THE PROTO-ONCOGENES A -, B - AND C-RAF IN ACUTE MYELOID LEUKEMIA

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Constitutive activation of the RAS-RAF-MEK-ERK signaling cascade plays an important role in the pathogenesis of several malignancies, including acute myeloid leukemia (AML). We were interested whether abnormalities of the serine-threonine kinases A -, B - and C -RAF occur in AML and constitute potential targets for antisense and small molecule inhibitors. Expression analysis of these genes by real-time quantitative (rtq) PCR was assessed in 102 patient samples of AML, three AML cell lines and normal CD34+ cells of seven individuals and results were correlated with the phosphorylation status of ERK 1/2, as measured by immunoblot analysis. Furthermore, we screened for gene amplifications by rtq PCR and mutations by PCR and sequence analysis. In samples with RAF mutations we additionally searched for point mutations off the N-RAS gene and internal tandem repeats of the FLT3 gene 45/82 (54,9%) of AML cases showed ERK phosphorylation indicating constitutive activation of the pathway. However, there was no correlation to the expression levels of either A-, B- or C-RAF. Deletions of the B-RAF gene as revealed by rtq PCR were observed in 20/99 (20%) of AML cases and were consistent with cytogenetic results but did not have pathogenetic relevance on ERK activity, either. However, in therapy-related AMLs one A-RAF gene duplication and two novel C-RAF missense mutations were detected: S427G in one and I448V in the other patient. Both mutations are located within the protein kinase domain and associated with strong ERK activation. Analysis of the primary tumors and buccal epithelial cells revealed these mutations of germline origin. In conclusion we have demonstrated that the frequently observed constitutive activation of the RAS-RAF-MEK-ERK signaling cascade in AML cannot be explained by RAF abnormalities alone. Nevertheless, our results suggest a role of RAF abnormalities, in particular C-RAF mutations, in the subgroup of therapy-related AML which may have therapeutic implications.

418 RAPID INDUCTION OF APOPTOSIS VIA A NOVEL DUAL PATHWAY BY 1-ACETOXYCHAVICOL ACETATE IN HUMAN LEUKEMIC CELLS

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1-acetoxychavicol acetate (ACA) was obtained from rhizomes of the commonly used ethno-medicinal plant *Laguas galangal* (Zingiberaceae). Recent extensive studies revealed that it has potent chemopreventive effects against several tumors. In this study, we evaluated the efficacy of the anticancer activity of ACA in leukemia cells in vivo as well as in vitro. Low dose of ACA (10µM) dramatically inhibited cellular growth of leukemic cells by very rapid induction of apoptosis. Cultivation with ACA rapidly increased the population of cells in the G0/G1 phase with a reduction of cells in the S phase, and a strong induction of apoptosis was shown by the appearance of a hypodiploid DNA peak with sub-G1 DNA contents for 6 h after treatment. Caspase assay revealed that ACA-induced apoptosis was almost completely blocked by the treatment with Z-VAD-FMK, and it was also partially inhibited by Z-IETD-FMK and LEHD-CHO. Treatment with 10µM ACA in NB4 cells within 0.5 h showed a dramatic increase in intracellular ROS compared to control cells, corresponding to reduction of intracellular glutathione (GSH). Treatment with a thiol antioxidant, N-acetyl-L-cysteine (NAC), an excellent supplier of glutathione (GSH), significantly attenuated ACA-induced apoptosis with complete inhibition of ROS generation in NB4 cells. However this inhibition was partial, and continued until 12 h after exposure. These results indicate that ROS plays a key role in ACA-induced apoptosis, but other pathway also contributes in this apoptosis. Expression analysis revealed that Fas was dramatically increased in a time-dependent manner and the death-inducing signaling complex (DISC) was formed in NB4 cells treated with ACA. ROS generation inhibitor, N-acetyl-L-cystein (NAC), and antagonistic anti-Fas antibody, ZB4, inhibited ACA-induced apoptosis respectively. However

NAC did not inhibited caspase-8 activation and ZB4 did not block ROS generation. NAC and ZB4 additionally inhibited ACA-induced apoptosis. Bid protein, which links caspases-8 and -9, did not alter during the treatment with ACA. These results strongly indicate that ACA induced two different apoptosis signalings; the first pathway acts via reactive oxygen species (ROS) generation, leading to mitochondria dysfunction and activation of caspase-9, and the second pathway is mediated by the cell surface receptor, Fas, resulting in activation of caspase-8.ROS and Fas function independently in ACA-induced apoptosis.

In addition, ACA remarkably inhibited cellular growth of freshly isolated cells from patients by induction of apoptosis corresponding to induction of Fas and Fas-L, and ROS generation. In contrast, same dose of ACA did not affect cellular growth of cells from healthy volunteers. These results strongly supported that Fas and ROS generation play a key role in the effects of ACA, and ACA has the possibility on treatment methods for myeloid leukemia. Our in vitro data prompted us to examine whether the effects of ACA are equally valid in vivo. ACA effectively inhibited tumor growth and induced apoptosis in vivo by using NOD/SCID mice with survival advantage and no toxic effects. We conclude that ACA induces rapid induction of apoptosis via novel dual pathway, ROS-generation and Fas, and ACA has potential as a novel therapeutic agent for the management of myeloid leukemia.

419 MUTATION PATTERNS IN CEBPA GENE IN ADULT PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA AT DIAGNOSIS AND RELAPSE

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Background: Transcription factor CCAAT/enhancer binding protein alpha is essential for granulocytic differentiation. Recently, CEBPa mutations have been described in 7-10% of adult patients with de novo acute myeloid leukemia (AML) and were associated with a favorable prognosis. The role of CEBPa mutations in the relapsed AML was not clear. Aims: We aimed to determine the role of CEBPa mutations in the relapse of AML and to determine whether the mutation patterns at relapse differ from findings at diagnosis in the same cohort of patients. Patients and Methods: CEBPa mutational analysis was performed on matched bone marrow samples obtained from 126 adult patients with de novo AML at diagnosis and first relapse. CEBPa mutations were assessed using polymerase chain reaction-single strand conformation polymorphism analysis followed by direct sequencing. Results: CEBPa mutations were identified in 20 patients (15.9%) at diagnosis. Eighteen patients had mutations located in the N-terminal part of the protein, resulting in frameshift and an eventual termination. Termination of translation by introducing a nonsense codon was found in another one patient. Nineteen patients had mutations occurred in the basic-leucine-zipper (bZIP) domain. The bZIP mutations included duplication (n=9), deletion (n=5), and insertion (n=5); all but one mutations were in-frame. Taken together, 18 patients had both N-terminal and bZIP mutations. Of the 20 patients harboring CEBPa mutations at diagnosis, 18 patients relapsed with identical patterns, one of them had a second relapse which also exhibited the same pattern as at diagnosis and first relapse. Two patients lost CEBPa mutations at relapse, whereas none of the AML patients who lacked CEBPa mutations at diagnosis, acquired the mutations at relapse. CEBPa mutations were not detected in the complete remission samples obtained from 15 patients who had mutations in the N-terminal and/or bZIP domains at diagnosis. The frequencies of CEBPa mutations according to FAB subtype were 6 in 23 patients with M1, 12 in 70 patients with M2, 1 in 11 patients with M4, and 1 in 4 patients with M6. None of the 6 patients with M0, 7 patients with M3, and 5 patients with M5 had CEBPa mutations. CEBPa mutations were found in 14 of the 59 patients with intermediate cytogenetics, but were not observed in the 35 patients with good cytogenetics including t(8;21), t(15;17) or inv(16), 4 patients with poor cytogenetics (-7/7q- or complex karyotypes), and 8 patients with MLL rearrangements. There were no significant differences in age, sex, hemoglobin level, platelet count, WBC count, or percentage of bone marrow blasts between patients with and without CEBPa mutations. Conclusion: The present study shows that 90% of adult patients with AML who carried CEBPa mutations at diagnosis, relapsed with identical mutation patterns, suggesting that these mutations contribute to the leukemogenesis in this subset of patients.

420 RELATIVE QUANTIFICATION OF TELOMERASE REVERSE TRANSCRIPTASE (hTERT) BY REAL-TIME POLYMERASE CHAIN REACTION (PCR) IN TWENTY-THREE ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS

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The activity of hTERT, is increased in many haematological disorders, including acute myeloid leukaemia (AML).

The present study was aimed at establishing whether hTERT activity was associated with the presence of FLT3 internal tandem duplication (ITD), with any clinical parameters and with relapse risk in AML-M3 patients.

Twenty-three patients were analysed at diagnosis and during the follow-up. A relative quantification of hTERT transcripts was achieved by using SybrGreen I, a double-stranded DNA-binding fluorescent dye, in a real-time PCR. A standard curve for real-time quantification was obtained by using serial dilution of total RNA from the K562 cell line. hTERT expression was normalized to ABL and calibrated on K562. Statistical analysis was made after having dichotomised hTERT values so that M3 patients could be subdivided in those with low and high hTERT activity. Comparisons of hTERT values during the follow-up were made by using a regression model for repeated measurements and correlations with clinical parameters and relapse by applying the Spearman test and Cox regression.

Thirteen patients were males and ten females; median age was 39.7 years (range 19-67). A complete remission (CR) was achieved in all the patients after a median time of 35 days (range 30-60). Seventeen patients are now in an unmaintained CR after a median follow-up of 24 months (range 3-90), while six have relapsed after a median follow-up of 17 months (range 9-35). One of these last patients with high hTERT values and FLT3 ITD did not achieve a second CR, which was obtained in the remaining five patients. Two of these last patients underwent an allogeneic bone marrow transplantation, but the one with high hTERT values and FLT3 ITD relapsed for the second time. A significant correlation was found between high hTERT values and white blood cell (WBC) counts on clinical diagnosis and at relapse ($p=0.037$). High hTERT values were observed in five out of the six FLT3 ITD positive patients. hTERT values during the follow-up showed high and low values. Patients with low hTERT transcript levels on clinical diagnosis had a relapse rate of 4.7% (95% confidence intervals, CI: 0.6-33), while those with high hTERT levels of 15.9% (95% CI: 6.0-42). The relative risk of developing relapse for these last patients was 4.3% (95% CI: 0.48-39) ($p=0.14$). This significant correlation was also found when we compared hTERT values one hundred-eighty days from clinical diagnosis: relapse rate was 6.8% (95% CI: 0.9-48) for patients with low hTERT values versus 21.7% (95% CI: 7.0-67) for those with high hTERT values. The relative relapse risk for these last patients was 3.5% (95% CI: 0.37-34) ($p=0.23$).

In conclusion our study suggests that on clinical diagnosis high hTERT values, being observed in patients with high WBC counts, are associated with FLT3 ITD and that high hTERT values on clinical diagnosis and during the follow-up are predictive of disease recurrence.

421 DOWN-REGULATION OF THE PUTATIVE TUMOR SUPPRESSOR GENE GRAVIN IN ACUTE MYELOID LEUKEMIA, CHRONIC MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES

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BACKGROUND AND AIMS. The gravin/AKAP12 gene encodes a scaffolding protein and regulates cell signalling, cell adhesion, mitogenesis and development, and differentiation. There is evidence that gravin may function as a tumor suppressor and it may play an important role in tumorigenesis. The expression of gravin is strongly suppressed in several solid tumors. Using cDNA microarray technology we found that the gravin gene was down-regulated in the CD34+ cell populations of all 13 patients with myelodysplastic syndromes (MDS) studied. We have since evaluated the expression levels of the gravin gene in the CD34+/blast cell populations of a range of myeloid malignancies as compared to the CD34+ cell populations of a group of healthy individuals using real-time quantitative PCR.

METHODS. 41 patients with acute myeloid leukemia (AML), 33 patients with chronic myeloid leukemia (CML) and four patients with MDS were included in the study. Seven patients with CML were in chronic phase (CP), three additional CP patients were in cytogenetic remission following successful treatment with imatinib (STI571), two were in accelerated phase (AP) and 24 in blast crisis (BC). Bone marrow samples were obtained and CD34+ cells isolated using MACS magnetic cell separation columns from all MDS patients and CML patients in CP, AP and 10 healthy controls. Peripheral blood or bone marrow samples were obtained from patients with AML and BC CML and the leukemic blast cells isolated by Histopaque density gradient centrifugation. Total RNA extracted from each CD34+/blast cell sample was reverse-transcribed to cDNA and used for real-time quantitative PCR.

RESULTS. The gravin gene was down-regulated in the leukemic blast cells of 41 out of 41 patients with de novo AML, a proportion of which showed absent expression. Gravin was also down-regulated in the CD34+/blast cell populations of 30 out of 30 CML patients and at all phases of the disease. The expression levels of gravin were reduced in AP and BC as compared to CP and thus a progressive reduction in the expression levels of gravin may be associated with disease evolution in CML. In the CD34+ cell populations of the three CP CML patients in remission following successful treatment with imatinib the gravin expression levels were similar to those observed in healthy individuals. We have also shown that gravin was down-regulated in the CD34+ cell populations of 4 out of 4 patients with MDS. There was no overlap between the expression levels of the patient groups and the control group (see Table). Mutation analysis of the gravin gene in five MDS patients and 16 AML patients identified several novel polymorphisms, but no point mutations in the promoter region(s) or exons of the gene.

CONCLUSIONS. We have shown that the gravin gene was consistently down-regulated in the CD34+/blast cells of patients with myeloid malignancies and in several cases showed absent expression. We speculate that this phenomenon may play a role in myeloid leukemogenesis. These findings warrant further investigation because of the potential therapeutic implications for myeloid leukemia. This study has been funded by the Leukaemia Research Fund.

GRAVIN GENE EXPRESSION LEVELS			
	Median	Range	P-value (Mann-Whitney U test)
Controls (n= 10)	0.895	0.61 - 2.29	---
MDS (n= 4)	0.095	0.03 - 0.18	0.0047
AML (n= 41)	0.02	0 - 0.42	< 0.0001
CML - CP (n= 7)	0.19	0.09 - 0.22	0.0006
CML - AP (n=2)	0.05	0.02 - 0.08	0.0317
CML - BC (n= 24)	0.025	0 - 0.52	< 0.0001
CML - CP STI (n= 3)	3.3	1.98 - 4.06	0.018

Caption 1:

422 ACUTE MYELOID LEUKEMIA OF ELDERLY (>60 YEARS) IS AN HETEROGENEOUS DISEASE: COAGULOPATHY AND FLT3 MUTATIONS ARE INDEPENDENT PROGNOSTIC FACTORS FOR SURVIVAL

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From December 2001 to April 2003, 80 patients aged > 60 years with acute myeloid leukemia (AML) were consecutively seen at the Department of Hematology, University 'La Sapienza' of Rome. At diagnosis, all patients were extensively characterized by a multiparametric approach for immunophenotype, cytogenetic and molecular analyses. These latter included the search of the BCR/ABL, AML1/ETO, CBFbeta/MY11 fusion genes and of the FLT3 status. Moreover, patients were divided into two aged-stratified groups (group I = 60-70 yrs.; group II >71 yrs)

to evaluate whether distinct biological features might be detected within these two groups. There were 38 and 42 patients in group I and II, respectively. Male/female distribution was 23/15 and 30/12 ($p = .01$). A previous myelodysplastic phase (MDS) was present in 11 (31%) and in 7 (16%) patients of the group I and II, respectively ($p = .01$). With regard to immunophenotype, combining the expression of the CD117, CD34 and CD15 molecules, we defined 5 different sub-groups: a) = CD117+ve, CD34+ve, CD15+ve; b) = CD117+ve, CD34+ve, CD15-ve; c) = CD117+ve, CD34+ve, CD15+ve; d) = CD117+ve, CD34-ve, CD15+ve; and e) = CD117-ve, CD34-ve, CD15+ve. The patients distribution within the two age stratified groups respect to the immunophenotypic category was as follows: a= 3/7 cases in group I and II, respectively; b= 12/26; c= 6/8; d= 6/0 and e= 8/1 ($p = .009$). Moreover, 54 patients (83%) had an evaluable karyotype. The low risk karyotype (i.e. = t(15;17); t(8;21); inv(16)) was never detected in any patients of the two age stratified groups; the intermediate risk changes (i.e. normal karyotype, 11q23, +8, del(7) or other numerical/structural abnormalities) were detected in 31/19 patients of group I/II, respectively; the poor risk changes (i.e. -5, del(5), -7, t(3;3) or inv(3), t(6;9), t(9;22) or complex karyotype) were present in 1/5 patients ($p = .003$). Molecular analyses performed in 45 cases did not show any of the fusion transcripts tested. By contrast, the FLT3ITD alteration was detected in 6 (11.5%) patients (group I/II = 3/3 patients) whereas the FLT3 D835 point mutation was detected in three additional cases. A statistically significant correlation was found between FLT3 and more immature immunophenotypic classes ($p = .001$). FLT3 positivity was significantly associated with higher WBC count at diagnosis ($p = 0.01$), high percentage of peripheral blood blasts ($p = 0.04$), presence of coagulative disorders (hyperfibrinolysis) ($p = 0.01$), worse conditions at presentation (WHO > 2, $p = 0.01$), low level of surface antigen CD34 ($p = 0.005$). Factors associated with a significantly worse overall survival were the presence at diagnosis of coagulative disorders ($p = .00001$), FLT3 abnormalities ($p = .00001$). In the multivariate analysis, absence of coagulative disorders ($p = .0004$) and of FLT3-ITD ($p = 0.02$) remained as independent prognostic factors for significantly longer OS. This study highlights the existence of biological heterogeneities within the elderly AML and shows the prognostic value of the FLT3 gene status in this leukemic subset. FLT3 gene expression should thus be more extensively evaluated in AML of elderly especially considering that FLT3 expression represents a target for recent molecular adapted strategies.

423 CEBPA MUTATIONS, FLT3 ITD AND BAALC EXPRESSION DEFINE DISTINCT PROGNOSTIC SUBSETS IN NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA (AML)

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Background: Approximately half of all newly diagnosed cases of acute myeloid leukemia (AML) have a normal leukemic karyotype. The prognosis of normal karyotype AML has been defined as being of intermediate-risk, however, individual disease courses vary considerably. Therefore, there has been much interest in identifying molecular markers in order to improve the prognostic stratification of patients with normal karyotype AML but distinct molecular AML entities.

Patients and Methods: Leukemic blasts purified at diagnosis from 67 consecutive AML patients with normal cytogenetics from a single University institution were analyzed for mutations in the myeloid transcription factor CEBPA (CCAAT/enhancer binding protein- α), for internal tandem duplications of the tyrosine kinase receptor FLT3, and for expression of the BAALC gene (Brain And Acute Leukemia, Cytoplasmic). Mutation screening was carried out using PCR amplification and direct sequencing. BAALC expression was analyzed by real-time RT-PCR.

Results: 28.4% of normal karyotype AML (19/67) had FLT3 ITD and 20.9% had mutations in the CEBPA gene (14/67). Of the remaining 50.7% of patients, 17 had high BAALC expression and 17 had low BAALC expression. Patients with FLT3 ITD had significantly shorter median disease-free ($p = 0.0003$) and overall-survival ($p = 0.0004$) than patients with wildtype FLT3. Patients with CEBPA mutations had a very favourable course of their disease. Median DFS and OS after 36 months of follow-up were not reached as compared to 10.4 and 11.9 months in patients without CEBPA mutations, respectively ($p = 0.0066$; $p = 0.0130$). High BAALC expression predicted for a significantly shorter DFS ($p = 0.0001$) and OS ($p = 0.0001$) as compared to AML with low BAALC expression.

Conclusions: Normal karyotype AML can be split into clinically relevant entities defined at the molecular level which are characterized by mutations in the CEBPA gene, tandem duplications of the FLT3 gene, and the expression level of the BAALC gene. We propose that these three molecular markers should be incorporated into the panel of diagnostic procedures as they provide a basis for risk stratification and, possibly, risk-adapted treatment in patients with normal karyotype AML.

424 COMPLETE MUTATIONAL ANALYSIS OF THE COMMONLY DELETED REGION UNDERLYING DEL(9Q) ACUTE MYELOID LEUKAEMIA

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BACKGROUND Interstitial deletions of 9q are found at diagnosis in approximately 2% of cases of acute myeloid leukaemia [del(9q) AML]. Del(9q) may occur as an isolated cytogenetic abnormality, in association with the t(8;21)(q22;q22) abnormality or in association with other abnormalities. We have previously analysed diagnostic bone marrow material from patients with del(9q) AML using FISH and LOH analysis to define a 2.5Mb commonly deleted region for del(9q) AML.

AIMS In order to identify a potential tumour suppressor gene that underlies the pathogenesis of del(9q) AML, mutational analysis of genes lying within the CDR has been performed. All coding exons have been evaluated to look for the presence of inactivating mutations according to Knudson's two-hit hypothesis.

METHODS A transcript of map of this region was characterised using the Ensembl gene-identification software (<http://www.ensembl.org/>). RT-PCR was performed on cDNA from CD34-purified cells in order to confirm expression within the haematopoietic lineage and to confirm the authenticity of prediction of novel gene sequences. Mutational analysis of these genes was performed upon diagnostic DNA extracted from 12 patients at the time of diagnosis with del(9q) AML. Coding exons were amplified by PCR and analysed using a combination of DHPLC (Transgenomic WAVE system) and direct cycle-sequencing. Analysis of NTRK2 was performed on a separate cohort of patients using direct cycle-sequencing only.

RESULTS Analysis of the del(9q) CDR using Ensembl revealed the presence of 13 genes within this region. The order from the centromeric to telomeric boundary is as follows:

ENSG00000165104, Q96N04, Band4.1-like protein, ENSG00000148057, UBQLN1, GKAP42, KIF27, Q9BT18, HNRPK, Q9H9A7, SLC28A3, Q9UMX4, NTRK2. Ten of the 13 genes were found to be expressed in CD34 cells (ENSG00000165104, Q96N04 and Q9UMX4 were not expressed). Mutational analysis revealed a combined total of 56 nucleotide changes within the 12 patient samples analysed. A single base change was found in exon 11 of SLC28A3 which was not present in 70 controls that were analysed. All of the other nucleotide changes were either also found in controls or were transcriptionally silent.

CONCLUSIONS Mutations within the coding region of genes within the del(9q) CDR do not represent the mode of inactivation of the putative tumour suppressor gene that lies within this region. This indicates that the method of leukaemogenesis may not due to the classical two-hit hypothesis and other mechanisms such as haploinsufficiency may be important in the pathogenesis of AML.

425 ELDERLY PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA (AML): PROGNOSTIC FACTORS IN 113 CASES

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Aim: Determine the value of karyotype in 113 patients >65 years old in a series of 346 patients with de novo AML.

Patients and Methods: 52 females and 61 males with median age 71 years (65-91) The FAB subtype were: 13(11.5%)LAM0, 17 LAM1, 30 LAM2, 13 (11.3%)LAM3, 15 LAM4, 17 LAM5, 7 LAM6, 1 LAM7(vs 3.4%LAM0, 26.2%LAM3 in <65 years $p = 0.008$). Karyotypes were assigned according to ISCN 1995. The prognostic significance of initial clinical, pathologic and cytogenetic factors was evaluated by Kaplan-Meier estimate and compared by log-rank, Breslow and Tarone test, for overall survival (OS) and continuous complete remission (CCR). Comparisons of remission rates were done by Chi-square analysis. The impact of prognostic factors was studied using Cox regression model. $P < 0.01$. M=median, m=months

Results: Cytogenetic abnormalities were seen in 62.8% of cases including: 26(23%) complex abnormalities (vs 5.6% in <65 years, $p < 0.001$) 11 t(15;17), 5 trisomy 8, 3 trisomy 21, 3 trisomy 11, 2 t(9;22), 2 del(5q), one del(7q), one monosomy 7, one 3q21q26 rearrangement, one inv h (16) and 13 with other abnormalities. Karyotype was classified by SWOG and MRC (Medical Research Council) classification. They showed trilineage myelodysplasia (TMDS) 20.4% (vs 7.7% in >65 years, $p = 0.001$). 65 patients received intensive chemotherapy (10 LAM3 also received ATRA). Only 50.8% (33/65) achieved CR (vs 76.3% in <65 years, $p = 0.001$) and this was lowest in complex karyotype (15.4%). The 3-year OR and CCR probability was 11.5% ($M = 2.6m$) and 18% ($M = 1.5m$). The longest OS was for t(15;17) was 45% surviving at 3 years (M not reached); normal karyotypes survive 4% and other had very short survival ($p = 0.0018$). The probability of relapse at 3 years for t(15;17) was 47% (M not reached), and this was highest in trisomy 8 (92%) normal karyotypes (96%) and complex abnormalities (100%) ($p = 0.0011$). A longer OS was seen in patients with leucocytes $< 20 \times 10^9/L$ ($p = 0.006$), subtype LAM3 ($p = 0.009$) who received ATRA ($p = 0.0001$) and without TMDS ($p = 0.0002$). LAM3 ($p = 0.003$) who received ATRA ($p = 0.0002$) and haemoglobin $> 100g/L$ ($p = 0.03$) were favorable prognosis for CCR. Multivariate analysis showed that karyotype, TMDS and leucocytes are independent factors for OS. Only unfavourable karyotypes and chemotherapy without ATRA are independent factor for relapse risk.

Conclusions: The elderly novo AML has a similar clinical- biological profile that the secondary AML, because of high frequency in indifferenciate subtypes, the presence of TMDS, high percentage of complex abnormalities and poor CR, CCR and OS. This matter suggest that their aetiology in probably a lengthy exposure to environmental toxins. That's the reason because its essential the cytogenetic study to decide induction chemotherapy or palliative support. Elderly patients with de novo AML which shown complex abnormalities, t(9;22), r3q21q26 or involvements of 5/7 failure to achieve CR, relapse promptly and have short survival. In this age group, today only therapy designed to target specific molecular rearrangements has good prognostic.

426 PROGNOSTIC FACTORS IN PATIENTS DIAGNOSED DE NOVO ACUTE MYELOID LEUKEMIA (AML) WITH <65 YEARS OLD

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Aim: To detect the prognostic factors into de novo LAM age <65 years. Patients and Methods: 346 patients were diagnosed de novo AML with an evaluable karyotype, but we excluded 113 elderly patients, because they received chemotherapy age adapted. 97 females and 136 males with median age 42 years (1-64). FAB subtypes: 3.4% AML0, 17.6% AML1, 21.9% AML2, 26.2% AML3, 10.7% AML4, 16.3% AML5, 3.4% AML6, 0.4% AML7. Karyotypes were assigned according to ISCN 1995. The prognostic significance of initial clinical, pathologic and cytogenetic variables were evaluated by Kaplan-Meier estimate and compared by log-rank, Breslow and Tarone test, for overall survival (OS) and continuous complete remission (CCR). Comparisons of remission rates were done by Chi-square analysis. The impact of prognostic factors was studied using Cox regression model. $p < 0.005$. $M = \text{median}$, $m = \text{months}$. Results: 128/233 (54.9%) showed clonal abnormalities including: 55 t(15;17) (90.2% of AML3), 13 (5.6%) complex karyotypes, 11 t(8;21) (21.5% of AML2), 11 trisomy 8, 8 inv h (16) (all AMLEo), six 3q21q26 rearrangements, 3 trisomy 21, 2 trisomy 11, one monosomy 7, one del(7q) and 17 with other abnormalities. Karyotype was classified by SWOG and MRC (Medical Research Council) classification. 7.7% showed trilineage myelodysplasia (TMDS). 219 patients received intensive chemotherapy (consolidated with autologous BMT 57 cases, allogeneic BMT 23 cases, and 36 AML3 also received ATRA). Groups of age <40 vs >40 years were similar with regard to analysed variables, except for treatment (20/23 youngs received consolidation with alloBMT, $p < 0.001$). 167/219 (76.3%) achieved CR but only 35.3% in unfavourable groups ($p < 0.001$). 54.8% (120/219) relapsed and 71/233 (30.5%) continued alive to the end of study. The 5-year OS and CCR probability was: 28% ($M = 13.2m$) and 38% ($M = 14.5m$). The longest OS was t(8;21), t(15;17) and inv h(16) with 62.3% (M not reached), 46% ($M = 30.9m$) and 33% ($M = 7.3m$) surviving at 5 years; normal karyotypes survive 24% ($M = 14.4m$), 13.5% trisomy 8 ($M = 8.7m$) and other abnormalities had very short survival ($p < 0.0001$). At % years, the relapse risk was only 22.1% for t(8;21), 37.3% for t(15;17) (M not reached), and 50% for inv h(16) ($M = 3.7m$) whereas for trisomy 8 and normal karyotypes were 58% ($M = 3.70m$) and 71% ($M = 13.8m$); every complex rearrangements and 3q21q26 relapse at 2 years ($p = 0.0001$). The survival of cytogenetic groups according SWOG and MRC classification was significantly different ($p < 0.0001$ in both) for OS and CCR. In univariate analysis, other factors predictive

(or probability predictive) of shorter OS and CCR were: chemotherapy treatment ($p < 0.0001$), FAB subtype not AML3 ($p = 0.02$ for OS, $p = 0.0019$ for CCR), TMDS ($p = 0.03$ for OS, $p = 0.01$ for CCR) and leucocytes $> 20 \times 10^9/L$ ($p = 0.04$ for OS, $p = 0.05$ for CCR). Multivariate analysis showed that treatment, karyotype, and FAB subtype are independent factor for OS and CCR. The higher risk of relapse in for unfavourable ($OR = 6.01$) cytogenetic SWOG groups and chemotherapy treatment ($OR = 6.3$).

Conclusions: Our study confirms that patients diagnosed de novo AML with <65 years are a homogenous group regard to clinical-biological characteristics. For unfavourable cytogenetic groups its necessary to desing new therapy that allow these patients access consolidation with BMT.

427 FREQUENT ABERRANT BRCA1 METHYLATION IN ACUTE MYELOID LEUKEMIA (AML) SECONDARY TO BREAST CANCER TREATMENT

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Background.

Aberrant DNA methylation has been observed in many human tumors, in particular in the CpG islands of tumor suppressor genes. BRCA1 is a tumor suppressor gene that encodes a 1863 amino acid protein that is important for regulation of cell proliferation, participation in DNA repair/recombination processes related to the maintenance of genomic integrity, induction of apoptosis in damaged cells and regulation of transcription. Recent data reveal that promoter hypermethylation is frequently associated with BRCA1 inactivation in non-inherited breast and ovarian carcinomas.

Methods and results.

Using a methylation-specific PCR, we studied BRCA1 promoter hypermethylation in 118 patients (59 females, 59 males, median age 62 years, range 16-85 years), with acute myeloid leukemia (AML). BRCA1 was hypermethylated in 63/118 (53.4%) AML samples. No differences were noted when looking at age, gender, karyotype and FAB-subtype. Eighteen patients had a therapy-related acute myeloid leukemia. All 6 patients (100%) treated for a previous breast cancer were hypermethylated for BRCA-1.

Summary/conclusion.

This study points to a possible link between genetic instability related to BRCA1 inactivation in non-inherited breast cancer and secondary leukemic transformation.

428 DIFFERENCES IN ANTI-APOPTOTIC AND MULTIDRUG RESISTANCE PHENOTYPES IN ELDERLY VS YOUNGER ACUTE MYELOID LEUKEMIA PATIENTS ARE RELATED TO THE MATURATION CHARACTERISTIC OF BLAST CELLS

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Elderly patients with acute myeloid leukemia (AML) have a less favorable outcome as compared to younger AML patients, which has been related, among others, with a higher frequency of unfavorable karyotypes, multidrug resistance (MDR) phenotypes and CD34 expression on the blast cells, although discrepant results have been reported. In the present study we have quantitatively analyzed by flow cytometry the expression of apoptosis (bcl-2, bax, APO2.7) and MDR (P-gp, MRP, LRP) associated proteins in three blast cell subpopulations, defined according to their maturation stage, in elderly ($n = 150$) and a control group of younger ($n = 25$) de novo AML patients. From the phenotypic point of view three different populations of leukemic blast cells with an increasing maturity were defined: CD34+, CD34-/CD32-/dim/CD33+ and CD34-/CD32+/CD33+. Once the distribution of these blast cell subsets was analyzed in the two patient groups, elderly AML showed a higher frequency of cases displaying CD34+ homogeneous blast cell population vs young patients (25% vs 8% of cases having only one

blast cell population that expressed CD34, $p = 0.04$). No statistically significant differences were detected between both age groups in the expression of both apoptosis and MDR associated proteins, except for slightly higher quantities of the LRP protein observed in the more immature CD34+ blast cell subset in the elderly AML cases ($p = 0.04$). Interestingly, when different blast cell populations were compared, immature (CD34+) blast cells were characterized by higher levels of bcl-2 ($p < 0.01$) and lower levels of APO2.7 ($p < 0.003$) in both age groups. In addition, higher P-gp levels ($p = 0.005$) were found in CD34+ vs CD34-blast cells in elderly AML patients together with a lower reactivity for LRP in both elderly ($p < 0.001$) and younger ($p = 0.01$) groups. No differences in bax, nor MRP expression were detected between the different blast cell subsets in the two groups of AML patients. In summary, our results suggest that the higher resistance to chemotherapy observed in elderly AML patients could be related to a higher incidence of cases with CD34+ homogeneous blast cell population, since these blast cells frequently display a more pronounced anti-apoptotic and MDR1 phenotype.

429 POLYMORPHISMS OF CYP1A1 AND GLUTATHIONE S-TRANSFERASE AND SUSCEPTIBILITY TO ADULT ACUTE MYELOID LEUKEMIA.

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BACKGROUND: The origin of acute myeloid leukemia (AML) may be explained by the combination of genetic susceptibility factors and environmental exposure. The carcinogenic effect of xenobiotics is influenced by a series of genes codifying enzymes involved in oxidation/activation (Phase I) and the conjugation/detoxification (Phase II) of these compounds. Polymorphisms of these genes, resulting in functional allelic variants of the corresponding enzymes, were shown to influence the risk to develop solid tumors and hematological malignancies.

AIMS: The cytochrome P450 CYP1A1 is a phase I enzyme involved in the bioactivation of several chemical carcinogens, including polycyclic aromatic hydrocarbons and endogenous and exogenous estrogens. Several CYP1A1 alleles have been described resulting from point mutations in the gene sequence. These alleles may have different enzymatic activity compared to the wild type allele. The glutathione S-transferase GSTM1 and GSTT1 are phase II enzymes involved in the detoxification of environmental carcinogens but also anti-cancer drugs. Homozygous deletions of GSTM1 and GSTT1 are present in a large proportion of individuals as a genetic polymorphism which causes absence of the specific enzymatic activity. We studied the polymorphisms of CYP1A1, GSTM1 and GSTT1 as risk factors for adult AML.

METHODS: The prevalence of CYP1A1 *2A, *2B and *4 alleles and of GSTM1/GSTT1 deletions was examined in 193 patients with AML and 273 normal individuals with negative history for cancer. We used PCR-based methods.

RESULTS: A higher prevalence of the CYP1A1*4 allele was found in AML patients compared to controls (19.1% vs 9.9%, O.R.=2.2, 95% C.I. 1.3-3.7, $p=0.006$), and the prevalence of this allele was even higher in female AML patients under 60 years of age when compared to control of the same age and sex (24.4% vs 7.1%, $p=0.02$, O.R. 4.2, 95% C.I. 1.2-14.5). GSTT1 deletions were also more frequent in AML patients (29% vs 19%, O.R.=1.7, 95% C.I. 1.1-2.7, $p=0.02$). The combination of GSTT1 deletions and CYP1A1 *2B or *4 alleles further increased the risk of AML (O.R.=10.2, 95% C.I. 1.2-83.9, $p=0.01$, and O.R.=7.0, 95% C.I. 2.0-24.8, $p=0.001$, respectively). Moreover the CYP1A1*2B allele was more frequent in AML patients with multilineage dysplasia (5 of 30 AML patients with myelodysplasia, corresponding to 16.7%), then in other AML subtypes where the *2B allele was present in 7 of 145 patients, corresponding to 4.8% (O.R.= 3.9, 95% C.I. 1.2-13.4, $p=0.03$). No differences in the frequency of CYP1A1 alleles and GST deletions were found by grouping patients according to history of previous cancer and karyotype.

CONCLUSION: Polymorphic variants in xenobiotic-metabolism genes, including CYP1A1 and GSTT1, particularly when combined, may increase the risk of adult AML, by leading to enhanced activation and reduced detoxification of environmental and endogenous carcinogens.

430 DEVELOPMENT OF THE EFFECTIVE TUMOR ANTIGEN SENSITIZATION METHODS FOR DENDRITIC CELLS DERIVED FROM PATIENTS WITH MYELOGENOUS LEUKEMIA

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Background: Dendritic cells (DCs) are the most effective antigen-presenting cells that initiate primary immune responses and

immunotherapy using DCs loaded with tumor antigens may represent a potentially effective method for inducing antitumor immunity.

Aims: We evaluated the effectiveness of DCs-based antitumor immune response in various culture conditions.

Methods: DCs were cultured from peripheral blood mononuclear cells (PBMC) in myelogenous leukemia (ML) and lysates of autologous leukemic cells were used as tumor antigen. The effectiveness of interleukin-12 (IL-12), CD40L (CD154) and/or prostaglandin E2 on the antigen presenting function of lysates-loaded DCs were analyzed by proliferation, cytokine production, and cytotoxicity tests with activated PBMC (mainly lymphocytes). For generating antigen-loaded DCs, direct fusion of DCs with MLs by polyethylene glycol and capacity to stimulate PBMC with proliferative activity were studied.

Results: Antigen loaded DCs induced significantly effective antitumor immune response against autologous leukemic cells. IL-12 was potent stimulator in antigen-loaded DCs based antitumor immune response. Fused cell has a potent antitumor immune response, but there were some technical problems including poor fusion rate etc.

Summary/ conclusion: We conclude that lysates-loaded DCs with IL-12 may be effectively utilized as inducer of antitumor immune reaction in ML and in vivo application with DCs-based antitumor immunotherapy or tumor vaccination seems to be feasible.

431 TUMOR NECROSIS FACTOR (TNF) PATHWAY IS DOWN-REGULATED IN APL: A GENE EXPRESSION PROFILING STUDY

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Acute promyelocytic leukemia (APL) is a singular type of acute myelogenous leukemia (AML) genetically characterized by a fusion gene transcript including the retinoic acid receptor α (RAR α). The most frequently translocation, t(15;17), involves the promyelocytic leukemia (PML) gene, with the result of the fusion gene PML/RAR α . This fusion product maintains the ligand-binding domains of RAR α and the functional domains of PML and blocks transcription of genes required for myeloid differentiation. To determine APL gene expression pattern, we have analyzed the expression profile of 53 de novo homogeneously treated APL patients included in the APL96 and APL99 PETHEMA protocols (27M/26F; median age: 44 yr. (range: 12-78); median WBC count $\times 10^9/L$: 2.45 (range: 0.51-128); median platelet count $\times 10^9/L$: 19 (range: 4.4-135); FAB subtype: 40 M3 / 13 M3v). The experiments were performed using the OncoChip[®] (<http://bioinfo.cnio.es/data/oncochip/>), a cDNA microarray that has been especially designed for analyzing genes involved in cancer, containing 6,386 genes of which 2,489 are cancer-relevant genes, others are involved in drug-response, or are tissue-specific and control genes. Bone marrow from three healthy donors was used to normalize the results. Using unsupervised analysis, hierarchical clustering revealed a highly homogeneous expression profile for all the series. After array processing, a total of 373 and 250 known genes were ≥ 2 fold down-regulated or up-regulated, respectively, in at least 40% of the patients. Of interest is the observation of tumor necrosis factor (TNF) signaling down-regulation, confirming a role of TNF pathway in APL. So, the subset of down-regulated genes implicated in TNF pathway includes TNFRSF6 also known as FAS, TNFAIP3, TNFAIP6, TNFSF10 or TRAIL, TNFSF13B, TRAF6, FIP2 and ARTS-1. Related to TNF signaling deregulation, we also found down-regulation of hematopoietic cell kinase (HCK) and v-src-1 Yamaguchi sarcoma viral-related oncogene homologue (LYN), implicated in TNF production in murine cells and human monocytes, as well as interleukin 2 receptor-beta (IL2RB), Janus kinase 2 (JAK2) and STAT4. An interaction between TNF and retinoic acid (RA) signaling pathways has been previously suggested. In fact, a cooperative effect of ATRA and TNF α in the induction of differentiation of APL cell lines and primary blasts from APL patients has been supported by recent reports. Also interesting is the down-regulation of genes implicated in the routes connecting TNF with NF- κ B pathways. These genes included TRAF6, TBK1, NFKBIA, TNFAIP3 and BCL10. In conclusion, our results demonstrate that TNF signaling pathway is down-regulated in APL, probably by the effect of the PML-RAR α fusion protein. So, addition of ATRA could induce the expression of genes involved in this pathway leading to terminal differentiation of APL cells. Moreover, our data also support the idea of a potential interaction between NF- κ B and RA pathways in APL.

This work was supported in part by Grant for Research Groups No. 03/225 from Generalitat Valenciana and by contract No. QLGI-CT-2001-01935 from the Fifth Framework Program of the European Commission.

CHRONIC MYELOPROLIFERATIVE DISORDERS

432 PERICYTE COVERAGE OF ABNORMAL VESSELS IN THE MYELOFIBROSIS BONE MARROW

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Background: Similar to findings in solid tumours, pathologic angiogenesis is a recognized feature of myelofibrosis (MF). However, the pathogenic mechanisms are not fully understood.

Aims: Since pericyte deficiency is noted on vessels of solid tumours we wanted to determine if pericyte (PC) deficiency also is occurring in the bone marrow of patients with MF.

Methods: By means of immunohistochemistry, single and double stainings were performed on bone marrow biopsies, where vessels were visualized with antibodies against CD34 and PC against α -smooth muscle actin (SMA). PC had to be positioned on the abluminal surface of endothelial cells in arterioles, capillaries and venules. Moreover, mean vascular density (MVD) and vessel morphometry (two perpendicular vessel diameters) was assessed in randomly chosen high power fields (HPF).

Results: In accordance with previous reports, mean MVD (SD) was significantly increased in the MF marrows, compared with controls (30.2 vs 8.3 / HPF, respectively; $p=0.006$). In patients 17 % of all vessels were PC coated, compared with only 4.5 % in controls. A general finding was that PC coated vessels had a larger diameter as compared with non-coated vessels (mean maximum diameter 20.6 vs 9.4; $p=0.001$). Interestingly, the diameters of PC coated MF vessel showed a larger mean diameter when compared with controls: this was true both for the maximum diameter (24.6 m vs 10.6 m; $p=0.087$ and the perpendicular one (15.8 vs 3.6, $p=0.01$). In contrast, non-coated vessels exhibited similar mean diameters in patients and controls (9.9 m vs 10.4 m; $p=0.83$).

Thus, the aberrant vessels seen in MF marrows were PC covered and conceivably mature. We suggest that PC are recruited preferentially to the aberrant MF vasculature, a finding that might have therapeutical implications.

433 ANALYSIS OF XCIP AND TELOMERASE ACTIVITY IN ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

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Background

Essential Thrombocythemia (ET) and Polycythemia Vera (PV) are generally considered chronic myeloproliferative disorders with a stem cell origin and a clonal development. However their heterogeneous nature is indicated by the finding of a small but sizable proportion of patients with polyclonal hematopoiesis. Analysis of X-chromosome inactivation patterns (XCIPs) with the HUMARA X-linked marker provides a useful method to investigate clonality of haematopoietic cell populations. We decided to test for an additional neoplastic marker, i.e. telomerase activity (TA), which is generally undetectable in normal somatic cells, but is expressed in 85% of the most common cancers.

Aims:

Although many studies have demonstrated the clonal origin of chronic myeloproliferative disorders using X-linked polymorphic markers, very little is known about telomerase activity in these diseases. The relationship(s) between these two markers therefore deserves investigation. XCIPs and TA were analysed in two peripheral blood cell populations, granulocytes (PMN) and mononuclear cells (MN), obtained from patients with ET and PV and informative at the X-linked HUMARA locus.

Methods:

PMN and MN were purified from peripheral blood according to standard methods. Primers sequences for XCIPs analysis were as follows: forward 5-TCCAGAATCTGTTCAAGAGCGTGC-3, and 5GCTGTGAAGTTGCTGTTCCTCAT-3 reverse. The 5'end of the forward primer was labeled with FAM fluorescent dye. The PCR products were analysed on a capillary electrophoresis system. Telomerase activity was determined with the aid of the TRAPeze telomerase detection kit (Intergen Co.), and expressed in TPG units. The assay has a linear range of 1 to 300 TPG, which is equivalent to the TA of approximately 30-10,000 control cells.

Results:

Twenty eight out of 34 patients tested (82%) were informative at the HUMARA locus. In 5 cases excess skewing prevented further analysis. Clonality status was then determined in PMN and MN by XCIPs from 23 subjects, and the results were as follows: PMN were clonal in 9 out of 13 patients with ET (69%) and 6 out of 10 with PV (60%). MN were constantly polyclonal. One patient showed loss of heterozygosity at the HUMARA locus in PMN, underscoring the presence of a monoclonal proliferation.

TA was measured in all female patients informative at the HUMARA locus. In clonal PMN TA ranged between 47.6 and 212.5 TPG units (mean 118.245.5), whereas in polyclonal PMN was between 1 and 24.7 TPG units (mean 8.79.2). The difference between TPG values in the two groups is highly significant ($p<0.001$, unpaired Students t-test). TA in MN was similar to that of polyclonal PMN.

Conclusions:

ET and PV are associated with a variable risk of thrombohemorrhagic complications and leukemic transformation. Moreover, the choice and timing of treatment can markedly influence the outcome of the disease. Our findings of heterogeneity of stem cell origin and clonal development in ET and PV, together with the striking correlation between high TA and clonality, hopefully will contribute to a better understanding of the pathophysiological and molecular mechanisms of these disorders and provide a useful tool in defining more effective therapeutic strategies.

434 COMPARISON BETWEEN ANAGRELIDE AND HYDROXYUREA IN THEIR ACTIVITIES AGAINST HAEMATOPOIETIC CELL GROWTH AND DIFFERENTIATION

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BACKGROUND AND AIMS: Anagrelide (ANA) and hydroxyurea (HU) are two distinct pharmacological agents with recognized platelet lowering properties, used to treat thrombocythaemia associated with myeloproliferative disorders. Both drugs inhibit bone marrow megakaryocytopoiesis but in addition have other distinctive biological effects. For this last reason comparisons of their specificity and mode of action are important to help in the evaluation of their relative risk to benefit ratio. However, surprisingly, comparative studies of this kind are still lacking. In an effort to fill this gap we have compared the activities of ANA and HU on the growth and differentiation of human haematopoietic progenitor cells in liquid culture. **METHODS:** CD34+ cells were purified from human umbilical cord blood and cultured for up to 12 days in IMDM-based medium supplemented with plasma and diverse haematopoietic growth factors, in the absence or presence of various concentrations of ANA or HU. Megakaryocytic, erythroid or monocytic differentiation was determined by flow cytometry using lineage specific antibodies. Cell death was evaluated using trypan blue exclusion and Annexin V/propidium iodide staining. **RESULTS:** Both ANA and HU inhibited TPO-induced megakaryocytic differentiation in a dose-dependent manner, but with strikingly different potencies. Thus, while ANA was effective at nanomolar concentrations ($IC_{50} \sim 30nM$), equivalent inhibition with HU was observed at 1000-fold higher doses ($IC_{50} \sim 30\mu M$). At these levels HU also inhibited erythroid or monocytic differentiation induced by erythropoietin or granulocyte-macrophage colony-stimulating factor, respectively, and showed marked cytostatic and cytotoxic effects under a variety of culture regimes. In contrast, ANA at a dose which caused maximal inhibition of megakaryocytopoiesis ($1\mu M$), did not affect either erythroid or monocytic differentiation, nor CD34+ cell expansion stimulated by stem cell factor, IL-3 and Flt-3 ligand. Furthermore, ANA showed no cytotoxic activity (necrosis or apoptosis) even when tested at 10 times higher doses. At $10\mu M$, however, ANA had a marginal ($\sim 10\%$) cytostatic effect. **CONCLUSIONS:** Taken together these results demonstrate that the anti-megakaryocytic activity of HU cannot be dissociated from the more general cyto-reductive and cytotoxic actions that emanate from its known effects on DNA synthesis. This mode of action of HU stands in sharp contrast to the effects of ANA, which was demonstrated to be a potent and selective inhibitor of megakaryocytopoiesis, having no significant cytostatic or cytotoxic effects on haematopoietic progenitor cell expansion or differentiation into other lineages. These differential in-vitro cellular activities suggest that in-vivo, compared to HU the platelet lowering action of ANA may be accomplished with a lesser degree of cytotoxicity.

435 ACUTE LEUKAEMIA/MYELODYSPLASTIC SYNDROME [AL/MDS] TRANSFORMATION RATES IN ANAGRELIDE TREATED PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA [ET] AND POLYCYTHAEMIA VERA [PV]

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Platelet cyto-reductive therapy is now well established in the management of patients with the chronic myeloproliferative disorders ET and PV. However concern regarding the potential of increasing the background AL/MDS transformation rate persists with the various agents being used. Anagrelide, a megakaryocyte maturation modulator is an alternative cyto-reductive therapy to older cytotoxic treatments and Interferon alpha. A database was constructed from 4 open label studies evaluating the efficacy and safety of anagrelide in patients with myeloproliferative disorders [MPDs]. This was retrospectively analysed to investigate the AL/MDS transformation rates in those patients with ET and PV. The safety population consisted of 2654 ET patients [median age 63yrs range 5 - 95 yrs] and 572 PV patients [median age 66yrs range 17 - 92 yrs]. Of the 2654 ET patients, 2264 had been treated previously with other cyto-reductives and their transformation rate was 2.3% [53pts of 2264pts]. These patients had a median duration of disease of 3.8yrs with a maximum of 32yrs. The median duration of anagrelide therapy was 1.1 yrs with a maximum duration of therapy of 11.0yrs and 10% [229 pts of 2264pts] received anagrelide for longer than 5 yrs. The remaining 390 ET patients were cyto-reductive naive and received anagrelide as first line therapy and their transformation rate was 0.3% [1pt of 390 pts]. These patients had a median duration of disease of 2.0yrs with a maximum of 13.3 yrs. The median duration of anagrelide therapy was 1.3yr with a maximum duration of therapy of 9.2yrs and 8.7% [34 pts of 390 pts] received anagrelide for longer than 5 yrs.

Of the 572 PV patients, 508 had been previously treated with other cyto-reductives and their transformation rate was 2.8% [14pts of 508pts]. These patients had a median duration of disease of 1.0yr with a maximum of 25 yrs. The median duration of anagrelide therapy was 0.8yrs with a maximum of 11.4 yrs and 5.7% [29pts of 508pts] received anagrelide for longer than 5 yrs. The remaining 64 PV patients were cyto-reductive naive and received anagrelide as first line therapy and their transformation rate was 0%. These patients had a median duration of disease of 1.0yr with a maximum of 13.0yrs. The median duration of anagrelide therapy was 1.0yr with a maximum of 8.0 yrs and 4.7% [3pts of 64pts] received anagrelide for longer than 5 yrs.

In conclusion, anagrelide in this relatively large ET and PV population did not appear to increase the AL/MDS transformation rate compared to that reported in the literature. Indeed the early signal from this analysis is that anagrelide monotherapy may be associated with a very low rate of transformation and those transformations that were recorded here may be partly explained by the use of prior cyto-reductive agents. Longer follow up of ET/PV patients is required, particularly of those on anagrelide monotherapy, to confirm or refute these findings.

436 NEUTROPHIL PRV-1 GENE EXPRESSION IN MYELOPROLIFERATIVE, MYELOYDYSPLASTIC AND REACTIVE BLOOD DISORDERS

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Background: The diagnosis of PV is often difficult and there is no single gold standard diagnostic test. Several previous studies have shown a high expression of the polycythemia rubra vera 1 (PRV-1) gene in the granulocytes of PV patients, suggesting that PRV-1 might be a useful molecular marker for clinical diagnosis of PV. Aims: In order to assess the clinical utility of expression levels of the PRV-1 gene, we investigated the mRNA expression of PRV-1 in various types of hematological (including myeloproliferative, myelodysplastic disorders) and reactive disorders. Methods: We quantified neutrophil PRV-1 levels in 20 PV, 13 essential thrombocythemia (ET), 9 chronic myelogenous leukemia (CML), 19 myelodysplastic syndromes (MDS), 9 secondary erythrocytosis (SE), 4 idiopathic erythrocytosis (IE), and 12 secondary leukocytosis (SL) by TaqMan real-time RT-PCR. The relative expression of PRV-1 for each patient sample, in which the Ct values of PRV-1 were normalized to β 2-microglobulin and compared with healthy controls,

was calculated as 2^{-Ct} . Results: Our results showed that the levels of PRV-1 expression were elevated in all groups. Among them, SL group had extraordinarily high expression of PRV-1 (mean 1260.98), and was significantly higher than any other groups ($p < 0.05$) except the PV group. PRV-1 mRNA expression was increased by 380.06-fold (mean) in PV patients. Although the PRV-1 mRNA levels were higher in PV than other polycythemia (SE or IE) or other CMPD (CML or ET), the differences were not statistically significant. Most MDS patients showed high levels of PRV-1 expression in their neutrophils even though the levels were significantly lower than PV ($p < 0.05$). Conclusions: Our data suggest that the PRV-1 gene expression is not a specific marker for the diagnosis of PV because elevated PRV-1 mRNA expression in neutrophils could be detected in various disorders ranging from myeloproliferative and myelodysplastic conditions to reactive conditions. The quantitation of PRV-1 mRNA expression cannot distinguish PV from any of the above conditions. It is clear that new molecular markers must be sought for the diagnosis of PV.

437 CIRCULATING ENDOTHELIAL PROGENITOR CELLS ARE INCREASED IN PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA

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Background. It has been shown that endothelial progenitor cells (EPCs) circulate in the peripheral blood (PB) of normal subjects. These cells co-express on their surface the CD34, VEGFR-2, and CD133 antigens and are able to form colonies in vitro. We have previously reported that patients with Myelofibrosis with Myeloid Metaplasia (MMM) had an increase number of circulating CD34+ cells compared to other Ph-negative myeloproliferative disorders. Moreover, we have shown that MMM is characterized by enhanced angiogenesis both in the bone marrow and in the spleen. Aims. In this study we have tested whether an increased number of circulating EPCs is present in patients with MMM compared with patient with PV or ET and to healthy subjects. Methods. Ten-50 ml of PB were obtained from patients with MMM, PV, ET, and healthy subjects. FACS analysis was performed either on whole PB or on immunomagnetic selected CD34+ cells. The following monoclonal antibodies were used: FITC anti-CD34, PE anti-CD133, and PerCp anti-VEGFR-2. Endothelial colonies were obtained by plating PB mononuclear cells in 24 well plates coated with fibronectin in the presence of a commercial medium specific for endothelial cell growth. Colony identity was confirmed by in situ staining with monoclonal antibodies directed towards CD31, vWf, VE-cadherin, CD34, and CD45. Results. Patients with MMM (n=52) had a median percentage of CD34+VEGFR-2+CD133+ cells (1.2%, range 0-21.5) higher than patients with PV or ET (n=8) (0%, 0-3.2) and higher than normal subjects (n=12) (0%, 0-3.6) ($p=0.012$, and $p=0.001$, respectively). This observation was confirmed by the analysis of immunomagnetic selected CD34+ cells. Indeed, the percentage of CD34+ cells co-expressing the VEGFR-2 and the CD133 antigens was higher in MMM patients (n=12) (median 1.65%, range 0.3-33.8) than in normal subjects (n=6) (0.5%, 0.21-0.76) ($p=0.026$). EPC cultures showed that the number of VE-cadherin+CD31+ colonies in 10 MMM patients was higher (median 80, range 56-100) than in 6 PV or ET patients (9, 4-16) and than in 6 healthy subjects (21, 5-37) ($p < 0.05$, and $p < 0.02$, respectively). However, in other 20 MMM patients the number of EPC colonies was in the range of those of controls. Summary. All together, our data suggest that in patients with MMM the circulating EPCs are increased compared to other Ph-negative myeloproliferative disorders and to healthy subjects. This finding may be related to the angiogenic process that characterize the disease.

438 POLYCYTHEMIA AND HIGH SERUM ERYTHROPOIETIN IN YOUNG PATIENTS: OCCURRENCE OF VHL MUTATION

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Background: The Chuvash polycythemia is a familial congenital polycythemia with increased EPO, caused in several affected individuals by homozygosity for the VHL mutation R200W. Aims and patients: We have studied the VHL gene in seven young with congenital sporadic polycythemia and high EPO. All of them are from north-eastern Italy.

Results: In only one patient and in his mother, who does not have erythrocytosis, a heterozygous VHL gene mutation, never reported in literature, was detected (c. 430G>A; G144R). No signs of VHL disease have been demonstrated in either individual. In two cases, other genetic conditions were associated with erythrocytosis (NF2 and Down syndrome).

Conclusion: The causal role of this new VHL mutation in determining the erythrocytosis of this patient is certainly questionable; it is possible that some unknown factors contribute to the phenotype. The VHL mutation might not be the only genetic alteration inducing polycythemia due to disturbed hypoxia-sensing mechanisms.

439 UTILITY OF HUMARA PCR ASSAY IN A COHORT OF SPANISH PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA.

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BACKGROUND.

Essential thrombocythemia (ET) and polycythemia vera (PV) are clonal myeloproliferative disorders (MPD) that lack consistent karyotypic and/or molecular markers. Human androgen receptor (HUMARA) PCR analysis, a X-chromosome inactivation pattern (XCIP) based-assay, provides a means of assessing clonality in female ET and PV patients. There is controversy about the percentage of clonal hematopoiesis in ET and little is known in female PV patients. The aim of the present study was to determine the heterozygosity rate for HUMARA locus and the rate of clonality in a Spanish cohort of ET and PV patients.

PATIENTS AND METHODS.

Thirty-nine ET and 14 PV patients were included in the study. Diagnosis of PV and ET was made according the PVSG criteria. Median age of ET and PV patients was 58 (range 32-79) and 59 (range 30-86) years, respectively.

At the time the HUMARA assay was performed, 19/39 ET and 3/14 PV patients were receiving therapy with myelosuppressive/platelet lowering agents as follows: hydroxyurea +/- ASA (n=12); anagrelide +/- ASA (n=8) and busulphan (n=1). Eleven patients received ASA. In PV patients, phlebotomy was performed when packed red cell volume >0.42 L/L. Peripheral blood samples were obtained from 26 healthy females as normal controls.

XCIP by HUMARA-PCR assay was performed on DNA of purified granulocytes and CD3+ lymphocytes. PCR product was analyzed with an automated DNA sequencer. Percentage of clonality was calculated after correcting for the degree of lyonization in CD3+ cells.

RESULTS.

Six of the 53 studied patients could not be tested by the HUMARA assay because their homozygous XCIP. In our series, the heterozygosity rate for the HUMARA gene was 88.6%.

Of the 39 ET patients, 5 were non informative (12.8%) and 5 were homozygous (12.8%); from the 29 ET remaining patients, 11 were clonal (38%) and 18 polyclonal (62%). Among the 14 PV patients, no patient was non informative and only one was homozygous; from the other 13 PV patients, 6 were clonal (46%) and 7 polyclonal (54%). Five of the 26 controls were homozygous and one non informative; the 20 remaining controls were polyclonal. The relationship between clonality status and age is depicted in table 1.

In ET patients, we did not observe any correlation between thrombotic or bleeding complications and clonality patterns. For the whole group (ET and PV patients) no relationship was found between clonal or polyclonal hematopoiesis and platelet lowering treatment.

COMMENTS.

Present data show that the heterozygosity rate for HUMARA gene in the studied Spanish ET and PV female patients is 88.6%. ET and PV are heterogeneous disorders in terms of clonality (38% and 46% showed a clonal pattern, respectively). Our results did not show neither a significant relationship between age and clonality in ET and PV patients nor a correlation between clonality status and thrombotic or hemorrhagic events in ET patients.

Larger and prospective series of HUMARA clonality assays in ET and PV patients are needed to ascertain the possible clinical implications of clonal hematopoiesis.

ACKNOWLEDGEMENTS. Grant PI030394

	Age	Clonal	Polyclonal	Non informative	Homozygous
ET	58 (32-79)	11	17	5	2
PV	59 (30-86)	6	7	0	1
Controls	58 (32-79)	5	15	0	1

Caption 1:

440 LEUKOCYTE AND PLATELET ACTIVATION IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV)

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Background. ET and PV are characterized by high incidence of thrombotic events. The pathogenesis of these complications is not completely clarified. Recently, we explored the activation status of circulating polymorphonuclear leukocytes (PMN) in these disorders. In this study, in ET and PV patients, we evaluated simultaneously the markers of activated PMN, activated platelets, and the circulating levels of PMN-platelet aggregates. In addition, we evaluated the PMN/platelet aggregating response and the PMN activation in vitro after stimulation with f-MLP.

Aims. Aim of this study was to assess the relative contribution of PMN activation, and or platelet activation to PMN/platelet aggregates generation.

Methods. PMN, platelets and PMN-platelet aggregates were analyzed by flow cytometry in whole blood samples from 43 patients with ET, 30 with PV and 50 control subjects (C). These parameters were studied in both basal condition and after PMN in vitro stimulation with f-MLP. PMN activation was evaluated by CD11b expression whether platelet activation was measured by CD62P expression. PMN-platelet aggregates were defined as the percentage of CD11b-positive PMN co-expressing a platelet-specific marker (i.e. CD42b or CD62P).

Results. Significantly ($p<0.01$) higher CD11b levels on PMN from ET and PV patients compared to controls were found. PMN/platelets aggregates (identified by CD11b/CD42b and CD11b/CD62P positivity) were significantly increased ($p<0.01$) in the two groups of patients compared to controls. Analysis of platelet surface showed no variations in CD42b expression, whereas increased CD62P levels, a surface antigen specifically exposed on platelet activation, were expressed by ET and PV platelets, compared to controls. f-MLP in vitro stimulation, increased CD11b expression by PMN, and simultaneously increased CD11b/CD42b aggregates in samples from all groups, and CD11b/CD62P aggregates only in ET and control groups. No significant differences in platelet CD62P and CD42b expression were found after in vitro f-MLP stimulation in any group. The analysis of the results on the basis of the different treatments, showed that, in ET patients receiving aspirin, the increase in f-MPL induced expression of CD11b on PMN surface and the percentage of PMN/platelet aggregates were significantly lower compared to non-aspirin treated patients.

Summary and Conclusions. In conclusion, increased PMN/platelet aggregates were found in association with the occurrence of PMN and platelet activation in ET and PV in vivo. However, the in vitro study suggests a major role for PMN in increasing the leukocyte/platelet interactions.

441 A NEW PROGNOSTIC MODEL (MPI) FOR PATIENTS WITH MYELOFIBROSIS

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Background: Several prognostic models for patients with chronic idiopathic myelofibrosis (CIMF) have been developed, with the LILLE score now most commonly used. The latter and other previous scoring systems, however, showed limited power to consistently separate patients with intermediate and poor prognosis.

Methods: We have studied 107 patients with MMM (median age 68, range 24-87) and correlated clinical parameters at the time of diagnosis with survival by multivariate analysis.

Results: First, previous scoring systems were applied to allocate patients to subgroups with distinct prognosis. Surprisingly, however, most previous scoring systems failed to clearly separate an intermediate and poor prognostic group, including the LILLE model. Instead, age and hemoglobin level emerged as most significant parameters in multivariate analysis ($p<0.1 \times 10^{-5}$,

$p < 0.1 \times 10^{-6}$, respectively). By allocating one risk point each for $Hb < 10$ g/dL and age > 60 years, three subgroups of patients with significantly distinct prognosis could be identified in our cohort of patients (median survival not reached, 64 and 31 months, $p < 0.1 \times 10^{-6}$). The overall model and the difference between each of the subgroups were statistically significant in a training group, a test group, the overall cohort of patients and the group of patients with agnogenic MMM.

Conclusion: We have established a new, simple prognostic index (MPI) for patients with CIMF which discriminates three prognostic groups with highly significant different survival. Our data also confirm a previous report by Kvasnicka et al. who have shown the prognostic relevance of age and hemoglobin before, but used in addition leukocyte and platelet counts for separation of patients into different prognostic subgroups.

442 CYTOGENETIC ABNORMALITIES IN MYELOFIBROSIS DETERMINED BY FISH

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Background: An abnormal karyotype has been correlated with poor prognosis in chronic idiopathic myelofibrosis (CIMF) in several recent studies. This may be due to an increased proliferative index in these cases. However, conventional cytogenetics is usually hampered by the difficulty of obtaining sufficient numbers of analyzable metaphases from bone marrow aspirates in MMM. This obstacle can be overcome by fluorescence in situ hybridization (FISH), which can be performed on interphase nuclei from archival material.

Methods: In this study, the bone marrow of 107 patients with MMM was analyzed using FISH and chromosomal probes 7cen, 7q, 8cen, 11cen, 12p, 13q, 17cen, 17p, 21q, 20q. Cases with myelodysplastic features or bcr/abl were excluded from analysis. The prognostic impact of individual cytogenetic lesions on survival was evaluated using multivariate analysis.

Results: In univariate analysis, -7/7q- was significantly associated with inferior outcome (median survival 99 vs. 25 months, $p < 0.001$). This abnormality remained significant in a multivariate analysis including all cytogenetic parameters, our own myelofibrosis prognostic index (MPI) and the LILLE prognostic score. When a cytogenetic risk indicator (CPI), based on several cytogenetic variables, was used, patients were clearly divided into two groups with a significantly distinct outcome.

Conclusion: Detection of loss of 7/7q by FISH was found to be significantly associated with inferior outcome in MMM. Multivariate analysis including established prognostic factors identified deletion of 7/7q as independent prognostic factor in patients with MMM. By using cytogenetic findings only, patients can be allocated into poor and good risk groups.

443 SECOND MALIGNANCIES IN ET: A RETROSPECTIVE ANALYSIS OF 306 PATIENTS WITH PROLONGED OBSERVATION TIME FROM A SINGLE INSTITUTION

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Background: Alkylating agents (ALK) has been demonstrated to increase risk of acute leukaemia and myelodysplastic syndromes in patients with myeloproliferative disorders. Hydroxyurea (HU) is the most used cytoreductive drug in essential thrombocytemia (ET), having a lower leukemogenic potential.

Aims: To retrospectively investigate long-term development of haematological and non haematological second malignancies in patients with ET, analyzing possible associations with cytoreductive treatments.

Methods: Data from 306 patients with ET referred to our Institution from January 1977 to April 2001 were retrospectively collected, particularly with respect to development of second malignancies. Diagnoses were confirmed according to the WHO criteria. Estimates of survival were based on the Kaplan-Meier method. Continuous independent variables between patient groups were compared by Wilcoxon two-sample test.

Results: The patient population included 197 females and 109 males, with a M/F ratio of 0.55. Median age was 61 yrs (range 18-87). At the time of analysis, 239 patients were still alive. Median follow-up of the alive patients was 108 months (range 21-276). Median survival of the whole population was not reached, with 57% of patients still alive 228 months after diagnosis. At diagnosis, median platelet count was $828 \times 10^9/L$ (range 489-3200), median hemoglobin value was 14 g/dL (range 9-17), and median WBC count was $8.7 \times 10^9/L$ (range 4-22.2).

After referral, 131 patients did not receive any treatment, whereas 175 were treated with chemotherapy; 100 patients received only HU, 38 only ALK (Melphalan/Busulphan), and 37 ALK followed by HU. A second malignancy was detected in 39 patients, with a median time from diagnosis of 96 months (11 acute leukemias, 4 NHL, 24 solid tumors).

Treatments administered for ET before development of second malignancy were as follows: 10 (26%) no treatment, 10 (26%) HU, 10 (26%) ALK only, and 9 (22%) ALK followed by HU.

Of the 267 patients who did not develop secondary malignancies, 121 (45%) were never treated, 90 (34%) received HU only, 28 (10.5%) received ALK only, and 28 (10.5%) received ALK followed by HU. A comparison between drug doses for patients who developed second malignancy and patients who did not is shown in the table.

Among patients who were treated with HU or ALK single agents, average drug cumulative doses were significantly higher in those who developed second malignancy compared to those who did not. In contrast, when patients were treated with ALK followed by HU, no significant differences between the two groups were observed in drug cumulative doses. When preceded by ALK, average HU cumulative dose among patients who developed second malignancy was significantly lower than when administrated as first line therapy.

Conclusions: Our study, including a large population of unselected ET patients with long observation times, suggest that chemotherapy treatment is associated with higher risk of developing second haematological and non haematological malignancies. Furthermore, such risk seems to be enhanced by relatively low cumulative doses of HU in patients who received previous alkylating agents.

Second Malignancy (n)	Second Malignancy (%)	First Malignancy (n)	First Malignancy (%)
Acute Leukemia	11 (28.2%)	11 (4.1%)	4.1%
Non-Hodgkin Lymphoma	4 (10.3%)	4 (1.5%)	1.5%
Solid Tumors	24 (61.5%)	24 (9.4%)	9.4%
Total	39 (100%)	39 (100%)	100%

Caption 1:

444 DIFFERENCES IN GENDER AND CLINICAL-HEMATOLOGICAL FINDINGS BETWEEN THE 4Q-/CHRONIC EOSINOPHILIC LEUKEMIA AND IDIOPATHIC HYPEREOSINOPHILIC SYNDROMES

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Background: WHO criteria for diagnosis of idiopathic hypereosinophilic syndrome (HES) include: eosinophilia exceeding 1.500/mm for more than six months; exclusion of a secondary eosinophilia; signs and/or symptoms of organ involvement. Detection of clonality is critical for diagnosis of chronic eosinophilic leukemia (CEL).

Aim: The aims of our study were: 1) to investigate selected genes rearranged in myeloid malignancies with eosinophilia in a series of cases diagnosed as HES according to the WHO criteria; 2) to compare the 4q-/CEL to the other HES.

Methods: We selected 34 HES for an interphase fluorescence in situ hybridization (FISH) study using the following probes: BAC 120K16 and BAC 3H20 in double color experiments (cryptic deletion of 4q12/CHIC2), BAC 586A12 (C-KIT/4q12), cosmid 4-1 and 9-4 (PDGFRbeta/5q33), BAC 350N15 (FGFR1/8p12), PAC 1132H12, PAC 913J14, PAC 888H11, and PAC 835J22 (ABL1/9q34), cosmid 148B6 and 179A6 (ETV6/12p13).

Results: Eight cases showed a cryptic 4q12 deletion which is consistent with a FIP1L1-PDGFRalpha fusion transcript. Nested RT-PCR confirmed the presence of the FIP1L1-PDGFRalpha fusion transcript in two cases. All the other candidate genes for eosinophilia in myeloproliferative disorders (PDGFRbeta, FGFR1, ABL1, and ETV6) were not rearranged in this FISH study.

Clinical-hematological findings gave the following results: 1) Sex distribution showed a male/female ratio of 7/1 in 4q-/CEL and of 1/1 in HES. 2) Hepatomegaly was observed in 6/8 (75%) of 4q-/CEL and in 5/26 (19%) HES, splenomegaly in 6/8 (75%) 4q-/CEL and in 6/26 (23%) of HES. 3) Involvement of heart and central nervous system in 2/8 (25%)

4q-/CEL and in 3/26 (11%) HES, whereas involvement of skin was seen in 4/8 (50%) 4q-/CEL and 10/26 (30%) HES and involvement of lung in 1/8 (12.5%) 4q-/CEL and 3/26 (11%) HES. 4) Clinical-hematologic response to imatinib was obtained in 7/7 cases of 4q-/CEL and in 0/4 cases of HES.

Conclusion: 4q-/CEL was highly prevalent in males. Hepato-splenomegaly was significantly more frequent in 4q-/CEL. Other genes rearranged in myeloid malignancies with eosinophilia were not involved in HES. Response to imatinib in 4q-/CEL is confirmed.

STEM CELL TRANSPLANTATION ALLOGENEIC

445 CD8+ T CELL DOSE AFFECTS DEVELOPMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE FOLLOWING ALLOGENEIC TRANSPLANTATION WITH ANTITHYMOCYTE GLOBULIN-BASED REDUCED-INTENSITY CONDITIONING REGIMEN

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Acute graft-versus-host disease (aGVHD) remains an important cause of morbidity after reduced intensity conditioning (RIC) allogeneic transplantation (allo-SCT). It has been shown that antithymocyte globulin (ATG) dose infused during RIC is a major determinant for the likelihood of developing aGVHD. The ATG modulation on aGVHD is likely related to in-vivo T cell depletion. We therefore investigated the relationship between the cellular composition of the allograft and clinical outcome in 57 patients who received allogeneic peripheral blood stem cells from HLA-identical siblings following an ATG-based RIC. The CD8+ T-cell dose infused was the only parameter associated with the risk of aGVHD ($P=.037$). Interestingly, when looking at the extremes, patients experiencing grade III-IV aGVHD received a median of 143 x106/Kg (range, 72-241) CD8+ T-cells, while patients without aGVHD received a median of 96 x106/Kg (range, 54-312) CD8+ T-cells ($P=.021$). Overall, the cumulative incidence of grade I-IV aGVHD was significantly higher among patients who received the higher CD8+ T-cell doses as compared to patients who received the lower doses (90% versus 64%; $P=.022$). In the 44 patients evaluable for cGVHD, 12 developed limited cGVHD, whereas 26 developed clinical extensive cGVHD for a cumulative incidence of cGVHD of 67% (95%CI, 55%-79%). In univariate analysis (including demographic factors, graft composition and aGVHD), only clinically significant grade II-IV aGVHD was associated with an increased probability of developing cGVHD ($P=.068$). When controlling for all prognostic factors, patients with grade II aGVHD had a significantly better overall survival in comparison to patients with grade 0-I or grade III-IV aGVHD ($P=.009$). Overall, the role of early immune reactions would tend to be more evident in truly RIC. In comparison to myeloablative allo-SCT, tailoring of the number of cells infused, at least in the context of ATG-based RIC, may have a significant impact on the probability of a favorable outcome.

446 GRAFT-VERSUS-MYELOMA EFFECT FOLLOWING REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

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Despite the development of high dose chemotherapy, there has been little progress in improving the cure of patients with multiple myeloma (MM). This report describes the results of 41 MM patients who received an antithymocyte globulin, fludarabine and busulfan-based reduced intensity conditioning (RIC) for allogeneic stem cell transplantation (allo-SCT) from HLA-identical siblings. 29 patients (70%) were in PR, one patient in CR, and 11 (27%) in progressive disease at time of allo-SCT. 34 patients (83%) received at least one autologous transplantation in the course of their disease prior to allo-SCT, but this was not part of the allo-SCT strategy. Median time between allo-SCT and autologous transplantation was 4 (range, 2-57) months. Median time between

MM initial diagnosis and allo-SCT was 24 (range, 6-146) months. The cumulative incidences of grade II-IV and grade III-IV acute graft-versus-host disease (GVHD) were 40% (95% CI, 24%-56%) and 8% (95% CI, 2%-22%) respectively. 10 patients developed limited chronic GVHD, whereas 7 developed an extensive form giving a cumulative incidence of chronic GVHD of 51% (95% CI, 34%-68%). With a median follow-up of 389 days, the overall cumulative incidence of transplant-related mortality (TRM) was 17% (95% CI, 6%-28%). In addition to the one patient who was in CR at time of allo-SCT, 21 other patients (53%; 95% CI, 38%-68%) could achieve an objective disease response during the follow-up period (complete remission, n=10; partial remission, n=11). 2 patients remained in stable disease, 3 patients were not assessable for disease response because of early death, and 14 patients (34%; 95% CI, 20%-48%) did not have any disease response and progressed after allo-SCT. Among the patients who survived beyond day 100 after allo-SCT and who had an objective disease response, 13 (65%) experienced limited or extensive chronic GVHD as compared to only 4 (31%) in the rest of the series ($P=.05$). The Kaplan-Meier estimate of overall survival (OS) at 2 years was 62% (95% CI, 47%-76%). Progression-free survival (PFS) and OS were respectively significantly higher in patients with chronic GVHD as compared to patients without chronic GVHD ($P=.005$ for PFS and $P=.01$ for OS). Collectively, these data demonstrate that RIC allo-SCT can mediate a potentially curative graft-versus myeloma effect with a low rate of toxicity and TRM.

447 CLINICAL FOLLOW-UP INDICATES DIFFERENTIAL OUTCOME OF EXTRAMEDULLARY VERSUS MEDULLARY RELAPSE AFTER AUTOLOGOUS OR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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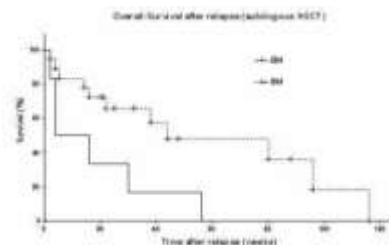
Background: Risk-adapted treatment of multiple myeloma (MM) includes autologous (auto-) and allogeneic (allo-) hematopoietic stem cell transplantation (HSCT). Casuistic reports on extramedullary (EM) as compared to bone marrow (BM) relapse after HSCT suggest a dismal prognosis.

Aims: The purpose of this study was to compare the outcome of 41 MM patients (pts) relapsing after auto- (group A: n=24) or allo- (group B: n=17) HSCT, stratified into those with BM versus EM (+ BM) relapse.

Methods: The groups were also compared with respect to adverse prognostic factors, including age, 2-microglobuline (2-MG), pre-treatment, cytogenetics and disease stage. Relapse eventually occurred in 41 pts, who had initially responded (CR/PR) to auto- and allo-HSCT in 10/24 (42%) and 3/17 (18%), respectively. EM relapse sites were lungs (5), soft tissue (4), pericard (2), bone (1), skin (1) and central nervous system (1).

Results: As of December 2003, the overall survival (OS) after HSCT in pts relapsing from EM sites was lower as compared to BM relapse, both after auto- (0% versus 39%) and allo-HSCT (13% versus 33%). The median follow-up was 30 months after auto-, and 13 months after allo-HSCT. With analysis of OS after relapse or progression following HSCT, this was lower in pts relapsing in EM sites as compared to BM after auto-HSCT (0% versus 38%) at a median follow-up of 44 weeks (range: 1-96).

Conclusions: Although MM pts relapsing after auto- or allo-HSCT were formerly regarded to have few therapeutic options, we observed encouraging results with various treatment approaches, with 14/22 (64%) and 7/12 (58%) pts responding after auto- and allo-HSCT, respectively. These observations suggest the need for individualized treatment approaches in MM during clinical follow-up after HSCT.



Caption 1:

448 REDUCED INTENSITY TRANSPLANTATION FOR PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background: conventional allogeneic stem cell transplantation (SCT), although curative for MDS patients, is associated with high incidences of severe acute GVHD and transplant-related mortality (TRM), resulting in poor overall survival.

The aim of present study was to evaluate the efficacy of a reduced-intensity conditioning (RIC) consisting of fludarabine and TBI in patients with MDS.

Methods: between March 2000 and October 2003, 12 patients (median age 57 years; range 20-70) with MDS were treated with a RIC regimen based on fludarabine (30 mg/m² x 3-5 days) and 200 cGy TBI followed by alloHSCt because of advanced age (n=10), extensive prior therapies (n=1) or comorbidities (n=1). GVHD prophylaxis consisted of cyclosporine and mycophenolate mofetil. The source of stem cells was blood in all patients. The median CD34+ and CD3+ contents of the grafts were 7.2 x 10⁶/Kg and 2.2 x 10⁸/Kg respectively. Three patients had received a prior allograft. The median time interval from diagnosis to transplant was 254 days (range 44-1465). Six patients had active disease at transplantation and 6 were untreated.

Results: Primary neutrophil engraftment with ANC > 0.5 x 10⁹/l was achieved in 10 (83%) patients at a median of 16 days after transplantation (range 12-30); 1 patient who died early (day +5) failed to reach this threshold and 1 patient never became granulocytopenic. Eighty percent of the patients had > 90% donor chimerism on day +30. Among 11 evaluable patients, 5 (45.5%) developed grade I acute GVHD, 5 (45.5%) grade II and 1 (9%) grade III acute GVHD; grade IV acute GVHD did not occur. Extensive chronic GVHD was observed in 3 of 9 patients (33%) surviving more than 100 days. Seven patients (59%) are alive (n=6 in CR, n=1 with relapsed disease); 1 patient (8%) died of treatment-related complications, 4 patients (33%) died as a result of relapse. At a median follow-up of 405 days (range 106-1179), the 1-year DFS was 54%. Two patients received dose-escalating DLI for disease relapse; none of these obtained a CR. One patient received DLI for mixed chimerism and converted to full donor chimerism after 2 doses of lymphocytes.

Conclusion: Although the limited number of patients and early follow-up preclude any definitive conclusions, our preliminary results suggest that RIC for allogeneic SCT in high-risk MDS patients achieves successful donor engraftment and disease remission with minimal transplant toxicity.

449 INDUCTION OF AGVHD AFTER ALLOGENEIC HEMATOPOETIC STEM CELL TRANSPLANTATION FOR REFRACTORY OR RELAPSED ACUTE LEUKEMIA

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BACKGROUND & OBJECTIVE: Patients with refractory or relapsed acute leukemia also have a poor prognosis with death due to relapse or transplant-related mortality (TRM) after allogeneic hematopoietic stem cell transplantation. The purpose of this paper was to clarify the effect of inducing acute graft-versus-host disease (aGVHD) during transplantation to prevent relapse and improve disease-free survival (DFS). METHODS: Thirty adult patients (age 12-48) with refractory or relapsed acute leukemia at our hospital were acute lymphoblastic leukemia (ALL) 16 cases, acute myelogenous leukemia (AML) 10 cases and acute mixed-leukemia 4 cases. They were in first complete remission (CR1) 4 cases, second complete remission (CR2) 9 cases, partly remission (PR) 12 cases and non-remission (NR) 5 cases at the time of transplantation. 30 patients underwent allogeneic peripheral blood stem cell transplantation (allo-PBSCT) from HLA-identical siblings (n=21), mismatched siblings donors (n=3), and allogeneic bone marrow transplantation (allo-BMT, n=5), all-PBSCT (n=1) from unrelated HLA matched donors. All patients received more intense myeloablative regimens consisting of total body irradiation, cyclophosphamide, etoposide and cytosine arabinoside. And the patients with PR or NR even received myeloablative regimens following a salvage chemotherapy (fludarabine, mitoxantrone and cytosine arabinoside) for

eliminating residual leukemic cell. For aGVHD prophylaxis the patients with HLA-identical siblings donors received cyclosporine (CSA) alone, and the patients with mismatched siblings or unrelated donors received CSA, methotrexate and low-dose ATG (2.5mg/kg/d, -3d to 1d). For inducing aGVHD at early stage after transplantation, patients were scheduled to be quickly reduced the maintenance dose of CSA at 20% to 30% off every week and treated with pre-emptive donor leukocyte infusion if there was no appearance of aGVHD at +30d to 60d after transplantation. RESULTS: After a median follow-up of 18.1 months, there were 24/30 (80%) patients developed aGVHD (grade 3 or 4 had 4/30, 13.3%), which was more frequent than we had in other standard risk leukemia patients with general protocol at same period (aGVHD 23/42 53.5%, p value less than 0.01). There were 11/19 (57.9%) patients developed chronic GVHD (cGVHD), with 3/19 (15.8%) had extensive cGVHD. 18 patients are alive with DFS (18/30, 60%) and 12 patients have died of relapsed leukemia (5/28, 17.9%) and TRM (7/30, 23.3%). The 2-year probability of DFS is 54.3%. CONCLUSIONS: Induction of aGVHD after transplantation is feasible and effective to prevent relapse and improve DFS in patients with refractory or relapsed acute leukemia

450 RECOVERY OF SPERMATOGENESIS IN LONG-TERM SURVIVORS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) often results in impaired male fertility. Azoospermia occurs mostly in patients undergoing total body irradiation. The extent to which chemotherapy, immune dysregulation and GVHD affect spermatogenesis and whether time after HSCT allows some recovery is still unclear. Aims: to assess spermatogenesis recovery we did a cross-sectional study in males who had undergone HSCT at Basel University Hospital. Methods: All consecutive males above 18 years old (yrs), in complete remission, > two years after HSCT and with full donor chimerism of hemopoiesis were included. Seminal fluid analysis on fresh samples was performed by the department of Andrology according to WHO protocol. Results: Between April 2003 and January 2004, 44 patients were asked to participate in the study. Three of them were excluded due to previous vasectomy and 12 declined testing. Twenty nine patients were available for analysis. Age at HSCT ranged from 13 to 56 (median 25) yrs, age at the time of semen analysis ranged from 22 to 59 (median 35) yrs. Post-transplant follow-up ranged from 2 to 20 (median 10) yrs. Indications for HSCT were severe aplastic anemia (3), acute lymphoblastic leukemia (1), acute myeloblastic leukemia (13), chronic myelocytic leukemia (10), myelodysplastic syndrome (1) and multiple myeloma (1). Twenty five out of 29 (86%) patients were conditioned with high intensity regimen, 4/29 received a low intensity regimen. Nine out of 29 (31%) patients had chronic GVHD. Nine out of 29 (31%) patients recovered some degree of sperm counts, of those 3/9 were normozoospermic, 1/9 oligozoospermic (between 5 and 20 x 10E6/ml) and 5/9 severely oligozoospermic (<5 x 10E6/ml). In patients below 25 yrs, 8/16 recovered spermatogenesis compared to 1/13 in patients over 25 yrs (p=0.02). Six out of 25 (24%) recipients of a high intensity regimen versus 3/4 (75%) recipients of a low intensity regimen (p=0.07) recovered spermatogenesis. One out of 9 (11%) patients with cGVHD versus 8/20 (40%) without cGVHD recovered spermatogenesis (p=0.20). Donor type and length of follow up was not correlated with recovery of spermatogenesis. Two patients with SAA, conditioned with cyclophosphamide alone, fathered a normal child 5 and 16 years respectively after HSCT. Conclusions: our results indicate that one third of the patients recover some degree of spermatogenesis after HSCT. Factors associated with recovery are age and conditioning regimen intensity. There was a positive trend toward spermatogenesis recovery in absence of cGVHD. This supports the practice of semen cryopreservation in these patients prior to HSCT. New insemination techniques might be considered even for patients with severe oligozoospermia.

451 ALLOGENEIC STEM CELL TRANSPLANTATION FROM UNRELATED DONORS IN SWITZERLAND. IMPACT OF MOLECULAR HLA MATCHING

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This is a report on all consecutive 214 unrelated donor transplants (HSCT) using peripheral blood (N=51) or marrow (N=143) as a stem cell source in Switzerland since 1990. All transplants were facilitated by the Swiss bone marrow donor registry and were performed at four institutions. Median age was 28 (1-61) years, and 132 (62%) patients were male. Ninety-eight patients were transplanted in the 1990s, 116 since 2000. Twenty-four patients with malignancy underwent transplants using reduced intensity conditioning regimens. GvHD prophylaxis was by T-cell depletion in 45. Patients had CML (62), AML (53), ALL (36), MDS/MPN (31), lymphoid malignancy (14), acquired or congenital aplastic anemia (11), other inherited disorders (7). Sixty-two patients with AML, ALL or CML were transplanted at an early disease stage, i.e. CR1 or 1st chronic phase, the remainder with more advanced disease. All donor recipient pairs underwent high resolution HLA typing at the Swiss national reference laboratory for histocompatibility in Geneva (LNRH). Donor/recipient pairs were HLA-A, -B, -C, -DRB1/B3/B5, -DQB1 matched (131), or were mismatched at the HLA-A or B (10), HLA-C (28) HLA-DR (14), HLA-DQ (16) or at multiple (15) loci. Median follow-up of surviving patients was 34 months.

Survival probabilities (+ 95% CI) at 2 years were, in decreasing order 61 + 9% for recipients of HLA 10/10 matched transplants, 46 + 26% with DQ mismatch, 44 + 20% with HLA-C mismatch, 30 + 27% with HLA-DR mismatch, 15 + 26% with HLA-A or B mismatch and 13 + 18% with multiple mismatches, $p < 0.0001$. These differences remained highly significant after adjustment for disease type and stage, patient age, conditioning intensity and GvHD prophylaxis in multivariate analysis. Unrelated donor transplantation is increasingly used in Switzerland to treat a variety of disorders. Long term survival is possible, especially in recipients of transplants from HLA-A/B/C/DR/DQ-matched donors, identified by molecular typing. Mismatches at some loci may be more acceptable than others. Without an adequate donor, alternative methods to unrelated donor HSCT should be considered.

452 RESPIRATORY VIRUSES ARE DETECTED FREQUENTLY IN ADULT RECIPIENTS OF STEM CELL TRANSPLANTATION WITH RESPIRATORY TRACT DISEASE

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Background: Respiratory virus infections have increasingly been recognized as important causes of severe pneumonia in patients who have undergone SCT. However, the role of respiratory virus infections as the cause of pulmonary complications in patients undergoing SCT is not yet clarified and may have been underestimated by traditional detection methods.

Aim: To determine the incidence and severity of respiratory virus infection and to assess the diagnostic value of real-time PCR compared to viral culture, 72 recipients of autologous or allogeneic stem cell transplantation were prospectively monitored during a 6-month period.

Methods: In case of suspected upper or lower respiratory tract infection, nose-throat (NT) swabs or broncho-alveolar lavage (BAL) samples were taken for virus culture and quantitative PCR. In addition, NT samples from all SCT recipients were taken at predetermined intervals, in the absence of any clinical symptoms.

Results: A total of 52 episodes of upper (n=41) or lower (n=11) respiratory tract disease (RTD) were observed in 40 of 72 patients. A respiratory virus was detected by viral culture in 11 of 52 (21%) episodes of RTD, and by real-time PCR in 33 of 52 episodes (63%, $P < 0.0001$). In lower RTD, real time PCR was much more sensitive than viral culture (73% versus 9%, $P = 0.008$) in the detection of respiratory viruses. Rhinovirus was detected most frequently (19 out of 33 episodes, 58%), predominantly in upper RTD. In 64 % of follow-up samples obtained >1

week from patients with a respiratory virus disease, the initial respiratory virus could still be detected by real-time PCR, but not by viral culture ($P < 0.0005$). From samples taken from SCT recipients at predetermined moments without clinical symptoms of RTD, a respiratory virus could be detected in 9% of recipients of SCT by real-time PCR and in 1% by viral culture ($P < 0.0001$), suggesting that shedding of respiratory viruses in SCT recipients can occur.

Conclusion: We conclude that respiratory viruses are being detected frequently in case of upper and lower RTD in recipients of SCT and that real time PCR is superior to viral culture in the detection and follow-up of respiratory virus disease.

Respiratory viruses detected either by virus culture or by real time PCR in 52 episodes of respiratory tract diseases.

	episodes of URTD n=11		episodes of LRTD n=11		Total episodes of RTD n=22	
	virus culture	PCR	virus culture	PCR	virus culture	PCR
respiratory virus detection	10 (91%)	26 (81%)	1 (9%)	9 (73%)	11 (50%)	35 (61%)
rhinovirus	7 (73%)	19 (59%)	-	5 (45%)	7 (32%)	24 (42%)
coronavirus	1 (10%)	2 (6%)	-	1 (8%)	1 (5%)	3 (5%)
parainfluenza	1 (10%)	3 (9%)	1 (9%)	1 (8%)	2 (9%)	4 (7%)
EBV	1 (10%)	3 (9%)	-	3 (27%)	4 (18%)	6 (11%)
herpes simplex	1 (10%)	2 (6%)	-	2 (18%)	3 (14%)	5 (9%)
adenovirus	1 (10%)	1 (3%)	-	-	1 (5%)	1 (2%)

URT=upper respiratory tract disease, LRT=lower respiratory tract disease, RTD=respiratory tract disease.
SD=standard deviation, * $P < 0.005$, ** $P < 0.001$, *** $P < 0.0001$. * P patients had a respiratory virus infection with a respiratory virus. RTD=detection, rhinovirus, coronavirus, parainfluenza, adenovirus.

Caption 1: Table 1

453 ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN MYELODYSPLASTIC SYNDROME AND SECONDARY ACUTE MYELOID LEUKEMIA: OUTCOME IS DEPENDENT ON DISEASE BIOLOGY

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Allogeneic stem cell transplantation (alloSCT) is a potentially curative therapy for patients with myelodysplastic syndromes (MDS) and secondary acute myeloid leukemia (2AML). Historically, patients with these diagnoses had a poor outcome after SCT when given at an older age or with a donor other than a matched sibling. Since 1/2000 32 patients with MDS/ 2AML were given alloSCT from an HLA-matched sibling (n=15), 1-antigen mismatched relative (n=5) or matched unrelated donor (n=12) in a single institution. The median age was 52 years (range, 28-66). Eight patients have been previously treated with chemotherapy for acute lymphatic leukemia (n=2), non-Hodgkins lymphoma (n=4, 3 after autologous SCT), and breast cancer (n=2, both after autologous SCT). Twenty-four patients had MDS (at least with excess of blasts) or AML in patients with a prior history of MDS. Cytogenetic abnormalities were documented in 16 patients. Complex karyotype (n=3), -7 (n=4), and -5 (n=1) were considered poor-risk cytogenetics. The other abnormalities included 11q- (n=2), +8 (n=2) and others (n=4). Sixteen patients had diploid karyotype. By the time of alloSCT 25 patients have already progressed to AML and 7 still had MDS. Disease status at alloSCT was CR1/2 (n=10), untreated (n=12), untreated relapse (n=2), and refractory disease (n=8). Fifteen patients had more than 20% blasts in the marrow at alloSCT. Seventeen patients had myeloablative conditioning consisting of intravenous busulfan and cyclophosphamide and 15 patients had reduced intensity conditioning consisting of fludarabine and a reduced dose of intravenous busulfan. With a median follow-up of 18 months (range, 1-45) 20 patients are alive and disease-free, with an estimated 2-year overall survival (OS) of 54.11%. Treatment related mortality occurred in 7 patients with an estimated risk of 25.8%, and 5 patients died of disease relapse. Acute GVHD grade II-IV occurred in 11 patients (34%) and chronic GVHD in 9 of 22 evaluable patients (41%). Eighteen of 20 patients alive have very good performance status. The most significant factor predicting for survival was cytogenetics. OS of the 8 patients with poor-risk cytogenetics was 16.14% compared with 66.12% of all others ($p = 0.005$). Interestingly, most deaths in this group were treatment-related. Patients with chemorefractory disease also fared less with OS of 31.18% compared to 61.13% in patients with chemosensitive or untreated disease ($p = 0.05$). Age, donor source, history of chemotherapy for another malignancy, and the type of conditioning were not significant in predicting survival in the univariate analysis. In conclusion, alloSCT can cure selected groups of patients with MDS or AML secondary to MDS or prior chemotherapy. The recent advances in allogeneic SCT allow application of a curative approach to elderly patients and those with only alternative donors with similar survival rates. Outcome has become mostly dependent on parameters of disease biology such as cytogenetics and chemo-sensitivity.

454 RECONSTITUTION OF CYTOMEGALOVIRUS (CMV)-SPECIFIC CD4+ T CELLS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION- IMPORTANCE OF CD4 RECOVERY, SOURCE OF STEM CELL AND HOST FACTORS

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CMV infection is one of the main causes of morbidity and mortality following allogeneic stem cell transplantation. Developing the most effective immunotherapy depends on a clear understanding of immune reconstitution following stem cell transplantation. CMV-specific CD4+ T cells are important for the maintenance of the CMV specific CD8 T cell response and have inherent anti-viral activity.

We studied CMV-specific CD4+ T cell reconstitution following allogeneic stem cell transplantation in a cohort of 32 patients at risk of CMV reactivation. Patients had been treated with a myeloablative (53.2%) or non-myeloablative (46.8%) conditioning regimen for haematological malignancies. CMV viremia was monitored by quantitative PCR. CMV-specific CD4 T cells were identified by intracellular cytokine staining before transplantation and every two weeks thereafter. CMV serology of recipient/donor was (+/+) in 53.1%, (+/-) in 34.4% and (-/+) in 12.5%. All (+/+) patients but only 70% of (+/-) and 25% of (-/+) patients developed CMV-specific CD4+ T cells in the first 6 months post transplantation. Patients with myeloablative conditioning had a lower frequency of CMV reactivation. CD4+ T cell count was significantly lower in patients who had CMV reactivation in both the first 100 (p=0.03) and the second 100 days (p=0.04). Patients who experienced acute GVHD (50%) with or without chronic GVHD had impaired reconstitution in both the first 100 days (median of 8300/ml vs. 25000/ml, p=0.04) and the second 100 days (median of 9100/ml compared to 29000/ml, p<0.01). Patients who had received a graft from a sibling (72%) had a higher CMV-specific CD4 T cell count compared to patients with an unrelated donor (median of 23000/ml vs 420/ml, p<0.001). There was a positive correlation between the absolute number of total CD4+ T cells and the CMV-specific CD4+ T cell subpopulation and CD4+ T cell count in first 100 days was significantly associated with lower CMV-specific CD4 count (p=0.003). In conclusion, nonmyeloablative conditioning and poor recovery of CD4+ T cells were associated with higher rate of CMV reactivation. Use of an unrelated donor, poor CD4 reconstitution, GVHD and donor CMV seronegativity all had a negative impact on CMV-specific CD4+ T cell reconstitution following allogeneic stem cell transplantation.

455 COMMUNITY RESPIRATORY VIRUS INFECTIONS (CRV) IN ADULT RECIPIENTS OF A HAEMATOPOIETIC STEM CELL TRANSPLANTATION (SCT) FOR A HEMATOLOGICAL MALIGNANCY: RESULTS FROM SINGLE-CENTER STUDY

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BACKGROUND AND AIMS: Infections by CRV can cause severe morbidity in recipients of a SCT. However, there is little data on the incidence and risk factors for the various CRVs in adults who have undergone a SCT or a hematological malignancy. **METHODS:** We performed a prospective sample collection of all adult SCT patients seen in the outpatient and inpatient setting during a 4-1/2-year period (September 1999 to February 2004) and analyzed nasopharyngeal aspirate (NPA) or bronchoalveolar (BAL) samples from all patients with clear symptoms of an upper respiratory (URT) or lower respiratory tract (LRT) infections. Immunofluorescence tests and viral culture was done in all samples searching for influenza A and B (IV-A/IV-B), respiratory syncytial virus (RSV), parainfluenza 1 to 4, adenovirus (ADV), rhinovirus and enteroviruses. All patients transplanted between September 1999 and October 2003 (at least 4 months follow-up in all) were analyzed for incidence and risk factors. Allogeneic and autologous SCT recipients were analyzed separately (181 and 241 cases, respectively). **RESULTS:** Seventy-two episodes of CRV were identified during the study period. The most common CRV were IV-A/B (n=41) and RSV (n=18). The 12- and 24-month estimated incidences of any CRV infection in allo vs. autoSCT were at least 24.4 % vs. 10.5 % (12-mo.) and 33.6% vs. 20.8%, respectively (P<0.0001). The 24-mo. incidence of IV-A/B and RSV were 18.8 % vs. 7.2 % (P=0.01) and 8.5% vs. 2.1%, respectively (P=0.04). In allograft recipients, the only risk factor for being diagnosed a CRV was close household contact with children < 12 y.o. (P=0.001), and in autoSCT also close household contact with children < 12 y.o and male sex (P=0.001 and P=0.04). There was a trend for a higher risk of progression to LRTI from prior URTI (n=71 with initial URTI) in allografts than autografts (33% vs. 16%, P=0.1). In allograft recipients with a

CRV (n=47), risk factors for development of LRTI at any time (n=25, 54%) were: grade 2-4 acute GVHD (P=0.02), lymphocytopenia (<500) (P=0.03) and infection by RSV (P=0.003). In autografts, the risk factors were (n=6, 24%): lymphocytopenia (<500) (P=0.03) and advanced disease phase (P=0.06). In all 72 cases, the mortality attributable to LRTI by the CRV was 7/47 (15%) in allografts and 0% in autografts (P=0.04). **CONCLUSION:** Although our data probably represent an underestimation of the true incidence of CRV infections, our results show that the incidence is high in the area of Barcelona (Spain). The main risk factor for the diagnosis of a CRV infection is being in close contact with children, while the risk of having a severe LRTI was higher among allografted patients with a very debilitated immunity.

456 T-LYMPHOCYTE RECOVERY FOLLOWING NON-MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION IS FASTER THAN AFTER CONVENTIONAL TRANSPLANTATION

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Background: Non-myeloablative conditioning regimens allow the engraftment of allogeneic stem cells from related and unrelated donors with lower early transplant-related mortality and morbidity. However, the immune recovery following this procedure has not been studied in detail. **Aims:** To compare the recovery kinetics of lymphocytes and their subsets in two groups of patients submitted to allogeneic stem cell transplantation (allo-SCT): those receiving non-myeloablative (NMA) and those receiving myeloablative allo-SCT (MA).

Methods: Fifty-one consecutive allo-SCT performed in two centres were evaluated: 24 non-myeloablative allo-SCT (median age 51 years, range 23-71, 14 M/10 F) and 27 myeloablative allo-SCT (median age 34 years, range 17-51, 12 M/15 F). Patients were conditioned with fludarabine + melphalan or busulphan (NMA), and with cyclophosphamide + total body irradiation (MA). All patients received G-CSF mobilized peripheral blood stem cells, except for seven patients in MA group that received bone marrow stem cells. Graft-versus-host disease prophylaxis consisted of cyclosporine A and methotrexate for both groups. Patients were followed with serial assessments of lymphocyte subset counts using multiparametric flow cytometry. Median follow-up was 6 months (range 1-12 months) for both groups.

Results: During the first 6 months post-transplant, CD3+ and CD8+ cell counts were significantly higher in the NMA group as compared to the MA group (p < 0.05 at 1, 3, and 6 months), whereas there was no difference at 9 and 12 months. The number of total and nave (CD45RA+CD62L+) CD4+ cells was also higher in non-myeloablative allo-SCT throughout the study (p < 0.05 at 1, 3, 6, 9, and 12 months). Moreover, total CD4+ cell counts were higher than 200 cells/mL in all patients in NMA group at 9 and 12 months. There were no statistical differences in B lymphocyte (CD19+) and NK cell (CD3-CD16+CD56+) recovery at any time point after transplant between groups.

Conclusions: These results suggest that T-cell reconstitution following non-myeloablative allo-SCT is faster than after conventional allo-SCT, which might contribute to the reduced morbidity in patients receiving non-myeloablative transplants.

457 T-CELL DEPLETION WITH LOW-DOSE ALEMTUZUMAB IN REDUCED INTENSITY ALLOGENEIC TRANSPLANTS IS ASSOCIATED WITH LOW INCIDENCE OF GRAFT-VERSUS-HOST DISEASE (GVHD) AND TRANSPLANT-RELATED-MORTALITY (TRM).

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Background: Although reduced-intensity conditioning (RIC) regimens induce engraftment of allogeneic stem cells with a relatively low toxicity, GVHD remains a significant cause of morbidity and mortality. In vivo T cell depletion with alemtuzumab (100 mg total dose) has been shown to reduce the incidence of GVHD. However, the in vivo persistence of alemtuzumab at lympholytic concentrations impairs the immunoreconstitution and limits the efficacy of the graft-versus-tumor (GVT) effect. **Aims:** The purpose of our trial was to test the effect of lower dose of alemtuzumab on GVHD and GVT effect. **Methods:** We have enrolled 21 patients receiving allogeneic stem cell transplantation (SCT) from HLA identical (n=18) or one-antigen mismatched (n=3) family donor. Patients were conditioned with a RIC regimen including thiopeta

(10 mg/kg), fludarabine (60 mg/sqm) and cyclophosphamide (60 mg/kg). GVHD prophylaxis consisted of short-course MTX and CSA (2 mg/kg). Alemtuzumab was given at 7.5 (n=11) or 15 mg/ms (n=10). Patients with progressive disease (PD), without GVHD, were considered eligible for donor lymphocyte infusions (DLI). Median age was 57 years (range: 41-65). Diagnosis were: MM (n=9), NHL (n=8), HD (n=1), MDS/AML (n=3). All patients received a median of two previous lines of therapies and 52% received a previous autograft. Before SCT, 20% of the patients had chemorefractory disease. Results: All patients engrafted and the majority (88%) were full donor (peripheral blood T cells and granulocytes) at day +30 after SCT. Chimerism analysis on myeloid and lymphoid dendritic cells (DC) has been performed on 10 samples: by day +30 more than 95% of DC were of donor origin. Sixteen patients have a minimum follow-up of 4 months (median follow-up 400 days) and are evaluable for toxicity, incidence of GVHD and disease response. The estimated 1-year TRM was 7%. Nine patients (42%) received DLI for PD (n=8) or for persistence of CMV infection (n=1) with a median of two infusions per patient. The incidence of grade II-IV acute GVHD pre- and post-DLI were 6% and 37%, respectively (no grade IV GVHD occurred). The incidence of chronic GVHD pre- and post-DLI were 0% and 31%, respectively. We observed a high incidence of CMV reactivation (95%). The median value of CD4+ and CD8+ at day +90 were 126/uL and 180/uL, respectively. Currently, 12 of 16 patients (75%) are alive, 11 of whom in CR (n=8) or PR (n=3). Four of 16 patients (25%) died from their underlying disease (n=3) or from TRM (n=1). The estimated 1-year progression-free survival and overall survival were 47% (95% CI, 20%-74%) and 69% (95% CI, 44%-94%), respectively. Summary: Our results suggest that: (1) low doses of alemtuzumab reduces the incidence of acute GVHD; (2) the encouraging results in terms of OS suggest that relapsing patients can be rescued with DLI.

458 HIGHLY EFFECTIVE TOPICAL TACROLIMUS TREATMENT IN SEVERE ORAL CHRONIC GRAFT-VERSUS-HOST DISEASE FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Oral involvement of chronic graft-versus-host disease (GVHD) is a most distressing and disabling complication of hematopoietic stem cell transplantations, for which systemic immunosuppression as well as topical corticosteroid treatment may offer only limited symptomatic relief. Clinical manifestations of chronic GVHD often resemble those seen in autoimmune diseases (e.g. scleroderma and Sjögren's syndrome), including skin thickening, xerostomia, lack of tear production, and mucosal involvement with erythema, lichenoid changes, atrophy, or ulcerations.

Patients, Methods and Results: Here we report encouraging preliminary results with the application of tacrolimus (FK-506) as a 0.1% ointment in three patients with severe oral chronic GVHD after hematopoietic stem cell transplantation from HLA-matched related or unrelated donors. All three patients, one female and two males, 23, 41 and 63 years of age, had received extensive treatment for oral GVHD with limited or no improvement. Before topical tacrolimus, all three patients suffered severe impairment in their quality of life, with pain, reduced oral intake, and consecutive weight loss. Topical tacrolimus 0.1% ointment (Protopic) was manually applied to mucosal lesions twice daily by the aid of a gauze. Patients were instructed not to eat or rinse their mouths for at least one hour after application. After self-administration of topical tacrolimus, patients experienced rapid, consistent, complete or at least marked, subjective and objective improvement. Pain was significantly reduced or resolved altogether. Symptomatic relief ensued as early as two weeks after beginning topical tacrolimus. Treatment was well tolerated with no systemic side effects.

Conclusion: To the best of our knowledge, this is the first report of topical treatment of oral chronic GVHD with tacrolimus. These encouraging results suggest that topical tacrolimus may become an important new adjunctive treatment for severe oral GVHD.

459 TREATMENT OF REFRACTORY CHRONIC GRAFT-VERSUS-HOST DISEASE WITH RAPAMYCIN

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Background: Chronic graft-versus-host disease (cGVHD) is a problem of increasing importance in allogeneic transplantation mainly due to the

more frequent use of peripheral stem cell grafts, unrelated donors, and transplantations of older patients. Treatment options for patients failing first line immunosuppression are limited and new agents for effective second or third line therapy are urgently needed.

Patients, Methods and Results: We report on the use of rapamycin (sirolimus; SRL) for refractory cGVHD in 9 patients. Median age was 42 years (range: 27 - 58); seven patients were male and four of them had received a transplant from a female donor. HLA-matched peripheral blood stem cell (n = 7) or bone marrow (n = 2) transplants from sibling (n = 2) or unrelated (n = 7) donors had been performed for acute leukemia (n = 7) or CML (n = 2) after myeloablative (n = 4) or reduced intensity conditioning (n = 5). GVHD prophylaxis consisted of cyclosporin A (CSA) plus methotrexate (n = 5), prednisolone (n = 2), or mycophenolate mofetil (MMF; n = 2). Initial diagnosis of cGVHD was made at day 136 (range: 101 - 334), and 8 out of 9 patients had had preceding acute GVHD. Organ manifestations of cGVHD were skin (n = 8), mucous membranes (n = 8), liver (n = 7), lung (n = 3), gut (n = 3), fasciitis (n = 3), and eyes (n = 4). SRL was started at a median of 23 (range: 5 - 40) months after transplantation and 17 (2 - 37) months after first diagnosis of cGVHD. All patients were refractory to first line treatment with prednisolone and CSA. Additional therapy prior to SRL included ursodesoxycholic acid (n = 7), MMF (n = 5), extracorporeal photopheresis (n = 2) and PUVA (n = 1). SRL was given in combination with CSA in 3 patients and as a single agent in 6 patients. Three out of 9 patients showed an objective response to SRL and one patient reported subjective improvement of oral cGVHD. Importantly, in 2 out of 3 patients with cGVHD of the lung, pulmonary function was dramatically improved by SRL in combination with CSA. No response was seen in the 3 patients with severe fasciitis receiving SRL as a single agent. SRL had to be discontinued due to profound bone marrow toxicity (hypoplasia, thrombocytopenia) in 3 patients and due to massive edemas in one patient. An increased incidence of infectious complications was not observed. We conclude that rapamycin may be effective in some patients with severe, refractory cGVHD, especially when involving the lung. However, due to the potentially serious toxicity, its role in refractory cGVHD must be evaluated in prospective controlled trials.

460 REDUCED-INTENSITY CONDITIONING FOLLOWED BY ALLOGENEIC TRANSPLANTATION: POOR OUTCOME IN SECONDARY VERSUS DE-NOVO ACUTE MYELOID LEUKEMIAS

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Patients with secondary AML (sAML) usually have a worse prognosis than de-novo AML. After myeloablative conditioning, the transplant-related-mortality (TRM) in sAML is higher than in patients with de-novo AML. We report the results of a phase II study employing reduced-intensity conditioning (RIC) followed by allogeneic peripheral blood stem cell (PBSC) or bone marrow (BM) transplantation in 31 patients with de-novo (n=14), or secondary to MDS (n=6), or therapy-related AML (n=11). Median age was 53 years (range: 22 - 67). Disease status at transplant was categorized as low risk (n=15 CR1 or CR2), high risk (n=5 PR, n=8 PD) and up-front (n=3). The median time from diagnosis to allograft was 6 months (range: 1-80 months). After conditioning with thiopeta (10 mg/kg), fludarabine (60 mg/ms) and cyclophosphamide (60 mg/kg), pts received BM (n= 10) or lenogastim-mobilized PBSC (n= 21) from HLA-matched/ 1ag mismatched siblings (n=21), or HLA-matched unrelated donors (n=3). GVHD prophylaxis consisted of cyclosporine A and short course methotrexate. All patients engrafted. Acute GVHD grade II-IV occurred in 48% of the pts (n=2 post-DLIs, n=1 for early CSA tapering), chronic GVHD developed in 36% of pts (n=2 post-DLIs). Overall non-relapse mortality was 20% (n=6); non-relapse mortality was higher in sAML(35%) than in de-novo AML (0%) (P<0.02). The 2-year event-free survival (EFS) was 45% (95%CI, 12% to 78%) in patients with de novo-AML versus 11% (95%CI, 1% to 26%) in patients with sAML (p<0.01). Patients with de novo AML in CR at the time of transplantation had a favorable outcome with an EFS of 71% (95%CI, 36% to 99%). At a median follow-up of 24 months (range: 8 to 50 months), 11 pts are alive: 9 in CR, and 2 with PD. In conclusion, RIC is an effective strategy for

de-novo but not sAML. Results are particularly disappointing in patients with therapy-related leukemias.

461 TUMORAL EPSTEIN-BARR VIRUS (EBV) VIRAL LOAD IN POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS AND HIV-ASSOCIATED LYMPHOMA

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BACKGROUND: Most of post-transplantation lymphoproliferative disease (PTLD) and lymphoma of HIV-infected patients are associated with Epstein-Barr Virus (EBV); blood EBV viral load (genome copy number) has a high predictive value in PTLD. **AIMS:** to explore prognostic value and pathologic specificity of tumoral EBV viral load in this type of neoplasms, and any correlation with immunodepression intensity. **METHODS:** 25 frozen (-80°C) tumoral samples were considered. Quantitative tumoral EBV viral load (TEVL) was determined in 11 PTLD (6 kidney transplantations, 2 liver transplantations, 2 bone-marrow transplantations and 1 lung transplantation) and 14 HIV-associated lymphomas. We used real-time quantitative LightCycler® polymerase chain reaction technology (Roche Molecular Biochemicals). **RESULTS:** Patients with a TEVL less than 5.5 Log per DNA microgram had a better survival than patients with an higher TEVL ($p=0.0344$). TEVL was not linked to immunodepression intensity. Indeed, TEVL was not linked to intensity and duration of TCD4+ lymphopenia of HIV-infected patients, and kidney recipient TEVL was not different from other recipient TEVL, although immunosuppression schedules in kidney transplantation are less intensive than in other organ transplantation. The TEVL of the 2 populations (HIV-infected patients and graft recipients) were not different. There was no association between TEVL and pathologic diagnose. **CONCLUSION:** lack of association between TEVL and immunodepression in HIV-positive patients may explain the persistent high risk of developing lymphoma after CD4+ T lymphopenia correction under highly active antiretroviral therapy. Prognostic value of TEVL in PTLD and HIV-associated lymphoma needs further confirmation.

462 NON-MYELOABLATIVE SIBLING ALLOGRAFTS IN ADULTS WITH INTERMEDIATE PROGNOSIS ACUTE MYELOID LEUKAEMIA (AML) IN FIRST COMPLETE REMISSION (CR1)

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BACKGROUND

The role of myeloablative allografts for adults with AML in CR1 is controversial with the potential benefit of aggressive conditioning and graft vs leukaemia (GVL) effects balanced by treatment related mortality (TRM) due to organ dysfunction and graft vs host disease (GVHD). Non-myeloablative conditioning may compromise leukaemic cell kill by direct cytotoxicity but improve outcome due to less TRM, including less GVHD as a consequence of less mucositis/cytokine storm and less rapid establishment of complete donor chimerism.

AIMS

To evaluate the outcome of sibling donor peripheral blood stem cell allografts after non-myeloablative conditioning in adult patients with AML in CR1 (excluding those with favourable cytogenetics) in a study under the auspices of the ALLG.

METHODS

Patients received cyclophosphamide 60-mg/kg x 2 and fludarabine 25mg/m² x 5 and cyclosporin-short course methotrexate as GVHD prophylaxis. Serial T cell and myeloid chimerism studies were performed post-engraftment.

RESULTS

Twenty-one patients have been enrolled; data is available on 19. No patient had adverse cytogenetics. Median age is 45 years (19-60). Six were transplanted after two cycles of induction-consolidation, 12 after 3 cycles and one after 4. Median time from diagnosis to transplant is 148 days (78-370) and to neutrophil ($>0.5 \times 10^9/L$) and platelet recovery ($>20 \times 10^9/L$) is 15 and 14 days respectively. No patient developed > grade 2 non-haematological toxicity or required opioid analgesia or parenteral nutrition for mucositis. Fifteen patients remain in CR1 a median of 333 days (36-994) post-transplant. Four patients relapsed by d140, one of whom is in a durable CR2 after withdrawal of immunosuppression. Most platelet had >90% T cell donor chimerism at d30; mixed myeloid

chimerism was observed early but evolved to complete donor chimerism by 6 months. Grade 3-4 acute GVHD was seen in only one patient. Most patients have had limited (5/12 evaluable) or no chronic GVHD. Fertility has been preserved in 4 evaluable patients.

CONCLUSION

These preliminary results suggest that non-myeloablative allografts in AML CR1 are well tolerated with a low incidence of acute GVHD and apparent preservation of GVL effects.

463 COMPARISON BETWEEN CONVENTIONAL AND REDUCED INTENSITY CONDITIONING REGIMENS: GVHD AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Background: Chemotherapy-induced toxicity significantly decreases after reduced intensity conditioning regimens (RIC). This decreased toxicity would also reduce the inflammatory cytokine production associated with the high doses of chemotherapy, thus diminishing 'cytokine storm' which contributes to the development of graft-versus-host-disease (GVHD). **Patients And Methods:** In the current study we have compared the incidence and characteristics of GVHD between patients receiving RIC allo-transplant (n=60) vs myeloablative conditioning regimen (MCR) (n=49) in the same period of time. All patients received the same GVHD prophylaxis, based on CsA plus Methotrexate and folinic rescue. RIC consisted of Fludarabine plus Busulphan for myeloid or Melphalan for lymphoid malignancies.

Results: Median age was 51 and 37 years for RIC and MCR recipients, respectively ($p<0.001$). Concerning disease status at transplant 18 % of patients receiving RIC were considered at low risk as compared to 45 % among patients receiving MCR ($p=0.01$). Also, CD34 cell dose infused significantly differed between both subgroups (median of 4.79 vs 5.98 x 10⁶/Kg among RIC vs MCR patients respectively, $p=0.007$).

No differences between both groups were found in terms of follow up (median of 842 and 1035 days for RIC and MCR patients, respectively, $p=0.1$), transplant related mortality (14 % for RIC patients and 21 % for MCR, $p=0.23$), overall (OS) and event free survival (EFS): 49 % vs 42 % OS in RIC and MCR patients ($p=0.42$), and 37 % vs 35 % EFS in RIC and MCR patients ($p=0.91$).

In terms of GVHD, cumulative incidence of acute graft-versus-host-disease (aGVHD) was lower in RIC as compared to MCR patients (45 % vs 63 % respectively, $p=0.01$), but no differences in TRM directly attributed to aGVHD were observed ($p=0.54$). Cumulative incidence of chronic graft-versus-host-disease (cGVHD) was similar in both groups (76 % vs 72 % in RIC vs MCR patients, respectively, $p=0.84$). Response to GVHD (both acute and chronic) first line treatment was similar in RIC and MCR patients.

Conclusion: This study shows that RIC significantly decrease incidence of aGVHD as compared to MCR using the same GVHD prophylaxis even through RIC patients are significantly older.

464 TOPIC THERAPY ON GUT ACUTE GRAFT VERSUS HOST DISEASE (GVHD)

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Background: Treatment of gut GVHD relies on systemic immunosuppressive therapy such as glucocorticoids which increase the risk of infections after transplant, among other complications. Beclometasone dipropionate (BDP) is a topically active corticosteroid with a low absorption thus limiting systemic effects. Previous studies suggest its efficacy in the treatment of gut GVHD in association with systemic steroids and even as a single agent in a low number of patients.

Methods: DBP was administered po at a dose of 2 mg q6h for 28 days in 18 patients undergoing allogeneic peripheral blood stem cell transplantation which had a biopsy proven gut GVHD in all cases. Eleven received reduced intensity conditioning regimen based on fludarabine plus melphalan or busulphan, 4 received CyTBI and 3 BuCy. Median age was 42 years (14-67). Median follow up was 420 days (159-1154). GVHD flared at a median of 38 days after transplant (18-90). Ten patients had gut aGVHD, 7 had skin and gut and 1 liver and gut aGVHD. Gut aGVHD symptoms consisted of nausea (n=10), diarrhea (n=1), both (n=5) and anorexia plus skin involvement (n=2).

Results: DBP was started at a median of 46 days after transplant. Patients received a median of 28 days of DBP. Thirteen patients reached complete remission (CR), 2 partial response (PR) and 3 had no response. The only patient who had liver GVHD did not respond. By contrast 86% of patients who had skin and gut aGVHD reached CR. No differences in terms of response were observed between patients who had upper (nausea, vomiting) or lower (diarrhea) aGVHD symptoms. Moreover, among six patients who had diarrhea five reached RC (n=5) or PR (n=1). Among 17 patients who responded to DBP 4 relapsed later on. Thus, 7 patients out of 18 required systemic glucocorticoids. Conclusions: BDP was well tolerated. In the present study BDP has shown an important efficacy in the treatment of gut GVHD with a high percentage of patients reaching complete or partial response. In these patients systemic glucocorticoids are avoided thus decreasing toxicity related to immunosuppressive therapy.

MYELODYSPLASTIC SYNDROMES

465 THE PRION-LIKE PROTEIN DOPPEL IS EXPRESSED IN BLAST CELLS OF ACUTE LEUKEMIAS AND MYELODYSPLASTIC SYNDROMES

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The PRND gene, located on human chromosome 20pter-p12, 27 Kb downstream from PRNP, the prion protein gene, encodes for the Doppel protein (Dpl), that has many biochemical and structural properties in common with the prion protein. Dpl is about 179 amino acid residues long, it is characterized by an alpha-helical conformation, and it is presented on the cell surface by a glycosylphosphatidylinositol anchor. Whereas prion protein is ubiquitous, Dpl is expressed only during embryogenesis and in adult testis and does not seem to be required for prion disease progression. Its physiological role is unknown. Since preliminary studies have demonstrated a significant level of Dpl expression in several types of cancers of different histologic origin, we investigated the Dpl distribution in bone marrow cells from patients with acute leukemia (AL) and myelodysplastic syndrome (MDS), in order to evaluate its possible overexpression in these disorders. Molecular and immunocytochemical studies were carried out on bone marrow samples from 11 normal controls, from 28 patients with AL, 16 myeloid (AML) and 12 lymphoid (ALL), at the onset, and from 37 patients with MDS (13 RA, 9 RARS, 5 RAEB, 5 RAEB-t and 5 CMML) not previously treated. A goat polyclonal antibody raised against a peptide mapping near the aminoterminalus of Dpl of human origin was used for immunocytochemistry and Western blotting. In AL and MDS the number of positive cells and intensity of staining were heterogeneous, whereas normal samples were negative or showed very weak expression in rare immature myeloid cells. Dpl protein was detected in 13/16 cases of AML (81%), in 9/12 cases of ALL (75%) and in 35/37 cases of MDS (94.5%), with median percentages of positive blasts respectively of 20 (range 0-38), 4.5 (range 0-29) and 10 (range 0-40). Dpl was localized on the blast surface or, seldom, in a cytoplasmic perinuclear area. Semi-quantitative RT-PCR demonstrated rather high mRNA levels in almost all AL and MDS cases, but barely detectable levels in normal bone marrow (p=0.004). These differences were confirmed by the results of in situ hybridization studies; they were likely not related to gene amplification. In AL and MDS there was no correlation between Dpl expression levels and clinical or laboratory findings such as age, leukocyte count or karyotype nor did Dpl levels predict disease progression in MDS. In conclusion, for the first time the expression of PRND has been demonstrated in human bone marrow cells. The molecular mechanism responsible for its overexpression in transformed cells is unknown; however, the differential expression of the Dpl protein in AL and MDS versus healthy subjects makes it a possible leukemia associated antigen with a potential attractive role as target for immunotherapy; on the other hand, Dpl could be used to quantitate minimal residual disease after treatment.

466 HEMATOPOIETIC MATURATION ABNORMALITIES DETECTED BY FLOW CYTOMETRY ARE USEFUL FOR DIFFERENTIAL DIAGNOSIS BETWEEN MYELODYSPLASIA AND NON-CLONAL DISORDERS

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Background: In myelodysplastic syndromes (MDSs), hemopoiesis present abnormalities in proliferation, maturation and apoptosis. These can be detected by the evolution of co-expression of several markers during maturation by flow cytometry (FCM).

Aims: to study the utility of the analysis of the maturation pattern of granulocytes and erythroblasts in order to differentiate MDS from non-clonal disorders

Material and methods: Patients with MDS were compared with normal BM (BM transplantation donors) and patients with pancytopenia due to non-clonal diseases. BM cell populations were separated in the CD45 / side scatter (SSC) plot. CD13/CD16, CD11b/CD16 and CD64/CD10 plots were used to follow the granulocyte maturation and the erythroblast maturation was analyzed in the CD71/glycophorin plot. The results were compared with those from BM routine cytology.

Results: We analyzed 28 cases of MDS (16 RA, 4 RARS, 8 RAEB), 10 controls and 11 patients with pancytopenia and non-clonal disorders. In MDS, hypogranulation of granulocytes was observed in cytology and FCM in 12 cases, only in cytology in 1 case and only in FCM in 10 cases. Expression of CD13 was abnormal in 12 cases, CD11b in 9 cases, and CD16 in 13 cases. CD64 percentage was increased in MDS patients. Deficiency of CD10 was observed in 4 cases. Patients with non-clonal diseases presented a normal pattern of granulocyte maturation. In erythroblasts, a deficient expression of CD71 was seen in 12 cases of MDS (7 RA, 2 RARS, 3 RAEB). All non-clonal disorders had a normal expression. An abnormal granulocyte maturation pattern was more frequent in RAEB than in RA/RARS but alteration of the erythroid maturation was not associated to any particular FAB type.

Conclusion: FCM is useful for detection of maturation abnormalities in the granulocytic and erythroid series which are characteristic for MDS and frequent in these diseases. This is an useful tool for differential diagnosis between clonal and non-clonal disorders.

Supported by FAPESP and CNPq.

467 TRISENOX (ARSENIC TRIOXIDE) IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS): PRELIMINARY RESULTS OF A PHASE I/II STUDY

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of chronic hematopoietic disorders with no approved curative treatment. Arsenic trioxide exhibits multifaceted mechanisms of action including induction of tumor cell differentiation, apoptosis, and angiogenesis inhibition. Recent reports have documented clinical activity of arsenic trioxide in MDS patients, leading to improvements in hematologic parameters and to transfusion independence. We report the preliminary results of an ongoing European multicenter phase I/II study of Trisenox® (arsenic trioxide) in MDS patients.

Aims: The purpose of this study is to evaluate the efficacy and toxicity of Trisenox® in patients with MDS.

Methods: Trisenox® is given as a 1-hr IV infusion, loading dose 0.30 mg/kg/day for 5 days, and maintenance doses 0.25 mg/kg/day twice a week for 15 weeks as an out-patient treatment. Disease assessments are every 8 weeks (modified IWG response criteria).

Results: Twenty-five hematologic responses were observed in 18 of 76 (24%) evaluable patients. Eighty-six patients (66 M/ 20 F) have been enrolled: median age is 67.5 years (range 31-89) and median time from diagnosis to first dose is 10.2 months (range 0.2-117.6). FAB categories are RA (8 patients), RARS (9), RAEB (56), RAEB-t (11), and CMML (2). Fifty-five patients have higher-risk (HR) and 31 have lower-risk (LR) MDS. Evaluable patients (n=76) received study drug and had at least one assessment (includes patients removed from study for AEs or PD). Sixty-four of the evaluable patients were transfusion-dependent.

Responses were as follows: 9 major erythroid (including 2 who also had major neutrophil response, 2 who also had major platelet, and 2 who also had minor platelet); 4 major neutrophil (including 1 who also had minor platelet); 1 major platelet; 2 minor erythroid; and 2 minor platelet.

The hematologic response rate among LR patients was 7/28 (25%) and among HR patients, 11/48 (23%) (all responses were maintained ≥ 56 days). Among the 18 responding patients, 9 became transfusion-independent while on study.

Responses were seen after 57 to 176 days of treatment. Fifteen of the 18 responders had ongoing responses at the last assessment; thus, true median response duration is unknown at this time, but is >102 days.

Trisenox® toxicity is manageable and mostly mild. Forty-three patients experienced grade 1 or 2 drug-related toxicities; 9 had grade 3 (febrile neutropenia, neutropenic sepsis, disorientation, hallucinations, hypokalemia, pruritus, edema, increased LFTs, or pain); and 1 had grade 4 neutropenia and thrombocytopenia. QTc prolongation was reported in only 1 patient, who experienced 2 isolated episodes that were considered clinically insignificant.

Conclusions: These preliminary results confirm the safety and tolerability of this out-patient Trisenox® regimen in MDS patients. Responses were seen across all hematologic lineages in HR patients, and in 2 lineages in LR patients. Hematologic responses were seen in 18 of 76 (24%) patients, with 15/18 patients (5/7 LR and 10/11 HR) still responding at their most recent assessments. Median response duration is >102 days. Combinations of Trisenox® with other agents are currently under investigation in MDS patients.

468 REPRODUCIBILITY OF FAB AND WHO CLASSIFICATIONS FOR THE DIAGNOSIS OF MYELODYSPLASTIC SYNDROMES

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Myelodysplastic syndromes (MDS) are generally diagnosed according to morphologic criteria using either the French-American-British (FAB) classification or, more recently, the World Health Organization (WHO) classification. However, the interpretation of the defined morphologic criteria of both classifications has a component of subjectivity. To evaluate the reproducibility of both FAB and WHO classifications, bone marrow and peripheral blood samples from 100 patients with MDS were reviewed by five experienced hematologists from different centers. Samples were stained with standard May-Grünwald-Giemsa and Perl's techniques. Reproducibility of diagnosis according to both FAB and WHO classifications was evaluated as follows: 1) good, the agreement of 4-5 observers in diagnosis; 2) intermediate, the agreement of 3 observers; and 3) bad, the agreement was achieved by 2 or less observers. In addition, reproducibility for assessing the presence or absence of dysplastic features, as were defined by the WHO classification, was also evaluated. Overall a good reproducibility was observed in 53% of samples with the FAB classification, ranging from 63% for RA to 41% for CMML. With regard of the WHO classification, a good reproducibility was only observed in 22% of samples, ranging from 41% for CMML to 0% for RSA. A good reproducibility was observed for the vast majority of specific dysplastic features, except for hyposegmentation (54%) and hypogranulation (55%) of the myeloid lineage, as well as for megaloblastosis (62%), and nuclear dysplastic features (60%-67%) of the erythroid lineage. Our study shows important discrepancies between five different qualified observers to classify MDS according to both FAB and WHO classifications. This lack of reproducibility was more obvious using the WHO classification.

469 BONE MARROW ANGIOGENESIS AND SERUM LEVELS OF ANGIOGENIN IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background:

There is evidence that angiogenesis plays a role in the progression of myelodysplastic syndromes (MDS). Neovascularisation is mediated

by various angiogenic molecules released by tumor cells themselves and accessory host cells. Angiogenin (ANG) is a basic single-chain protein involved in early angiogenesis by activating capillary endothelial cells. ANG is a member of the ribonuclease superfamily and is also a type 2 acute phase protein. Interleukin 6 (IL-6) has been recognized as the principal regulator of many acute phase proteins. Elevated serum IL-6 values have been found in patients with high-risk myelodysplastic disease and in transition to overt leukemia.

Aim:

The aim of the present study was to measure the serum levels of ANG in patients with MDS and to evaluate a possible relationship with IL-6 and bone marrow microvessel density.

Methods:

67 patients with newly diagnosed MDS, 46 males and 21 females (median 71.5 and 74 years respectively) were included in the study. According to the FAB classification 10 patients were classified as CMML, 23 as RA, 14 as RARS, 14 as RAEB and 12 as RAEB-t. Patients with RA and RARS were considered as having low-risk MDS for the development of acute non-lymphocytic leukemia (ANLL) while patients with CMML, RAEB and RAEB-t as having high-risk disease.

Serum levels of interleukin-6 (IL-6) and ANG were measured by ELISA using commercially available kits (R & D Systems Inc, Minneapolis MN USA) in all patients and 15 controls.

Immunostaining of bone marrow microvessels was performed with the monoclonal antibody to CD34 (Immunotech, clone QBEND 10). Microvessel density (MVD) was then assessed in the three most vascular areas (hot spots).

Individual microvessels were counted on a X400 magnification. The countings were performed on a field-part of the whole field of view, corresponding to the projection of a calibrated eyepiece graticule of a 0.0625 mm² surface area. After microvessel counting, the mean MVD (per field of 0.0625 mm²) of the three hot spots was calculated.

Results:

In the MDS group ANG concentrations were higher than controls (meansd 407.53232.82 versus 247.60151.28 pg/ml ($p<0.01$). ANG was significantly higher in the RAEB, RAEB-t and CMML group in comparison to the RA and RARS groups ($p<0.01$). IL-6 was higher in MDS patients in comparison to controls (10.696.29 versus 1.210.65 pg/ml, $p<0.0001$). MVD was significantly higher in MDS patients in comparison to controls ($p<0.0001$). Among the FAB-related subsets MVD was significantly higher in the RAEB, RAEB-t compared to the RA and RARS subsets ($p<0.01$). Serum ANG levels correlated significantly with serum IL-6 ($r=0.387$, $p<0.0001$). We also found a positive correlation between MVD and serum IL-6 levels ($r=0.278$, $p<0.05$).

Conclusions:

Serum angiogenin and IL-6 are increased in high risk MDS patients in comparison to low risk. Microvessel density is significantly higher in high risk MDS patients but does not correlate with serum angiogenin levels. However, progression of MDS to forms with increased blasts is accompanied by an increase in bone marrow angiogenesis and angiogenic cytokines.

470 PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) IMMUNOSTAINING IN MYELODYSPLASTIC SYNDROMES AND ITS PROGNOSTIC SIGNIFICANCE

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Introduction

The myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal haematologic malignancies with frequent evolution to secondary leukemia. The precise cause of this leukemic transformation is still unknown, however, one property of the immature blastic cells that distinguishes them from their more mature counterparts is their higher proliferative potential. The proliferating cell nuclear antigen (PCNA) is a nuclear acidic protein, the levels of which correlate directly to the rate of cellular proliferation and DNA synthesis. MDS are also associated with alterations in the serum levels of various cytokines. TNF- α has been attributed a causative role for excessive intramedullary hematopoietic cell apoptosis in MDS.

Aim

The aim of the present study was to evaluate the bone marrow PCNA and TNF- α serum levels in MDS patients and to question the prognostic significance of PCNA in this group of patients.

Methods

A total number of 51 patients (35 men and 16 women with a median age of 73 years) with primary (de novo) MDS were analysed. The patients were diagnosed between 1996 and 2002. Classification was performed according to the French-American-British (FAB) criteria and included 19 patients with RA, 6 with RARS, 12 RAEB, 5 with RAEB-t and 9 patients with CMML. Patients with RA and RARS were grouped as patients with low risk MDS for leukemic transformation whereas patients with RAEB, RAEB-t and CMML as high risk MDS. We also studied 15 normal bone marrow specimens. TNF- α was measured with a commercially available enzyme-linked immunosorbent assay (ELISA) (Quantikine, RD systems Minneapolis MN (USA)) in all patients and 15 controls. Immunohistochemical staining of bone marrow samples for PCNA was performed using a monoclonal antibody to PCNA. Results were expressed as percentile of nucleated myeloid cells.

Results

The mean⁷ levels of PCNA in the MDS group was 44.57 $\pm$ 26.9 and thus higher compared to level (2.47 $\pm$ 1.7) found in the control group ($p < 0.0001$). PCNA expression was higher in RAEB-t, RAEB and CMML in comparison to RA and RARS ($p < 0.001$). In MDS serum TNF- α concentrations (meanSD: 17.19.6 pg/ml) were significantly higher than controls (7.22.3 pg/ml, $p < 0.0001$). Elevated serum TNF- α levels were seen in patients of the high-risk in comparison to the low risk MDS group ($p < 0.001$). PCNA correlated significantly with TNF- α ($r = 0.37$, $p < 0.008$). Using the median PCNA of 43 as a cut-off point it was shown that patients with PCNA > 43 had significantly lower survival times than patients with PCNA < 43 ($p < 0.001$).

Conclusions

In conclusion, our study demonstrated that high bone marrow PCNA levels are associated with a shortened survival in MDS and that high proliferative activity is associated with increased serum TNF- α . Taken together, PCNA might be considered as an additional prognostic factor in this group of patients.

471 EFFICACY AND SAFETY OF DARBEPOETIN IN MYELOYDYSPLASTIC SYNDROMES

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Darbepoetin-alpha (DPO) is a Novel Erythropoiesis Stimulating Protein (NESP) which, compared to human alpha or beta recombinant erythropoietin (r-EPO), has augmented sialylated carbohydrate content permitting a prolonged serum half-life and a possible increased in vivo biologic activity. Recent data from clinical trials have indicated that DPO is effective in alleviating anemia and improving quality of life in neoplastic patients undergoing chemotherapy, using long intervals of administration. Such dosing schedules may clearly optimize patients convenience and compliance. However, no data are currently available about the efficacy of DPO as single agent in myelodysplastic syndromes (MDS).

Fifteen anemic (Hb < 9.5 g/dl) patients (eight transfusion-dependent) with low-to-intermediate risk MDS, (10 males and 5 females, mean age 69.9 years), were entered into this study. According to WHO classification, there were four refractory cytopenias with trilineage myelodysplasia (RCMD), eight refractory anemias (RA), two of which with ring sideroblasts (RARS), two RA with excess of blasts type I (RAEB-1) and one 5q- syndrome. Two patients had a moderate degree of renal failure, two were MDS secondary to chemotherapy for solid tumors. All patients received DPO s.c. at the dose of 150 mcg once-a-week (q.w), for at least 12 weeks.

Ten patients, including two subjects who had previously received r-EPO without benefit, did not show any improvement in Hb levels or transfusional support and interrupted the study. According to IWG criteria, three patients achieved a major erythroid response (increase of hemoglobin levels > 2 g/dl and/or complete interruption of transfusions), while two patients obtained a minor erythroid response (increase in hemoglobin levels between 1 and 2 g/dl and/or reduction of transfusional support $> 50\%$). Thus, intention-to-treat overall response was 33.3 (3 RA, 1 RARS and the patient with 5q- syndrome). Four out of responders currently maintain stable hemoglobin levels > 9.5 g/dl after 3, 8, 9, and 17 months of treatment, respectively, without modifications of initial DPO dose. The fifth responder died after 5 months, due to causes unrelated to DPO treatment. No other relevant adverse events or significant side effects were recorded during the study period. All

responders had baseline serum levels of endogenous EPO < 200 IU/l, (four < 100 IU/l), no or very low red-cell transfusion requirement, no excess of blast in bone marrow, a diagnosis of MDS performed no more than 4 months before treatment with DPO, a single lineage (erythroid) involvement.

The data of the present study suggest that single, weekly doses of DPO are safely given and may be effective in a proportion of MDS patients which seems to be at least comparable with those achieved by using r-EPO. The characteristics of responders were not different from those of MDS patients who usually respond to r-EPO.

472 STUDY OF THE EFFECT OF CA2 ANTI-TNFA MONOCLONAL ANTIBODY THERAPY ON THE BONE MARROW (BM) STEM/PROGENITOR CELL RESERVES AND BM STROMAL CELL FUNCTION IN PATIENTS WITH MYELOYDYSPLASTIC SYNDROMES (MDS)

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Background: Increased local production of TNF α in the BM has been implicated in the apoptotic depletion of haemopoietic progenitor cells and their BM progeny in MDS patients.

Aim: To evaluate the effect of the treatment with cA2 (Infliximab) on the number and functional characteristics of BM stem/progenitor cells and on the haemopoiesis supporting capacity of BM stroma in MDS patients and to investigate the efficacy of this therapy on peripheral blood (PB) cytopenias and disease progression.

Methods: Four patients with MDS (1 RA, 2 RARS, 1 RAEB) received cA2 on days 0, 15, 45, 85 (3mg/kg). At the initiation (day-0) and end (day-120) of the treatment we studied: (a) BM stem/progenitor cell reserve and function using a limiting-dilution assay of purified CD34+ cells for the evaluation of the long-term culture initiating cells (LTC-ICs), clonogenic assays for the enumeration of the myeloid (CFU-GM), erythroid (BFU-E) and megakaryocytic progenitors (CFU-Meg) in the BM mononuclear cell (BMMC) fraction and flow-cytometry for the quantification of the CD34+ cell subpopulations (CD34+/CD38+, CD34+/CD33+, CD34+/CD71+, CD34+/CD61+ cells), the proportion of apoptotic (7-amino-actinomycin-D+) CD34+ and non-CD34+ BM cells and the percentage of Fas+ cells in the CD34+ cell fraction. Apoptosis was also evaluated in the CD34+/CD71+, CD36+/ClycoA+ and CD36-/GlycoA+ BM cells representing the early, intermediate and mature erythroid cells. (b) BM stroma function in terms of the capacity to sustain autologous or normal haemopoiesis using standard long-term BM cultures (LTBMCs) or irradiated LTBMCs recharged with normal CD34+ cells.

Results: The frequency of LTC-ICs did not change significantly following treatment (3.394.37/5x10³CD34+cells) compared to baseline (2.092.09/5x10³CD34+cells). A significant improvement, however, was obtained in the committed progenitor cells following cA2 administration compared to pre-treatment values indicated by the proportion of the CD34+/CD38+ ($P < 0.05$), CD34+/CD33+ ($P < 0.05$), CD34+/CD61+ ($P < 0.05$) BM cells and the frequency of CFU-GM and CFU-Meg in the BMMC cell fraction ($P < 0.05$ and $P < 0.05$, respectively). This improvement was paralleled with a significant reduction in the proportion of Fas expressing CD34+ cells and the proportion of apoptotic cells within the CD34+ and non-CD34+ cell fraction compared to baseline ($P < 0.05$, $P < 0.05$, $P < 0.05$ respectively). The number of CD34+/CD71+, CD36+/ClycoA+ and CD36-/GlycoA+ BM erythroid cells and the proportion of apoptotic cells in the above cell compartments did not change significantly compared to baseline. Nor the BFU-E content of the BMMCs changed significantly following treatment in comparison to pre-treatment, although a trend towards an improvement was obtained (275221/107BMMCs versus 200141/107BMMCs). The capacity of patient BM stromal cells to support autologous and normal hemopoiesis was improved significantly following treatment as indicated by the CFU-GM number in standard LTBMCs ($P < 0.05$) and in recharged LTBMCs (comparison with normal cultures $P < 0.05$ pre-cA2 and $P = 0.30$ post-cA2) over a period of 5 weeks. Patient PB counts remained stable all over the period of the study with no disease progression.

Conclusion: cA2 may have a beneficial effect on haemopoiesis in MDS patients by down-regulating the TNF α -induced apoptotic mechanisms. The limited response of the erythroid development, however, suggests that mechanism(s) other than TNF α are also implicated in MDS impaired haemopoiesis.

473 ANGIOGENESIS AND VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN THE BONE MARROW OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: Angiogenesis is a process of creating the new blood vessels from existing ones, and has been associated with the growth and metastases of malignant diseases. It was also connected with the pathogenesis of premalignant blood disorder, myelodysplastic syndromes (MDS). Vascular endothelial growth factor (VEGF) is the principal stimulator of angiogenesis.

Aims: The main aims of this study were to determine the intensity of angiogenesis by measuring the microvessel density (MVD), and VEGF expression in bone marrow in the patients with MDS, and control group, and to evaluate the association of these factors and clinical, cytological, laboratory and prognostic characteristics of the MDS patients.

Methods: The study was conducted in 55 MDS patients (18 RA, 2 RARS, 22 RAEB, 3 RAEB-T and 10 CMML patients) and 20 patients of control group with normal bone marrow biopsies (9 non-Hodgkin lymphomas, 9 Hodgkin lymphomas and 2 patients with other diseases). Microvessel density (MVD) in the paraffin embedded bone marrow trephine biopsies was determined by measuring the number of blood vessels stained with CD34 antibody and labeled streptavidin biotin method on magnification 400 x, and the results were expressed as average values. The VEGF expression was determined in the paraffin embedded bone marrow trephine biopsies by application of VEGF antibody and labeled streptavidin biotin method, and the result was expressed as percentage of positive cells. The differences in MVD and VEGF expression between groups were analyzed by Student T test, permutational T test and Mann-Whitney test. Survival analysis was done using Caplan-Meier and log-rank test.

Results: MVD was significantly higher in the MDS patients (3,8) compared with controls (2,27), ($p < 0,05$). The highest MVD was found in CMML (6,6), then in RAEB-T (4,36), RAEB (3,31), RA (3,07), and RARS (0,55). MVD was significantly higher in CMML compared with RA ($p < 0,01$), and RAEB ($p < 0,05$). The VEGF expression was not significantly different in MDS (12,11%) and controls (13,60%). The highest VEGF expression was found in RA patients (15,17%) and the lowest in RAEB patients (9,72%). The VEGF expression was significantly higher in RA compared with RAEB patients ($p < 0,01$), and in the low risk compared with advance MDS ($p < 0,01$). Significant adverse prognostic factor in univariate statistical analysis was low VEGF expression. MVD was not statistically significant as a prognostic factor in survival analysis.

Summary/Conclusions: The microvessel density is significantly increased in bone marrow biopsies in MDS patients comparing with control group and in CMML patients comparing with RA and RAEB patients. There was no significant difference in VEGF expression between MDS patients and control group. However, there was a significantly higher VEGF expression in RA patients comparing with RAEB patients. The low VEGF expression was an adverse prognostic factor in univariate survival analysis.

474 CD55 AND CD59 EXPRESSION ON GRANULOCYTES IN MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal disorder of the pluripotent stem cell resulting in a deficient expression of membrane-bound glycosyl-phosphatidylinositol (GPI)-anchored proteins in different cell types. CD55 and CD59 antigens are the most common GPI-anchored proteins that are used for the diagnosis of the presence of PNH clone. The association of MDS with PNH is rather controversial. There are few published individual cases of PNH arrived from a previous MDS or MDS following PNH.

Aim: Recent studies have demonstrated the existence of PNH clone in some MDS patients. These studies were performed mostly on erythrocytes and to the best of our knowledge they are only 4. Granulocytes appear to be more sensitive markers of PNH clone existence than erythrocytes and therefore we investigate the expression of PNH clone on granulocytes of patients with MDS.

Material-Methods: A total number of 56 patients 25-80 yrs old with MDS {A:15 with refractory anaemia (RA), B:5 with refractory anaemia with ring sideroblasts (RARS), C:13 with refractory anaemia with excess blasts (RAEB), D: 8 with refractory anaemia with excess blasts in transformation (RAEB-t) and E: 15 with chronic myelomonocytic leukemia (CMLL)} and 15 healthy donors were included in our study. The presence of GPI-anchored protein-deficient granulocytes (CD55, CD59) was examined by flow cytometry. For the detection of the PNH clone the commercial kit by Beckman Coulter (cellquant CD55/CD59) was used. This is a single colour flow cytometric analysis of CD55 and CD59 antigens on granulocytes. The method establishes an analysis region into which CD55 and CD59 deficient granulocytes migrate and can be differentiated from normal granulocytes. Statistical analysis was made by ANOVA.

Results: Table 1 shows the percentages of CD55 and CD59 deficient granulocytes in the 5 groups of pts with MDS comparing with the control group.

a) There is statistically significant difference in the comparison of the mean value of CD55 and CD59 between the fifth patient's group ($p < 0,005$ and $p < 0,0001$ respectively). b) There is no statistically significant difference in the comparison of the mean value of CD55 between each group of pts with the control group ($p > 0,10$) except a very weak one ($p < 0,10$) observed in the comparison between group C of pts with control group. c) On the contrary there is a very statistically significant difference in the comparison of the mean value of CD59 between the three last groups (C, D and E) of pts with the control group ($p < 0,003$, $p < 0,001$ and $p < 0,001$ respectively).

Conclusions: These preliminary data showed that a) there are definitely relatively large populations of CD59 deficient-PNH granulocytes at a proportion between 14,1% and 38,9% in the last three groups (RAEB, RAEB-t, CMML) of MDS patients. On the contrary the two low risk forms (RA, RARS) revealed no difference in antigens expression with the control group. b) CD59 antigen on granulocytes is a more sensitive marker than CD55 antigen of the presence of PNH clone.

Groups	N	CD55		CD59	
		Mean Value	Std deviation	Mean Value	Std deviation
A (RA)	15	1,9047	1,7798	3,2907	3,2104
B (RARS)	5	1,3700	1,4377	5,2280	4,4621
C (RAEB)	13	5,5423	5,2862	14,1131	4,7911
D (RAEB-t)	8	4,2738	3,1698	27,0125	6,9950
E (CMML)	15	4,1447	3,6961	38,9933	16,5063
Control	15	3,3940	2,9411	4,2560	2,9828
Total	71	3,5879	3,5784	15,8283	16,3871

Caption 1: Percentages of CD55 and CD59 in pts with MDS

475 LOW-DOSE DECITABINE RE-TREATMENT IN OLDER MDS PATIENTS

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Background: Low-dose decitabine (DAC) has significant single-agent activity in MDS and is being studied in older patients with AML.

Aim: To assess the feasibility and efficiency of the re-treatment with DAC in previously DAC-responsive patients.

Results: 11 patients (pts) with MDS have received both initial and re-treatment with low-dose DAC at a single center. Between 3/97 and 12/02, 11 pts (f:m = 4:7), median age 71 years (range, 51-79) enrolled in 2 phase II trials of DAC received re-treatment after loss of the initial response. Characteristics of MDS at time of initial treatment according to FAB: 1 pt with RA, 4 pts with RAEB, 5 pts with RAEB-t (i.e. AML according to WHO) and 1 pts with CMML. Patients initially received a median of 6 DAC courses (range, 2-6), in 5 pts resulting in a complete remission (CR), in 2 pts in a partial remission (PR) and in 4 pts in hematologic improvement (HI). The re-treatment of relapse started a median 12 months (range,

3-27) after the last course of initial treatment. Characteristics of MDS at time of re-treatment: 5 pts RAEB, 5 pts RAEB-t and 1 pt sAML. During re-treatment, pts received a median of 3 courses (range, 1-6), in 1 pt resulting, as at initial treatment, in CR, in 3 pts resulting in HI (1 pt CR at initial treatment, 1 pt PR, 2 pts HI). 6/11 pts did not show objective responses to re-treatment, including 2 with primary resistance to the first re-treatment course. Median time from second response to relapse was 4 months (range, 1-16), thus on average three times shorter than first response. The pt with CR both at initial treatment (9 months duration) and at re-treatment (5 months duration) received a second re-treatment with 3 courses of DAC (total of 15 courses), without achieving another response. Median survival of all pts (n=11) from start of first DAC course was 28 months (range, 15-44) and from end of re-treatment 6 months (range, 1-15). 6/11 pts transformed to AML.

Conclusions: re-treatment with DAC resulted in objective responses in only 45% of previously DAC-responsive patients and second remissions were markedly shorter, implying that DAC-responsive patients might benefit from continuation of the initial treatment.

An ongoing multi-institutional trial thus allows continuation of DAC treatment beyond 6 courses.

476 ACQUIRED ALPHA THALASSAEMIA (HAEMOGLOBIN H) IN MYELODYSPLASTIC SYNDROME: HAEMATOLOGICAL AND MOLECULAR ANALYSES

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BACKGROUND: Since the first reports of the detection of haemoglobin H (HbH) in cases of 'erythroleukaemia' and 'Di Guglielmo syndrome' in 1960, acquired alpha thalassaemia has become recognized as a rare complication of haematologic neoplasia. This interesting association is now commonly called 'alpha thalassaemia - myelodysplastic syndrome' (ATMDS). Our minimal ATMDS case definition includes: (1) the presence of HbH, detected by electrophoresis and/or supravital staining; (2) no evidence of pre-existing (i.e., congenital) thalassaemia or family history of thalassaemia; and (3) the presence of haematologic neoplasia.

METHODS AND RESULTS: We have compiled data on 37 ATMDS cases published in abstract or manuscript form and 28 unpublished cases (total 65). Most cases are Northern European men (51 Europeans, 8 Asians, 6 ethnicity unreported; 56 males) with a median age of 68 years (range, 10-87). Patients with ATMDS almost always have hypochromic and microcytic red cell indices; the mean MCV and MCH are 75.2 fL and 22.4 pg, respectively, in contrast to MDS without thalassaemia (97.3 fL and 32.3 pg). The amount of HbH in ATMDS is variable but can be considerable; among 45 cases where brilliant cresyl blue (BCB) staining was quantified, 16 had >50% BCB-positive erythrocytes and 34 had >10%. Automated analyzers have mistaken HbH-containing cells for reticulocytes. The alpha/beta globin chain synthesis ratio is strikingly abnormal in ATMDS; 25 patients were analyzed and the ratio was <0.2 in 13 and <0.75 in 23 (normal 0.9-1.2). Associated hematologic disorders have included MDS or probable MDS (52 cases), myeloproliferative or other chronic myeloid disorders (10), ALL (1), and AML (2 at diagnosis; 16 MDS cases transformed to overt leukaemia - in all but one MDS case HbH was not demonstrable after transformation.) There is no consistent ATMDS-associated karyotype.

Archival genomic DNA from 19 ATMDS cases was available for molecular analysis. In 1 case the telomeric region of a single copy of chromosome 16p was found to be deleted in a clonal fashion, but in all other cases analyzed the globin genes themselves were intact. In 12 ATMDS cases we detected inactivating point mutations in the ATRX gene at Xq13.3, which encodes a member of the SWI/SNF family of helicase-ATPases. The remaining 5 cases had relatively small amounts of HbH (<10%) and it is possible that current techniques are insensitive to very small ATRX-mutant clones. Surprisingly ATRX mutations associated with ATMDS were associated with a much more severe haematologic phenotype than identical or comparable germline ATRX mutations associated with congenital ATR-X syndrome (mental retardation, alpha thalassaemia, and facial/urogenital dysmorphism). This suggests a common interacting factor in MDS that exacerbates the phenotype associated with ATRX mutations. In view of the known effects of germline ATRX mutations on DNA methylation we are currently analyzing the methylation status of key regions of the alpha globin cluster in ATMDS. Fresh cases will be needed to study associated chromatin changes.

CONCLUSION: Alpha thalassaemia can develop in patients with neoplastic myeloid disorders and is strongly associated with ATRX mutations. Hypochromic or microcytic anaemia in such patients should prompt a search for HbH inclusions.

477 MOLECULAR PATHWAYS OF RHUEPO RESPONSE IN MDS PATIENTS

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Background: Myelodysplastic syndromes are clonal disorders characterized by cytopenias and a variable frequency of leukaemia progression. Nevertheless, in the cases with a low-intermediate 1 risk classes, as assessed by IPSS, the main clinical problems are represented by infections, due to leukopenia, and transfusion need, namely in the case of thrombocytopenia and anemia. Recently, promising results have been obtained in the treatment of MDS related anemia, by high dosage recombinant human erythropoietin (rHrEPO) treatment in the cases with low endogenous EPO. We have reproduced these clinical results in our patients.

Aims: Based on these premises, in order to evaluate the molecular basis of the rHrEPO response in MDS patients, we chose to investigate the differential gene expression in the bone marrow erythroid cells expressing glycophorin (Gly+) of EPO responders (ER) and EPO non responders (ENR) MDS patients, using commercially available microarrays (Clontech).

Methods: We analysed 3 ER and 3 ENR MDS patients (RA and RARS), and compared the gene expression to a physiologic baseline represented by Gly+ erythroid cells from bone marrow of 2 healthy BM donors. BM erythroid cells, after Ficoll density gradient, were separated by positive selection using an immunomagnetic procedure (MACS, glycophorin isolation kit; Miltenyi Biotec, Auburn, CA). The purity of Gly+ cells after magnetic immunosorting was tested by flow-cytometry and was superior to 97% in all the cases. RNA extraction, cDNA synthesis and microarrays hybridization and analysis were performed following manufacturer instructions (Clontech Laboratories, Palo Alto, CA). The data were validated on 4 differentially expressed genes by real time RT-PCR TaqMan technology, obtaining results that were superimposable to the microarrays ones.

Results: We obtained a differential pattern of expression in ER and ENR patients, compared to the normal, unstimulated BM Gly+ erythroid cells. In particular all the ER patients presented the up-regulation of genes described to be involved in the normal erythroid differentiation process in *in vitro* systems, namely HMG-I, xeroderma pigmentosum group C repair complementing protein, transferrin receptor, hEGR-1. Interestingly, hEGR-1, known to directly regulate CDKN2D transcription, and this latter gene, were both consistently down modulated in ER patients. In ER, also the expression of MEK, involved in EPO induced STAT3 phosphorylation in erythroid cells, is consistently down regulated. In ENR patients, we observed the consistent up-regulation of genes such as EHK1 ligand, NUC, glutathione peroxidase, rap1 GTPase activating protein 1, LYL-1, and the down-regulation of thymosin beta 10, prothymosin alpha, and p55CDC.

Summary/Conclusions: ER MDS patients appear to maintain a molecular pattern near to the physiologic one, when compared to ENR patients. These results may provide the basis for early testing of the genes differentially expressed in ER and ENR patients in order to elucidate their role in the prognostication of rHrEPO response in MDS patients with low endogenous EPO levels.

478 DISEASE-SPECIFIC PROGNOSTIC FACTORS FOR OUTCOME OF PATIENTS WITH POOR-RISK MYELODYSPLASTIC SYNDROME (MDS) AND ACUTE MYELOID LEUKEMIA (AML) TREATED WITH INTENSIVE ANTILEUKEMIC THERAPY

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It is questionable whether MDS and AML are distinct disorders or part of a disease with a continuous spectrum. The FAB classification

distinguishes MDS from AML through blast percentages and the presence of dysplastic features. However, multilineage dysplasia is also identified in de novo AML and a blast percentage of 30% for the definition of AML is arbitrary. The purpose of the present analysis is to identify disease specific prognostic factors for MDS and AML, which may influence outcome after treatment with intensive antileukemic therapy. This analysis combines the data of 692 patients (pts) included in the AML-10 study (EORTC 06931) between 1993 and 1993 with the data of 289 pts with high-risk MDS or secondary AML (sAML) included in the CRIANT study (EORTC 06961) between 1996 and 2002. Both patient groups received identical remission-induction therapy consisting of idarubicin, cytarabine and etoposide (ICE), followed by a consolidation course with intermediate dose of cytarabine and idarubicin (IDIA). In both studies post-consolidation therapy consisted of alloSCT for pts with age < 45-55 years if an HLA-identical sibling donor was available. The remaining pts received autologous stem cell transplantation (ASCT) in the AML-10 study, and were randomized between ASCT and a second consolidation course with high dose cytarabine in the CRIANT study. The AML study included pts with de novo AML (N= 659), sAML after MDS of less than 6 months duration (N=16) and therapy-related tAML (N=17). The CRIANT study included pts with high-risk MDS (N=197), with sAML after MDS of more than 6 months duration (N=55), with de novo AML (N=8), and with tMDS/tAML (N=29). Median age was 44 yrs (AML-10) versus 52 yrs (CRIANT). The performance status was better in the CRIANT study: 6% WHO 2/3 vs. 25% in the AML-10. The complete remission rate was 68% in AML-10 vs. 59% in the CRIANT study. Median follow-up was 5.3 yrs vs. 3.5 yrs. Median survival was 1.5 yr. (AML-10) vs. 1.3 yr. (CRIANT). The 5-year survival rates were 33.5% (SE=1.9%) vs. 26.9% (SE=3.2%), p=0.26. For pts who reached CR, the 5-year DFS rates were 39.7% (SE=2.3%) vs. 27.8% (SE=4.0%), p=0.02. In multivariate analyses we tested the following variables: age, WBC, number of cytopenias, cytogenetics, antecedent hematological disorder, study and presence of a donor (for DFS analysis only). For the duration of survival, cytogenetic risk group (according to IPSS), age, WBC and initial performance status were in this order the most important independent prognostic factors. Duration of antecedent hematological disorders, number of cytopenias, and inclusion in one of the studies were not prognostic. In patients aged less than 56 years cytogenetic risk group, WBC and the presence of an HLA-identical sibling donor appeared independent prognostic factors for DFS.

In conclusion: the present analysis identifies a subgroup of MDS and AML patients, who have a poor prognosis despite intensive antileukemic treatment, mainly characterised by the presence of poor prognostic cytogenetic features. New treatment strategies should be offered to these patients in the context of clinical trials.

BLEEDING DISORDERS

479 EFFICACY AND RESPONSE TO INTRAVENOUS ANTI-D IMMUNOGLOBULIN IN REFRACTORY CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background:

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by persistent thrombocytopenia due to autoantibody binding to platelet antigen(s) causing their premature destruction by the reticuloendothelial system. It has been long known that anti-D treatment is effective for Rh (D) positive patients who are not splenectomized, however it is not recommended for refractory patients following splenectomy.

Aims:

We record our experience in using anti-D (Partobulin & WinRho) for treatment of chronic ITP and how response can be achieved in both splenectomized and non-splenectomized patients.

Methods:

A total number of 9 patients with chronic ITP were investigated retrospectively over a period of 6 years. There were 7 females and 2 males. The median age was 20 years. The presence of other medical disorders which may be responsible for thrombocytopenia were excluded. AntiD was given as a short IV infusion whenever platelet count dropped below 30X10⁹ /L or the patient was haemorrhagic. Response

was defined as an absolute platelet count above 30X10⁹ /L or an increment by 20X10⁹ /L or more. Average anti-D dose was 50 microgram/Kg.

Results:

Total number of courses administered was 40 and post treatment platelet count was documented in 36 cases. The response was obtained in 32 treatments ~ 91% . There was no difference in response to Anti-D between males and females. Average lag period was 2 weeks. Average duration of response was 5 months. The improvement in platelet count lasted for more than 8 weeks in 91% of patients who responded to Anti-D. 1 patient has not required any further treatment with ongoing remission since august 2000. We report 2 patients with previous splenectomy for ITP who responded to Anti-D therapy. The possibility of an accessory spleen has not been explored in both cases. Both patients were females and 6 doses of Anti-D were administered with a response rate of 66%. The average platelet increment was 120 x 10⁹ /l. With regard to the side effects, drop in haemoglobin by an average of 1 g/dl was documented in 55% of treatments.

Conclusion:

The response rate of our patients to Anti-D was as high as reported in literature (~90%) for non-splenectomized patients and 66% for splenectomized patients. The duration of response was much higher than many other reported series as 91% of patients had an improvement lasted for more than 8 weeks. Despite the recommendation that Anti-D should not be used in post splenectomy cases, we report 2 cases who had a good response to Anti-D therapy. The side effects profile was very mild with no cases required red cell transfusion, because of the safety, ease of administration and effectiveness of anti-D, we recommend extending its use in chronic ITP to include refractory cases. Whether it is effective in post-splenectomy cases or not remains to be answered preferably by conducting appropriate clinical trials.

480 STUDY ON THE MECHANISM OF HUMAN PLATELET AGGREGATION INDUCED BY SJAMP

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Background: Acidic Mucopolysaccharide from *Sticopus Japonicus* Selenka (SJAMP) is a kind of acid mucopolysaccharide. It can induce platelet aggregation, but the exact mechanism is not clear. Prof. Shen used it as a new agent in the research of platelet aggregation and found a new platelet disease. The disease manifests gum bleeding, recurrent ecchymoses and petechiae in the skin and mucosa, epistaxis and hypermenorrhea, platelet count and coagulant tests are normal. The platelets from the patients have normal reaction to ADP, Adr and collagen, but have a SJAMP induced aggregation defect.

Aims: To investigate the mechanism of platelet aggregation induced by SJAMP

Methods: Using spectrofluorimeter to measure the change of platelet cytoplasmic calcium, Western blotting to examine protein tyrosine phosphorylation of platelets and platelet lumi-aggregometer to observe platelets aggregation and ATP release.

Results: SJAMP(100µg/mL) can induce normal human platelets [Ca²⁺]_i increase to 94.5610.24nmol/L (n=10). In the presence of extracellular Ca²⁺(1mmol/L), the magnitude of platelets [Ca²⁺]_i responding to SJAMP was increased and can reach 116.7210.66nmol/L (n=10). If platelets were first incubated with cyclo-oxygenase inhibitor, the rise of [Ca²⁺]_i evoked by SJAMP could be inhibited. The protein tyrosine phosphorylation of resting platelets and platelets induced by agonists indicated that only a few proteins were phosphorylated in resting platelets and after stimulated with collagen or SJAMP, 85 kD proteins become phosphorylated. But those phosphorylation induced by SJAMP happened in 5 minutes while collagen in 2 minutes. The aggregation curve induced by SJAMP(100µg/mL) was a typical two phase aggregation. There existed a certain lag between platelet aggregation and secretion(ATP release). The platelet maximum aggregation rate and ATP release induced by SJAMP(100µg/mL) were 553.31% and 0.690.22nmol/108platelets, respectively. If platelets were incubated with cyclo-oxygenase inhibitor, the SJAMP induced secondary phase aggregation was inhibited and had no ATP release.

Conclusions: SJAMP can induce human platelet aggregation and ATP release. It is related with the elevation of platelet cytoplasmic calcium and protein tyrosine phosphorylation. The aggregation induced by SJAMP might be dependent upon the generation of prostaglandin endoperoxides and TXA₂.

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481 MOLECULAR ANALYSIS OF HAEMOPHILIA B IN IRANIAN PATIENTS: THREE NEW MUTATIONS IN THE FIX GENE

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Background: Haemophilia B is a X-linked recessive bleeding disorder caused by a deficiency of factor IX affecting 1/30 000 males. There are about 800 haemophilia B patients in Iran and genotyping of these patients can be very useful in carrier detection and prenatal diagnosis.

Aims: The aim of this study was the identification of the causative mutation in the factor IX genes of haemophilia B patients and their families leading to precise carrier detection and prenatal diagnosis in affected families.

Methods: DNA samples were obtained from 29 unrelated haemophilia B patients and some of their female relatives. The whole of FIX gene sequence, including intron/exon flanking regions and promoter, was analysed by conformation sensitive gel electrophoresis (CSGE). Products demonstrating CSGE bandshifts were directly sequenced.

Results: Only the exons with abnormal band pattern were sequenced and a total of 25 mutations were identified. However in four of them, no abnormal band was detected by CSGE. For these patients, all the exons and flanking regions have been sequenced and a mutation was found in all 4 patients. The identified mutations included 28 base substitutions and an isolated one base deletion. Three of these mutations (31267deltaA, G93S, Q246K) have not been previously reported, but the others have been previously reported and present in the database. It was interesting to note that more than 85% of these mutations have been found in exon 2, 5 and 8 (30%, 15% and 42% respectively) suggesting that mutations in these 3 exons are very frequent in our haemophilia B patients.

Conclusion: Analysis of factor IX mutation in the rest of our haemophilia B patients is now in progress for precise carrier detection and possible use in prenatal diagnosis.

482 DEMOGRAPHIC DATA, OUTCOME AND PROGNOSIS OF CHILDREN WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP) IN ARGENTINA. RESULTS FROM A MULTICENTERED STUDY

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Background: Demographic data, clinical course, outcome and management of ITP in children present differences between countries.

Aim: To establish valuable data for our population

Methods: We have been collecting information in a database since 1997. Data regarding 844 children with ITP, treated in 8 centers, has been recorded to date. The analysis of these data is presented.

Results: Mean age was 59.2 months, with the peak incidence at first 2 years. M:F ratio was 1.1, but a male predominance was observed in patients up to 24 months (M:F 1.8). The greatest frequency was seen in spring (34.8% of cases). The antecedent of preceding viral illness or immunization was detected in 369 children (45.3%). 590 children had dry purpura and 254 wet purpura. Therapies received included: no treatment (426), steroids (335), IVIG (129), IgG anti-D (2), Interferon (6) and/or Vincristine (3).

The outcome was evaluated on 696 children with adequate follow-up: 467 (67.1%) children had acute ITP (thrombocytopenia resolving within 6 months), 194 (27.9%) patients had chronic ITP, 34 (4.9%) children had recurrent ITP, and 1 (0.1%) child died (intracranial hemorrhage). Three age groups with different recovery rates ($p < 0.001$) could be established: Group A (2 to 12 months) showed an 84.4% recovery rate, Group B (2 to 8 years) had a recovery rate of 67.8%, and in Group C (9 to 15 years) the recovery rate was 47.3%. Time needed to achieve remission was shorter in group A than in groups B and C ($p < 0.05$). Platelet count at diagnosis did not modify the outcome in group A; on the other hand, platelet count $< 10 \times 10^9/L$ was associated to favorable prognosis in groups B and C ($p < 0.05$). Gender, antecedent of preceding illness, and dry or wet purpura had no influence on the final outcome.

The analysis of recovery rate in chronic ITP showed that 53 of 201 non-splenectomized patients (26.4%) achieved remission spontaneously

between 6 months and 10 years from diagnosis. It is worth noting that 30 of them (56.6%) recovered between the 6th and the 12th month from the diagnosis. The recovery rate was higher in groups A (46.7%) and B (29.3%) than in group C (14.6%) ($p < 0.05$). Therefore, the total remission rate without splenectomy for our population was 74.7%. Splenectomy was performed on 28 patients, achieving a 71.4% successful rate. Thus, if we included the splenectomy-induced remission, the total recovery rate for our population reached 77.6%.

Conclusions: A) In our population, the peak incidence is at first 2 years of age. B) Age is the main prognostic factor, with infants less than 1 year having the best prognosis; most patients over 8 years develop chronic disease. The definition of groups based on age could be useful to establish differential guidelines for management. C) In chronic ITP spontaneous long-term remission can occur at any time, mainly between 6 and 12 months. Therefore, the time to define chronic ITP should be at least 12 months instead of 6 months.

483 MANAGEMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA OF THE LEBANESE CHILDREN

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The management of idiopathic thrombocytopenic purpura is a controversial entity.

We conduct a descriptive study of patient hospitalized or presenting at the pediatric out clinic at Hotel Dieu de France Hospital and Lebanese Hospital from October 1995 to December 2003. We studied the clinical presentation, the biological aspect and the therapy given.

There were 74 patients, 40 boys and 34 girls. Mean age was 4.2 years [2.5month-15years]. 71 consulted for purpura. 4 of the 71 had gastrointestinal bleeding. One had isolated epistaxis and one convulsion without bleeding.

Predisposing factor were viral infection 42 cases, antibiotherapy 2 cases and vaccination 6 cases.

Thrombocytopenia $< 20000/mm^3$ is found in 53 cases (71%). 50 of them were hospitalized. Of the other 21, 12 were hospitalised.

A bone marrow aspiration was done in 64 cases (86%) and was normal. The reason for performing a bone aspiration was for starting steroid in 46 cases, non response to immunoglobulin in 7 cases and systematically in 8 cases. Immunological study done showed Comb's positive in 3/44 of cases and ANA in 5/42 of cases.

Initial treatment was steroid in 38 patients, immunoglobuline in 26, and no treatment in 10 cases.

Given as first line treatment, the response to steroid, evaluated at one week, (4mg/kg/d, 5 days or 1mg/kg/d, 21 days) was 73% (28/38). 57% (16/28) relapse when steroid were stopped. The response to immunoglobulin (1g/kg), evaluated at 72 hours, was 53% (14/26). 57%(6/14) relapse 3 weeks after.

As second line treatment 16 take steroid and 23 immunoglobulin.

The response to steroid was 62% (10/16, and 60% (6/10) relapses when steroid were stopped. For the immunoglobulin the response was 91% (21/23) and 61% (13/21) relapse 3 week later.

Evolution is characterized by 36% (27/74) of cure at 1month, 44%(33/74) at 6 months, 44% (33/74) at one year and 27% (20/74) involves to chronic status.

Severe complication was noted in 4 cases: 2 intra cerebral hemorrhage, with one death, one pharyngeal hematoma and one hematuria. All had then mucosal or humid purpura.

The patients with chronic status had a mean age of 6 at diagnosis with 8 girls and 12 boys. The mean level of platelets at diagnosis was below 30000/mm³ (5000- 30000) and the level varies between 10000/mm³ and 650000/mm³ during the follow up. 80% of these patients did not received any treatment after the trial of steroid and immunoglobulin.. The management of idiopathic thrombocytopenic purpura is variable. At diagnosis laboratory study are not so useful when the complete blood count and the physical exam is normal. The apparently same efficacy of treatment may let choose the cheaper. Severe complication is rare, and can be predictable. High level of chronic state is to noted, even if there is a recruitment bias.

Larger study at a national level should be conducted.

484 CARTILAGE DAMAGE AND SYNOVIUM CHANGES IN HAEMOPHILIC ARTHROPATHY: EVALUATION WITH MAGNETIC RESONANCE IMAGING

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Introduction: Haemophilia is an X chromosome linked disease characterized by an increased tendency to hemorrhage mainly into joints. Due to recurrent haemarthroses specific changes occur in synovium and cartilage. This process is called haemophilic arthropathy.

The aim of our study was to determine the value of magnetic resonance imaging (MRI) in the evaluation of early pathologic changes of haemophilic arthropathy.

Material-Methods: We investigated 53 joints (27 knees, 14 elbows, 10 ankles and 2 shoulders) in 18 patients with Haemophilia A aged 5-57 years old (mean age 21) who all had at least one episode of previous acute haemarthroses. In all patients plain film radiographs (anteroposterior and lateral) and MRI evaluation was performed when there was no clinical signs of acute haemarthroses. MRI protocol consisted of T1 SE, T2*GRE, T2 FSE and whenever necessary STIR in three perpendicular planes. We used intravenous contrast medium (Gadolinium DTPA) in only 3 cases to better delineate the hypertrophy of the synovium.

Results: Synovial hypertrophy and hemosiderin deposition was detected with MRI in 36 patients, a finding with a very important role for the pathogenesis and evolution of the disease, which is not detected with plain X-rays. Current concepts about the pathogenesis of haemophilic arthropathy hold that the synovium becomes catabolically active because of the exposure to blood components and as a result it induces cartilage destruction. Focal or diffuse destruction of the cartilage was observed in 27 joints with MRI while plain radiographs gave only indirect signs of cartilage destruction in 17 joints. Bone changes were equally well estimated with both methods.

In conclusion, we believe that MRI imaging, with specific sequences, is a non-invasive tool for the assessment of early joint cartilage and synovium pathological changes still undetectable by clinical examination or conventional X-rays in haemophilic patients.

485 THERAPY OF PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP): EFFICACY, TOLERABILITY, AND SAFETY OF A NEW LIQUID INTRAVENOUS IMMUNOGLOBULIN

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Background/Aims: Therapy of bleeding complications in patients with chronic immune thrombocytopenic purpura (ITP) is commonly done with intravenous immunoglobulins (IVIgG). Patients should be provided with IVIgG of maximum safety, tolerability and convenience. We have developed a novel liquid intravenous immunoglobulin (Sandoglobulin® NF Liquid). It consists of a nanofiltered 12% immunoglobulin G (IgG) solution, stabilized by the vitamin nicotinamide together with the amino acids L-proline and L-isoleucine. The formulation minimises the formation of IgG dimers, assuring good clinical tolerability.

The primary endpoint (response rate) of this Phase II/III study was the number of patients whose platelet count rose to $50 \times 10^9/L$. Secondary efficacy endpoints included (i) time to platelet response and (ii) regression of hemorrhages. Methods: A randomised, double-blind, controlled study in patients with chronic ITP (platelet count $< 20 \times 10^9/L$). A total of 33 adult patients were randomised (Sandoglobulin® NF Liquid: 16, Sandoglobulin®: 17; ITT-population). Study medication was infused at a dose of 0.4 g IgG/kg body weight (study drug or comparator) on five consecutive days (total dose 2.0 g IgG/kg body weight per patient). Adverse events were recorded until Day 30 after the last infusion. Viral safety was assessed serologically, supplemented with nucleic acid testing (NAT) for B19 virus. Results: The response rates were 75% (12/16) in the Sandoglobulin® NF Liquid and 71% (12/17) in the Sandoglobulin® group ($p=1.000$; Fisher's exact test). Mean time to response was five days (range: 3-15 days) in the Sandoglobulin® NF Liquid recipients, and four days (range: 2-8 days) in

the Sandoglobulin® group. Severity of bleeding symptoms decreased in both treatment groups. Improvement until Day 8 was seen in 8/16 (50%) patients of the Sandoglobulin® NF Liquid group, and in 6/17 (35%) of the Sandoglobulin group ($P=0.344$, Mantel-Haenszel Chi-Square test). The number of adverse events and profile of safety were comparable. Tests for virus markers (anti-HCV, HBsAg, anti-HIV) in serum samples were negative up to six months after the last infusion. No seroconversion for B19 virus nor evidence of viral transmission were detected. Summary/Conclusions: Sandoglobulin® NF Liquid was efficacious, well tolerated and safe in the treatment of chronic ITP patients. As a ready-to-use solution, Sandoglobulin® NF Liquid has a similar efficacy, safety and tolerability profile as its parent product Sandoglobulin®.

486 HAEMATOLOGICAL FINDINGS AND TREATMENT OF HAEMORRHAGIC DENGUE IN VENEZUELA

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Dengue is an acute febrile viral disease transmitted by the mosquito *Aedes aegypti*. In Venezuela, dengue is a public health problem. In July 2001 an epidemic of dengue was detected with a morbidity of 337.7 per 100000 inhabitants. Materials. A diagnosis of dengue was done in 210 patients, whose age ranged from 15 to 45 years. The hemorrhagic form developed in 77 patients and was diagnosed according to the clinical characteristics (fever plus bleeding) and two abnormal laboratory tests: thrombocytopenia (platelet count less than 100000/ul) and hemoconcentration (increases of the haematocrit in more than 20%, 4 to 5 days after beginning of the symptoms. Methods. Routine hematology, blood chemistry (BUN, creatinine, liver tests), IgG and IgM dengue blot tests, coagulation tests (prothrombin time, PTT, thrombin time, fibrinogen, fibrin degradation products). In 45 cases the levels of protein S and C antigenic tests, antithrombin, and plasminogen were determined. Results. The main clinical characteristics were: fever during 2 to 7 days (92.6%), a sharp drop in platelet count occurred in 95% of patients, headache (76%), muscular pain (77%), fatigue (39.4%), erythematous macular rash (39.3%), arthralgia (37.7%), petechias and purpura (37.2%). Hematology tests showed thrombocytopenia in 77 cases. CBC on admission showed Hb: 14.9 g/dl $\pm$ 1.2, crit: 44.4%, the relation crit/Hb was less than 3.5 in 98.9 % of patients, WBC 3340 $\pm$ 1010. Differential count: neutrophils (67% $\pm$ 12.4), lymphocytes (22 % $\pm$ 11, monocytes (8.3% $\pm$ 4.5), eosinophils (1.7% $\pm$ 4.8), basophils (0.02% $\pm$ 1.7). Thrombin time was increased in 60% of cases, prothrombin time increased in 10% of cases, activated PTT increased in 65% of cases, level below normal range of fibrinogen in 25% of cases, high fibrinogen levels in 10% of cases. Dengue blot test was positive for IgM in 91% of patients. Protein C level was normal or above normal range in 60 -160% (normal values: 60 -120%). Protein S level decreased in 23 patients (21%), antithrombin levels were found below normal range in 3 cases and the plasminogen levels below the normal range in only one case. The patients were treated with blood components when platelet count dropped to less than 20000/ul and the PTT difference between control and patient was greater than 10 seconds. 18 patients received blood components plus methylprednisone (250 mg daily until the platelet count increased to 80000/ul); 44.6% of the patients without steroid treatment required platelet transfusion while only 27% of patients who received steroids required it. There was no difference in the requirement of fresh frozen plasma and in the average time of hospitalization between patients with and without steroid treatment. CONCLUSION. The clinical characteristic of 77 patients with hemorrhagic dengue are reported. 18 patients had abnormal coagulation tests and required administration of blood components. Some patients had low levels of Protein S indicating the establishment of a procoagulant state. The use of steroids decreased the use of platelets and no complication of steroid therapy was found.

VASCULAR BIOLOGY

487 THE EFFECT OF A NEW VASOPRESSIN ANALOG TERLIPRESSIN AND AMINO ACID GLYCINE ON FVIII AND T-PA RELEASE

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BACKGROUND. It is known that arginine vasopressin and its analog desmopressin cause the release of factor VIII (FVIII) and tissue plasminogen activator (t-PA) in vivo. In our previous work we showed that a vasopressin analog des-glycylamid-arginine vasopressin induced the release of FVIII in blood but decreased fibrinolysis and t-PA activity. Moreover, we supposed that the presence of glycine at the C-terminal in the molecules of vasopressin analogs leads to an increase in their profibrinolytic effects. Besides, small glycine-containing peptides have fibrinolytic and anticoagulant activities.

AIMS. The aim of the present investigation was to study the influence of a new vasopressin analog terlipressin (lys-vasopressin analog with three amino acid glycines at the N-terminal in the molecule of a natural hormone) and amino acid glycine on procoagulant activity and fibrinolysis in experiment on white rats (intravenous injection of drugs).

METHODS. Fibrinolytic activity (FA) and t-PA activity in blood plasma were measured by a fibrin plate method and by determination of clot lysis time. APTT and FVIII activity were detected by a one-stage clotting method.

RESULTS. Our experiments demonstrate that terlipressin induces a dose-dependent increase in APTT and FVIII activity (on 15-18% from the initial level), FA and t-PA activity (on 65-100%). Besides, we observe correlation between the effects on the haemostasis system and the rise of systemic blood pressure after terlipressin administration. Intravenous injection of glycine led to the increase in FA and t-PA activity too (on 35-40% from initial value).

CONCLUSIONS. Thus, we confirm that modification of amino acid sequence in the vasopressin molecule changes its effects on the haemostasis system and the presence of glycine at any terminal of the natural hormone is responsible for the increase in t-PA activity. Besides, amino acid glycine itself promotes the increase in FA and t-PA release from endothelium. Thus, synthesis of peptides with definite properties for direct correction of blood coagulation disorders is possible.

488 METABOLIC SYNDROME AND ENDOTHELIAL DYSFUNCTION

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Background: Metabolic syndrome (MS) is defined as cluster of cardiovascular risk factors, including impaired fasting glucose (IFG) or type 2 diabetes mellitus (DM2), obesity, dyslipidaemia and hypertension (HT). Endothelial dysfunction (ED) is considered to be a part of MS, but only 5-15% of the variability of endothelial markers levels is explained by MS components and the relation of insulin resistance to ED is weak. Mechanism of ED in MS is not fully understood.

Aims: The aim of this study was to analyse the influence of MS components on endothelial markers levels, including von Willebrand factor (vWF) as marker of endothelial activation and thrombomodulin (TM) as marker of early endothelial injury.

Methods: 122 patients (69 males, 51 y.) with at least one component of MS were included to study. MS was defined by modified NCEP ATP III criteria with recommended methods for examination of IFG/DM2, obesity, dyslipidaemia and HT. ED was examined using vWF and TM levels and together with VCAM-1 only in subgroup of DM2 patients. ED was defined by levels of vWF (>1.5 IU/ml) and/or TM (>50 ng/ml). Patients with new diagnosed DM2 were followed 3 months on antidiabetic treatment and vWF, TM and glycosylated HbA1c levels were examined before and after treatment.

Results: MS (3-4 components) was associated with increased prevalence of ED (61 vs 44%) and increased vWF levels (1.410.54 vs 1.290.36 IU/ml, $p < 0.01$). IFG/DM2 was associated with increased prevalence of ED (59 vs 41%) and increased levels of vWF (1.400.50 vs 1.270.38 IU/ml, $p < 0.01$) and TM (52 vs 42 ng/ml, $p < 0.01$). There was relation of HT only to vWF levels ($p < 0.01$). Obesity and dyslipidaemia were not associated with additional increasing of vWF and TM levels. There was relation of vWF levels to tertiles of VCAM-1 ($p < 0.01$) levels and not to obesity parameters in DM2 patients. Improved metabolic control in DM2 with decreased HbA1c levels was accompanied by parallel decreasing of TM levels (from 75 to 55 ng/ml, $p < 0.01$) and not vWF levels.

Conclusion: MS is associated with endothelial dysfunction with the strongest influence of IFG/DM2 on vWF and TM levels. Increased vWF levels are probably related to low grade inflammatory reaction independent on glucose control, while increased TM levels such marker of early and reversible endothelial injury are closely related to hyperglycaemia.

Study was supported by Grant of Ministry of Education - VEGA 1/1189/04 and National Diabetology Program of Slovak republic.

489 THROMBOMODULIN AND INTERCELLULAR ADHESION MOLECULE-1 LEVELS IN PATIENTS WITH THALASSEMIA INTERMEDIA AND SICKLE CELL SYNDROMES

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Background and Aims: Vascular endothelium separates the subendothelial tissue from blood constituents and maintains vascular integrity and vascular tone by expressing vasoprotective and thromboresistant molecules. Thrombomodulin (TM) is one of the important vasoprotective molecules. TM possesses several properties to protect arterial walls and reduce thrombotic tendency. It is a transmembrane protein expressed constitutively primarily in endothelial cells. It has a large extracellular region comprising a thrombin binding site. The extracellular region of TM is cleaved constitutively into several fragments collectively called soluble TM (sTM). Intercellular adhesion molecule-1 (ICAM-1) is expressed on endothelial surface after inflammatory stimulation, and the extracellular region of ICAM-1 is cleaved which circulates as soluble ICAM-1 (sICAM-1). sICAM is considered as an important marker of inflammation. Thus, a high level of sTM signifies protection and has a lower risk of coronary heart disease (CHD), whereas a high sICAM level signifies an inflammatory state and increases risk of CHD. It is postulated that sTM interacts with sICAM in predicting CHD risk.

Patients and Methods: In this study we measured sTM (Asserachrom, Diagnostica Stago, France) and sICAM-1 (Diacore Research, France) levels in 28 patients with thalassemia intermedia (TI) (group A), 26 patients with Sickle Cell Syndromes (SCD) (group B), while 50 healthy subjects (no inflammation, diabetes, nephropathy etc), served as controls (group C).

Results: We found that sTM levels were significantly higher in TI and SCD patients compared to normal controls [53.2 54.2mg/l (median 42.0mg/l), 43.6 35.4mg/l (median 33.0mg/l) and 38.2 19.0mg/l (median 34.3mg/l) respectively], ($p < 0.01$). Similarly sICAM-1 levels were significantly higher in TI and SCD patients compared to normal controls [670 91.1mg/l (median 687.5mg/l), 727.0 59.1mg/l (median 710.5mg/l) and 259.0 69.0mg/l (median 258.0mg/l) respectively], ($p < 0.001$). Furthermore, we found a significant negative correlation between sTM and sICAM-1 levels in TI patients ($r = -0.42$, $p < 0.02$), while this type of interaction between these molecules was absent in SCD patients and normal controls ($r = 0.19$, $p > 0.35$ and $r = 0.38$, $p > 0.1$ respectively).

Conclusions: These findings demonstrated that TI and SCD patients even when they are in steady phase appear to have endothelial inflammation indicated by the increased sICAM-1 levels. The negative interaction of sTM and sICAM-1 in TI patients is of particular interest as this relationship has been proposed as marker of increased risk for CHD in normal individuals (Circulation, 107: 1729-1732, 2003).

490 TAFI AND METABOLIC BALANCE IN DIABETIC NEPHROPATHY

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Background: Thrombin activatable fibrinolysis inhibitor (TAFI) provides a link between coagulation and fibrinolysis. Activated TAFI (TAFIa) mediated down regulation of fibrinolysis is dependent on the concentration of TM. The increased circulating plasma levels of TAFI were reported in type 2 diabetes mellitus (DM), obesity, insulin resistance and in hypercholesterolemic subjects. TAFI plasma level was significantly higher in diabetic patients with microalbuminuria than those in diabetic patients with normoalbuminuria and normal subjects. These data suggest that increased plasma level of TAFI may be involved not only in hypofibrinolysis but also in the mechanism of vascular endothelial damage in patients with type 2 DM.

Aims of study: We evaluated the activity of TAFIa and its relation to another hemostatic and metabolic parameters in patients with diabetic nephropathy (DN).

Patients and methods: Eighteen patients (pts) with diabetic nephropathy (DN) 14 pts type 2 DM / 4 pts type 1 DM, age 54,113,3 years (12 men, 6 women), fourteen pts with non-diabetic nephropathy (NDN), age 49,715,6 years (7 men, 7 women) and twenty healthy subjects were enrolled in the study. TAFIa (Actichrome Activity TAFI, American Diagnostica), soluble TM (sTM), von Willebrand factor (vWF), tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), homocysteine (Hcy), D-dimers plasma levels and metabolic parameters including glycosylated hemoglobin (HbA1c) were measured.

Results: TAFIa plasma levels in DN pts were significantly decreased compared to NDN pts (7,22 3,54 vs. 12,67 6,99 ug/ml, $p<0,01$, respectively). After analysis the significant TAFIa decrease was only in those DN pts with hypercreatinemia and proteinuria. The sTM levels showed significant increase in DN pts with hypercreatinemia ($p<0,0001$). There was a significant positive correlation between TAFIa and HbA1c ($r=0,50931$, $p<0,05$).

Conclusion: Decreased TAFIa plasma levels in our DN pts with proteinuria and hypercreatinemia compared to NDN group may be due to the secondary (compensatory) increased fibrinolysis as a consequence of the known hypercoagulability in pts with DM. TAFIa was in a close relation to the metabolic balance of DM (HbA1c) that can be of clinical importance for DN pts.

491 ENDOTHELIAL FACTORS AND ADHESION MOLECULES IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL DISEASE

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BACKGROUND: Activation of the vascular endothelium plays an important role in the pathophysiology of sickle cell disease (SCD). It is known from several studies that interactions of endothelium with sickle cells may alter the expression of several adhesion molecules and endothelial factors.

AIM: Based on that knowledge, the aim of this study was to determine the activation markers of vascular endothelium in children and adolescents with SCD during steady state and thrombotic crisis, targeting in their possible prognostic value and therapeutic intervention.

METHODS: Forty one patients with SCD were studied (38 S β -thalassemia and 3 S/S), aged 2-22 years (mean age: 14,3 years), 22 girls and 19 boys. In 38 patients during steady state and in 16 during thrombotic crisis, as well as, in 28 healthy individuals, the levels of endothelial factors (NO, ET-1, PGDF, vWF, Thrombomodulin), adhesion molecules (sVCAM-1, LFA-1, LFA-2, LFA-3, sP-selectin), and acute phase reactants (ESR, CRP, IL-6), were determined.

RESULTS: From all parameters determined, abnormal findings were as follows: a) in patients during steady state significantly increased levels of ET-1 (16,28, pg/ml $p<0,05$) and sP-selectin (293,68 ng/ml, $p<0,001$), and decreased levels of integrins LFA-1 (64,8%) and LFA-3 (64,4%), compared with those of the control group were found, b) in patients during thrombotic crisis significantly increased levels of ET-1 (27,24 pg/ml, $p<0,005$), sP-selectin (220,25 ng/ml, $p<0,01$), vWF (186,97%, $p<0,001$), ESR and CRP, and significantly decreased levels of integrins LFA-1 (57,5%) and LFA-3 (55,6%), compared with those of the control group were found.

CONCLUSIONS: Abnormal values of the parameters determined confirms the serious dysfunction of the vascular endothelium, especially during thrombotic crisis. On the other hand, significantly increased levels of ET-1 and sP-selectin during steady state shows the presence of a chronic inflammation and activation of the vascular endothelium in SCD patients. According to these findings, we would speculate that periodic assessment of ET-1 and sP-selectin along with evaluation of other parameters, such as frequency of thrombotic crises and presence of silent cerebral infarctions, could be used as predictors of serious complications and contribute to the decision of preventive therapeutic intervention.

492 VARIABLE PROTECTION FROM THROMBOTIC STIMULI IN P-SELECTIN GLYCOPROTEIN LIGAND-1 DEFICIENCY

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Background: In thrombosis and inflammation, vascular cells interact with flowing blood cells via multiple receptors. During the association of leukocytes with platelets and endothelial cells, P-selectin glycoprotein ligand-1 (PSGL-1) binds to P-selectin expressed on activated platelets and endothelial cells.

Aims: We intended to evaluate the response to various thrombotic stimuli in wild type and PSGL-1 knockout mice.

Methods: Three different groups were formed during the study. Twelve to twenty week old mice were challenged by tail vein injection of (i) 15 ug collagen plus 2 ug epinephrine (ii) thromboplastin (5x diluted Innovin) or (iii) saline. After 3 minutes, retro-orbital samples were drawn to ACD anticoagulant and platelet and leukocyte counts were measured and plasmas were frozen for thrombin-antithrombin III (TAT) complex. TAT measurements were carried out by an ELISA assay. Animals were sacrificed after 30 minutes and the kidneys, liver, spleen and lungs were investigated for fibrin deposition by immunohistochemistry.

Results: The proportion of neutrophils and monocytes were significantly higher in unchallenged PSGL-1 knockout mice. After collagen/epinephrine and thromboplastin challenge mice of both genotypes decreased significantly platelet and leukocyte number. The degree of thrombocytopenia was milder after thromboplastin challenge, while the mean TAT complex values were 10-fold elevated in this group compared to saline injected animals (244 mg/L versus 23 mg/L). TAT values after thromboplastin challenge were also significantly elevated compared the collagen/epinephrine treated animals, but the wild type and PSGL-1 knockout group showed no significant difference. The major difference between the two genotypes could be observed by investigating lung and liver sections for fibrin deposition by immunohistochemistry. After collagen/epinephrine challenge the lungs of the PSGL-1 knockout animals contained less thrombi and in the liver no central vein involvement could be observed.

Summary/conclusions: Thromboplastin challenge produces more thrombin generation while collagen/epinephrine challenge results in a more pronounced thrombocytopenia. For the applied doses, the deficiency of PSGL-1 defended for neither effects, however the difference in the fine structure of the formed fibrin refers to the importance of this molecule in multicellular interaction during thrombus formation.

493 KINETIC OF MOBILIZATION OF ENDOTHELIAL PRECURSORS AFTER ACUTE MYOCARDIAL INFARCTION

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BACKGROUND It has been recently demonstrated that bone marrow houses also endothelial progenitors, able to give rise, in vitro and in vivo, to terminally differentiated cells. These progenitors can be detected in peripheral blood following tissue damage, but little information exists regarding the kinetic of their mobilization. **AIM** With this aim we evaluated the expression of endothelial and hematopoietic stem cell specific antigens on peripheral blood mononuclear cells at different time points after AMI and in normal blood donors. **METHODS** Peripheral blood samples were obtained from AMI patients (mean age 54 years) at 24 hours, after 3, 7 and 14 days from infarction and from normal control subjects (mean age 41 years). AMI patients met the following criteria: chest pain lasting for 30 minutes that was not relieved by sublingual nitroglycerin, abnormal Q waves on the ECG, and elevated serum creatine kinase levels. Angiography revealed the total occlusion of one of the 3 major coronary artery branches. The patients underwent angioplasty and were treated with heparin, isosorbide dinitrate, aspirin and GpIIb/IIIa antagonists. 100 μ L of peripheral blood were labeled for 30 minutes at 4°C with the following antibodies: CD105-FITC (SN6, Serotec, UK), P1H12-PE (Chemicon International, USA) and CD133-PE (293C3, Miltenyi Biotec, Germany). The samples were treated with FACS Lysing Solution (Becton Dickinson, USA) for 10 minutes. Cell fluorescence was analyzed on FACSCalibur (Becton Dickinson, USA) using Cell Quest Software

and 150,000 total events were acquired for each staining. **RESULTS** The percentage of immature CD133+/CD105+ progenitors showed the highest value in AMI patients after 3 days from infarction (0.58%, 0.41% in control subjects). On the other hand the mean percentage of P1H12+ mature endothelial circulating progenitors increased already in the first 24 hours (1.11% in AMI patients versus 0.78% in control subjects) and reached the highest value after 7 days (2.68%). **CONCLUSIONS** The mobilization of immature progenitor cells, defined by the co-expression of both CD133 and CD105 antigens should be considered a primary, rapid and transient response to ischemic myocardial injury. Approaches based on selective therapeutic administration of high dose purified autologous cells could exploit and enhance the mechanism of physiologic endothelial repair occurring in the early phase of AMI.

494 VASCULAR/LYMPHATIC ENDOTHELIAL PROGENITOR CELLS ARE MORE ENRICHED IN HUMAN CORD BLOOD IN EARLY GESTATIONAL WEEKS

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Background and Aims: 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 CD34 or CD133 positive cells derived from cord blood (CB) of mature infant. The hematopoietic stem/progenitor cell like LTC-IC (long term culture-initiating cell) or CFC (colony forming cell) is 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.

Methods and Results: CB were obtained in accordance with institutional guidelines including guardians informed consent. We classified preterm group as gestational age less than 35 weeks (n=16) and term groups as more than 36weeks(n=18). Mononuclear cells (MNCs) of CB were subjected to flowcytometry analysis to examine surface expression of CD34, CD133, VE-cadherin, CD117, 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 are more enriched in preterm CB (P<0.05), which is consistent with phenotypic analysis. In addition, CD34(+) flt-4(+) cells, which are thought to be presumptive lymphatic EPC were also significantly 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. Moreover, CB of preterm infants seems to be a much more robust source for not only vascular EPCs but also lymphatic EPCs.

PLATELET DISORDERS

495 THROMBOPOIETIN (TPO) LEVELS IN HEPATIC PATIENTS WITH THROMBOCYTOPENIA

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Thrombocytopenia is a common problem that complicated the course of liver disease. The aim of present study was to evaluate the role of TPO in the occurrence of thrombocytopenia. Serum TPO levels was determined by ELISA in 40 patients with liver disease (11 with hepatitis C; 10 with liver cirrhosis; 19 with hepatosplenic schistosomiasis), beside 14 normal healthy subjects as a control group. The sTPO levels were unevenly distributed among liver disease subgroups being the highest among HCV group (median 1232.0, range 154.7-2042.0 pg/ml) followed by cirrhosis group (556.5; 342.0-1497.0 pg/ml) and the lowest among schistosoma hepatosplenic group (130.0; 227.0-204.0 pg/ml) ($P < 0.01$). While, sTPO levels in HCV and cirrhotic group were significantly higher as compared to control group (130.0; 22.0- 204.0 pg/ml); its levels in schistosoma hepatosplenic group was insignificantly elevated as compared to control group ($P > 0.05$). There is insignificant negative correlation between sTPO levels and splenic size ($R = -0.3$, $P = 0.043$); but was not with platelet counts ($R = 0.09$, $P > 0.05$). In addition, sTPO levels was significantly higher in patients with platelet counts $\geq 60109/L$ as compared to those

with platelet counts $< 60 \times 10^9/L$ ($P = 0.04$). Using ROC curve at sTPO cut off value ≥ 368 ng/ml, most of HCV and cirrhotic patients have higher sTPO levels (81.8%, 80.0% respectively), while all Schistosomiasis group (100%) had lower sTPO levels. In conclusion, sTPO levels had no role in the occurrence of thrombocytopenia in liver disease patients and that other factors might act. Also the mechanism controlling sTPO levels might be differ in cirrhotic patients than schistosomiasis patients.

496 DECREASED PLATELET ACTIVATION AND ENDOTHELIAL DYSFUNCTION AFTER PERCUTANEUS MITRAL BALLON VALVULOPLASTY

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Objective: This study was conducted to assess the changes in platelet activation and endothelial dysfunction in patients with mitral stenosis (MS) and sinus rhythm (SR) following percutaneous mitral balloon valvuloplasty (PMBV).

Background: Systemic thromboembolism is a serious complication in patients with valvular heart disease, and its incidence is highest in those with mitral stenosis. A hypercoagulable state has also been reported in patients with mitral stenosis and sinus rhythm. A recent study has shown that patients with previous PMBV had a lower incidence of thromboembolism.

[illegible]

497 ADRENALINE AND LYSOPHOSPHATIDIC ACID ACTS SYNERGISTICALLY TO INCREASE PLATELET ADHESION TO ALBUMIN

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Background: There is experimental evidence indicating that lysophosphatidic acid (LPA) is a potentially atherogenic and thrombogenic molecule. Adrenaline is a weak platelet activator that has the ability to increase the effect of other activators. We have previously shown that the combination of LPA and adrenaline synergistically increase platelet aggregation. The response was however highly variable between individuals with some donors not showing any synergistic effect at all.

Aims: The purpose of this study was to examine the occurrence of synergistic activation by LPA and adrenaline on platelets using a newly developed method for measuring platelet adhesion in platelet rich plasma (PRP). We also wanted to characterise signalling pathways important for achieving this synergistic effect.

Methods: Platelet adhesion to albumin and collagen was measured in PRP. Briefly, PRP together with adrenaline and/or LPA were added to wells in microtiter plates, previously coated with albumin or collagen. Platelets were allowed to adhere and the amount of platelet adhesion was detected utilizing the reaction between p-nitrophenyl phosphate and the intracellular enzyme acid phosphatase.

Results: In our system LPA (10 M) alone had no effect on platelet adhesion. However, the combination of LPA and adrenaline (0.1 M) caused a synergistic increase of adhesion to albumin but not to collagen. The synergistic effect of LPA and adrenaline was analysed for a total of 50 healthy donors. The degree of synergy varied between individuals

and 7 of the donors (14%) did not show any synergistic effect at all. The synergistic effect could be blocked by the α 2-adrenoceptor antagonist yohimbine (1 M) and inhibited by the ADP scavenger system creatine phosphate (0.01 M)/creatine phosphokinase (20 U/ml). Conclusions: We conclude that signalling through α 2-adrenoceptors and simultaneous autocrine stimulation by ADP are involved in the LPA/adrenaline-induced synergistic increase on platelet adhesion to albumin. We also confirm our previous findings that the synergistic platelet activation achieved with LPA and adrenaline show considerable individual variability.

498 PLATELET LARGER CELL RATIO (P-LCR) IN PATIENTS WITH DYSLIPIDEMIA

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BACKGROUND: Platelets play an important role in the pathogenesis of thrombosis and atherosclerosis. Activated platelets interact with endothelium and other inflammatory cells by the action of different molecules present on the platelet surface and/or stored in platelets granules, as P-selectin. Platelet volume reflects platelet reactivity and has been suggested as an independent risk factor for ischemic events in cardiovascular disease.

The Sysmex XE-2100 (Sysmex Inc., Kobe, Japan), provides a parameter that refers to the percentage of large platelets - P-LCR- Platelet Larger Cell Ratio.

AIMS: Individuals with dyslipidemia have more tendency to form atherosclerosis plaques with a consequent increasing consumption of platelets. We have shown preliminary results on production of larger platelets by P-LCR determination in a group of patients with lipid profile abnormalities.

METHODS: Forty-one patients samples showing one or more of the following values: total cholesterol > 200 mg/dL, LDL-C > 100 mg/dL, triglycerides > 150mg/dL. Controls: Twenty normal individuals, whose lipid profile and blood cell counts did not show abnormalities.

All the patients and controls were seen at School Hospital of State University of Campinas, Brazil and they had normal platelet counts (between 150 and 400 x10⁹/L).

P-LCR, Platelet Distribution Width (PDW) and MPV were determined by the Sysmex XE-2100. The P-LCR is calculated as a ratio comparing the number of particles detected by impedance between the 12 fl fixed discriminator and the upper discriminator, to the number of particles between a lower and an upper discriminator. The result is represented in %. Differences between groups were evaluated by one-way ANOVA and for assessment of correlations, coefficients of correlation were determined. A level of $p < 0.05$ was considered significant.

RESULTS: VPM, PDW and P-LCR were significantly higher in dyslipidemic patients than in controls ($p < 0.00001$). Correlation between variables (VPM x P-LCR and PDW x P-LCR) were significant in both groups ($p < 0.001$).

CONCLUSIONS: A practical and reliable index of platelet activation has been tested, as measurements of platelet number and size, the tendency to form aggregates and the concentration of released substances stored in platelet granules. A larger MPV is an indicator of in vivo platelet activation and it is increased in vascular diseases as myocardial and cerebral infarction. However, some limitations have been reported in MPV determination: age, gender and number of circulating platelets can influence MPV distribution; as well as anticoagulants used for the collection of blood, storage time and temperature; and the resolution of the cell counters, once minimal alteration in platelet size may be not detected by routine hematologic counters.

We have studied a new parameter - P-LCR - and our results showed that larger platelets are more frequent in patients with abnormalities in lipid profile. Because larger platelets are more reactive, they can contribute to an increased risk for cardiovascular diseases. Further studies will elucidate the reactivity of the larger platelets and the utility of the new parameter in assessing qualitative abnormalities in platelets.

499 MULTIPLE PLATELET FUNCTION DEFECTS IDENTIFIED BY FLOW CYTOMETRY IN A CONSECUTIVE COHORT OF 50 PATIENTS AT DIAGNOSIS OF AML

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In acute myeloid leukaemia (AML) megakaryocytopoiesis is characterized by maturation arrest strongly indicating that functional

defects exist. The aim was to examine platelet function by flow cytometry in a consecutive cohort of patients with AML at diagnosis. Fifty patients with AML and 20 healthy volunteers (HV) were included in this study evaluating the expression of platelet activation markers by three-colour flow cytometry analysis following in vitro stimulation by platelet agonists. To examine whether platelets were activated in vivo, we measured soluble P-selectin (sP-selectin) by ELISA. Results: A significantly lower expression of vWF receptor (CD42b) in resting platelets was found in patients when compared to HV (mean 31.9 17.7 vs. mean 41.2 19.5 MESF x 10exp3; $P < 0.05$). Strong platelet agonist stimulation resulted in significantly lower expressions of platelet membrane activation markers P-selectin (CD62P) (mean 40.2 28.8 vs. 116.029.2 MESF x 10exp3; $P < 0.0001$) and granulophysin (CD63) (mean 12.06.6 vs. 51.022.4 MESF x 10exp3; $P < 0.0001$) in patients compared to HV. The percentage of stimulated platelets expressing the activated fibrinogen receptor (PAC-1) was significantly reduced in patients compared to HV (mean 45%31% vs. 79%25%; $P = 0.0001$). Reduction of CD42b upon stimulation was more pronounced and more homogenous in HV than in patients (mean 70%9% vs. 39%28%; $P < 0.0001$). To study the effect of thrombocytopenia on the expression of platelet membrane activation markers, whole blood from HV was diluted 1:8 in phosphate buffered saline (N=16) to obtain a median platelet count of 25 x 10exp9/L (range 13-63 x 10exp9/L). Strong platelet agonist stimulation resulted in significantly lower expression levels of CD62P ($P < 0.0001$) and CD63 ($P < 0.001$) in patients compared to diluted blood from HV. The plasma levels of sP-selectin (fg/platelet) were not significantly different in AML patients compared to HV indicating that the decreased platelet membrane expression of P-selectin measured by flow cytometry was not a consequence of recent in vivo degranulation. In conclusion, flow cytometry is a feasible method for analysing platelet function in thrombocytopenic patients, demonstrating multiple platelet defects in AML involving adhesion, aggregation and secretion.

500 NO EPISODE OF THROMBOCYTOPENIA AFTER FOUR-WEEK ADMINISTRATION OF FONDAPARINUX, A NEW SYNTHETIC AND SELECTIVE INHIBITOR OF FACTOR XA, IN THE PENTHIFRA-PLUS STUDY

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Type II immunoallergic thrombocytopenia is an important and potentially life-threatening side-effect of heparin therapy. Fondaparinux is a synthetic selective inhibitor of factor Xa. No cross-reactivity between fondaparinux and the antibodies responsible for type II immunoallergic thrombocytopenia, i.e. anti-platelet factor 4/heparin antibodies, was reported. In four studies in patients undergoing major orthopedic surgery, fondaparinux 2.5 mg once daily reduced the incidence of venous thromboembolism (VTE) by 55.2%, as compared with enoxaparin, without significantly increasing the risk of clinically important bleeding. No episode of severe thrombocytopenia was reported in these clinical studies where more than 3500 patients were treated with fondaparinux. However, fondaparinux was administered for 5 to 11 days and therefore the risk of type II thrombocytopenia was limited. In addition, in trials with peri-operative measurement of platelet count and using an active control, it is difficult to determine whether the variations of platelet count observed are related to the surgical context or induced by study drugs. In the randomized, double-blind PENTHIFRA-PLUS study performed in 656 hip fracture surgery patients, 2.5 mg fondaparinux was administered up to four weeks postoperatively: after an initial open-label treatment of fondaparinux for 71 days, patients were randomized to receive fondaparinux or placebo for 212 days. The incidences of all VTE and symptomatic VTE were reduced to very low absolute incidences of 1.4% and 0.3%, respectively, compared to 35.0% and 2.7% with placebo (relative risk reductions: 95.9% and 88.8%, $p < 0.001$ and $p = 0.021$). Blood platelet count was measured at randomization and at the end of the study. No patient experienced a platelet count <100 x 10⁹/L, and the number of patients presenting a drop in platelet count >40%, as compared to the value at randomization, was similar between the two groups (Table). In addition, no cases of white clot syndrome or type II immunoallergic thrombocytopenia were reported. Thus, extended administration of 2.5 mg fondaparinux from one to four weeks did not induce significant decreases in platelet count in comparison to placebo.

	Placebo n/N	Fondaparinux n/N
Patients with platelet count $<100 \times 10^9/L^1$	0/279 (0.0%)	0/283 (0.0%)
Patients with drop in platelet count $>40\%^2$	20/274 (7.3%)	15/274 (5.5%)

¹For patients with value at randomization $\geq 100 \times 10^9/L$ or missing.

²As compared to value at randomization

Caption 1: Table

501 RAPID DETECTION OF INHIBITORS OF THE VON WILLEBRAND FACTOR-CLEAVING PROTEASE (ADAMTS-13) USING A NOVEL SEMI-AUTOMATED ASSAY

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Introduction: The classical clinical entity of thrombotic thrombocytopenic purpura (TTP) has been closely associated with a severe deficiency of the von Willebrand factor (vWF)-cleaving protease (ADAMTS-13). Particularly levels below 5 % are rather specific for TTP. Deficiency of ADAMTS-13 can either be inherited or, much more frequently, caused by specific inhibitory IgG autoantibodies to ADAMTS-13. Since the presence or absence of ADAMTS-13 inhibitors may result in different therapeutic approaches, and inhibitor titers may give insights into the disease activity, a rapid and reliable assay for both ADAMTS-13 activity and ADAMTS-13 inhibitors would be very useful in clinical routine.

Methods Results: We have therefore designed a rapid, sensitive and simple assay for the determination of ADAMTS-13 activity and ADAMTS-13 inhibitors. Set up as a semi-automated assay, the test is based on the measurement of the ristocetin-cofactor activity of purified urea-denaturated vWF concentrate after incubation with diluted patient plasma that has been preactivated with barium chlorid. Concentration- and incubation-finding studies with barium chloride and urea allowed to minimize the time of vWF digestion to only 60 minutes at room temperature (RT). Moreover, by using a calibration curve obtained by serial dilutions of pool plasma with ADAMTS-13-inactivated plasma, discrimination of ADAMTS-13 levels was even made possible in the range of 0-6 %. Extensive mixing studies with patient plasma and normal pool plasma revealed that a relatively short period of only 30 minutes at RT is sufficient but necessary for most samples to detect inhibitors. Some patients, however, may have &#8222slowly acting' inhibitors requiring a longer incubation time. In one case we observed a continuing decrease of ADAMTS-13 activity over a period of 3 hours.

Conclusion: Using this novel assay determination of ADAMTS-13 activity and ADAMTS-13 inhibitors can be performed easily, rapidly, and accurately. At the University Hospital of Zurich, this assay has now been introduced as routine laboratory test, whereby ADAMTS-13 activity and ADAMTS-13 inhibitors are measured simultaneously in order to provide rapid information (turn-around time less than 2 hours) not only about the severity of ADAMTS-13 deficiency but also about its cause. In addition, since the test can discriminate ADAMTS-13 levels even below 6 %, it may help to get further insights into the clinical significance of the severity of ADAMTS-13 deficiency.

502 PLATELET ACTIVATION DURING STRESS IN HYPERHOMOCYSTEINAEMIC RATS

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BACKGROUND: Hyperhomocysteinaemia (HHcy) has been associated with arterial and venous thrombosis especially under stress possibly by causing platelet dysfunction. The molecular mechanisms involved in the platelet activation observed in HHcy are not known. Therefore we

investigated platelet function in rats with mild hyperhomocysteinaemia under stress conditions. **METHODS AND RESULTS:** Stress was induced by free swimming of rats in water at 22 C for 40 min. Platelet aggregation, thrombin-induced formation of malondialdehyde (MDA) and nitric oxide production were studied in control and HHcy rats after stress. Total plasma homocysteine was analyzed by using high-performance liquid chromatography with fluorescence detection. Male rats that consumed an amino acid defined diet containing 8 g of folate per kg of chow (control animals, n=10) or folate-deplete (HHcy, n=10) diet for 6 weeks had total plasma homocysteine concentration of 8.1 ± 0.9 and 22.2 ± 1.1 mmmol plasma folate concentration (5.7 ± 0.6 versus 7.7 ± 1.1 ng/ml) was observed in HHcy versus control animals. After stress platelet aggregation induced by ADP, collagen or thrombin was higher in HHcy versus control rats ($p < 0.05$). There was increase also in thrombin-induced MDA formation (in 48%) by platelets from HHcy rats under stress conditions in comparison with control animals. Basal platelet nitric oxide production was lower in HHcy rats than in control animals. **CONCLUSIONS:** These findings suggest that mild hyperhomocysteinaemia evoked by folate depletion may contribute to the development of thrombosis under stress conditions by inhibiting platelet nitric oxide production and enhanced peroxidation of arachidonic acid with the subsequent increased platelet activation and aggregation.

503 SEVERE CONSTITUTIONAL ADAMTS-13 DEFICIENCY IN CONGENITAL THROMBOTIC THROMBOCYTOPENIC PURPURA PRESENTING WITH APPARENT ADAMTS-13 INHIBITOR DUE TO HEMOGLOBIN IN HEMOLYTIC PLASMA

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Background: Severe ADAMTS-13 deficiency in thrombotic thrombocytopenic purpura (TTP) is either constitutional and caused by ADAMTS13 gene mutations, or acquired and most often due to ADAMTS-13 inhibitory autoantibodies. Non-neutralizing autoantibodies causing increased clearance of ADAMTS-13 from the circulation, or interfering with its interaction with endothelial cells may be additional mechanisms. Although temporary decrease of ADAMTS-13 activity was reported after stimulated release of von Willebrand factor from endothelial cells, the physiological regulation of ADAMTS-13 activity is poorly understood. **Patients and results:** In strongly hemolytic premortem serum of a pediatric patient who had suffered from repeated episodes of TTP since his early infancy, ADAMTS-13 activity was found to be $<3\%$, and $\sim 50\%$ in plasma of his parents. Sequencing of the ADAMTS13 gene revealed a hitherto not described A-G splice site mutation between amino acids 228 and 229, affecting exon 7 which codes for part of the catalytic domain of ADAMTS-13. No other mutation was detected in any of the 29 exons of the ADAMTS13 gene, including their adjacent intron/exon boundaries. The propositus was homozygous for this mutation and his parents heterozygous, confirming constitutional ADAMTS-13 deficiency and congenital TTP. Surprisingly, ADAMTS-13 activity of normal plasma was inhibited by incubation with the propositus serum. This inhibition was not caused by ADAMTS-13 antibodies. Total immunoglobulin purified from serum had no ADAMTS-13 inhibitory effect. In contrast, the immunoglobulin-depleted hemolytic serum continued to be inhibitory indicating a causal role of hemolysis products, primarily hemoglobin. Experiments involving incubation of normal plasma with hemoglobin, recombinant and from lysed erythrocytes, revealed a concentration- and temperature-dependent ADAMTS-13 inhibitory capacity of the hemoglobin molecule which started from a concentration of ~ 2.5 mg/mL and was more pronounced with increasing hemoglobin concentration. The inhibitory effect of hemoglobin depended on the incubation temperature, and was maximal at 37C but markedly reduced at 4C.

Conclusions: Although no clear genotype-phenotype correlation has so far been observed in congenital TTP, the described splice site mutation is apparently associated with early-onset and frequently relapsing TTP. Abnormal splicing most likely led to a frame shift and premature termination of the polypeptide. Inhibition of ADAMTS-13 in normal plasma could be observed at relatively low hemoglobin concentrations that may occur during in vivo-hemolysis. It would therefore seem important to determine whether this may contribute to microvascular occlusion in TTP, or whether it is first of all an in vitro-phenomenon. A practical conclusion that can be drawn from these observations is that results of an immunoglobulin inhibitor screening will not be valid in hemolytic samples, since very likely false positive. The finding of an

ADAMTS-13 inhibitory capacity of the hemoglobin molecule might in addition be representing structural homologies with a hypothetical physiological inhibitor of ADAMTS-13, and thereby facilitate its identification. This would, however, require the localization of the inhibitory capacity to specific structural elements of the hemoglobin molecule, as well as the identification of the ADAMTS-13 inactivating mechanism.

504 THROMBOTIC THROMBOCYTOPENIC PURPURA: 24 YEARS OF EXPERIENCE AT THE AMERICAN UNIVERSITY OF BEIRUT MEDICAL CENTER

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BACKGROUND

Thrombotic thrombocytopenic purpura (TTP) is a hematological syndrome defined by the presence of thrombocytopenia and microangiopathic hemolytic anemia without a clinically apparent etiology. Patients may also suffer from fever in addition to neurologic and renal impairment. Treatment should be initiated as soon as possible otherwise this rare disease can be fatal. The main treatment options include therapeutic plasma exchange, fresh frozen plasma infusion and adjuvant agents such as steroids and antiplatelet drugs.

STUDY DESIGN AND METHODS

A search of patient records was carried out at the American University of Beirut Medical Center looking for patients who developed TTP over a 24-year period extending from 1980 to 2003. Relevant information was collected and analyzed.

RESULTS

A total of 47 records were found. All presented with anemia and thrombocytopenia, 61.7% had fever, 83% had neurologic symptoms and 34% had renal impairment. All patients were treated with a multimodality regimen including therapeutic plasma exchange, FFP infusion, steroids, antiplatelet agents, vincristine and others. 38 (81%) cases achieved complete remission. Out of these, 12 (31.6%) relapsed and responded to treatment. Patients who did not receive plasma exchange were more likely to relapse ($p=0.032$). A second relapse was observed in 6 cases. The overall mortality rate from TTP over 24 years was 21.3%.

CONCLUSIONS

TTP remains a fatal disease. A high index of suspicion should therefore always be present. Treatment options should be further developed and patients should directly be referred to tertiary care centers.

505 TREATMENT OF MULTIPLE RELAPSING THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP) WITH AZATHIOPRINE

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TTP is a microangiopathic disease characterized by the lack of functional activity of vonWillebrand-cleaving protease (ADAMTS-13). Patients with the classic form of TTP have less protease levels while in sporadic form, recent studies suggest the presence of an IgG autoantibody against metalloprotease. This data support the biologically plausible role of immunosuppression in TTP. Conventional treatment with plasmaexchange plus steroids increases patient survival but, despite this, relapses may occur in, as many as, 40% of cases. We evaluated the efficacy of treatment with azathioprine (AZA) in four patients with relapsing TTP.

Methods. From 1996 to 2003 we treated 21 patients (6 males and 15 females) with median age of 34 years (25-63). All patients were treated with plasmaexchange (PEX) plus steroids (1 mg/Kg) and ASA (when platelet count was $> 30 \times 10^9/L$). Azathioprine (1mg/Kg) was started at the time of the third relapse and in those patients in which relapse criteria were satisfied. Relapse was defined as the recurrence of any of the following conditions: recurrence of initial symptoms, microangiopathic haemolytic anemia with presence of fragmented erythrocytes and elevated lactate dehydrogenase (LDH), thrombocytopenia (platelet count $< 100 \times 10^9/L$) and creatininemia $< 2mg/dl$. We evaluated patients follow-up in a minimum of six months after AZA treatment starting.

Results. Four patients (2 males and 2 females) presented three relapses (see table). All of them were treated with standard protocol (PEX plus steroids and ASA) and with AZA. ADAMTS-13 activity and presence of inactivating inhibitor were measured in these four patients. All of them had

a reduced metalloprotease activity (normal range: 46-168%) and anti-ADAMTS-13 autoantibodies were found in three of these. All four patients have maintained remission from 6 to 10 months without recurrence and without any side effect. In this period blood count parameters, LDH levels were normal with absence of clinical symptoms. Relapsing TTP remains a problem in terms of management. Given recent evidence that this disease is antibody-mediated, therapies based on immunosuppression seem logical. Our study, even if a short follow-up, shows an important promise for azathioprine which may have a role in inducing long-term remission in relapsing TTP.

Case	Patients	Diagnosis (HUS)	Age (years)	No. of relapses	Underlying condition	Concomitant drug treatment	AZA treatment	Outcome	Follow-up (months)
1	1	2002	40M	3	—	Plasma exchange	Relapsing TTP	remission	6
2	1	1998	30M	3	—	Plasma exchange	Relapsing TTP	remission	6
3	1	1998	30F	3	—	Plasma exchange	Relapsing TTP	remission	6
4	1	2002	30F	3	—	Plasma exchange	Relapsing TTP	remission	10

Caption 1:

COST EFFECTIVENESS & QUALITY OF LIFE

506 RESULTS OF A DIGITAL WEB BASED EXERCISE FOR UK NEQAS(H), BLOOD CELL AND BONE MARROW MORPHOLOGY SCHEME.

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In collaboration with UK NEQAS(H) and local universities we have conducted pilot studies to explore the use of digital imaging for blood cell morphology utilising the www. We now present results from a fourth national exercise. Participants of UK NEQAS(H), General Haematology Scheme were invited to log on to www.manlab.co.uk or via NEQAS(H) site) to review a blood film Case 0301BF1, of myelodysplastic syndrome with megakaryoblastic features and unusually complex morphology. Digital images were presented in three formats.

Part 1 had 12 single static images with expert morphological comment and participants consensus comments from the original national survey using a traditional glass slide preparation. Part 2 provided four images with an interactive educational tutorial in which participants were asked to identify specific cells using a the UK NEQAS(H) given coding list for blood films. Part 3, was a composite of nine Stitched images prepared using Panavue Image Assembler with software features that allowed movement across the image and zoom facility. Of 426 participants 128 (30%) returned completed questionnaires and results sheets to UK NEQAS(H).

Part 1: Over 90% of responders were positive on questions regarding image quality, access to the site, presentation of expert opinion and agreed that the single images represented the morphological features they had originally reported from their glass slide.

Part 2: Over 95% responded positively to the educational element and commented that the tutorial element was a useful tool.

Part 3: Of the 128 responders 34 were unable to download the software necessary to view the Stitched image due to their Trusts policy or firewall. Of the remaining 94 responders 85 thought image quality adequate and representative. A high proportion (71%) thought the composite Stitched image offered advantages over single static images, including ease of navigation, overview of morphology and control of magnification. Other comments included insufficient area of view, and that the stitched image complemented the single images.

This positive feedback supports our intention to post images of all six annual blood film surveys on the www together with expert comment and the top five features reported by participants. We aim to expand the tutorial aspect by utilising specific surveys to highlight interesting cases. Whilst software problems are to be addressed, the development of 'Stitched' images into a virtual slide is ongoing. The potential educational aspect of digital morphology is creating keen interest and future development will provide a mechanism for continuing professional development as part of external quality assessment and education.

507 DIFFERENCES IN TREATMENT PRACTICE, RESPONSE RATES AND COST OF EPOETIN ALFA AND DARBEPOETIN ALFA TREATMENT FOR ANEMIC CANCER PATIENTS: A RETROSPECTIVE ANALYSIS FROM SWEDEN

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BACKGROUND: A historically, standard therapy for both acute and chronic anemia has been red blood cell (RBC) transfusions. An effective alternative to frequent RBC transfusions for the treatment of anemia in patients with cancer is the administration of an erythropoiesis-stimulating agent. Epoetin alfa (EPREX or ERYPO) and Darbepoetin alfa (Aranesp), are two alternatives to increase Hb levels, reduce transfusion requirements, improve quality of life (QOL) in patients with solid or nonmyeloid hematologic cancers who develop anemia during chemotherapy. An improved understanding of which patients to treat, when to initiate treatment, and response rates at different Hb levels are important factors in achieving optimal use of these agents in anemic patients with cancer. **PURPOSE:** To document and compare actual treatment practices, outcomes (hematopoietic response rates, Hb changes, transfusions, hospital care), and costs of Epoetin alfa and Darbepoetin alfa in the treatment of chemotherapy-related anemia in cancer patients in Sweden. **METHOD:** A retrospective chart review was performed at three Swedish hospitals. A total of 59 patients with cancer who developed chemotherapy-related anemia and received erythropoiesis-stimulating agent treatment were identified: 29 patients initially received Epoetin alfa and 30 patients initially received Darbepoetin alfa. Data were collected on dosage and duration of treatment with these agents, hemoglobin (Hb) response measures, red blood cell transfusions, and health care resource consumption and analyzed at follow-up time points of 28, 56, 84, and 112 days. **RESULTS:** A significantly faster Hb response and increase in Hb levels was observed in patients treated with Epoetin alfa compared to patients treated with Darbepoetin alfa. The results also suggest that lower dosages are used in actual clinical practice than recommended in Swedish treatment guidelines. While the costs are in favor of Epoetin alfa, they are not significantly different for the two treatment groups. No significant difference in resource utilization or in health care costs between Epoetin alfa- or Darbepoetin alfa-treated patients were found. At a follow-up of 112 days, the mean treatment cost per patient was SEK74,701 (approximately US\$9,800 or €8,300) with Epoetin alfa and SEK85,285 (approximately US\$11,000 or €9,500) with Darbepoetin alfa. Drug acquisition and administration costs accounted for 81% and 67% of total costs for Epoetin alfa- and Darbepoetin alfa-treated patients, respectively, with remaining costs accounted for by hospitalization and transfusions. **SUMMARY/CONCLUSIONS:** Epoetin alfa was associated with a higher hematopoietic response rate, a significantly faster Hb response, and significantly higher increase in Hb levels compared with Darbepoetin alfa, as used clinically. While the costs are in favor of Epoetin alfa, they are not significantly different for the two treatment arms. The findings also suggest that lower dosages of both agents are used in actual clinical practice than recommended in Swedish treatment guidelines.

508 INCREASING INCIDENCE OF LYMPHOPROLIFERATIVE DISORDERS IS NOT ASSOCIATED WITH SICK LEAVE DUE TO DEPRESSIVE DISORDERS

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Background: A continuously increasing incidence of lymphoproliferative diseases, such as NHL (Non-Hodgkins lymphomas), mainly aggressive NHL, has been described from many countries. The reasons for this are unknown, but identified risk factors deals with altered or impaired immune functions. Multiple interactions between immune, nervous and hormonal systems have been identified, although their clinical relevance is unclear. Psychiatric diseases, mainly depression, is a large health problem. Few studies deals with the possible health effects of depression on the risk of developing malignant disease.

Aim of the study: The aim of the study was to see if depressive disorders through a potential effect of the immune system might explain part of the increase in incidence of lymphoproliferative diseases, mainly NHL.

Methods: All persons reported for an episode of sick leave for more than two weeks or receiving temporary disability pensions because of a psychiatric disease (ICD 300 to 360 according to ICD 7) since 1988 were identified by their individual ten digit personal number and the first episode of sick leave. A final cohort of 87.677 individuals put on the sick list between 1988 and 2000 with a medical certificate reporting depressive disease for more than two weeks were followed until a diagnosis of lymphoproliferative disease by linking to the Swedish Cancer registry for lymphoproliferative malignancies (ICD-code 200+2+2, 201 and 204 according to ICD 7), death, emigration or end of follow-up period (Dec 31st 2000). There were 373.135 years of follow up. Individuals identified as treated on an in-patient basis for psychiatric disease were excluded. The ratios of observed to expected numbers (SIR) served as a measure of the relative risk.

Results: Overall, there were 1673 cases of malignancies compared to 1649 expected, which yielded a SIR of 1.01 (CI 0.97-1.06). Totally 80 cases of NHL, CLL (Chronic Lymphocytic Leukaemia) and HL (Hodgkins Lymphoma) in the cohort which corresponded with the 70 cases expected. 65 cases of NHL were identified in the cohort along with 4 cases of HD and 11 cases classified as CLL. For NHL, an overall SIR of 1.27 (CI 0.98-1.62) was found. In a further stratification, latency periods of <1, 1-5 and >5 years were analysed. The SIR for men in the latency period of less than one year showed a SIR of 2.84 (CI 1.30-5.40), which might be due to the fact that the sick leave was caused by symptoms of the tumour rather than depression. For HL overall the SIR was 0.58 (CI 16-1.49). The SIR overall for CLL was 0.95 (CI 0.47-1.69). The results of analyses separating men and women showed results close to the expected.

Conclusions We could not show an excess risk of lymphoproliferative diseases among individuals with one or more episode of sick leave due to depression. It seems unlikely that an increasing incidence of depressive disorders have had an impact on the incidence of lymphoproliferative diseases.

509 CLINICAL RESEARCH IN THE GERMAN COMPETENCE NETWORK "ACUTE AND CHRONIC LEUKEMIAS"

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Leukemias are a challenge and a cost factor to society because of their frequency in all age groups. They also serve as a model for a variety of diseases and possess exemplary relevance for basic research and patient care. Leukemia research and therapy have achieved high standards and even a leading position in Germany with regard to clinical trials, standardization of diagnostics and molecular studies of signal transduction and gene expression. Progress is hampered, however, by fragmentation of leukemia trial groups, diagnostic approaches and treatment research activities. A network was therefore created to integrate the leading leukemia trial groups (CML, AML, ALL, MDS, CMPD) and their interdisciplinary partners (diagnostics, treatment research, biometry) in cooperation with basic research and pharmaceutical industry to foster advancements in leukemia-related research and health care. Through promotion of treatment optimization trials and prognosis research, application of advanced genomics and telematics to therapeutic progress and of translational research, introduction of standards for diagnostics, and treatment procedures, and development of evidence-based guidelines. Now the network covers over 1400 physicians and scientists in more than 400 institutions. Scientific highlights of accomplished work include establishment of information, communication and management structures, creation of an AML intergroup comprising five study groups, formation of a MDS study group and establishment of platforms for standardization of diagnostics, genomics and proteomics, and medical informatics. Exchange of scientific progress is mediated by intra- and internet, biannual network newsletters, regular project group meetings and annual network symposia. Already now, the network structures leukemia therapy and research in Germany supports the spread of scientific excellence in the field of leukemias. Lastly, because of the model character of the leukemias, advances in understanding gained are directly applicable to many other tumors.

510 CLINICAL SIGNIFICANCE OF DIFFERENCES AND CHANGES IN QUALITY OF LIFE SCORES: EVALUATION OF 424 MULTIPLE MYELOMA PATIENTS COMPLETING THE EORTC QLQ-C30 QUESTIONNAIRE

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Background: In clinical studies with large sample sizes, small differences in QoL scores may attain statistical significance without necessarily being clinically important.

Aims: To evaluate the clinical significance of 1) differences in QoL scores between newly diagnosed multiple myeloma patients and a reference population, and 2) changes in QoL scores after the initiation of treatment.

Methods: 221 patients <60 years treated with high dose chemotherapy and autologous stem cell transplantation and 203 patients >60 years treated with melphalan and prednisone in two trials carried out by the Nordic Myeloma Study Group (NMSG) completed the EORTC QLQ-C30 questionnaire at diagnosis and during follow-up. Their scores were compared to an age and sex adjusted Norwegian reference population (N=3000), using a multiple regression model. The magnitude of the score differences was evaluated A) as effect size, i.e. mean score differences divided by the standard deviation of the scores of the patient population (0.2-0.5 is conventionally considered small, 0.5-0.8 moderate, and 0.8 and above large); B) by 'clinical evaluation' as large (> 20 on the 0-100 scale), moderate (10-20) or small (5-10) according to data obtained by interviewing breast cancer patients; and 3) by calculating the percentage of the patients with scores similar to or worse than the 10th percentile of the reference population.

Results: 1) At diagnosis, multiple myeloma patients had a statistically significant impairment of all mean scores on the EORTC QLQ-C30 questionnaire except for diarrhea. The functioning scores most severely affected were physical functioning, role functioning and global QoL, while pain, fatigue and appetite loss were the most prominent symptoms. All these score differences were high or moderate, and there was excellent agreement between the rating methods based on effect sizes and clinical evaluation (Table 1). The proportion of patients that scored below the 10th percentile of the reference population was 67% for physical functioning and 43% for global QoL, and 46% and 33% for pain and fatigue, respectively. 2) During the first 12 months of treatment, there was a moderate score improvement for physical functioning, role functioning, global QoL and pain and a small improvement for fatigue, with complete concordance between the rating methods. Statistically significant improvement was also found for other QoL domains, but the effect size for these score changes was below 0.2 and they were also below the threshold for small differences by clinical evaluation (i.e. <5 on the 0-100 scale). The proportion with scores worse than the 10th percentile of the reference population was reduced to 48% for physical functioning, 20% for global QoL and 17% for pain and fatigue.

Conclusions: When reporting the results of QoL studies, it is not sufficient to present the magnitude and statistical significance of score differences. Although the methods used in this study have limitations, one should attempt to evaluate the clinical importance of QoL score differences. As for other end-points in clinical studies, a statistically significant improvement in QoL scores does not in itself warrant the conclusion that one treatment modality is superior to another.

511 WEB-BASED EUROPEAN EDUCATION TRAINING AND ACCREDITATION PROGRAM IN HEMATOLOGY: LEONARDO DA VINCI EC PILOT PROJECT (AGREEMENT NUMBER: 2002 4518 / 001 001)

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On behalf of European Hematology Association (EHA) and European School of Haematology (ESH).

The European Education, Training and Accreditation in Hematology online (EurETAH-on-line) project was designed to provide a readily accessible and constantly updated framework for Training, Education and Continuing Medical Education to scientists and clinicians involved in the field of hematology. It has four objectives:

- a) to develop a technically certified internet based area;
- b) to upload peer-reviewed scientific information;

c) to satisfy the need for a pan-European tool for harmonized, high-level Continuous Education and Training in Hematology;

d) to offer novel opportunities for exchange of knowledge among peers and for transfer of knowledge between junior and expert practitioners throughout an expanding Europe.

At the end of the project's first year (December 2003), the platform became freely available on the EHA and ESH websites. The public platform is available on internet: www.dcsweb.org. Since the December 17 on-line launch, more than 1500 connections and an estimated 894 single visitors have been registered.

This project has triggered a new mode of collaboration between medical doctors by integrating knowledge, skills and data within a software project. The process of collecting, editing, adapting and publishing hematology case studies has led to an improved understanding of doctors needs and how to effectively communicate scientific updated contents to them. The process has been repeated many times and has proved to be robustly efficient. The procedures for scientific content validation include a peer review system similar to the one in use for publications in high level international scientific journals. This peer review system relies on a pool of reviewers selected for their scientific expertise and not otherwise involved in the development of the project.

The training tool content presently includes 8 fully reviewed clinical cases, while others are under revision. Furthermore, the references listed for each clinical case are linked to the Medline website.

The Education tool currently includes: 1) a template for a morphological database containing all the images related to the edited clinical cases; 2) a list of the main on-line hematology-related scientific journals; 3) a glossary of the hematological terms used within the training tool.

Before the end of this project (December 2004) the web will be implemented with a new scientific content and interactive tests will be uploaded within the new Accreditation tool. Public users will be able to request a personal identification password to obtain European continuing medical education credit points for the time spent on EurETAH to improve their knowledge and skills.

512 THE COST-EFFECTIVENESS OF EPOETIN ALFA (EPREX) AND DARBEPOETIN ALFA (ARANESP) IN THE TREATMENT OF CHEMOTHERAPY-RELATED ANEMIA

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Background: Epoetin alfa is effective in increasing hemoglobin (Hb) in anemic cancer patients receiving chemotherapy. Darbepoetin alfa has a similar claim, and without comparative data it has been suggested that darbepoetin alfa is more cost-effective.

Aims: Using pooled clinical trial data from published and previously unpublished sources (Kurz 1997, Littlewood 2001, Dammacco 2001, Vansteenkiste 2002, Hedenus 2002), we determined the probability that epoetin alfa is more cost-effective than darbepoetin alfa at approved dosages from the perspective of national health insurance plans in the UK and France and a private health insurance plan in the US.

Methods: We reviewed Hb response (greater than/equal to 2-g/dL increase in Hb or Hb greater than/equal to 12 g/dL, unrelated to transfusions) to epoetin alfa and darbepoetin alfa reported for cancer patients from 3 epoetin alfa and 2 darbepoetin alfa multicenter, randomized, double-blind, placebo-controlled studies. We chose Hb response as our effectiveness measure because, unlike transfusion rates, it is not prone to differences in practice patterns. Darbepoetin alfa was administered at a starting dose of 2.25 mcg/kg/week; dose was doubled to 4.5 mcg/kg/wk if Hb had not increased >1.0 g/dL by week 6. Epoetin alfa was administered at a starting dose of 150 IU/kg 3 times/week; dose was doubled to 300 IU/kg if Hb had not increased >1.0 g/dL by week 4. Relative cost-effectiveness is determined by a Bayesian simulation model run over a 12-week treatment period that calculates the probability that epoetin alfa is more cost-effective than darbepoetin alfa, given that a payer is willing to pay for darbepoetin alfa. Among other sensitivity analyses, variances have been inflated by a factor of 2 to reflect additional uncertainty due to differing trial characteristics.

Results: Pooled Hb response to darbepoetin alfa was 65% vs. 24% for placebo (net response: 41%). Hb response to epoetin alfa was 74% vs. 24% for placebo (net response: 50%). 43% of darbepoetin alfa patients and 25% of epoetin alfa patients required dose-escalation. Epoetin alfa patients avoided a mean of 0.87 units of red blood cells (RBC) transfused, while darbepoetin alfa avoided a mean of 1.24 RBC transfusion units.

Based on unit cost of drug, available packaging, percent of patients requiring dose escalation, the number of RBC transfusion units avoided, and the cost of a physician office visit for drug administration (US only), mean 12-week per patient cost for darbepoetin was 3,553, 5,819, and \$11,193 (UK, France, and the US, respectively) and for epoetin alfa was 3,486, 5,161, and \$6,697. The probability that epoetin alfa is more cost-effective than darbepoetin alfa is 0.9207, 0.9787, and 0.9999 (UK, France, and the US, respectively). By contrast, the probability that darbepoetin alfa is more cost-effective than epoetin alfa is only 0.0813, 0.0137, and 0.0000 for these 3 countries.

Conclusions: Based on available data, it is far more likely that epoetin alfa is more cost-effective than darbepoetin alfa than vice versa in these 3 countries at approved doses. Head-to-head trials are necessary to confirm these findings.

513 EVIDENCE-BASED, NOVEL COMPARISON BETWEEN EPOETIN ALFA, EPOETIN BETA, AND DARBEPOETIN ALFA BASED ON DRUG USE, EFFICACY AND TREATMENT COSTS IN DAILY ONCOLOGICAL CLINICAL PRACTICE

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Background: The overall goal in the treatment of tumor/chemotherapy-induced anemia is the correction of lowered hemoglobin (Hb) levels as well as improvement in Quality of Life. For more than 10 years, anemia in cancer patients has been treated with a standard dosage of 10,000 IU epoetin alfa/beta three times weekly. In August 2002 darbepoetin alfa (dosed in g) was registered in Europe for the oncological indication with a new, and at first sight, more convenient once weekly dosage regimen.

Aims: This analysis is intended to confirm whether epoetin alfa/beta and darbepoetin alfa are equivalent in dosing and efficacy as well as in treatment costs with regard to the oncological indication.

Methods: The overall goal of this analysis was to compare epoetin alfa, epoetin beta, and darbepoetin alfa in daily clinical practice. Data related to prescriptions and treatment costs of all health-insured people, in a specific region in Austria, within a one-year time period (Oct. 2002 Sept. 2003) were gathered from the comprehensive customer-related database of the Local Austrian Public Health Insurance Association.

Results: The data analysis (n = 269) showed that anemia therapy with darbepoetin alfa increased treatment duration and/or EPO dosage, translating into a much higher overall cost of treatment (3,882.20) compared to standard epoetin treatments (3,409.47). These results also hold true after taking into consideration additional treatment variables such as early drop outs in each of the three groups. The results were also compared with retrospective Hb levels for patients included in this analysis in order to exclude the assumption that different relative Hb-increases might have resulted in different treatment costs.

Conclusion: In this evidence-based statistical analysis from the Austrian Local Public Health Insurance Association, assuming that darbepoetin alfa 150 g once weekly is not equivalent in efficacy to the standard treatment of epoetin alfa/beta 10,000 IU three times weekly, darbepoetin alfa leads to longer treatment duration and/or dosage increase resulting in an overall increased cost of therapy. Quality control precautions are highly recommended and will be executed prospectively for epoetin alfa-, epoetin beta-, and darbepoetin alfa-treated cancer patients in an ongoing Austrian EPO evaluation project.

514 EVALUATION OF THE COGNITIVE EFFECTS OF ONCE-WEEKLY EPOETIN ALFA IN ANEMIC PATIENTS WITH HEMATOLOGIC CANCERS RECEIVING CHEMOTHERAPY: PRELIMINARY RESULTS OF THE EPOLYM TRIAL

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Background: Chemotherapy-associated cognitive dysfunction has received relatively little attention despite the negative impact it can have on patients' social, educational, and professional activities, as well as their general quality of life (QOL). Clinical evidence suggests an association between chemotherapy-related decreases in hemoglobin (Hb) level and an increased risk of cognitive dysfunction.

Aims: To assess the impact on cognitive dysfunction of correcting Hb levels with a once-weekly (QW) regimen of epoetin alfa in anemic patients receiving chemotherapy for non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), Hodgkin's disease (HD), and chronic lymphocytic leukemia (CLL).

Methods: EPOLYM is a prospective, international, multicenter, open-label, Phase IIIb trial in anemic (Hb ≤ 12.0 g/dL) patients receiving chemotherapy (planned N = 1000). Epoetin alfa therapy was initiated at a dose of 40,000 IU QW administered subcutaneously, with dosage adjustments (increases or decreases) to be made based on clinical response (target Hb, 11.5-13.0 g/dL). Cognitive function was evaluated from baseline to Week 12 using the Cognitive Drug Research (CDR) Computerised Cognitive Assessment System. This system included tasks of attention (Simple Reaction Time, Choice Reaction Time, Digit Vigilance), working memory (Numeric Working Memory), and secondary memory (Immediate and Delayed Word Recognition, Picture Recognition). Two factors, Power of Attention and Picture Recognition-Sensitivity Index, were calculated as additional measures of attention and secondary memory (Picture Recognition). Because of the relationship between affect and cognition, depression and anxiety were assessed at the time of cognitive assessment using the Hospital Anxiety and Depression Scale (HADS). Changes from baseline in Hb levels, transfusion requirements, and QOL (Functional Assessment of Cancer Therapy-Anemia [FACT-An]; Linear Analog Scale Assessment [LASA]) were also evaluated (transfusion and QOL results not reported here).

Results: Preliminary (unscheduled) analyses were performed on the cognitive data for 383 patients. Cognitive function for all diagnostic groups was significantly poorer at baseline compared to age-matched healthy volunteers. Performance on attention tasks was slightly impaired over the duration of the study; however, the effect was small and not considered clinically meaningful. Improvement in memory was observed in all 4 diagnostic groups. Speed of memory improved by a mean 350 msec; combined speed of memory scores (numeric, word recognition, picture recognition) substantially improved 8% by Week 8. A slightly larger effect for memory improvement (mean 410 msec) was observed in the MM group, even though this group had the poorest scores at baseline. Anxiety and depression, which had HADS scores near the high normal range at baseline, improved slightly during the study. The clinical improvement in cognitive function was associated with an increase in Hb from 10.5 g/dL at baseline to ~ 12 g/dL beginning at Weeks 8-12 and sustained for up to 24 weeks.

Conclusion: Results showed improved cognitive function in anemic patients with NHL, MM, HD, and CLL. This improvement was associated with an increase in Hb level achieved with QW epoetin alfa. These findings are based on preliminary results and will need to be confirmed by findings from the full study cohort.

515 EPOETIN ALFA IMPROVES HEMOGLOBIN LEVELS AND QUALITY OF LIFE PARAMETERS IN ANEMIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES RECEIVING CHEMOTHERAPY: INTERIM RESULTS FROM THE EPOLYM TRIAL

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Background: Many patients with Hodgkin's disease (HD), non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM) are anemic from the disease and/or chemotherapy. Because cancer- and chemotherapy-related anemia is associated with fatigue, decreased cognitive function, and other symptoms of diminished quality of life (QOL), treatment of anemia is important.

Aims: To evaluate clinical outcomes (QOL, Hb levels, and transfusion requirements) of correcting anemia with a once-weekly (QW) regimen of epoetin alfa in patients with HD, NHL, CLL, or MM receiving chemotherapy.

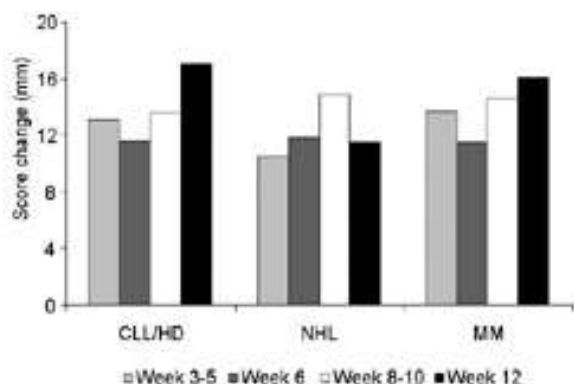
Methods: EPOLYM is an international, multicenter, open-label, Phase IIIb trial in anemic (hemoglobin [Hb] ≤ 12 g/dL) cancer patients receiving chemotherapy. Patients received epoetin alfa 40,000 IU QW subcutaneously to a target Hb of 11.5-13.0 g/dL, with dosage adjustments (increases or decreases) based on clinical response. QOL was evaluated from baseline to Week 12 by the Linear Analog Scale Assessment (LASA; also known as the Cancer Linear Analog Scale [CLAS]) for Energy Level, Daily Activities, and Overall QOL; and the Functional Assessment of Cancer Therapy-Anemia (FACT-An). Changes in cognitive function and mood from baseline to Week 12 were assessed with the Cognitive Drug Research Computerised Cognitive Assessment System. Changes from baseline in Hb level and transfusion requirements were also evaluated (transfusion requirements not reported).

Results: Interim data analyses are available for 382 patients: 165 NHL, 106 MM, 58 HD, and 53 CLL. QOL data are available from the LASA and preliminary cognitive function evaluations. Mean baseline LASA

scores ranged from 44.549.1 mm (normal is 70100 mm) indicating that all patients had large deficits in QOL at study initiation. Improvement in LASA scores was achieved in all diagnostic groups at all evaluations (i.e., Weeks 35, 6, 8, and 12). Mean changes at Week 12 for the 4 diagnostic groups combined were clinically significant [1]: Energy Level 12.7 mm, Daily Activities 10.1 mm, Overall QOL 14.3 mm. Changes in Overall QOL for individual diagnostic groups during treatment are shown in the figure. Performance on working and secondary memory tasks improved during the study by Week 8. Improvement in QOL parameters was associated with increases in Hb from 10.5 g/dL at baseline. Mean Hb increased 1.02 g/dL at Weeks 3-5 and 2.02 g/dL at Weeks 15-16. The greatest Hb increase was seen in MM patients (2.5 g/dL), who also had the lowest baseline Hb (10.0 g/dL).

Conclusion: Treatment with epoetin alfa resulted in improved QOL parameters and cognitive function in anemic patients with HD, NHL, CLL, and MM. This improvement was associated with increases in Hb attained with epoetin alfa 40,000 IU QW.

[1] Patrick DL, et al. Eur J Cancer 2003;39:335-345



CLL: chronic lymphocytic leukemia; HD: Hodgkin's disease; MM: multiple myeloma; NHL: non-Hodgkin's lymphoma; QOL: quality of life.

Caption 1: Overall QOL: Mean Change by Diagnosis

516 EPOETIN ALFA IMPROVES QUALITY OF LIFE IN CANCER PATIENTS: RESULTS OF A META-ANALYSIS

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Background: Anemia is common in cancer patients, particularly in those receiving chemotherapy. Numerous clinical trials have demonstrated that epoetin alfa significantly increases hemoglobin (Hb), reduces transfusions, and improves quality of life (QOL) in cancer patients receiving chemotherapy. Despite the high prevalence of anemia in this patient population and the high correlation between low Hb levels, poor performance status, and reduced QOL, many cancer patients do not receive anemia treatment.

Aims: To evaluate and combine the evidence from clinical trials for the impact of epoetin alfa on cancer patients QOL.

Methods: Medline, the Cochrane Library, and other databases were searched for published and unpublished randomized/controlled and single-arm studies that met quality inclusion criteria, included greater than/equal to 1 arm receiving epoetin alfa, and administered at least 1 of the following QOL instruments: Cancer Linear Analog Scale (CLAS), Functional Assessment of Cancer Therapy (FACT) scale, Eastern Cooperative Oncology Group (ECOG) scale, and/or Medical Outcomes Study Short-Form 36 (SF-36) scale. Pre/post analyses of QOL results from the epoetin alfa cohorts were compared with those from the control cohorts.

Results: The database search yielded 11,459 patients from 23 trials fulfilling the inclusion criteria for this meta-analysis. At baseline, the epoetin alfa and control cohorts were indistinguishable in terms of demographic, clinical, and QOL characteristics. The analysis demonstrated statistically significant beneficial effects of epoetin alfa on patients QOL across a number of scales; the most definitive results were observed in the cancer-specific subscales focused on anemia (Energy and Vitality). The improvement from baseline in epoetin alfa cohorts for

the 3 CLAS subscales was significant ($P < .05$) and substantial (20%-25%). Similarly, epoetin alfa-treated patients experienced statistically significant improvements in mean change from baseline FACT scores, whereas control patients remained unchanged or worsened. The most dramatic changes in epoetin alfa-treated patients were observed in the FACT-Fatigue (17%) and FACT-An (12%) subscales. Whereas no significant change in ECOG score from baseline was observed in epoetin alfa-treated patients, control patients worsened significantly ($P < .05$). Four SF-36 subscales (Vitality, Physical Function, Role Physical, and Social Function) improved significantly in epoetin alfa patients ($P < .05$). After adjusting for confounding factors and placebo effects, the observed improvements in QOL remained statistically significant and substantial (Table 1).

Summary/conclusions: In cancer patients receiving chemotherapy, the impact of both disease and treatment can be severe. Anemia-associated reductions in patients QOL are common. Results from clinical trials have demonstrated that epoetin alfa corrects cancer-related anemia and significantly improves QOL in cancer patients receiving chemotherapy. The pooling of these results in the current meta-analysis confirms that epoetin alfa has a consistent, significant, and positive impact on improving QOL in cancer patients, and highlights the difference in QOL between epoetin alfa-treated and control patients. The results further emphasize the need to manage anemia in this patient population.

Adjusted estimates of differences in mean QOL change scores from baseline (epoetin alfa vs control)

QOL measure	Overall mean (95% CI)	CLL/HD	NHL	MM
CLAS energy	12.7 (10.1, 15.3)	13.0 (10.0, 16.0)	10.0 (7.0, 13.0)	13.0 (10.0, 16.0)
CLAS activities	10.1 (7.5, 12.7)	11.0 (8.0, 14.0)	8.0 (5.0, 11.0)	11.0 (8.0, 14.0)
CLAS overall	11.4 (8.8, 14.0)	12.0 (9.0, 15.0)	9.0 (6.0, 12.0)	12.0 (9.0, 15.0)
FACT fatigue	-17.0 (-19.0, -15.0)	-18.0 (-20.0, -16.0)	-16.0 (-18.0, -14.0)	-17.0 (-19.0, -15.0)
FACT anxiety	-12.0 (-14.0, -10.0)	-13.0 (-15.0, -11.0)	-11.0 (-13.0, -9.0)	-12.0 (-14.0, -10.0)
FACT social	-10.0 (-12.0, -8.0)	-11.0 (-13.0, -9.0)	-9.0 (-11.0, -7.0)	-10.0 (-12.0, -8.0)
FACT physical	-15.0 (-17.0, -13.0)	-16.0 (-18.0, -14.0)	-14.0 (-16.0, -12.0)	-15.0 (-17.0, -13.0)
ECOG	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)
SF-36 vitality	15.0 (13.0, 17.0)	16.0 (14.0, 18.0)	14.0 (12.0, 16.0)	15.0 (13.0, 17.0)
SF-36 physical	10.0 (8.0, 12.0)	11.0 (9.0, 13.0)	9.0 (7.0, 11.0)	10.0 (8.0, 12.0)
SF-36 role physical	15.0 (13.0, 17.0)	16.0 (14.0, 18.0)	14.0 (12.0, 16.0)	15.0 (13.0, 17.0)
SF-36 social	10.0 (8.0, 12.0)	11.0 (9.0, 13.0)	9.0 (7.0, 11.0)	10.0 (8.0, 12.0)

Caption 1: Table 1

DRUG RESISTANCE / DRUG PHARMACOLOGY

517 ASSOCIATION OF MDR1 POLYMORPHISMS WITH DRUG RESISTANCE RISK IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS

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Background: P-glycoprotein, the gene product of MDR1, belongs to the ATP-binding cassette superfamily of membrane transporters involved in protection against xenobiotics. The overexpression of P-gp in malignant cells plays a major role in phenomenon called multidrug resistance, decreasing the intracellular concentration of anticancer drugs. Many antineoplastic agents including anthracyclines, Vinca alkaloids and taxanes are P-glycoprotein substrates. Functional genetic polymorphisms in the MDR1 gene influence on the distribution and bioavailability of substrates of P-glycoprotein and that may lead to alteration of sensitivity of tumour cells to chemotherapy.

Aim: We investigated MDR1 polymorphisms in intron 6 and exons 12, 21 and 26 in patients with lymphoproliferative disorders (non-Hodgkin's lymphoma, chronic lymphocytic leukaemia) and estimated association MDR1 gene polymorphism with response to chemotherapy.

Methods: Polymerase chain-reaction - restriction fragment length polymorphism assay was applied to assess C6/+139T, C1236T, G2677T and C3435T MDR1 polymorphisms in 53 patients after chemotherapy. Effect from treatment was defined in case of complete remission or partial response (decrease of amount of lymphocytes in a blood and the bone marrow, reduction of the lymph nodes size). Drug resistance was evaluated as absence of change in disease manifestation after the chemotherapy or in case of patient death. Odds ratio was applied to estimate association of MDR1 genotypes with risk of resistance to chemotherapy and was calculated for mt/mt in comparison to the combined group wt/mt+wt/wt.

Results: We have found the following frequencies of mutant alleles: 6/+139T, 0.33; 1236T, 0.36; 2677T, 0.44; 3435T, 0.45 in patients with lymphoproliferative disorders.

The risk of resistance to chemotherapy for patients with mutant T2677T genotype was a 6.8-fold higher in comparison with heterozygotes and subjects homozygous for G-allele. The carriers of T3435T genotype had a 10.08-fold increased risk of resistance to chemotherapy compared to the combined category (C3435C and C3435T). The T2677T T3435T haplotype conferred a statistically significant 17-fold increase in risk of resistance to chemotherapy relative other combinations genotypes. All differences described above were statistically significant. Mutant TT-genotype carriers in 6+139 and 1236 positions did not show strong association with response to chemotherapy.

Conclusion: The results of the present study provide evidence that carriers of the mutant TT-genotype in positions 2677 and 3435 are more resistance to chemotherapy than other individuals, whereas TT-genotype carriers in 6+139 and 1236 positions don't show strong association with response to chemotherapy.

Supported by RFBR grant N 02-04-48328.

Position SNP	Study odds	95% Confidence Interval	p-level
6/+139T	0.29	0.05 - 1.41	p=0.16
1236T	0.29	0.05 - 1.41	p=0.16
2677T	6.80	1.35 - 35.87	p=0.0004
3435T	10.08	1.88 - 53.79	p=0.004

Caption 1: MDR1 polymorphisms and drug resistance risk

518 EFFECTS OF IMATINIB MESYLATE ON NORMAL HAEMOPOIETIC PROGENITORS

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Background: The development of imatinib mesylate (Glivec®) has been a major advance in the management of chronic myelogenous leukaemia (CML). Although several clinical trials, revealed neutropenia, thrombocytopenia and/or anemia in a proportion of patients on imatinib treatment, little is known about the activity of the agent on normal haemopoietic progenitors. I

Aims: In the present study, we have analysed the in vitro effects of imatinib on normal haemopoiesis using a human healthy donor model.

Methods. Bone marrow mononuclear cells from healthy individuals and immunomagnetically purified peripheral blood CD34+ cells from normal donors undergoing G-CSF induced stem cell mobilisation were incubated with imatinib at final concentrations of 2 mM and 10 mM. Functional evaluation of haemopoietic progenitors was performed by myelomonocytic (CFU-GM) and erythroid (BFU-E) colony formation in semisolid medium, as well as Dexter type long term bone marrow cultures (LTBMC). Assessment of apoptosis in purified CD34+ progenitors was measured by flow cytometric analysis of phosphatidylserine expression using fluorescein isothiocyanate (FITC)-labelled Annexin V.

Results: Concentrations of 2 mM and 10 mM imatinib caused a 32.6%73% and 75.8%55% reduction in the number of bone marrow CFU-GM, respectively, compared to controls. Erythroid progenitors showed 91.9%8% inhibition at 2 mM, and 99.1%1% at 10 mM imatinib concentrations. When stem cell factor (SCF) was added to the culture system, imatinib induced inhibition of both CFU-GM and BFU-E persisted at the same magnitude. Treatment of purified CD34+ cells with imatinib induced a significant reduction of both CFU-GM (p=0.011) and BFU-E (p=0.003). Annexin V apoptosis assays showed no significant effect of imatinib on the survival fraction of CD34+ cells.

Conclusion: Our results demonstrate that imatinib inhibits the growth of committed normal hemopoietic progenitors, in a dose dependent manner. Imatinib also reduces the differentiation potential of CD34+ cells but does not induce apoptosis of these progenitors. The negative effects of imatinib on normal haemopoiesis are not reversed, by the addition of stem cell factor (SCF), suggesting that they are not due to blockage of the c-kit tyrosine kinase.

519 EFFECT OF DIOXIN ON HUMAN NORMAL HEMATOPOIETIC AND LEUKEMIC CELLS

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has a large number of biological effects such as long-lasting skin disease, cardiovascular disease, diabete and cancer. TCDD interacts with the aryl hydrocarbon receptor (AhR), a member of the erb-A family that also includes the receptors for steroids, thyroid hormones, peroxisome proliferators and retinoids. When bound to dioxin, the AhR can bind to DNA and alter the expression of some genes including cytokines and growth factors. In this study, we analyzed the effect of TCDD on human myeloid progenitor cells. The HL60 (promyelocytic leukemia) and K562 (chronic myelogenous leukemia) human myeloid leukemic cell lines, as well as CD34+ cells from the leukapheresis of normal donors stimulated with G-CSF, were exposed to escalating doses of dioxin for 12-24 hours, after which they were washed and plated in a semisolid medium in order to evaluate their clonogenic potential. The dioxin dose capable of inhibiting the growth of 50% of the colonies (IC50) was 33 nM for HL60, 28 nM for K562 and 20 nM for the CD34+ cells. The possible specific modulation of gene expression was then tested by means of microarray analyses of the HL60, K562 and CD34+ cells before and after exposure to 20 nM of TCDD. We identified a series of genes modulated by TCDD exposure. Among the up-regulated genes were LUCA15 putative tumor suppressor, cyclin-D binding Myb-like protein (hDMP1), MAPK/ERK kinase 6, ras-related protein RAB2, RalB GTP-binding protein, transcription factor ZFM1, BRCA1-associated ring domain protein (BARD1). Among the down-regulated genes we found c-myc, TNF alpha precursor, ICAM1, MIP1-beta, interferon gamma-induced protein precursor (gamma-IP10), granulocyte-macrophage colony stimulating factor (GM-CSF), monocyte chemotactic protein 1 precursor (MCP1). All of these genes are variously involved in the processes of proliferation, differentiation and transformation in haematological and tumoral cell models. Basing on these data we can hypothesized that the inhibition of clonogenic potential and the gene expression induced by TCDD exposure on the CD34+ normal progenitor cells provide the basis for a possible role of TCDD in the neoplastic transformation of hemopoietic stem cells and support the epidemiologic data of increased hematologic cancer risk in the population exposed accidentally to the substance.

520 DEVELOPMENT OF A NOVEL BIOSENSOR TO TO MONITOR ARA-C UPTAKE AND ASPECTS OF INTRACELLULAR METABOLISM BY AML CELLS

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The prognosis of AML patients is disappointing with approximately 30% of patients failing to achieve remission. Despite various accepted prognostic indicators, unpredictable responses to chemotherapy and often brief remission duration present a challenge to the clinical Haematologist. Ara-C, the mainstay of treatment for AML, features regularly in therapeutic protocols. Impaired drug uptake and subsequent intracellular concentration of its active derivative Ara-CTP, may contribute to chemoresistance in AML patients. The establishment of a model to indicate efficiency of drug uptake and metabolism would be of value both biologically and potentially clinically as resistance to chemotherapy remains a major obstacle to the effective treatment of AML patients. A previously constructed E.coli strain (SO5110) expressing the human deoxycytidine kinase gene has been shown to be sensitive to Ara-C (1). This strain has been further modified to express bacterial lux genes resulting in an organism in which conversion of Ara-C to Ara-CTP alters light output. Bacterial bioluminescence gene-reporter technology has been used to monitor the survival of bacteria within human cells (2) and previous work has demonstrated that bioluminescent E. coli can be internalised by the AML (M5) cell line THP1 (3). Modified bioluminescent E.coli were exposed to 10, 50 and 100 microM Ara-C and monitored over a six hour period. Bacterial growth diminished but measurable light output increased in response to all concentrations of Ara-C. This system which is currently used to monitor bacterial sensitivity to antibacterial agents may provide a rapid, robust and highly sensitive tool to allow the efficiency of drug uptake and conversion to Ara-CTP to be assessed in AML patient samples. In vitro assessment of Ara-C induced apoptosis, interference

with S phase activity or clonogenic potential all allow the effects of the drug to be studied but give no direct indication of the efficiency of uptake or conversion to Ara-CTP by leukaemic cells. The balance of pharmacokinetic and biochemical factors may vary considerably from patient to patient, thus the establishment of this model would enable assessment of these processes in leukaemic cells.

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521 THE USE OF ANAGRELIDE FOR CONTROL OF THROMBOCYTHAEMIA

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Background: Anagrelide is efficacious in controlling the platelet count in primary polycythaemia. The MRC PT1 study comparing anagrelide and aspirin to hydroxyurea (HU) and aspirin in high risk patients with polycythaemia was stopped prematurely because of the possible excess of haemorrhagic events and myelofibrosis in the anagrelide/ aspirin arm. Anagrelide is currently available off licence in the United Kingdom and Ireland for control of an elevated platelet count caused by primary marrow pathology. It is perceived that anagrelide may be preferable in some patients rather than them being exposed to the possible long term risks of HU.

Aims: Therefore the aim of this study was to review the indications for treatment, response and toxicity in a cohort of patients treated with anagrelide.

Methods: We accessed pharmacy records from January 2000 in two teaching hospitals and identified patients on anagrelide and collated their data.

Results: Twenty four patients were thus identified (6M:18F). The mean age was 60 years (range 27 to 82). Twenty of these had primary thrombocythaemia (PT), two had polycythaemia vera, one had a primary myeloproliferative disorder 'NOS' and one had a myelodysplastic syndrome. Seven patients were treated first-line with anagrelide, in the remainder anagrelide was used as a second line agent due to failure of adequate control with other drugs. One was treated with both anagrelide and HU. Side effects included headache, palpitations, fluid retention, diarrhoea and nausea, all of which declined over time suggesting long-term tolerability. In two patients the drug was discontinued due to vascular side effects, in one of these the interruption was temporary. Two patients required concomitant prescribing of recombinant erythropoietin. The range of dose to maintain a normal platelet count was from 500mcg to 7mg daily in divided doses, taking on average 4.5 months to achieve a platelet count less than 400x10⁹. Two patients achieved a platelet count <600x10⁹ but not <400x10⁹. There was no evidence of either haemorrhagic events or transformation to myelofibrosis.

Summary/ Conclusion: This cohort has been maintained on anagrelide as a second line agent or due to their young age with overall good control of the platelet counts and an acceptable side effects profile. This group of patients should however continue to be followed up.

522 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 the treatment of patients (pts) with relapsed or refractory acute promyelocytic leukemia (APL).

Aim: The aim of this analysis is to assess the safety of Trisenox® in the total exposed patient population, both in clinical trials and postmarketing studies.

Methods: Safety information was gathered from monitored clinical trials and from spontaneous reports in postmarketing studies.

Results: of As of Dec 2003, approximately 2888 pts had received commercial drug for a variety of hematologic and nonhematologic cancers. In clinical trials or compassionate programs since the beginning of the Trisenox® development program, 787 pts [with APL (265), multiple myeloma (102) MDS (174), and other hematologic cancers or solid tumors (246)] have been treated. The overall safety experience for Trisenox® is therefore based on exposure of approximately 3675 pts.

Postmarketing Experience: By January 2004, spontaneous postmarketing adverse events (AEs) were reported for 229 pts; 98 of these were serious AEs (SAEs), with 12 deaths. Ten deaths unrelated to Trisenox® were

due to complications of disease in pts with APL, AML, MDS, NHL, MM or ALL. One potentially related death was due to possible tumor lysis syndrome in a pt with MM, 1 AML pt died with no cause reported. Most reported AEs are expected events that are listed in the Trisenox® product labeling. AEs reported for >10 pts were cytopenia (anemia, leukopenia, neutropenia, thrombocytopenia, pancytopenia), edema/wt gain/fluid retention, pain, increased LFTs, rash/pruritus, dyspnea/hypoxia, leukocytosis, differentiation syndrome, prolonged QT interval, dysrhythmias, pyrexia. No postmarketing reports of torsade de pointes have been received.

Clinical Trial Experience: SAEs considered related to Trisenox® have been reported for 103 of 680 pts enrolled in clinical trials since US approval. This larger proportion of pts with SAEs likely reflects the monitored data in clinical trials compared to 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 186 pts with data received by CTI since US approval are nausea/vomiting (31%/12%), rash/pruritus (26%/18%), fatigue/asthenia (23%/8%), diarrhea (23%), cytopenia (16%), headache (13%), anorexia (12%), edema (12%), prolonged QT interval (10%), dyspnea (9%), pyrexia (8%), increased LFTs (8%), tachycardia (6%), abdominal pain (6%), and in 5%: dizziness, paresthesia, fluid retention, and cough. A recent report of transient arrhythmias in pts continuously monitored during treatment with a non-Trisenox® formulation of arsenic trioxide (Unnikrishnan, Br J Haematol 2004;124:610-7) does not reflect the safety experience in pts treated with Trisenox® and managed in accordance with the product labeling.

Conclusion: With >3675 pts exposed to Trisenox®, the postmarketing AEs reported are generally similar to those observed in clinical trials and listed in the product labeling. The risk/benefit profile of Trisenox® when pts are managed in accordance with the product label remains acceptable.

523 INFLUENCE OF FUNCTIONAL POLYMORPHISM IN MDR1 GENE ON GENETIC PREDISPOSITION AND PROGNOSIS IN ADULT ACUTE LEUKEMIA

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Background: P-glycoprotein (P-gp) is an ABC-family transporter involved in protection against environmental toxicants, multi-drug resistance and pharmacokinetics of anti-cancer drugs. Recently, functional single nucleotide polymorphism C3435T in P-gp encoding MDR1 gene was found to impact the outcome of adult acute myeloid leukemia (AML) and we shown that it may predispose to childhood acute lymphoblastic leukemia (ALL). **Aims:** In this study we aimed to verify whether the MDR1 C3435T polymorphism influence predisposition to adult AML or predisposition and outcome of adult ALL. **Methods:** We genotyped 172 adult leukemia patients (141 AML and 31 ALL) and 215 healthy subjects at MDR1 3435 site by PCR-RFLP method. Moreover, in leukemic cells from 62 of these patients MDR1 expression by real-time RT-PCR and P-gp expression and activity by flow cytometry assays were studied. **Results:** As compared to controls, higher frequency of mutant T allele (0.54 vs. 0.43, p=0.047) and a trend to higher frequency of CT/TT genotype (84% vs. 65%, p=0.060) were observed among ALL patients, while no significant differences were found regarding AML. Besides, in AML and ALL MDR1 expression, P-gp expression, P-gp activity and outcome did not differ as stratified by MDR1 3435 genotype. **Conclusion:** We found that MDR1C3435T polymorphism may be associated with predisposition to adult ALL, although confirming this hypothesis in larger cohorts is necessary. Our data do not support influence of C3435T MDR1 polymorphism on predisposition to AML or outcome of adult AML and ALL.

524 EXPRESSION OF THE MULTIDRUG RESISTANCE PROTEINS MDR1, MRP1 AND LRP IN RELATION TO DE NOVO ACUTE MYELOID LEUKEMIA TREATMENT OUTCOME

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Occurrence of therapeutic resistance at diagnosis of acute myeloid leukemia (AML) may contribute to the treatment failure.

The purpose of our study was to elucidate the relation of multidrug resistance (MDR) phenotype using MDR1 (encoding P-glycoprotein),

and an alternative proteins, which can be responsible for the increased drug efflux in cases with no significant MDR1 expression) eg. MRP1 (multidrug resistance-associated protein 1), and LRP (lung resistance protein) to the AML therapy outcome.

Twenty three previously untreated patients with de novo AML (M0-1, M1-3, M2-6, M4-11, M5-2), 11 females and 12 males, aged from 23 to 74 years, mean 47.1 yrs) were included into the study. The diagnosis of AML was based on cytological and cytochemical examination of bone marrow smears according to the FAB criteria. Immunophenotyping was performed using a immunoflowcytometry method.

MDR1 (Pgp) and MRP1 function were detected with Syto16/PSC833 and calcein-AM/probenecid, respectively. LRP expression of leukemic cells from peripheral blood were identified using leukemia-associated phenotypes combined with immunocytochemistry by means of monoclonal antibody fluorescent probe assay/modulator LRP-56.

At diagnosis elevated activity (shift 1.5) of MDR1 was found in 15 patients, MRP1 in 9 patients, and LRP in 3 patients. The MDR1 activity was found in all six elderly patients (≥ 60 yrs) and in 5/18 < 60 yrs, and was significantly higher than that in younger patients (median ratio of 4.14 vs respectively). In 11 AML cases the elevation of MDR1 activity was accompanied by the overexpression of MRP1. The high expression of LRP at diagnosis of AML was present in at diagnosis in 3 out of 23 cases.

In 11/23 AML patients the expression of multidrug resistance proteins has been examined at diagnosis and after first chemotherapy cycle (consistent with daunorubicine and cytosine arabinoside). Overexpression of MDR1 (Pgp) activity (shift ≥ 1.5) was detected at diagnosis in 7/11 pts, and after chemotherapy reversed in 1 (alive), was still normal in 4 (3/4 alive), become slightly elevated in 1 (alive) and remained elevated in 5 (4 of them died). Elevated expression of MRP1 activity at diagnosis was seen in 3/11 and after therapy remained elevated in 2, become elevated in one case, and reversed in another one.

Patients with without LRP expression at diagnosis responded better to chemotherapy than these with LRP expression (4/23 and 3 of them died).

These findings strongly suggest that in AML patients high MDR1, MRP1 and LRP activities at diagnosis contributes to treatment failure. And, persistent or appeared overexpression of these proteins after chemotherapy may also be a factor of bad prognosis.

525 THIOPURINE S-METHYLTRANSFERASE GENETIC VARIATION IN SERBIAN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes S-methylation (inactivation) of thiopurine drugs such as the anticancer agents 6-mercaptopurine and 6-thioguanine and the immunosuppressant azathioprine. Decreased activity of TPMT is associated with severe hematopoietic toxicity after using standard doses of these drugs. Activity of TPMT enzyme depends on genetic background.

There are several mutations in TPMT gene which give rise to low phenotypic activity. The wild type allele is designated as TPMT*1 and the mutant alleles are: TPMT*2 (G238C), TPMT*3A (G460A and A719G), TPMT*3B (G460A) and TPMT*3C (A719G). Genotypes were determined in 70 Serbian children with acute lymphoblastic leukemia (ALL), using polymerase chain reaction (PCR)-restriction fragment length polymorphism and allele-specific PCR assays. 60 patients had wild type genotype (85.8%), nine patients TPMT*3A/TPMT*1 genotype (12.8%) and one patient TPMT*2/TPMT*1 genotype (1.4%). In order to determine the frequencies of TPMT alleles in Serbian population, 100 volunteer blood donors were analysed as well. 96% of them had wild type TPMT genotype, 3% TPMT*1/TPMT*3A genotype and 1% TPMT*1/TPMT*3B genotype. Our results revealed that the frequency of mutated allele TPMT*3A is very high in children affected with ALL. In addition, statistically significant difference between the frequency of mutated allele TPMT*3A in children affected with ALL and in healthy individuals was observed.

All the patients with genetic variations revealed neutropenia during full dose of thiopurine administration. The doses were reduced regarding patients genotype characteristics. After the doses reduction none of these patients developed hematopoietic toxicity complications.

Recently, a variable number of tandem repeats (VNTR) within the TPMT promoter has been reported to modulate levels of this enzyme activity.

In this study, number of VNTRs of TPMT genes of children with ALL was determined using PCR assays and varied from 4 to 7. Using linkage analysis it was determined that there was no correlation between number of tandem repeats in TPMT promoter and the allelic variants of TPMT gene.

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526 BIFOSFINIC PALLADACYCLE COMPLEX DERIVED FROM N,N-DIMETHYL-1-PHENETHYLAMINE AND THE LIGAND 1,1'-BIS(DIPHENYLPHOSPHINE)FERROCENE INDUCES APOPTOSIS IN HL60 AND JURKAT CELL LINES IN THE PRESENCE OF NORMAL BCL-2 EXPRESSION.

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Recent studies demonstrated that a variety of widely used anticancer drugs kill cells through the induction of apoptosis. The Bcl-2 family plays a central role in the regulation of this process. The 26 kD Bcl-2 protein protects cells from the induction of apoptosis by diverse stimuli including the withdrawal of survival factors, heat shock and treatment with DNA damaging agents. In this study, we investigated the cytotoxic activity of the Bifosfinic Palladacycle Complex (BPC), a new organometallic compound with antitumour activity, on sensitive HL60 and Jurkat leukemia cell lines. Our results demonstrated that BPC induces apoptosis in both cell lines, even in the presence of normal Bcl-2 expression. By using the MTT tetrazolium reduction test, we obtained an IC50 of 5.9 μ M and 5.7 μ M for HL60 and Jurkat cell lines, respectively, after 72 h incubation. The trypan blue assay showed an IC50 less than 3.0 μ M for both cell lines. Moreover, the Feulgen reaction and DNA fragmentation assay suggested that BPC triggers apoptosis in these tumour cells. Interestingly, the Bcl-2 expression, evaluated by immunocytochemistry and RT-PCR, was similar to control non-treated HL60 and Jurkat cells. Our results points to BPC as a promising drug with cytotoxic activity in leukemia cells. Hence, it seems very important to further investigate the mechanisms involved in the induction of cell death.

Financial support: FAPESP, CNPq, FAEP/UMC.

527 SNP MINING BY NANOGEN DEVICE: A SENSITIVE TOOL FOR THE IDENTIFICATION OF BCR/ABL MUTATIONS IN CHILDHOOD PH+ALL

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Background: it was recently shown that resistance to Gleevec (STI571) in patients with CML or Ph+ALL, is associated with different single amino acid substitutions in distinct positions, known to be important for STI571 binding within the ABL kinase domain. The presence of mutations pre- and post-Gleevec treatment is currently included in the laboratory follow-up of CML and Ph+ALL patients receiving that drug. However most of the current available methods are either not enough sensitive or time-consuming.

Aim: to test the feasibility to use the SNP mining by Nanogen device for the rapid identification of BCR/ABL mutations.

Methods: Nanogen (San Diego, CA, USA) developed a method for the de novo discovery of genetic variations, including single nucleotide polymorphisms (SNPs) and mutations, on microelectronic chip devices. The method combines the features of electronically controlled DNA hybridization on open-format microarrays, with mutation detection by a fluorescence-labeled mismatch-binding protein. Electronic addressing of DNA strands to distinct test sites of the chip allows parallel analysis of several individuals. Viceversa, several SNPs/mutations can be tested in few individuals. This microelectronic chip-based mutation discovery assay may substitute time-consuming sequencing studies and will complement existing technologies in genomic research.

Results: in a preliminary series of experiments, we demonstrated that a point mutation in ABL could be identified in a 10 copies dilution of a plasmid carrying the mutation, and in a 10-4 BM sample dilution of a single patient resistant to Gleevec. The analysis of a larger patient series by parallel cloning/sequencing and SNP mining is still in progress.

Conclusion: for the sensitivity and the short time required for the analyses of different patients samples, or multiple time points during follow-up, the Nanogen device represents an alternative to DHPLC or cloning, for the rapid identification of BCR/ABL mutations and monitoring of BCR/ABL mutations during Gleevec treatment.

528 CD80 AND CD38 EXPRESSION AND EX-VIVO CHEMOSENSITIVITY TO PURINE ANALOGUES IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Ex-vivo chemosensitivity assays of peripheral blood chronic lymphocytic leukaemia (CLL) cells may provide useful information to guide treatment strategies in CLL. Recently, Kivekas et al (Leukemia Research 2002; 26:443-446) have suggested that CD80 antigen expression on CLL cells may predict ex-vivo chemosensitivity to the purine analogues cladribine and fludarabine (FDR). All ex vivo drug resistant CLL cases had low or negative CD80 expression. No correlation was observed with the expression of 31 other antigens tested, including CD38. CD80 recognises an immune co-stimulatory molecule expressed on dendritic cells, monocytes and activated B and T lymphocytes; it can be detected and/or up-regulated in vitro on CLL cells. CD38, widely expressed in haemopoietic cells, has recognised prognostic value in CLL.

Aims:

1. To analyse the correlation between CD80 and CD38 expression and ex-vivo chemosensitivity to purine analogues (FDR, cladribine and pentostatin) in CLL lymphocytes
2. To determine whether CD80-negative expression can identify CLL resistant cases.

Methods: Peripheral blood lymphocytes from 40 patients with CLL were tested for CD80 and CD38 expression by flow-cytometry using 3-colour immunostaining with directly conjugated monoclonal antibodies (CD5-FITC clone L17F12, CD80-PE, clone L307.4 and CD38-PE, clone HB-7, from BD Biosciences; CD19-TC, clone SJ25-C1, Caltag). A FACSCalibur flow cytometer was used with Cell Quest software for data acquisition and analysis. Immunophenotypic results were shown as percentage of CD80+ or CD38+ cells in the CD5+/CD19+ gated population. Ex-vivo chemosensitivity was assessed by published methods (Leukaemia Research 1996; 20:143-153) adapted for use in the Octospot microtitre system (Shandon). Chemosensitivity data were expressed as concentration of drug (µg/ml) required to kill 90% of cells (LC90). Both logarithmic and linear scales of LC90 were considered. Statistical analysis was performed by Pearson's correlation test.

Results: Cells from 40 CLL cases were tested independently for both ex-vivo drug sensitivity and marker expression. Results were similar to those obtained from other untreated CLL patients. No correlation was found between CD80 expression and chemosensitivity to FDR ($r=0.007$; 0.056 for linear and logarithmic scales respectively), cladribine ($r=0.228$; 0.012) or pentostatin ($r=0.143$; 0.235). All $p > 0.1$. There was also no correlation between CD38 expression and chemosensitivity to FDR ($r=-0.118$; 0.037), cladribine ($r=0.044$; -0.047) or pentostatin ($r=0.104$; -0.071). All $p > 0.2$.

Conclusions: It could be useful if a pattern of immunophenotypic markers could identify drug resistant CLL cases. However, we showed that neither CD80 nor CD38 antigen expression is predictive of ex-vivo chemosensitivity to purine analogues in CLL.

529 THE EXPRESSION OF MULTIDRUG RESISTANCE PROTEINS IN RELATION TO ZAP-70 EXPRESSION IN B-CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Resistance to cytotoxic drugs in many malignancies may be due to the over expression of the plasma membrane bound multidrug resistance (MDR) efflux proteins P-glycoprotein (P-gp), multidrug resistance protein (MRP1), breast cancer resistance protein (BCRP) or the nuclear membrane lung resistance protein (LRP). Chemoresistance at presentation or post treatment has been observed in CLL. CLL cells often express an MDR phenotype with over expression of P-gp. Although CLL has been described as an indolent disease, recent studies indicate that a sub group may present with aggressive disease which is resistant

to treatment. The malignant cells from this subgroup of patients have recently been shown to express zeta-associated protein (ZAP-70), a tyrosine kinase involved in T-cell signaling. We investigated the link between MDR proteins and ZAP-70 expression in cells from 38 B-CLL patients (22M/16F, 30 Stage A, 6 Stage B, and 2 Stage C) for (i) ZAP-70 expression using an antibody (clone 2F3.2)-Alexa Fluor 488 conjugate by flow cytometry and expressed as a ratio of B to T-cell mean cell fluorescence (MCF) with a ZAP -70+ve cut-off ratio of >0.75 , (ii) the expression of P-gp, MRP and LRP protein by flow cytometry using specific antibodies and expressed as MCF ratio to isotype controls, (iii) flow cytometric analysis of functional activity of P-gp expressed as MCF ratio in the presence and absence of verapamil (vpm) and (iv) the IC50 (µg/ml) for cytotoxic drugs 2-chlorodeoxy adenosine (CdA), chlorambucil and fludarabine using the MTT assay. Eleven (29%) patients were ZAP-70+ve and the remaining 27 (71%) ZAP-70-ve. P-gp expression and P-gp functional activity were not statistically different between the ZAP-70+ve and ZAP-70-ve groups (median P-gp expression 1.5 vs 1.65 respectively, $p=0.44$, median P-gp function 2.11 vs 1.91 respectively, $p=0.35$). There was no significant difference in the expression of MRP1 (median 2.37 vs 2.72, $p=0.11$) or LRP (median 4.87 vs 4.64, $p=0.11$) between ZAP-70+ve and ZAP-70-ve patients. In the ZAP-70+ve group there was a correlation between P-gp over expression (median 1.5) and its function (median 2.11, $r=0.71$) compared to the ZAP-70-ve group ($r=0.16$). The IC50 levels for CdA, chlorambucil and fludarabine between the ZAP+ve and ZAP-ve groups were not statistically different ($p=0.07$, 0.08, 0.12 respectively). Ten ZAP-70-ve and 8 ZAP-70+ve patients were sequentially investigated over a mean period of 3.8 years for expression of P-gp, MRP, LRP and P-gp function. No significant changes in P-gp expression and its function were noted between the two groups over the period of time. However, a significant increase in the expression of MRP1 and LRP ($p=0.00015$ and 0.05 respectively) was observed in the follow up samples of ZAP-70-ve group. In conclusion, although P-gp, MRP1 and LRP are frequently expressed in CLL cells, it appears that they do not contribute to the poor prognosis in ZAP-70+ve patients. The clinical significance of a higher functional P-gp in the ZAP-70+ve group and the increase in the expression of MRP1 and LRP with time in the ZAP-70-ve group is unclear.

530 ANALYTICAL METHODOLOGY AND PHARMACOKINETICS OF CAMPATH-1H IN PATIENTS WITH CLL AFTER IV OR SC ADMINISTRATION

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CAMPATH-1H is a humanized immunoglobulin G1 (IgG1) specific for CD52. It causes lysis of more than 95% of normal human blood lymphocytes, as well as of B and T lymphomas, by Complement Mediated Cytotoxicity and Antibody Dependent Cellular Cytotoxicity.

The aim of the present study was to validate a modified method (P. Rebello et al, JIM,260:285-302, 2002) for the quantification of CAMPATH in serum samples of patients treated with this drug by subcutaneous (SC) (D=10mg; 12 pts) or 2 hour-infusion (IV) (D=30mg; 8 pts) administration. Briefly, after serum samples incubation with targets cells HUT-78 (human T cell lymphoma) CAMPATH-1H levels were detected by a fluorescent-labeled anti-human IgG. Samples were analyzed by flow cytometry (FACScan) and the median fluorescence intensity (MFI) was plotted on a standard curve. Standard curve, ranged from 0.0024 to 50 mg/ml, was obtained by two-fold dilutions of CAMPATH-1H in 1:8 diluted AB serum. Low, medium and high quality controls concentrations were 0.39, 1.56 and 6.25 mg/ml, respectively. Inter-day accuracy was 99.2 %, 98.8 % and 99.1% and the inter-day CV% was 14.1%, 16.9 % and 12.8 % for the low, medium and high quality controls, respectively.

Peak serum levels, obtained 1 hour after SC administration, were superimposable to the CAMPATH-1H levels obtained at the end of the 2 hour-infusion; trough serum levels were ~ 30% the corresponding values observed after IV administration (data normalized to the same dosage).

531 TIME TO RESPONSE OF ASPERGILLUS ANTIGEN TO CASPOFUNGIN COMPARED TO AMPHOTERICINE B : A SMALL RETROSPECTIVE STUDY

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Background : In order to detect an invasive aspergillus infection in our neutropenic hematological patients, we studied routinely in our centre

for a period of one year the Aspergillus antigen two times a week (on Monday and Tuesday). We considered invasive aspergillus infection when we detected a positive Aspergillus antigen on two consecutive measurements as mentioned by EORTC criteria.

Aims : To compare respons of Aspergillus antigen on Caspofungin and Amphotericine B : time to response was measured.

Methods and results: Fourteen patients were treated with Caspofungin for an invasive aspergillosis, and we followed the response of the Aspergillus antigen to treatment. Patients were started on Caspofungin after two positive Aspergillus antigen tests. In some cases Amphotericine B had already been started earlier because of prolonged neutropenic fever. Most of the patients were males (10/13). Mean age of patients was 66.7 years, with a range from 28 to 84 years of age. The mean value of the Aspergillus antigen before start of treatment was 2.58 (range 1.1-5.5). 10/14 (71.4%) of patients were followed until negative values were obtained. 3/14 (21.4%) of patients deceived, 1/14 (7.1%) of patients did not respond to Caspofungin and continued having positive Aspergillus antigen tests, and needed other treatment. Time to response (first negative Aspergillus antigen after start of treatment) was situated between 1 and 11 days, with a mean response rate of 3.2 days. Aspergillus antigen was measured two times a week, which could even delay time to response in certain cases. Further study with daily measurement of Aspergillus antigen is necessary to define exact time to response.

To compare, we studied ten patients in a period of six months who were treated with Amphotericine B for prolonged neutropenic fever, and who had a positive Aspergillus antigen retrospectively. Eight out of ten patients were male. Mean age of patients was 66.9 (range 54-84). The mean value of Aspergillus antigen before start of treatment was 1.4 (with range from 0.1-3.4). Three out of ten patients were started with Amphotericine B before a positive Aspergillus antigen was detected. Seven patients with positive Aspergillus antigen before start of Amphotericine B were followed until negative values were obtained. 2/7 patients had a deterioration of Aspergillus antigen, and needed other treatment. Five patients responded on treatment. Their time to response was situated between 2 and 14 days, with a mean value of 6.4 days.

Conclusion : Aspergillus antigen response to Caspofungin seems more rapidly than to Amphotericine B, although further investigation is necessary. Daily measurements of Aspergillus antigen are therefore needed as well as controlled start of both medications after positive antigen testing.

SIGNALLING & TRANSCRIPTION

532 UBF EXPRESSION AND RIBOSOMAL SYNTHESIS IN PHA STIMULATED LYMPHOCYTES TREATED WITH N-BUTYRIC ACID

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Background: The nucleolar transcription of the ribosomal RNA precursor is governed by RNA polymerase I. The promoter selectivity factor SL1, and an enhancosome maker, DNA binding architectural factor UBF, contribute to the efficient formation of a transcription initiation complex. Previously, we have demonstrated that the blastic transformation of quiescent lymphocytes was accompanied by the early promotion of rRNA synthesis, early promotion of UBF mRNA production and by early phosphorylation of an inactive pool of UBF. In contrast, the translation of UBF was considerably delayed and paralleled the DNA replication. The question of the existence of a regulatory pathway submitting the UBF synthesis under control of rDNA replication arose.

Aim: By Studying the translation of UBF and the transcription of rRNA in stimulated lymphocytes incapable of the first nuclear cycle progression, we aim to elucidate the problem of linkage between ribosomal gene mobilization and DNA replication.

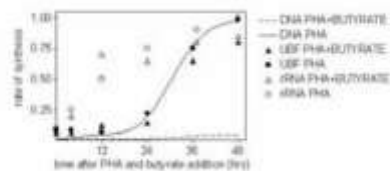
Methods: Peripheral blood lymphocytes were stimulated to growth with 10 µg/ml of phytohemagglutinin and simultaneously arrested (or not) in G1 phase with 1 mM n-butyrate. In time intervals up to 50 hours aliquotes of cells were taken for: 1) BrdU labelling of neosynthesized DNA, 2) 35S-amino acid labelling of neosynthesized UBF and 3) 32P-UTP labelling of rRNA synthesized in nuclear Run-On assays. The synthesis of DNA was monitored on microplates by anti-BrdU ELISA and UBF synthesis by anti-UBF immunoprecipitation followed by SDS-PAGE and filmless autoradiography of gels. The rate of rRNA synthesis was measured by hybridization of labelled Run-On transcripts to rDNA fragments immobilized on Zeta-Probe and filmless autoradiography of nylon strips. Autoradiographs were scanned and quantified using PhosphorImager

FLA 2000 and Raytest Software AIDA 2.1. Graphs were generated using GraphPad Software Prism 2.01.

Results: Data shown in fig. 1 demonstrate that in human T lymphocytes subjected to the mitogenic transformation, the synthesis of chromosomal DNA started 24 hours after the addition of phytohemagglutinin and culminated after approximately 48 hours of total PHA treatment. Lymphocytes treated with PHA and butyric acid didn't duplicate their content of DNA in the interval up to 48 hours. The translation of UBF protein in PHA-stimulated lymphocytes was significantly active despite the presence of butyric acid which abolished the DNA replication. The transcription of ribosomal RNA started immediately after the addition of PHA and reached its maximal rate within 24 hours. The presence of butyric acid didn't influence the time course of synthesis. **Summary/conclusions:** In cells entering the growth-division cycle an increase of cellular content of regulatory protein, UBF, parallels the increasing amount of DNA generated by replication. However, within 48 hours following the mitogenic signal, the synthesis of rRNA as well as synthesis of the UBF enhancer is independent of the amount of rDNA copies. Another regulatory mechanism, more likely a process comprising the early phosphorylation of UBF, upregulates the synthesis of rRNA.

Acknowledgements: Supported by Grants IGA NC/5933-3 and CEZMZ 0023736001

of Ministry of Health of the Czech Republic.



Caption 1: Fig.1

533 A ROLE OF MUTATION OF CXCR4 GENE IN TWIN SISTERS WITH WHIM SYNDROME

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Background: It has been shown that the responsible gene for WHIM syndrome (warts, hypogammaglobulinemia, immunodeficiency and myelokathexis) is the mutant CXCR4 gene. The purpose of this study is to elucidate a role of the mutant CXCR4 gene in twin sisters with WHIM syndrome.

Case: The subjects studied were 11-year-old dizygotic twin sisters. The diagnosis of WHIM syndrome was made at the age of 8 years. Nobody of the family was similarly affected.

Method: PCR and RT-PCR using primers at the region of CXCR4 gene amplified DNA and RNA isolated from leukocytes, respectively. The PCR products were subsequently analyzed by a direct sequencer. CXCR4 proteins in leukocytes were detected by immunoblotting by using anti-CXCR4 monoclonal antibody. The surface expression of CXCR4 was studied with flowcytometry.

Result: We found a mutation that occurred on CpG dinucleotide, 1000c-T in both patients and their father. Their brother, mother, grandfather and grandmother were all normal in regard to the CXCR4 sequence. Sequence of CXCR4 from m-RNA by RT-PCR disclosed the same mutation as DNA only in the patients. The other family members were of normal CXCR4 sequence. The mutant protein of CXCR4 in leukocytes was detected at the molecular 27kd, which was lower than normal CXCR4 protein. These mutant proteins were detected in only the patients. Surface expression of CXCR4 in the patients were the same as that in normal control. Inhibitory apoptotic function in the patients neutrophils stimulated with SDF-1 were also detected.

Conclusion: Somatic mutation as CpG dinucleotide, 1000c-T in the CXCR4 gene in father may have been transmitted to the patients as a heterozygote, resulting in WHIM syndrome in the pedigree. No occurrence of WHIM in father may probably be due to mosaicism of his cells. The mutation of the CXCR4 gene may cause mutant the CXCR4 proteins, which suppress the production of normal CXCR4 protein, resulting in normal expression of the mutant CXCR4 on patients leukocytes. These phenomena are thought to be related to the etiology of the immunological disorders in WHIM syndrome.

534 AN INVESTIGATION OF THE PRO-APOPTOTIC EFFECT OF THE MEK INHIBITOR U0126 ALONE AND COMBINED WITH ARA-C IN ACUTE MYELOID LEUKAEMIA, NON-LEUKAEMIC NEOPLASTIC DISORDERS AND NORMAL HAEMOPOIETIC PROGENITORS

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Constitutive activation of the MAPKinase signaling pathway at the p44/42 MAPK level in acute myeloid leukaemia (AML) has been associated with poor clinical outcome. Interruption of the pathway with MEK inhibitors can induce pro-apoptotic signals. This study examined the pro-apoptotic potential of the MEK inhibitor U0126 on AML cell lines (KG1a, HEL, TF-1, M-07e, THP-1, MV411, ML-2, HL60), marrow samples from 14 newly diagnosed AML patients and patients with non-leukaemic neoplastic disorders (NHL n = 7; myeloma n = 6; other n = 2). Normal marrow samples (n=8), from femora of hip replacement patients were also included. Cells were exposed to U0126 (50 microM) and alone or in combination with cytarabine (Ara-C) (100 microM) to determine whether these agents might act synergistically. Apoptosis was detected flow cytometrically using monoclonal antibodies Apo 2.7-RPE directed against the mitochondrial protein 7A6 and AnnexinV-FITC which has high affinity for exposed phosphatidylserine on the outer cell surface membrane. The pro-apoptotic effect of U0126 was inconsistent in cell lines however a significant correlation between sensitivity and CD34 positivity was noted. This was particularly apparent in the primitive M0 cell line KG1a. Ara-C induced apoptosis in cell lines was variable but with no evidence of enhancement of pro-apoptotic effect with U0126. Leukaemic patient samples exhibited inconsistent sensitivity to U0126 and a similar association with CD34 expression but no synergy with Ara-C. In non-leukaemic samples a varied response to U0126 was apparent whilst normal marrow samples showed no significant sensitivity to the agent and again no enhancement of the apoptotic effects of Ara-C. These results suggest that U0126 may have potential as a targeted therapeutic agent particularly in primitive AML and that there is no synergism between the inhibitor and Ara-C. Further work is required to further characterise differential sensitivity of AML, non-leukaemic neoplastic and normal marrow progenitors to U0126 induced apoptosis.

535 BCL-2 INHIBITORS SENSITIZE TRAIL-INDUCED APOPTOSIS BY UNCOUPLING OF MITOCHONDRIAL RESPIRATION IN HUMAN LEUKEMIC CELLS

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Previous studies have shown that the lymphoblastic leukemia CEM cell line is resistant to TRAIL-induced apoptosis due to a low expression of caspase-8. Bcl-2 inhibitors, BH31-2 and HA14-1, are small cell-permeable non-peptide compounds and are able to induce apoptosis by mediating cytochrome c release and also lead to dissipation of the mitochondrial membrane potential (DYm). This study aimed to use the Bcl-2 inhibitors to sensitize CEM cells to TRAIL-induced apoptosis by switching on the mitochondrial apoptotic pathway. We found that a low dose of BH31-2 or HA14-1, which did not induce cytochrome c release, greatly sensitized CEM cells to TRAIL-induced apoptosis. In a similar manner to the classical uncoupler carbonyl cyanide m-chlorophenylhydrazone (CCCP), both BH31-2 and HA14-1 induced a reduction in DYm, a generation of reactive oxygen species (ROS), an increased mitochondrial respiration, and a decreased ATP synthesis. This uncoupling function of the Bcl-2 inhibitors was responsible for the synergy with TRAIL-induced apoptosis. CCCP per se did not induce apoptosis but again sensitized CEM cells to TRAIL-induced apoptosis by uncoupling mitochondrial respiration. The uncoupling effect facilitated TRAIL-induced Bax conformational change and cytochrome c release from mitochondria. Inhibition of caspases failed to block TRAIL-mediated cell death when mitochondrial respiration was uncoupled. We observed that BH31-2, HA14-1 or CCCP can overcome resistance to TRAIL-induced apoptosis in TRAIL-resistant cell lines, such as CEM, HL-60 and U937. Our results suggest that uncoupling of mitochondrial respiration can sensitize leukemic cells to TRAIL-induced apoptosis. However, caspase activation per se does not represent an irreversible point of commitment to TRAIL-induced cell death when mitochondrial respiration is uncoupled.

536 ROLE OF STROMAL CELLS IN REGULATION OF THE HUMAN PRIMITIVE HEMATOPOIETIC STEM CELLS

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The bone marrow microenvironment provides support of hematopoiesis in vivo and consists of heterogeneous cells populations, including reticular cells, fibroblasts, osteoblasts, adipocytes, endothelial cells and others cells.

The purpose of the present study was to determine the influence of various bone marrow stromal cells on proliferation and differentiation of the human early progenitors of the hematopoietic stem cells (HSC). Stromal and hematopoietic cells were obtained from bone marrow aspirates of 6 normal volunteers. We have produced in vitro and used the various stromal feeder layers: of 3-4 week long-term cultures (LTC) after radiation (48 Gy), fibroblasts non-stimulated, fibroblasts stimulated to osteogenic differentiation by dexamethasone (10-7M) and MS-5 cell line as control. Stromal cells achieved confluency before the coculture in vitro with hematopoietic cells. The activity of stromal cells to the HSC we defined by the number of the cobblestone areas-forming cells (CAFC) on different stromal feeder layers in LTC bone marrow by limiting dilution the assay. The early (5-9 weeks incubation) and late (1-4 weeks incubation) CAFCs were estimated.

Results: In our experiments we found that all examined human bone marrow stromal cells (LTC, fibroblasts, stimulated fibroblasts) supported growth of the late and early CAFCs up to 9 week incubation in absence of any added exogenous factors by direct cellular contacts. We have detected that number of the early and late CAFCs on feeder fibroblasts and late CAFC on the feeder of LTC was the same as ones on MS-5. The number of the early CAFCs (5-9 week) was significantly higher on LTC and on fibroblasts stimulated to osteogenic differentiation than on fibroblasts non-stimulated and MS-5.

Thus, we have shown, that in vitro activities of the stromal bone marrow cells are different. The stromal cells stimulated to osteogenesis or forming complex structure as long-term cultures support proliferation and differentiation of the early CAFCs progenitors of the HSC better than fibroblasts and MS-5 feeders.

537 THE PROMYELOCYTIC LEUKEMIA ZINC FINGER (PLZF) PROTEIN DOWN-REGULATES APOPTOSIS AND EXPRESSION OF THE PRO-APOPTOTIC BID PROTEIN IN LYMPHOCYTES

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Background. The promyelocytic leukemia zinc finger (PLZF) gene, involved in rare cases of acute promyelocytic leukemia, encodes a Kr ppe-type zinc finger transcription factor. It has been reported that PLZF affects myeloid cell growth, differentiation and apoptosis. However, the function of PLZF in the lymphoid compartment, where PLZF is also expressed, remains largely unknown. We have previously shown that a significant proportion of B-cell chronic lymphocytic leukemia (B-CLL) patients exhibit down-regulation of PLZF expression compared to normal lymphocytes, and this pattern of expression correlated with a better prognosis (Parrado et al, Hematol J 2000, 1: 15-27). As B-CLL progression is likely associated to decreased apoptosis of the malignant cells, such a correlation suggests a potential anti-apoptotic function of PLZF in B-CLL.

Aims. To investigate a potential relationship between PLZF expression in lymphocytes and programmed cell death.

Methods. An inducible model of stable clones of the lymphoid Jurkat cell line was created using the tet-off system. Apoptosis was induced by serum deprivation, and measured by dual annexin V/propidium iodide staining, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labelling (TUNEL), and loss of mitochondrial transmembrane potential ('m). A semiquantitative reverse transcription polymerase chain reaction (RT-PCR) method that uses β-actin as an internal control was developed to detect modulation in the levels of mRNA expression of a variety of apoptosis regulators or effectors, including BCL2 family members, caspases, transcription factors amongst others. Northern blot, western blot and flow cytometry analysis were performed to confirm gene regulation. Electrophoretic mobility shift assays (EMSA) were performed using Jurkat nuclear protein extracts

Results. Although induction of PLZF expression by itself did not produce changes in the basal levels of apoptosis, PLZF had a significant anti-apoptotic effect in Jurkat cells cultured in conditions of serum starvation. These results support an anti-apoptotic function for PLZF in the lymphoid compartment. We sought to identify potential apoptosis-related PLZF targets for transcriptional regulation in Jurkat cells. Only BID, a pro-apoptotic member of the BCL2 family, was shown to be significantly repressed in the PLZF-expressing clones upon doxycycline removal. Furthermore, PLZF was found to bind the sequence TTACATACAGTAAA located at 2 kb upstream of BID transcriptional start site. Summary/conclusion. These findings suggest for the first time that BID is a target for PLZF repression, and this could be a candidate mechanism to mediate the anti-apoptotic effect of PLZF in these cells. We hypothesize that BID repression by PLZF may play a role in lymphoid malignancies where PLZF is dysregulated, such as B-CLL.

538 EVIDENCE FOR RAD51-MEDIATED DNA REPAIR UP-REGULATION IN AML PATIENTS WITH FLT3 INTERNAL TANDEM DUPLICATIONS

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The presence of the activating internal tandem duplication (ITD) mutation in the FLT3 receptor confers a worse disease free survival and overall survival in patients with AML. Increased relapse risk may result from drug resistance secondary to upregulated DNA repair following sub toxic chemotherapeutic agent exposure. The activated FLT3 receptor signals via STAT5, which is a transcriptional activator for many genes including the DNA repair enzyme RAD51 and the anti-apoptotic protein Bcl-xL. We have investigated the expression of FLT3, RAD51 and Bcl-xL in a cohort of AML patients using a sensitive semi-quantitative real-time PCR method with sybr green detection. Similar to other groups we have shown differences in the expression of FLT3 between patients with WT (n=48), ITD (n=27) and point mutations (n=4): mean qFLT3/2M ratio; wildtype 0.273, ITD 0.390, point mutation 0.730 (Mann-Whitney U test: ITD compared to WT p=0.05; point mutation compared to WT p=0.01). We have also demonstrated a strong correlation between the levels of Bcl-xL and FLT3 in FLT3 ITD positive patients (n=23; Spearman correlation coefficient p=0.001), however this correlation is not related to the phosphorylation level of STAT5 (detected using a quantitative flow cytometric method) and therefore appears to act, at least in part, via a separate, STAT5-independent signaling pathway. We also observed a statistically significant correlation between transcript levels of Bcl-xL and RAD51 in FLT3 ITD samples (n=22; Spearman correlation coefficient p=0.03). This supports recent work demonstrating that over-expressing Bcl-xL enhances homologous recombination of DSBs and suggests that the level of FLT3 transcripts in FLT3 ITD patients is an important factor in enhancing DNA repair.

To provide support for the hypothesis that activated FLT3 up-regulates DNA repair, we examined DNA repair in a cell line with a FLT3 ITD (MV4-11) and a FLT3 WT cell line (HL60) using the comet assay and examined the effect of inhibiting FLT3 signaling. The cell lines were incubated with the FLT3 inhibitor PKC412 for 24 hours, then exposed to cytostatic doses of daunorubicin for 1 hour. The MV4-11 cells showed loss of early DNA repair (within 1 hour) when treated with PKC412 and daunorubicin, however this was not seen in HL60 cells. Furthermore FLT3 ITD AML patient cells demonstrated a reduction in repair kinetics following treatment with PKC412, whilst there was no effect in the WT FLT3 AML cells. This loss of early repair in PKC412-treated FLT3 ITD cells was also associated with the down-regulation of several DNA repair proteins, including RAD51, as determined by real time PCR. Our data suggests that in cells with a FLT3 ITD dysregulation of a pathway involving RAD51, and possibly Bcl-xL, may enhance homologous DNA repair. This pathway may actively promote cell survival following cytotoxic therapy leading to an increased risk of disease relapse.

539 ACTIVATED GAMMA/DELTA T CELLS EXPRESS THE NATURAL CYTOTOXICITY RECEPTOR NKP44

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Background gamma/delta T cells account for up to ten percent of T lymphocytes in the peripheral blood of healthy donors. They can be activated by cytokines like interleukin(IL)-2 and IL-15, express natural killer (NK) cell markers

such as NKG2D and show cytotoxic activity against several tumor cells. Natural cytotoxicity receptors like NKP44 have so far been regarded as specific NK receptors. NKP44 is expressed by activated NK cells only, resting NK cells do not express NKP44. To date, only two gamma/delta T cell clones expressing NKP44 have been described. There is no description of polyclonal gamma/delta T cells expressing NKP44.

Aims

The aim of the presented study was to evaluate the expression of activating NK cell markers including NKP44 on activated gamma/delta T cells.

Methods

gamma/delta T cells were isolated to a purity of >90% by magnetic activated cell sorting and cultured in the presence of anti-CD3, IL-15 and high dose IL-2. On days 0, one and seven the expression of activation markers including NKG2D and NKP44 on gamma/delta T cells were evaluated immunophenotypically. For further confirmation, RT-PCR was performed on a light cycler for mRNA of beta-actin, NKP44 and DAP 12, the signal transduction molecule of NKP44.

Results

On day 0 there was no expression of NK cell markers NKP44 and NKG2D on isolated polyclonal gamma/delta T cells. Expression of NKG2D was up to 85 % after one day of stimulation with cytokines IL-2 and IL-15 and did not change significantly during further culture. Although there was still no expression of NKP44 after one day of activation with cytokines, on day 7 a surface expression of up to 26 % could be determined (n = 22, mean 8.7 %). In contrast isolated CD4+ and CD8+ alpha/beta T cells were consistently negative for surface expression of NKP44 after culture with high dose IL-2 and IL-15. These results could be confirmed by detection of NKP44 and DAP12 mRNA by RT-PCR from RNA from gamma/delta T cells but not from alpha/beta T cells.

Summary and conclusion

To our knowledge this is the first description of NKP44 on polyclonal gamma/delta T cells. NK cells markers are known to play an important role in the cytotoxic activity of gamma/delta T cells. NKP44 has so far only been described on two gamma/delta T cell clones, however, in these, an activating role could also be determined (Cantoni et al., J Exp Med, 1999). From our results we conclude, that NKP44 is not only expressed by activated NK cells but also by activated gamma/delta T cells. In further studies the role of NKP44 in the cytotoxic activity of gamma/delta T cells should be evaluated.

540 THE EXPRESSION OF CHEMOKINE RECEPTOR CXCR3 IN LYMPH NODES OF LYMPHOMAS AND LYMPHOID REACTIVE TISSUE

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Background: Chemokines are small soluble 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 CXCR3 have been shown to be present on activated T cells, particularly those of Th1 lineage. The data concerning the expression of CXCR3 in B cell malignancies are confusing. There is report that CXCR3 is expressed on a subset of peripheral blood B cells and in distinct subtypes of B-cell lymphoma, especially CLL/small lymphocytic lymphoma, mantle cell lymphoma (MCL), hairy cell leukemia (HCL).

Aim: The aim of the study was evaluation of expression of CXCR3 in lymph nodes of different types of lymphomas and lymphoid reactive tissue.

Methods: Samples of the studied lymphomas (18) and reactive lymph node tissues (15) 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 CXCR3 (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 CXCR3 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 CXCR3 expression were counted. For each slide mean number of CXCR3 was calculated per

1.0 mm². Statistical analysis was made with the U Mann - Whitney test. Differences were considered statistically significant at $p < 0.05$.

Results: Mean expression of CXCR3 in lymph nodes in lymphomas was 57.20 40 per mm² and 84.88 60.25 per mm² in reactive lymph nodes. Statistical analysis showed no significant differences between both groups. In reactive lymphoid tissue CXCR3-expressing cells were mainly T cells (CD3+). In lymphoma tissues a strong correlation between CXCR3 expression and the subtype of lymphoma was observed. CXCR3 expression was seen in all T-cell lymphoma, marginal zone lymphoma, HCL, 2/3 MCL, 2 follicular G1 but not in follicular G3, Burkitts lymphoma and diffuse large B-cell lymphoma (DLBCL).

Conclusion: CXCR3 expression in lymphoid tissues is observed. In reactive lymph nodes and T-cell lymphomas this expression is associated mainly with the presence of T-cells. In B-cell lymphoma tissues a strong correlation between CXCR3 expression and the subtype of lymphoma was observed: higher expression in indolent than in aggressive lymphomas, which may explain different biological behavior.

541 HUMAN MASK, A MULTIPLE ANKYRIN REPEAT SINGLE KH DOMAIN PROTEIN INTERACTS WITH SHP2 IN K562 CELL LINE

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Background: MASK, Multiple Ankyrin Repeat Single KH domain protein was first identified in *Drosophila*. MASK was isolated through a genetic screen designed to identify proteins that interact with the protein tyrosine phosphatase Corskew (CSW). The phenotypic characterization of MASK in *Drosophila* suggested that MASK is a novel protein involved in receptor tyrosine kinase signaling (RTKs) and MASK activity is required for differentiation, cell survival and cell proliferation in *Drosophila* eyes. However, the mechanism by which MASK mediates signaling downstream of RTKs has not been determined. Several lines of evidence suggest that Shp2, the vertebrate homolog of CSW, can transduce signals from RTKs. Shp2 acts to transduce signals that are delivered by growth factors, cytokines, hormones, antigens, and interactions with the extracellular matrix. Although the biological role of Shp2 has been much appreciated, the mechanism for its activity is yet to be uncovered. Human MASK (hMASK) has been recently reported and the protein has been identified only in prostate cancer cell line LNCaP. Aims: In the present study, we investigated the expression of hMASK in a human leukemia cell line, K562, as well as the association of hMASK with Shp2 in this cell line. Material and methods: Samples containing 2x10⁷ of the human leukemia K562 cells were pelleted and resuspended in lysis buffer. Equal amounts of protein were used for immunoprecipitation with specific antibodies against hMASK and Shp2, followed by SDS-PAGE and Western blot analysis. Results: Our results demonstrated that hMASK is abundantly expressed in the K562 cell line and it is associated with Shp2. By immunoprecipitation of K562 cells lysates and immunoblotting with anti-hMASK antibodies, an expected 270kDa band was detected, corresponding to hMASK. By immunoprecipitation of K562 cells with anti-Shp2 antibodies and immunoblotting with anti-hMASK, a 270kDa band was also detected, indicating the association between these two proteins. By reverse immunoprecipitation, immunoprecipitation with anti-hMASK and immunoblotting with anti-Shp2, a band corresponding to Shp2 was clearly detected, corroborating the association between hMASK and Shp2. Control negative immunoprecipitations and immunoblottings with Rabbit polyclonal normal IgG were performed and no band was detected. Conclusions: Our findings are the first to identify hMASK in a human leukemia cell line, and describe a new protein association, hMASK and Shp2. The presence of close homologs in other systems indicates that hMASK is a conserved, essential signaling molecule. The presence of multiple ankyrin repeats suggests a role for hMASK as a scaffolding protein, bringing together many signaling molecules. The identification of proteins that interact with hMASK and which might direct hMASK to various signal transduction pathways will be essential to understand the role of hMASK in the signaling pathways in general. The exact role of the association between hMASK and Shp2 in transducing the RTKs will be determined with further studies.

542 TRANSFORMING GROWTH FACTOR-BETA1 (TGF-BETA1) ENHANCES ADHESION OF HAIRY CELLS TO BONE MARROW FIBROBLASTS VIA VLA-4 / VCAM-1

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Bone marrow (BM) infiltration with the malignant hairy cells (HCs) is a characteristic finding in patients with hairy cell leukemia (HCL). In the

BM, the hairy cells are found in association with fibroblastic cells and surrounded with fine reticulin meshwork. Such selective tissue localization may allow the HCs to receive signals for survival and proliferation. We have recently reported that BM of HCL patients contain high levels of transforming growth factor-beta 1 (TGF-beta1) as compared to healthy donors (HDs) (Shehata et al. JCI 2004). Therefore, we investigated the role of TGF-beta1 in regulating the interaction between HCs and BM fibroblasts (BMF) in vitro.

BM fibroblasts were isolated from patients with HCL and from HD. Peripheral blood mononuclear cells from HCL patients or purified HCs were co-cultured with BM fibroblasts. Microscopic examination demonstrated a striking adhesive property of the HCs to BMF. The adhesion of HCs to BM fibroblasts was associated with enhanced survival of the leukemic cells. Adhesion assays revealed that TGF-beta1 further increases the adhesion of HCs to BM fibroblasts while neutralizing anti-TGF-beta1 antibody significantly inhibits this process. In spite of the known inhibitory effect of TGF-beta on the proliferation of normal B cells, exposure of HCs to TGF-beta1 did not lead to growth inhibition of the leukemic cells. To get further insight into the interaction between HCs and BM fibroblasts, we investigated the pattern of expression of adhesion molecules on the HCs. FACS analysis revealed a high expression of CD49d (VLA-4) and CD49e (VLA-5) on the HCs. Immunofluorescence studies in co-cultures demonstrated the co-localization of VLA-4 and VLA-5 with their counterreceptors CD106 (VCAM-1) and fibronectin respectively. Preincubation of the HCs with antibodies against the alpha-4 chain of VLA-4 integrin or preincubation of the BM fibroblasts with anti-VCAM-1 (CD106) Ab inhibited the adhesion of HCs.

We conclude that the adhesion of hairy cells to bone marrow fibroblasts through binding of VLA-4 integrin to its ligand VCAM-1 may represent a key step in bone marrow infiltration with the malignant cells and that TGF-1 may promote this process.

543 EARLY HEMATOPOIETIC ZINC FINGER (EHZF), A MULTIFUNCTIONAL TRANSCRIPTION CO-FACTOR EXPRESSED IN HUMAN EARLY HAEMATOPOIETIC CELLS AND MYELOID LEUKAEMIC BLASTS

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The concerted expression of specific repertoires of regulatory genes among which many encode transcription factors' is essential to ensure the balance between maintenance of the immature hematopoietic cell compartment and continual supply of differentiated blood cells in response to the requirements of the organism.

We have used Representational Differential Analysis (RDA) to identify genes expressed in human CD34+ cells, but not in peripheral blood mononuclear cells. This approach led to the identification of a transcript encoding a 30-zinc-finger protein strongly abundant in human CD34+ cells but not in mature leukocytes. The predicted product that we have named EHZF (Early Haematopoietic Zinc Finger protein) - is the human homologue to the murine Evi-3 protein and shares high sequence similarity with the zinc finger multi-functional transcription co-factor, OAZ. OAZ is known to interact with the neural and hematopoietic transcription factor OLF-1/EBF-1 and to inhibit its activity; in addition, it has been implicated in the signalling pathway of the Bone Morphogenic Proteins (BMP) 2 and 4 where - in the presence of these BMPs - it associates with Smad1 and Smad 4, binds to, and activates the transcription directed by, BMP-responsive elements (BREs).

EHZF is expressed in several human tissues, including brain, placenta, spleen, lymph nodes, thymus and foetal liver. In human CD34+ cells, its mRNA levels decrease rapidly following exposure to cytokines that induce proliferation and differentiation. Significant amounts of transcript were detected in human hematopoietic cell lines of leukaemic origin. RT-PCR, and hybridisation of a commercial 'blood disease profiling array' containing cDNA from blood cells of normal individuals and patients with a variety of hematological diseases, revealed positivity for EHZF in over 50% of the acute myelogenous leukaemias (AMLs) and some of the chronic myeloid leukaemias (CML) tested, but not in any of the normal blood cell populations. Transfection experiments have shown that: i) EHZF is localised predominantly in the nucleus, ii) EHZF interacts with SMAD-1 and SMAD-4 in the presence of BMP4, iii) its expression specifically enhances the transcriptional activity of a BRE in response to BMP-2 and BMP-4 whereas it iv) inhibits the transcriptional

activation of Olf1/EBF1-responsive elements (Bond et al., 2004. Blood 103:2062-2070).

Preliminary experiments suggest that enforced overexpression of EHZF cDNA in human CD34+ cells causes an increase in total cell expansion and the accumulation of myeloid progenitors in Dexter-type co-cultures, and affects the cell cycle status.

Taken together all the above data support the notion that EHZF might be a relevant factor in the regulation of normal and neoplastic human haematopoiesis. The further characterisation currently in progress - of the biological effect of EHZF, of its molecular interactions and of the regulatory elements of its gene will provide insight into its function in the development and the homeostasis of the human haematopoietic system and its potential involvement in mechanisms of leukaemogenesis.

ANEMIA - GROWTH FACTORS

544 ERYTHROPOIETIC ACTIVITY INTERFERES WITH RED CELL PARAMETERS IN HEMODIALYSIS PATIENTS

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Background The percentage of hypochromic red blood cells (%HYPO) is usually monitored to detect functional iron deficiency in hemodialysis patients treated with recombinant human erythropoietin (rHuEPO). Initiation of rHuEPO therapy is associated to erythropoietic stimulation and reticulocyte production. We have previously shown that the percentage hypochromic RBC was correlated to the reticulocyte count. Moreover, a younger age of the red cell population is associated to an increase in %HYPO.

Aims The aim of this study was to assess the specificity of %HYPO for iron deficiency in a situation of erythropoietic stimulation with appropriate iron supply.

Methods Six patients, free of inflammation and transfusion, completed the follow-up. rHuEPO was administered intravenously at the dose of 200 UI/kg/week in 3 doses. Iron supplementation was initiated at the start of rHuEPO therapy at the dose of 100 mg IV iron sucrose (Venofer®) once weekly. Iron and red cell parameters were measured weekly for 11 weeks before the first dialysis session of the week.

Results The treatment schedule was efficient for the correction of anemia. The Hb level was 12.1 ± 0.6 at the end of follow-up versus 8.3 ± 1.2 g/dL before rHuEPO administration. The reticulocyte crisis occurred at day 7. Despite IV iron supplementation, ferritin (FRT) and transferrin saturation (TfSAT) decreased respectively from 241.2 ± 130.4 to 112.0 ± 30.1 ng/mL and from 18 ± 3 to 15 ± 3 %. All along the follow-up, CHr remained constant (31.8 ± 2.1 vs 32.4 ± 0.7 pg; p=0.56). However, a progressive increase of %HYPO was observed (7.2 ± 4.1 vs 1.7 ± 0.8 %; p<0.05). This evolution is due to a slight decrease in Hb concentration (CHCM: 32.3 ± 0.5 vs 33.0 ± 0.7 pg/mL; p=0.14) secondary to increased RBC volume (90.1 ± 2.5 vs 85.7 ± 2.4 fL; p<0.001) and stable Hb content (CH: 28.6 ± 2.0 vs 28.7 ± 0.8; p=0.89). The evolution of reticulocyte and RBC volume and Hb concentration were similar.

Conclusion The administration of 100mg IV iron sucrose per week at rHuEPO initiation prevented iron deficient erythropoiesis according to the evolution of CHr. Despite CHr stability, %HYPO increased. This can be explained by the reticulocytosis and renewal of the red blood cell population interfering with red cell parameter determination. This may lead to overtreatment by IV iron with the risks of complications it may carry. The measurement of mature erythrocyte parameters with new cell counters could be of interest in this field since they could exclude the influence of the erythropoietic stimulation with increased reliability in detecting functional iron deficiency.

545 EFFECT OF ERYTHROPOIETIC ACTIVITY AND CELL VOLUME ON RBC PARAMETER DETERMINATION: COMPARISON WITH MATURE ERYTHROCYTE INDICES

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Background : Since the era of erythropoietin (rHuEPO), iron supply has been a major limiting factor for therapeutic response. Percentage hypochromic red blood cells (%HYPO) and reticulocyte hemoglobin content (CHr) have proved to be reliable parameters for functional iron deficiency. However, CHr shows acute changes following rHuEPO or iron administration and %HYPO presents a lack of specificity. Moreover, the latter is dependent on erythropoietic stimulation and age of the

erythrocyte population. New cell counters are able to measure volume and Hb concentration of individual reticulocytes and mature RBC. We have studied the effect of erythropoietic activity, cell volume and iron availability on RBC parameters. These parameters were compared to reticulocytes and mature erythrocytes indices.

Methods. Red cell parameters were measured by the Advia 120 (Bayer Diagnostics, Tarrytown, NY, USA). We determined reference values in 57 healthy subjects as the central 95 percentiles. The effect of erythropoietic activity was studied through cell parameter determination in 20 patients with auto-immune hemolytic anemia (HA). Cell volume effect was assessed in 11 heterozygous α -thalassemia (HT) and 6 beriberi's disease (BD). Influence of iron availability was studied in 20 patients with iron deficient anemia (IDA) and 21 patients with hemochromatosis (HC). **Results** : In healthy subjects, reticulocyte volume was 20.1% greater than in mature RBC, Hb content was 8.7% greater and Hb concentration was 9.6% lower. Stress reticulocytosis as observed in HC (11.6 ± 4.6 % reticulocytes) led to bias in RBC parameter determination with increased MCV, decreased CHCM and increased %HYPO. These parameters were normal when determined on the mature erythrocyte population. In all other studied pathologies, mature and routine measurements were similar since the number of reticulocytes was low. In microcytic anemia (HT), Hb content was low but Hb concentration was normal. The opposite situation was observed in macrocytic anemia (BD) with high Hb content and normal Hb concentration. In IDA, both Hb content and concentration were low. These parameters were high in HC.

Conclusion : Stress erythropoiesis leads to bias in red cell parameter determination (increased cell volume and decreased cell hemoglobin concentration). Such bias can be excluded through the measurement of mature erythrocyte parameters. Hemodialysis patients treated with erythropoietin represent a situation between hemolytic anemia (increased erythropoietic activity without iron deficiency) and iron deficient anemia (iron deficiency and normal to low erythropoietic activity), still more at rHuEPO initiation. In such patients, the use of mature erythrocyte indices could be more efficient for the monitoring of iron supplementation. As cell volume positively influence the Hb content (HA and BD) but not the Hb concentration, and iron availability influence both Hb content and concentration, Hb concentration and %HYPO of mature RBC should be the most specific and sensitive markers of iron deficiency among all available indices. Further studies should be performed to determine the efficiency these parameter in the prediction of response to iron supplementation.

546 ERYTHROPHAGOCYTOSIS BY HUMAN MACROPHAGES INTERFERES WITH THEIR ANTI-BACTERIAL EFFECTOR FUNCTIONS

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Background: Bacterial infections are well known complications of severe forms of thalassaemia. Possible mechanisms include impaired antibacterial effector functions of the mononuclear phagocyte system due to its involvement in ineffective erythropoiesis and hemolysis.

Aim: a. To examine the effect of erythrophagocytosis on bacterial ingestion and reactive oxygen species (ROS) generation by macrophages and b. to elucidate the mechanism of any compromise in these antibacterial functions.

Methods: THP-1 cells were phorbol myristate acetate (PMA)- induced to differentiate into macrophages and exposed to IgG anti-RhD coated human erythrocytes (ElgG). Following lysis of any extracellular ElgG, the macrophages were investigated by flow cytometry for 1. phagocytosis of unopsonised FITC-labelled E. coli 2. PMA-elicited generation of ROS, by 2,2'-dichlorofluorescein (DCFH) oxidation. In addition, macrophage lysates were prepared and assayed for the anti-oxidant enzyme catalase (by the ammonium molybdate procedure) and protein content (by the Bradford reagent)

Results: ElgG phagocytosis, in a dose-dependent manner, reduced macrophage bacterial ingestion and ROS generation by 25% (n=3) and 75% (n=10) respectively, with ElgG:macrophage ratio 20:1, but only the decrease in ROS reached statistical significance (p<0.001).

The impaired ROS generation by ElgG-treated macrophages was not due to decreased viability (control vs ElgG-treated macrophages, n=3: 79 vs 81% p>0.05, by propidium iodide). Neither was the reduction in ROS caused by excessive iron: the defect was not corrected by desferrioxamine (0.2-2.0 mM). Furthermore, the addition of arachidonic acid (1-20 μ M) previously shown to correct impaired ROS generation by erythrophagocytosing rat macrophages, did not improve ROS production by ElgG-treated THP-1 macrophages.

However, following ElgG phagocytosis THP-1 macrophage lysates showed a dose-dependent increase in catalase activity by 44% (n=6) with ElgG:macrophage ratio 20:1 (p<0.05), but not in total protein content.

Conclusions: The findings suggest that erythrophagocytosis compromises bacterial ingestion and ROS generation by human macrophages which is likely to contribute to the increased susceptibility to bacterial infections in thalassaemia as well as in other conditions of haemolysis and ineffective erythropoiesis. A possible mechanism of reduced ROS is their accelerated inactivation by erythrocyte-derived catalase.

547 INVESTIGATION OF ACTIVATED T-CELLS IN NON-HODGKIN LYMPHOMA PATIENTS

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Background. The immune system has several mechanisms to fight against developing malignant cell clones in the host, one of them is the activated T-cell response. Both CD4+ helper and CD8+ cytotoxic T-cells bear CD69 and HLA-DR molecules as important surface activation markers.

Our aim was to determine how the ratio of activated T-cells change in the peripheral blood of non-Hodgkin lymphoma (NHL) patients during the periods of polychemotherapy.

Methods. We used the blood samples of 35 NHL patients (17 females, 18 males, mean age 52.4 years). We determined the level of CD3+HLADR+ and CD3+CD69+ T-cell subsets before, during and after the periods of polychemotherapy, using the methods of immunofluorescence stain and flow cytometry.

Results. We found the ratio of HLADR+ cells significantly higher in NHL patients before treatment than in the blood of healthy controls (10.63% vs. 2.97%, p<0.001). During the period of polychemotherapy, this ratio began to increase significantly (10.63% vs. 16.94%, p=0.006). Looking at the level of CD69+ cells, we could not find any significant changes. After treatment, the ratio of activated T-cells decreased, however, learning the lesson of some clinical cases, we suppose that permanently high T-cell activity might refer to persisting disease or danger of relapse.

Summary. Investigation of activated T-cells might be a promising method to determine the immune defence and this way further refine the prognostics of lymphoma patients.

548 THE IMPACT OF BASELINE HEMOGLOBIN ON TRANSFUSION REDUCTION, HEMOGLOBIN RESPONSES AND QUALITY OF LIFE IN BREAST CANCER PATIENTS ON CHEMOTHERAPY RECEIVING ONCE WEEKLY 40,000 IU EPOETIN ALFA

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Background: A randomized controlled trial designed to address the impact of hemoglobin (Hb) maintenance at 12 g/dL using epoetin alfa was conducted in 350 adjuvant or metastatic breast cancer patients receiving chemotherapy with baseline hemoglobin $\leq$ 12 g/dL. Patients receiving 40,000 IU QW epoetin alfa showed significantly higher percentage of Hb responders (maintaining mean Hb $\geq$ 12 g/dL from week 4 to 12), lower transfusion rate and improved quality of life (QOL) compared to patients receiving standard of care treatment (SOC) (Chang et al, "Once weekly epoetin alfa improves quality of life." ASCO 2003)

Aim: The impact of baseline Hb on efficacy parameters, a planned secondary analysis, was performed to examine whether patients with certain baseline Hb levels exhibit better outcomes when receiving epoetin alfa as compared to SOC. Patients were divided into three Hb strata for the analysis: <10 g/dL, $\geq$ 10 g/dL and <11 g/dL and $\geq$ 11 g/dL and $\leq$ 12 g/dL.

Method: Breast cancer patients receiving chemotherapy were randomized in a 1:1 ratio to epoetin alfa 40,000 IU QW or SOC when their Hb fell below 12 g/dL. Baseline Hb and other demographics were balanced between the two groups.

Results: Data are shown in table below. Since patients were randomized when Hb fell below 12 g/dL, only a small number of patients (N=26) entered the study with Hb < 10 g/dL. The percentage of patients maintaining a mean Hb level of 12 g/dL in this stratum was therefore lower than in other strata, however a high percentage did demonstrate a $\geq$ 2 g/dL increase in Hb when treated with epoetin alfa. Patients with

baseline Hb $\geq$ 10 g/dL and < 11 g/dL responded best to epoetin alfa with an almost twenty-fold increase in response rate and a six-fold reduction in transfusion rate compared to SOC. Patients from all Hb strata benefited in terms of QOL improvement when receiving epoetin alfa with maximum benefit observed in patients with baseline hemoglobin from $\geq$ 10 g/dL. Conclusion: Epoetin alfa administered at 40,000 IU once-weekly benefited patients with different baseline Hb levels but early therapy appears to be most effective in reducing risk of transfusion.

Caption 1: Table 1

549 TREATMENT OF ANEMIA USING RECOMBINANT HUMAN ERYTHROPOIETIN (R-HUEPO) IN PATIENTS TREATED WITH CHEMOTHERAPY : PROSPECTIVE SURVEY TO CREATE A BELGIAN DATA BASE (BEPOS)

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The Belgian Erythropoietin survey (BEPOS) will prospectively and comprehensively document the use of recombinant human erythropoietin (r-Hu EPO) in adult cancer patients receiving chemotherapy at various stages of their disease. It is intended that data will reflect routine clinical practice.

Patients with age of 18 years or older, treated for non small cell lung cancer, breast cancer, multiple myeloma and lymphoma, as soon as erythropoietin therapy is started for the first time in this treatment, were enrolled in the period from January 2003 to June 2003.

Interim analyses available in January 2004 include data from the first for 208 patients (90 breast cancer, 3 Hodgkin, 24 non Hodgkin, 73 lung cancer and 18 multiple myeloma). These patients were treated by medical oncologist (109), haematologist (49), pneumologist (49) and gastroenterologist (1). They are 78 males and 132 females, the median age was 60 years old and 146 patients were treated with non-platinum chemotherapy, 126 patients (61%) started EPO at the first cycle for chemotherapy because of inadequate Hb level but also fatigue (54 %) or impairment of quality of life (34 %).

At baseline, mean and median Hb were respectively 9.9 $\pm$ 1.1 gr/dl and 10.1 $\pm$ 1.1 gr/dl, 47 % of patients had a Hb level between 10.0 and 10.9 gr/dl, in 29 % between 9.0 and 9.9 gr/dl and in 15 %, the Hb concentration was less than 9 gr/dl. This mean concentration was similar in patients with breast and lung cancers and lymphomas but somewhat lower in patients with multiple myeloma.

At the end of the survey period by which time 69 % of patients had completed their course of EPO, mean and median Hb level of the 201 patients studied had increased to respectively 11.6 $\pm$ 2, 11.7 $\pm$ 2. Sixty-three % of patients have Hb had completed their course of EPO, mean and median Hb level of the 201 patients studied had increased to respectively 11.6 $\pm$ 2, 11.7 $\pm$ 2. Sixty-three % of patients have Hb mean and median Hb at 4 weeks after EPO were respectively 11.1 $\pm$ 1.4, 11.9 $\pm$ 1.4 and the mean and median number of weeks to achieve 2gr/dl. Hb increase were 6.4 $\pm$ 4.8 and 4.6 $\pm$ 4.8 weeks.

550 EARLY INCREASE IN IMMATURE RETICULOCYTE FRACTION PREDICTS RESPONSE TO RHUEPO THERAPY IN VERY LOW BIRTH WEIGHT PREMATURE INFANTS

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Background: Premature infants, frequently develop anemia resulting at least in part from a relatively poor erythropoietic response to anemia primarily ascribed to a blunted erythropoietin production. These infants often receive multiple transfusions with the risk of disease transmission. Early administration of high doses r-HuEPO combined with iron supplements and blood withdrawal limitation significantly reduced the need for blood transfusion. However, almost half of infants still need red cell transfusions despite high dose rHuEPO. Reticulocyte count and morphological indices have proved to be useful in monitoring rHuEPO response in adult renal patients by early detection of functional iron deficiency using hemoglobin content of reticulocytes (ChR) and by assessment of erythropoietic stimulation using immature reticulocyte fraction (HFR).

Aims: The aim of this study was to assess the usefulness of reticulocyte indices as markers of response to rHuEPO and/or of iron deficient erythropoiesis.

Methods: Forty-six very low birth weight (VLBW) premature infants were treated from the first day of life until 36th week with rHuEPO (Eprex®) 750 UI/kg/week, s.c. and with iron 1mg/kg/day, i.v. (venofer®) from day 3 to day 15, then 7.5 mg/kg/day, p.o. (Fer-In-Sol®). Patients were transfused with 15 ml/kg of irradiated, leukocyte-reduced, CMVneg packed red cells when the following criteria were met: either HCT<20% or HCT<30% and FIO2<0.35. According to these criteria, 17/46 infants needed transfusion despite rHuEPO treatment. Hemoglobin, HCT, reticulocyte count, HFR and CHr were determined using Bayer Advia 120 hematology analyzer and were compared together with clinical parameters including 'Clinical Risk Index for Babies' (CRIB) between transfused and non-transfused infants. **Results:** Transfused infants had lower birth weight and gestational age and higher CRIB than non-transfused. Reticulocyte count increased shortly after rHuEPO treatment initiation equally in both groups. However, HFR at day 7 was significantly different between transfused and non-transfused babies, even when patients transfused during the first week were excluded (13.2 ± 3.1, n=14 vs 22.6 ± 2, n=26, mean ± SEM respectively in transfused and non-transfused patients, p<0.02). A HFR value less than 14.4% at day 7 identifies patients who will require transfusion with 71.4% sensitivity and 76.9% specificity. There was no difference for CHr and other iron parameters between both groups.

Conclusion: Despite rHuEPO therapy combined with iron supplementation, 37 percent premature infants still need red cell transfusion. As a sensitive marker of erythropoietic stimulation, immature reticulocyte fraction (HFR) may be helpful to monitor early response to rHuEPO therapy, and identify poor responders. As assessed by both biochemical parameters and CHr, these poor responses were not secondary to functional iron deficiency as reported in renal adult patients.

551 EPOETIN BETA (NEORECORMON) ONCE WEEKLY OR THREE-TIMES WEEKLY PRODUCES A RAPID HAEMOGLOBIN RESPONSE IN ANAEMIC PATIENTS WITH LYMPHOPROLIFERATIVE MALIGNANCIES

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Background: Time to achieve a clinically relevant haemoglobin (Hb) response to erythropoietic therapy is an important consideration in anaemic patients with cancer, especially those receiving myelosuppressive chemotherapy. However, very few studies assessing the efficacy of erythropoietic agents have reported Hb response as a function of time.

Aims: To assess rapidity of Hb response following treatment with epoetin beta (NeoRecormon) once weekly (q.w.) or three-times weekly (t.i.w.) in patients with lymphoproliferative malignancies.

Methods: This was an open-label, randomised, prospective clinical trial in which patients with lymphoproliferative malignancies received epoetin beta 30 000 IU q.w. or 10 000 IU t.i.w. (Cazzola et al. Br J Haematol 2003; 122: 386-393). Adults with low-grade non-Hodgkin's lymphoma, multiple myeloma or chronic lymphocytic leukaemia, Hb levels of 9-11 g/dl and serum erythropoietin levels of ≤100 mU/ml were enrolled. The time at which patients achieved a ≥1g/dl or ≥2 g/dl increase in Hb from baseline (confirmed by a 2nd measurement within 2 weeks) was determined.

Results: A total of 241 patients were enrolled, of whom 229 were evaluable for intent-to-treat analysis (q.w., n=115; t.i.w., n=114). Almost all patients were receiving concurrent chemotherapy or had received recent chemotherapy. Percentages of patients achieving a ≥1 g/dl or ≥2 g/dl response and median time to response with epoetin beta q.w. and three times weekly are reported below.

Conclusions: Epoetin beta treatment resulted in an Hb increase of ≥1 g in 4.1 weeks with both q.w. and t.i.w. treatment in the majority of patients. This increase represents a rapid and meaningful clinical benefit in patients receiving antineoplastic therapy.

	Epoetin beta 30 000 IU q.w. (n=115)	Epoetin beta 10 000 IU t.i.w. (n=114)
Hb increase ≥1 g/dl % of patients Median time, weeks (CI)	90% 4.1 (3.4, 4.7)	89% 4.1 (3.4, 5.1)
Hb increase ≥2 g/dl % of patients Median time, weeks (CI)	73% 6.1 (5.1, 10.1)	74% 6.1 (5.1, 8.1)

Caption 1:

552 RED BLOOD CELL AGING PATTERNS: IN VITRO OBSERVATIONS USING ATOMIC FORCE MICROSCOPY

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During the in vivo finite life span of human red blood cells (RBC), a variety of physicochemical processes are at work, influenced by many cellular parameters. This work presents results of observations of RBC in vitro using an atomic force microscope (AFM), and proposes a three or five state aging pattern. Since its invention in 1986, the AFM has found widespread applications in biological sciences. In hematology, the AFM has been used to analyze RBC, providing automated statistics of their size and shape, to observe mechanically traumatized erythrocytes, to distinguish between RBC groups A and O using functionalized tips with attached Helix pomatia lectin. Among the usual parameters employed nowadays as RBC characteristics, mobility, aggregation, and membrane glycolyx properties are age-related, as are the hematological (hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) and structural (size and shape) parameters. Increased release of free radicals and reactive oxygen affect membrane fluidity and cellular Ca²⁺ homeostasis, inducing lipid peroxidation and finally cell death. Investigation of membrane fluidity offers additional insight into the mechanisms by which different compounds, drugs and contact with foreign surfaces affect cellular function. The complex RBC life span is generally described in the literature in a simplified manner, in which RBC are classified as young or old according to standard hematological parameters, and a (disputed) relationship between RBC age and density. Counterflow centrifugation has been the usual method of RBC age-related separation for practical purposes, which is based on the density separation of the cells. In this work, an extended set of RBC structural parameters (size and shape related) were tested to establish an improved picture for the RBC aging pattern. The temporal evolution of the volume, deviation from a circular profile, degree of concavity, mean border slope (degree of border smoothness) etc of hundreds of in vitro RBC was followed individually, both with μm and sub-μm resolution, using an AFM from Digital Corporation, Nanoscope IIIa model. Analysis of the high resolution AFM images, including those recorded in real time films to be presented, indicates good statistical temporal correlation of most of the investigated structural parameters for the definition of time evolution patterns. The results suggest a three or five state evolution pattern for the complex RBC life span. The main physicochemical processes that might be assigned to each state are discussed, as well as the influence of disease on the time aging patterns. Finally, a strategy to implement in vivo characterization of the RBC aging patterns using the AFM is presented.

553 EFFECT OF ANAEMIA CORRECTION WITH EPOETIN BETA (NEORECORMON) ONCE WEEKLY ON PERFORMANCE STATUS AND DISEASE RESPONSE

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Background: Epoetin beta improves haemoglobin (Hb) levels, reduces transfusion requirements and improves quality of life in patients with cancer. Recent data also suggest that epoetin may have an anti-neoplastic effect.

Aims: To investigate the effect of anaemia correction with epoetin beta on performance status and treatment response in patients with haematological malignancies.

Methods: Anaemic patients (Hb 9-11 g/dl) with multiple myeloma, low-grade non-Hodgkin's lymphoma or chronic lymphocytic leukaemia were randomised to 16 weeks of treatment with epoetin beta 30 000 IU once weekly (q.w.) or 10 000 IU three times weekly (t.i.w.). Patients had a WHO performance status (PS) of 0-2 and life expectancy of >6 months. Hb response was defined as an increase of ≥2 g/dl. Changes in PS and treatment response (complete or partial response, stable disease or progressive disease) were assessed by Hb response (<2 or ≥2 g/dl).

Results: A total of 241 patients were randomised to receive epoetin beta q.w. (n=119) or t.i.w. (n=122). Most had a WHO PS of 0-1 and an advanced tumour stage at baseline. The vast majority of patients (93%)

received concurrent chemotherapy. Hb response rate in patients treated with epoetin beta q.w. was high and similar to t.i.w administration (72% vs 75%). Patients with a Hb response were more likely than patients without an adequate response to have an improvement in PS (q.w., 29% vs 11%; t.i.w., 26% vs 4%) and less likely to have a deterioration in PS (q.w., 10% vs 29%; t.i.w., 9% vs 24%). Over half of patients showed a complete or partial response to chemotherapy by study end (q.w., 60%; t.i.w., 55%) while very few patients had progressive disease (q.w., 8%, t.i.w., 13%). Overall, 96% of patients with a complete response (q.w., 100%; t.i.w., 92%) and 85% with a partial response (q.w., 90%; t.i.w., 81%) had a Hb increase of ≥ 2 g/dl.

Conclusions: Epoetin beta q.w. results in a high Hb response in patients with haematological malignancies. Hb response with epoetin beta is associated with an improvement in PS and improved response to chemotherapy. Anaemia correction is associated with treatment response in patients with haematological malignancies.

554 PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH CHRONIC RENAL FAILURE SUPPRESS ERYTHROPOIESIS IN VITRO VIA THE PRODUCTION OF TUMOR NECROSIS FACTOR ALPHA.

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Anemia is a frequent manifestation of chronic renal failure (CRF) and is probably caused by multiple factors. Apart from the low erythropoietin levels recent data suggest for a possible inhibitory effect of inflammatory cytokines on CRF erythropoiesis.

Aim: In order to investigate the role of cytokines in the pathogenesis of CRF anemia we studied the effect of peripheral blood mononuclear cell (PBMC) supernatants from CRF patients on haemopoietic colony formation in vitro.

Patients and methods: The effect of the PBMC supernatants from 111 CRF patients (41 without dialysis, 42 on hemofiltration and 28 on continuous ambulatory peritoneal dialysis) and 33 normal controls on normal erythroid progenitors (BFU-E) colony formation was studied. Mononuclear cells from CRF patients were separated and cultured at 37°C, 5% CO₂ for 48 hours with the addition of phytohemagglutinin. The PBMC supernatants were collected and stored. The formation of normal BFU-E colonies in methycellulose with or without PBMC supernatants at a concentration of 10% was scored on day 14. Furthermore, the modified effect of the PBMC supernatants after the addition of anti-TNF- α and anti-IL-1 β was studied concurrently. The levels of TNF- α , IL-1 β , and IL-3 in the PBMC supernatants were measured by ELISA.

Results: The PBMC supernatants from CRF patients were found to suppress the BFU-E colony formation (BFU-E with PBMC supernatants from controls: 9067,35 / 2X10⁵ cells vs BFU-E with PBMC supernatants from CRF patients: 50,6436,25/2X10⁵ cells, p=0,0006). The suppression of the colony formation (66% of the control) was similar in the three subgroups of CRF patients. The addition of a polyclonal antibody to TNF- α reversed almost completely this suppression (BFU-E with PBMC supernatants from CRF patients: 66% of the control colony formation vs BFU-E with PBMC supernatants from CRF patients + anti-TNF- α : 93% of the control colony formation, p<0.001). The addition of anti-IL-1 β did not alter the BFU-E formation (p=0.948). The TNF- α levels in the PBMC supernatants from CRF patients were elevated compared with the controls (2,23 1,29 ng/ml vs 1.551,20 ng/ml, p=0,006).

Conclusions: PBMC supernatants from CRF patients suppress the in vitro erythropoiesis via the production of TNF- α . The above data suggest that in the pathogenesis of CRF anemia, probably an additional mechanism, similar with the one of the anemia of chronic disease, could be implicated.

555 MECHANISMS INVOLVED IN FETAL HEMOGLOBIN ELEVATION IN ADULT ERYTHROID CELL CULTURES TREATED WITH HYDROXYUREA

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Hydroxyurea (HU) augments the production of fetal hemoglobin (HbF) in most sickle cell patients and is beneficial for some patients with β -thalassaemia. Various cell culture systems have been used to elucidate the molecular mechanisms by which HU mediates these effects. We have previously demonstrated in both K562 human erythroleukemic cells and human adult erythroid cells (hAEC) that HU increases the transcription of low-expressed globin genes as well as the splicing efficiency of primary globin transcripts; however, other possible post-transcriptional effects could not be excluded. The aim of this study was to understand further the role of HU in erythropoiesis and the molecular mechanisms involved in gamma globin gene elevation. Erythroid cells in phase II were exposed to HU at day 5 (proerythroblast stage) either continuously (1-4 days) or were pulse treated [initially exposed to HU (1-3 days), then washed and harvested at day 8-14 days of phase II culture] over a range of HU concentrations (100 and 300 μ M). The effect of HU on cell number was measured by flow cytometry. Continuous exposure had a dose- and time dependent effect on cell number, contrasting pulse treatment. HbF-cell numbers were determined from day 0 of phase II through phase II culture by flow cytometry and the immunocytochemical StreptABComplex/AP staining procedure (SAP). At day 0 of phase II no HbF cells were detected by either method; F-cell numbers increased 2-fold in cultures exposed to 100 μ M/ 300 μ M HU for 3 days. Hemoglobin measurement was done by ion exchange HPLC system while globin chain separation and quantification were done using a C-18 (RP) column on HPLC system. HU treatment caused a dose-dependent increase in HbF percentage but had no significant effect on G γ /A γ globin synthesis rates. Qualitative and quantitative RT-PCR evaluation of gamma-mRNA transcripts in normal hAEC showed that both HU concentrations caused a significant time- and dose-dependent increase in gamma globin mRNA (100 μ M HU, 1-4 days: 2.0 to 4.0-fold); nevertheless, HU at 300 μ M HU led to a more modest increase in gamma-globin mRNA (1.3 -1.5-fold) because of cytotoxicity. HU effects on the stability of gamma-globin mRNA were analyzed in hAEC cultures-treated with HU and actinomycin-D (5 μ g/ml) for 1 hr - 24 hrs. Actinomycin D+100 μ M HU-treated hAEC cells exhibited a 1.6-fold increase in gamma globin mRNA at 24 hrs post-actinomycin D addition. Exposure at higher HU concentrations and/or prolongation of treatment had a toxic effect (decrease in both gamma globin mRNA and as well as cell numbers). In conclusion, the present study provides evidence that HU can stimulate erythropoiesis and increase Hb production in a dose- and time dependent fashion; HU effects are mediated by transcriptional and post-transcriptional modulation of gamma globin gene expression.

556 IMPACT OF RHUEPO (EPOETIN BETA) ON SURVIVAL OF PATIENTS WITH LYMPHOPROLIFERATIVE MALIGNANCIES: LONG-TERM FOLLOW-UP OF A LARGE RANDOMISED STUDY

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Background: Recent clinical studies have suggested that treatment of anaemia with rHuEPO may impact on the survival of patients with cancer (Littlewood et al. J Clin Oncol 2001;19:2865-74; Glaser et al. Int J Radiat Oncol 2001;50:705-15; Henke et al. Lancet 2003;362:1255-60; Leyland Jones et al. Lancet Oncol 2003;4:459-60).

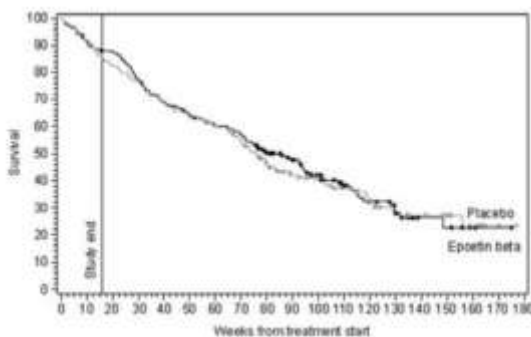
Aims: To conduct a long-term analysis of survival in patients with lymphoproliferative malignancies who were included in a previous randomised study (sterborg et al. J Clin Oncol 2002;20:2486-94) that

assessed the effects of treatment with epoetin beta (NeoRecormon) or placebo.

Methods: This was a randomised, double-blind trial in which transfusion-dependent patients with severe anaemia ($Hb \leq 10$ g/dl) and B-cell chronic lymphocytic leukaemia, low grade non-Hodgkin's lymphoma or multiple myeloma received either subcutaneous epoetin beta 150 IU/kg or placebo three times weekly for 16 weeks. Patients were scheduled to receive anticancer therapy, had a WHO performance status of 0-3 and a life expectancy of at least 4 months. Patient survival data were analysed by standard Kaplan-Meier methods and differences in survival between groups were assessed using a log-rank test.

Results: The intention-to-treat population consisted of 343 patients who received study medication (epoetin beta, $n=170$; placebo, $n=173$). There were no major differences between the two treatment groups in demographic or clinical characteristics/prognostic factors. More patients achieved a haematological response (>2 g/dl Hb increase without transfusion) with epoetin beta compared with placebo at 16 weeks (67% vs 27%; $p<0.0001$), which was associated with a significantly improved quality of life ($p<0.05$). A total of 110 (65%) patients died in the epoetin beta group (censored, $n=60$) and 109 (63%) died in the placebo group (censored, $n=64$) up to the end of long-term follow-up. Kaplan-Meier curves for survival were similar in both treatment groups (see figure) and median survival was 17 months with epoetin beta and 18 months with placebo. Log-rank test indicated no significant difference in survival ($p=0.76$).

Conclusions: This long-term follow-up analysis of a randomised study of patients with lymphoproliferative malignancies and severe, transfusion-dependent anaemia indicated that there were no differences in survival between the epoetin beta group and the placebo group. Further prospective clinical trials specifically designed to address survival endpoints are needed to investigate the long-term effects of anaemia correction with rHuEPO.



Caption 1:

557 PREDICTION OF RESPONSE OF ANEMIA TO RECOMBINANT HUMAN ERYTHROPOIETIN IN PATIENTS WITH MULTIPLE MYELOMA AND LYMPHOMA: EVALUATION AND INTERIM ANALYSIS OF TRADITIONAL AND NOVEL PREDICTIVE MARKERS

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Background: Treatment with recombinant human erythropoietin (rh-EPO) improves anemia in 60-70% of patients with multiple myeloma (MM) and lymphoma. However, about 1/3 of these patients will not respond. The commonly used predictive models often fail to identify those patients who will benefit from rh-EPO therapy. Furthermore, functional iron deficiency is reported as a cause of blunted response to rh-EPO in hemodialysis patients. The purpose of this study was to evaluate both traditional and novel predictive markers of anemia response to rh-EPO as well as to recognize early development of functional iron deficiency, during rh-EPO treatment.

Patients and methods: We studied 27 newly diagnosed patients with MM or lymphoma aged 29-83 (mean 67.714.7) all patients received rh-EPO at a dose of 30000U/wk for six consecutive weeks. In those who responded (Hb increase by 2g/dl after six weeks) rh-EPO was tapered and stopped after two weeks. Non responders received iron saccharate 200mg IV/wk for the next 4 weeks. Response to IV iron was defined as an increase in hemoglobin by 1g/dl within 4 weeks. The parameters studied included

hemoglobin, percentage of reticulocytes (retics%), absolute reticulocyte count (retics-ab), immature reticulocyte fraction (IRF), high fluorescence reticulocytes (HFR), reticulocyte hemoglobin (retics-Hb), reticulocyte Hematocrit (retics-Ht), percentage of hypochromic erythrocytes (HYPO%), reticulocyte hemoglobin content (ChR), reticulocyte mean volume (MCVr), measured in an ADVIA 120 analyser (Bayer). Levels of endogenous EPO and ferritin, soluble transferrin receptor (sTfR) and transferrin saturation (SAT%), were determined with standard techniques. Changes of parameters during follow up were also evaluated.

Results: Nineteen patients (70%) responded to rh-EPO. Eight (30%) did not respond and received IV iron. Six of them responded to iron treatment. Univariate analysis revealed statistical significance for HYPO% ($p=0.05$) at a baseline evaluation and retics% ($p=0.05$), retics-ab ($p=0.03$), retics-Ht ($p=0.01$) at week 2 of observation. Significant changes between baseline and week 2 values were found in retics-Ht ($p=0.01$), retics- Hb ($p=0.021$) retics% ($p=0.01$) and retics-ab ($p=0.02$). Multivariate analysis confirmed baseline HYPO% and a change between baseline and week 2 in retics-Ht as independent predictive markers of response to rh-EPO. A ROC curve revealed that by using a cut-of value of 5% for baseline HYPO% and an increment of 50% of retics-Ht between baseline and week 2 the sensitivity of the model reaches 100% and the specificity 85%. None of the markers studied revealed evidence for the development of functional iron deficiency.

Conclusions: Concerning prediction of response the present study shows that a) When baseline HYPO% is $<5\%$ and the increment of retics-Ht is $>50\%$ after two weeks of rh-EPO therapy there is a strong possibility for response, and b) High HYPO% baseline values ($>5\%$) may represent a probable indicator that co administration of IV iron will improve response of anemia to rh-EPO.

558 EFFECTS OF POPULATION DEFINITIONS AND STUDY ENDPOINTS ON EFFICACY OF EPOETIN ALFA VS PLACEBO IN ANEMIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES RECEIVING CHEMOTHERAPY

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Background: Recombinant erythropoietins (rHuEPOs) have been shown in numerous trials to raise hemoglobin (Hb), reduce transfusions, and improve quality of life (QOL) in anemic cancer patients. Because of high dropout rates in oncology studies and the potential for transfusions to confound Hb measurement, there is concern that some analytic techniques may overstate/understate actual Hb changes.

Aims: Reanalyze a randomized, placebo-controlled, double-blind study of epoetin alfa vs placebo to determine the effect of analytic techniques on results.

Methods: In the original trial, patients ($n=375$) receiving nonplatinum chemotherapy with Hb less than/equal to 10.5 g/dL or Hb decrease greater than/equal to 1.5 g/dL in a single month and Hb less than/equal to 12 g/dL were randomized 2:1 to epoetin alfa or placebo for up to 24 weeks. Mean Hb increase from baseline to last assessment was 2.2 g/dL with epoetin alfa vs 0.5 g/dL with placebo. Because the duration of the most recently completed trials of rHuEPOs have been shorter (12-16 weeks), we reanalyzed the data at these timepoints to allow for comparison with other trials. Analyses addressed the use of intent-to-treat (ITT) vs efficacy (EFF) vs per-protocol (PP) populations, the effect of censoring Hb values within 28 days of transfusion (CEN) vs including all Hb values regardless of transfusions (ALL), and the effect of study duration. Analyses were performed on the hematologic subset of patients in the original trial.

Results: Results are presented in the table. There was no apparent difference in Hb change estimates across populations analyzed. However, censoring Hb values within 28 days of transfusion resulted in higher Hb change estimates than not censoring for both epoetin alfa and placebo. Nevertheless, the differences were small, with a Hb change of 2.6 g/dL in the ITT/ALL group and 2.8 g/dL in the ITT/CEN group at 16 weeks. Variations in results from different analytic methods were equally pronounced when the difference between epoetin alfa and placebo was considered. Trial duration had a greater effect on Hb results: Hb change with epoetin alfa (ITT/ALL) was higher at 12- and 16-week assessments (2.4 g/dL and 2.6 g/dL, respectively) than that previously reported at last assessment (2.2 g/dL at up to 24 weeks). Trial duration also had an effect on Hb change in the ITT/CEN population (mean Hb change: 2.6 g/dL at Week 12 and 2.8 g/dL at Week 16).

Summary/conclusions: Censoring Hb values within 28 days of transfusion exaggerated reports of Hb change in both study arms, presumably because transfused patients have a much lower Hb throughout the study. Censoring Hb values may also bias results in favor of the active treatment group, given the higher transfusion rate in the placebo group. Study duration also appears to have an effect on results, with Hb change in the epoetin alfa group at 12 and 16 weeks higher than previously reported at up to 24 weeks, likely because of disease progression or decreases in epoetin alfa dose in patients whose Hb goals were reached. These factors must be carefully considered when designing or comparing trials.

Mean change in Hb (g/dL) from baseline to Week 16 in patients with hemato-oncologic malignancies

	ALL	ITD	GBM	BP
Response rate	0.8	0.8	0.8	0.8
	0.8	0.8	0.8	0.8
Placebo	0.8	0.8	0.8	0.8
	0.8	0.8	0.8	0.8
Difference between epoetin alfa and placebo groups	0.8	0.8	0.8	0.8
	0.8	0.8	0.8	0.8

Caption 1: Table 1

IRON / BONE MARROW FAILURE

559 TRANSFERRIN MODULATES TNF PRODUCTION ON HUMAN

IMMUNE CELLS: ROL OF TRANSFERRIN RECEPTOR

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Background: Transferrin (Tf) is the major iron (Fe) transport protein, and tumor necrosis factor (TNF) is a multifunctional cytokine mainly produced by blood monocytes and lymphocyte, which play a key role in both immunity and Fe metabolism. Animal studies have previously demonstrated that Tf induce cytokine production.

Aims: The capacity of Tf to modulate in vitro TNF production by human immune cells and the participation of transferrin-receptor (TfR) in this event were investigated.

Methods: Women 25-45 years old with normal iron status (n=10) or with iron deficiency anemia (IDA, n=10) due to gynecologic bleeding were studied. Blood mononuclear cells (BMNC) and purified monocytes (Mo) or lymphocytes (Ly) were in vitro stimulated with different Tf-Fe saturations (0-30-80%). The secretion and mRNA levels of TNF were measured by ELISA and colorimetric quantitative assays, respectively.

Results: Apo-Tf or holo-Tf induced equally well TNF secretion in the supernatants of the cells from both groups. However, cytokine levels in BMNC and purified Mo and Ly were significantly lower in IDA subjects. For all Tf-Fe saturations assayed, mean values varied between 1.4-1.6 ng/mL and 0.4-0.7 ng/mL in BMNC from normal and IDA women, respectively (p < 0.001). Importantly, Fe-deficient Mo and Ly produced 50% and 90% less TNF than respective normal Fe cells. Tf-treated BMNC from normal individuals expressed about 2-3 times more TNF mRNA than those from IDA subjects. Mean values ranged 96-110 atmol/mL in normal women and 24-31 atmol/mL in IDA women for all Tf-Fe saturation's tested (p < 0.001). Cells co-cultured with anti-TfR monoclonal antibody (mAb) decreased Tf-induced TNF secretion by about 70% compared to the isotype controls; by contrary, cytokine secretion in Fe-deficient cells was not influenced by addition of mAb to the culture.

Summary/conclusions: The present study shows that Tf-induced TNF secretion is transcriptionally regulated, where Ly are more sensitive than Mo to Fe status of subjects. Data also suggest that transferrin-receptor is involved in the signalling mechanism of the cytokine. The demonstrated linkage of an individual Fe status to monocytes and lymphocytes may be linked through this pathway to quality immune response and thus capacity to resist infections and malignancies.

This study was supported by Fondecyt grant N 1020693

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560 PREVALENCE OF C282Y, H63D AND S65C MUTATIONS OF HFE GENE IN 1146 NEWBORNS FROM A REGION OF NORTH SPAIN

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Background: Genetic hemochromatosis (GH) is a disease characterised by a progressive increase of body iron stores that may eventually cause tissue damage. In Spain, 85% of patients with GH are homozygous for the C282Y mutation of the HFE gene. H63D and S65C mutations of HFE may also play some role in the disease.

Aims: To establish the prevalence of these three HFE gene mutations in newborns in Catalonia (Spain)

Methods: 1146 newborn screening cards were randomly selected. DNA from these cards was extracted and HFE mutations were analysed with the commercial system FRET using LightCycler equipment

Results: In 1084 cases, C282Y mutation detection was possible. The allelic frequency of this mutation was 0.03 (IC 95% 0.023-0.037). In 1054 cases, H63D and S65C mutations detection was performed, with allelic frequencies of 0.2 (IC 95% 0.18-0.22) and 0.01 (IC 95% 0.006-0.015), respectively. The frequency of C282Y homozygous newborns was 0.001 (IC95% 0.0005-0.0014). The frequency of newborns doubly heterozygous for C282Y/H63D and C282Y/S65C was 0.01 (IC 95% 0.004-0.02) and 0.002 (IC 95% 0.0002-0.01). Serious alterations in HFE gene were seen in 0.06 of newborns.

Summary and conclusions: Newborn screening cards are useful for genetic testing for hemochromatosis, with 92-95% efficiency. The allelic frequency of C282Y mutation is similar to that observed in Southern France, in the Czech Republic and in some areas of Italy with inhabitants of northern European ancestry. The allelic frequency of H63D mutation in Catalonia is the highest reported to date. Nevertheless, S65C is infrequent. These data should be kept in mind in the design of genotypic screening programs for hemochromatosis in Catalonia

561 PHARMACODYNAMICS OF REPEAT DOSE ORAL

ADMINISTRATION OF GT56-252 IN IRON- LOADED CEBUS MONKEYS

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Background: GT56-252 is a novel orally available iron chelator under development for the primary treatment of iron overload resulting from transfusion therapy in thalassemia and sickle cell disease. Pharmacodynamic studies have been conducted over a range of doses and dosing regimens to measure iron clearing efficiency in a non-human primate model of iron overload.

Aims: The goal of these experiments was to expand the dose response relationship for iron clearing activity.

Materials and Methods: Eight animals which had been previously iron loaded to approximately 500 mg/kg were treated with GT56-252 at doses of 5, 15, 30 and 45 mg/kg once per day over a three day period and the iron clearance was measured. In addition, the relative iron clearing efficacy of administering a single dose of 30 mg/kg/day or administering 15 mg/kg twice a day was evaluated. Urine and fecal samples were collected over 12 hour intervals each day for 3 days prior to treatment and 3 days following treatment. Fecal samples were analyzed by ICP-MS for iron content.

Results: The drug was well tolerated in all of these studies. Iron clearing efficiency values were calculated to range from 0.06 mg/kg dose in the 5 mg/kg dose group to 0.53 mg/kg/dose in the 45 mg/kg dose group.

Conclusions: The data demonstrate good dose proportionality, and a minimal impact of dosing regimen (once a day vs. twice a day) on iron clearing efficacy.

562 THE STUDY OF THE COMPLEX FORMATION BETWEEN

SOLUBLE TRANSFERRIN RECEPTOR AND TRANSFERRIN

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BACKGROUNDS: Transferrin receptor (TfR) is a dimeric transmembrane protein that takes part in iron uptake by cells through complex formation with iron loaded transferrin (Tf) and its subsequent internalization into cells. Soluble transferrin receptor (sTfR) is an extracellular part of cell TfR

that is cut and released into plasma mainly from maturing red cells. It is supposed that sTfR has a dimeric structure like cell TfR although there are no disulfide bonds in sTfR.

AIMS. The aims of this work was to study a dimeric structure of sTfR through the immunological determination of complex formation between sTfR and Tf.

METHODS. Monoclonal antibodies for sTfR, Tf, and sTfR-Tf complex determination were from HyTest, Finland. FPLC gel filtration of Tf, sTfR and their complexes was performed using a Superdex 200 column and the AKTA-FPLC system (Amersham Pharmacia Biotech, Sweden).

RESULTS. FPLC gel filtration of purified sTfR showed that it eluted as a peak corresponding to a molecular weight of 237 kDa. FPLC gel filtration of sTfR mixed with Tf in various ratios showed that complexes with different molecular weights can be obtained. When a molar ratio between sTfR and Tf was 10:1, a small left shoulder appeared in the sTfR peak. This shoulder contained sTfR-Tf complex activity corresponding approximately to 291 kDa whereas the main peak of 237 kDa had only sTfR activity. When sTfR and Tf were mixed in a 1:2 ratio (the excess of Tf), the resulting peak corresponded to a weight of 345 kDa, and sTfR-Tf and sTfR activities coincided. Gel filtration of a 2:1 receptor-transferrin mixture (the excess of sTfR) resulted in a wide protein peak with a molecular weight between 291 and 345 kDa. No free sTfR was detected in this case, and both sTfR-Tf and sTfR activities coincided with the protein peak. Only 345 kDa sTfR-Tf complex was found in human plasma; the activity of pure sTfR corresponding to 237 kDa was not detected in plasma.

CONCLUSIONS. Our study shows that sTfR can form different complexes with Tf depending on their ratio in mixture. The molecular weights determined for sTfR-Tf complexes indicate that a triple 2:1 sTfR:Tf complex (MW 291 kDa), a quadruple 2:2 sTfR:Tf complex (MW 345 kDa), and their mixture can be obtained when sTfR and Tf are mixed in different ratios that confirms the suggestion about dimeric structure of sTfR. No free sTfR circulates in blood that is all sTfR in plasma is bound to Tf.

563 FUNCTIONAL CHARACTERISTICS OF DENDRITIC CELLS (DC) AND ACTIVATED LYMPHOCYTES IN APLASTIC ANEMIA (AA) PATIENTS

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Background: Characteristic increase in Th1/Th2 ratio in AA patients might be responsible for autoreactive T-cell mediated suppression of hematopoiesis. Immunoregulatory cytokines (IL-10 and IL-12) secreted by the activated DC and lymphocytes (LCs) mediate Th1 and Th2 immune response, respectively. Besides, IL-10 influences generation of CD4+CD25+ regulatory T cell. However functional characteristics of DC and activated LCs in AA patients remain unelucidated. **Aims:** to study the functional characteristics of DC and activated LCs in AA patients. **Methods:** We have carried out the flow cytometry analysis of the expression of costimulatory molecules by DC generated from peripheral blood mononuclear cells (PBMC) of 10 AA patients before and after the immunosuppressive therapy (IST). DCs from 4 healthy donors (HD) were studied as a control group. Supernatants (SNs) of LPS activated DC cultures and LC cultures with or without PHA stimulation were studied for IL-10 production by ELISA. CD4+CD25+ regulatory T cell population of AA patients and HDs has been evaluated by means of magnetic beads for the negative selection of CD4+ T-cells and immunofluorescence staining with anti-CD25 monoclonal antibodies. **Results:** DCs of 5/10 AA patients exhibited lower than HDs' DCs expression of CD83 - marker of mature DCs. Level of IL-10 production by DC from AA patients was significantly lower compared with HD; opposite to HD LCs, unstimulated LCs from untreated AA patients failed to produce IL-10, however, LPS stimulation resulted in IL-10 secretion though to significantly lower levels compared with HDs; IST enhanced IL-10 production by DCs as well as stimulated and unstimulated LCs from AA patients, though to lower amounts than those of HD. **Conclusion:** Altered expression of CD83 by the DC from AA patients might influence the activation of autolytotoxic T-cells. IL-10 downregulation might inhibit the generation of CD4+CD25+ regulatory T cell population and consequently, contribute to the expansion of autolytotoxic T-cells and to the development of AA. Data of CD4+CD25+ Treg cells will be presented.

564 TNF-ALPHA CAUSES AN AUTOCRINE IFN-GAMMA RESPONSE IN CD34+ CELLS

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Acquired aplastic anaemia is a haematological disorder characterised by peripheral blood pancytopenia with hypocellular bone marrow. The aetiology of most cases is unknown. As 70% of cases respond to immunosuppressive therapy with ALG or ATG, T-cell mediated destruction of CD34+ of bone marrow haemopoietic stem and progenitor cells (HSC) is implicated in some cases. The proinflammatory cytokines TNF- α and IFN- γ are suggested as factors involved in immune attack on bone marrow but their exact role is not clear. To try and further understand the effects of these cytokines on CD34+ HSC, we have studied their effect on the transcriptome of the CD34+ KG1 cell line using DNA microarrays. Cells were treated with TNF- α and IFN- γ separately and in combination. Total RNA was extracted and mRNA was amplified by the aRNA method. We have shown that aRNA amplification can be used to obtain quantitatively consistent results, as long as amplified RNA is compared with amplified RNA. Samples labelled with Cy3 or Cy5 were competitively hybridised to a 3360 gene IMAGE clone array (HGMP HuGen1). Results were analysed with ImaGene 5.1 and GeneSpring 6.1 software. We performed a time course experiment on KG-1 cells treated with TNF- α and genes with different expression patterns were explored by k-means clustering and by self organising maps. We noted a large gene set that was only induced from 4h of treatment and included typical IFN- γ responsive genes. Closer examination showed that IFN- γ mRNA was induced from 0.5h. We, therefore hypothesised that TNF- α is causing an autocrine IFN-production by KG1. This was confirmed by ELISA assay of IFN- γ and by western blotting and subcellular localisation of STAT1. We conclude that TNF- α has a dual effect, inducing autocrine production of IFN- γ and simultaneously sensitising the cells to IFN- γ . It will be important in the context of aplastic anaemia to examine whether TNF- α and IFN- γ have similar interactions on primary cells.

565 THE SAFETY AND PHARMACOKINETICS OF GT56-252, A NOVEL ORALLY AVAILABLE IRON CHELATOR

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Background: GT56-252 is an orally active, tridentate, iron chelator under development to treat chronic iron overload conditions resulting from transfusion therapy. The compound is derived from desferrithiocin specifically modified to minimize toxicity associated with the parent compound (Bergeron et al., 1999). GT56-252 effects iron excretion with an efficiency of 13-18% when administered orally to Cebus apella monkeys, a species whose iron metabolism closely resembles that of humans. Iron excretion is predominantly by the fecal route (80-90%). **Methods:** We conducted a study of 18 adult beta-thalassemia patients requiring chronic transfusion and chelation therapy. Three panels of patients received two doses of GT56-252 over two days. Group 1 received approximately a 3 mg/kg solution, with food or fasting. Group 2 received approximately 4.5 mg/kg with food, as the solution or as a capsule. Group 3 received approximately 8 mg/kg as capsules, with food or fasting. Blood samples were taken at intervals up to 24 hours to characterize the pharmacokinetics of GT56-252. Safety laboratories included chemistry and hematology panels, coagulation parameters, urinalysis, and urine beta 2 microglobulin. Additional safety evaluations included physical examination and Electrocardiogrammes (ECG).

Results: GT56-252 was well tolerated. There were no related serious adverse events or notable laboratory abnormalities. ECGs performed before and after dosing were unremarkable. GT56-252 was very well absorbed. The AUC for the fed and fasted state were similar, suggesting that bioavailability is not impaired by food. Further, the systemic exposure is similar for the capsule and liquid, although absorption was delayed and Cmax was considerably lower for the capsule. For all three dosing levels, a significant amount of GT56-252 was recovered in urine. Only a small percentage of the GT56-252 in the urine was complexed to iron, consistent with animal model observations that the majority of iron is excreted in stool.

Conclusion: In this initial clinical study, GT56-252 was safe, well-tolerated, and well absorbed with food and fasting in beta-thalassemia patients. Further studies will be needed to define the effect of GT56-252 on iron balance.

566 COMBINED CHELATION THERAPY IN THALASSAEMIA MAJOR: HOW LONG IT TAKES TO REDUCE BODY IRON OVERLOAD AT SAFETY MARGINS?

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Aims:

The use of intensive chelation therapy with deferiprone (Ferriprox®) and desferrioxamine (Desferal®) in thalassaemia major patients in order to obtain negative iron balance and a significant reduction of body iron overload.

Methods:

0-50mg/kg, 8-12h s.c. or i.v. 2-6 days/week), based on the patients overall clinical status and laboratory assessments. quarterly assessments of serum ALT/GPT, PTT and ECG. Cardiac function was evaluated by Echo annually or biannually. Cardiac and hepatic iron content was estimated by MRI. Compliance to chelation was determined by the Compliance Index.

Results

1) Group 1, 33 patients, mean age 29.2 years, 3 years on combination therapy, with an overall compliance of 88% (table1).

- Analysis of monthly measurements revealed a negative trend of serum Ferritin over the time ($p<0,0001$) with a rate of decline of $-85\mu\text{g/L/month}$.
- Patients with high baseline Ferritin showed a statistically significant higher rate of decline than those with lower baseline Ferritin.

- In 8 patients with pre-existing heart dysfunction symptoms reversed and cardiological medications stopped. Ventricular dimensions and function in Echo were normalized.

- MRI analysis of T2Heart T2Liver revealed a statistically significant reduction of iron overload. Mean T2H changed from moderate (25-30msec) to light (30-35msec) and mean T2L changed from severe ($<25\text{msec}$) to light (30-35msec).

2) Group 2, 8 patients, mean age 27.9 years, 2 years on combination therapy. Compliance was 87% (table2).

- There was a statistically significant decrease of mean serum Ferritin.

- In 2 patients with cardiac symptoms and abnormal cardiac function medication stopped following 2 years of combination therapy.

- MRI assessments showed a mean T2H change from moderate (25-30msec) to light (30-35msec) and a mean T2L change from severe ($<25\text{msec}$) to Free of Iron ($>35\text{msec}$) ($p<0,0001$).

3) Group 3, 9 patients, mean age 22.7 years, 1 year on combination therapy, and a compliance of 67% (table3).

- There was a statistically significant decrease of mean serum Ferritin levels.

- Chelation was not enough to reduce iron-induced cardiac failure in 2/9 patients.

- MRI assessments showed a statistical significant decrease in Liver iron concentration but not in Heart iron concentration yet.

Conclusions:

The combined use of Ferriprox® and Desferal® resulted in a significant decrease in body iron load as assessed by serum Ferritin levels and Liver and Heart iron concentration by MRI. effect in the reduction of body iron. penia (800-1.200) was observed 13-16 months after combination therapy, in 3 non-splenectomised patients leading to discontinuation of deferiprone. One of them with cardiac disease had already benefited from its advantages.

Average	Ferritin µg/L	Cardiac Symptoms	LVEF %	FS %	MRI T2H msec	MRI T2L msec
Group 1: N = 33						
Baseline	3439	24%	60,5	32,5	28,1	22,4
YEAR 2	473	0%	69,5	39,1	34	32,7
Change from Baseline	-2965 $p<0,0001$	YES $p<0,0001$	9 $p<0,0001$	6,6 $p<0,0001$	5,9 $p<0,0001$	10,3 $p<0,0001$
Group 2: N = 8						
Baseline	1435	25%	65,8	36,3	29,3	25
YEAR 2	133	0%	72	42,5	33,3	35,3
Change from Baseline	1302 $p<0,0001$	YES $p<0,0001$	6,2 $p<0,0019$	6,2 $p<0,0001$	4,6 $p<0,0001$	10,3 $p<0,0001$
Group 3: N = 9						
Baseline	3778	22%	62,1	33,7	27,7	20,7
YEAR 1	1948	22%	66,6	37,1	31	29,7
Change from Baseline	-1829 $p<0,0001$	NO $p<0,45$	4,5 $p<0,001$	3,4 $p<0,001$	3,3 $p<0,03$	5 $p<0,002$

Caption 1:

567 DO ANDROGENS AND CYTOGENETIC COULD EXPLAIN LOW INCIDENCE OF SECONDARY LEUKEMIAS IN ACQUIRED APLASTIC ANAEMIA TREATED WITH IMMUNOSUPPRESSIVE THERAPY

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Background : Immunosuppressive therapy in the treatment of aplastic anaemia (AA) consisting of horse antithymocyte globulin (ALG) has been incriminated in the emergence of secondary leukemias (s-MDS/AML). We previously reported a low incidence of secondary leukemias in AA treated with ALG (20 mg/kg/day x 7 days) associated to androgens (norethandrolone, 1 mg/kg/day during 6 months then progressively tapered) (abstract 467, EHA 2003).

Aims : We report the final results of this retrospective monocentric study including cytogenetic analysis, on 87 AA patients (pts).

Results : The median follow up was 120 months (mo.). The median age was 29.8 y and the sex ratio M/F was 0.93. Thirty seven (42%) pts had severe AA according to Camitta criterias, of them 17 (46%) had very severe AA. Sixty four (73.6%) pts received androgens with their first immunosuppressive treatment. Sixty seven (77%) pts reached a response after the first ALG, fifty six (64%) had a complete response (normal hemogram). Twenty (23%) pts presented with first line refractory disease and 27 (31%) pts relapsed at a median time of 21.3 mo. Forty four (50%) pts presented an event (relapse, refractory disease, death because of AA), the median event free survival (EFS) was 64 mo. (IC95% 0-158). Twenty two (25%) pts died, median survival was not reached, and the 20-year cumulative survival was estimated at 70%. In univariate analysis, the refractory disease to the first ALG and the androgens were prognostic factors for overall survival (OS) : 9 mo. (IC95% 0-37) if refractory vs median not reached for the responders ($p<0.00001$) and 106 mo. without androgens vs median not reached ($p<0.0042$). No variable had prognostic impact for EFS.

Two (2.3%) pts developed a late clonal complication, s-MDS=1pt and s-AML=1pt, occurring at 10 and 12 years, respectively. The estimated 10 and 20-year cumulative incidences were 3.1 and 6.5, respectively.

Forty nine (56%) pts had an informative cytogenetic at diagnosis. Of them, 3 (6.1%) had the following abnormal karyotypes (abnormal clone if ≥ 2 metaphase cells with the same abnormality) 46,XX,del(20)(q12)=1pt, 46,XX,del(5)(q22q32)=1pt and 47,XX,+mar(der6? der8?)=1pt. The former presented an early death and the two latter reached a persistent complete remission. None of the informative pts who relapsed or were refractory developed cytogenetic abnormalities (n = 22). One pt developed a monosomy 7 at time he presented with s-MDS while karyotype was normal at diagnosis. The pt who displayed s-AML had normal karyotype. Karyotype had no prognostic impact on OS, EFS and secondary leukemia incidence.

Conclusion : As previously reported, relapse have no significant impact on survival. In our study, being refractory to the first immunosuppressive treatment and having not receive androgens were associated with short survival. Camitta criterias failed to predict survival, as did the cytogenetic. In AA, cytogenetic abnormalities at diagnosis are infrequent but compatible with the diagnosis of AA regardless of marrow morphology, of the long follow-up, and with the achievement of a complete response. We have no explanation for the low secondary leukemias incidence but the possible protective impact of the androgens.

568 EXTRAHEPATIC HEPCIDIN SYNTHESIS IN

LIPOPOLYSACCHARIDE-TREATED MICE

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Background:

Anemia of chronic disease (ACD) is characterized by iron sequestration within macrophages, decreased plasma iron levels and decreased iron absorption. According to current concepts, the pathophysiology of ACD is related to increased synthesis of hepcidin, a small peptide which blocks iron absorption in the duodenum and iron export from macrophages. Hepcidin synthesis is induced during inflammation: urinary hepcidin levels have been documented to significantly increase in the course of bacterial infection, and human hepcidin has been actually characterized as an acute phase protein.

Aims:

We used male C57BL/6 mice treated with lipopolysaccharide (LPS, 1 mg/kg, 6 hours prior to euthanasia) to examine the expression of hepcidin mRNA in the liver, intestine and kidney.

Methods:

Gene expression was determined by real-time PCR using SYBR Green protocol. Specific primers were designed for mouse hepcidin1 (HAMP) mRNA, which is known to influence iron metabolism, and hepcidin2 (HAMP2) mRNA, which responds to iron overload, but has no effect on iron homeostasis. Target mRNA levels are calculated relative to β -actin.

Results:

Hamp gene was very strongly expressed in the liver; HAMP mRNA levels in the intestine and kidney were extremely low. As expected, administration of LPS dramatically elevated hepatic IL-6 mRNA (by two orders of magnitude), however, HAMP mRNA displayed only a slight increase. On the other hand, there was a significant increase of HAMP mRNA in the intestine and kidney, although it must be noted that the induced HAMP mRNA levels in these organs were still much lower than hepatic HAMP mRNA levels (control liver, 4.9 3.5, LPS liver 8.7 1.8; control intestine 0.00005 0.00003, LPS intestine 0.007 0.004, all values relative to β -actin mRNA). In contrast to HAMP mRNA, hepatic HAMP2 mRNA was decreased by LPS treatment.

Following LPS administration, there was a slight decrease in intestinal NRAMP2 and ferroportin1 mRNA; intestinal CYBRD1 mRNA decreased to about 10 % of control levels. As previously reported, LPS significantly downregulated hepatic ferroportin1 expression.

Conclusions:

Anemia of chronic disease results from both increased iron sequestration in macrophages and decreased iron absorption in the duodenum. In LPS-treated mice, there is a dramatic decrease of hepatic ferroportin1 mRNA and duodenal CYBRD1 mRNA. According to current concepts, expression of both these genes is modulated by hepatic hepcidin.

There is some controversy regarding plasma levels of hepcidin during infection. While it has been convincingly demonstrated that urinary hepcidin levels dramatically increase during inflammation, a similar rise in plasma hepcidin has not been found. This suggests that hepatic hepcidin could be rapidly cleared from the circulation. In this respect, it is interesting to note that the induction of murine HAMP by LPS is not restricted to the hepatocyte, since LPS increases HAMP mRNA levels in the pancreas, kidney and intestine as well. It can therefore be speculated that the increased intestinal hepcidin synthesis during inflammation could possibly contribute to local hepcidin concentration, which in turn probably downregulates intestinal Cybrd1 expression, and therefore leads to a decrease in iron absorption.

569 FREQUENCY AND PHENOTYPE OF MUTATION S65C IN 550 PATIENTS WITH SUSPECTED IRON OVERLOAD

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Background: Analysis of the common C282Y and H63D mutations in the HFE gene is widely used to diagnose hereditary hemochromatosis (HH). The role of HFE S65C mutation in the development of hepatic iron overload is unknown. The frequency of S65C mutation in populations is rarely described and the phenotype of S65C mutation is uncertain.

Aims: The aim of this study was to analyse the frequency of S65C mutation in 550 Belgian patients and to correlate the presence of the S65C mutation with the phenotype of these patients. **Methods:** In the last four years 550 patients samples were sent to our laboratory for the molecular analysis of C282Y, H63D and S65C. These patients showed increased serum iron indices or the samples were sent for family screening. Single PCR and Real-time PCR were applied to analyse C282Y, H63D and S65C mutations on the molecular basis. **Results:** Out of 550 investigated patients 348 were genetically affected for C282Y, H63D and/or S65C. Twenty-four percent of the 348 patients were homozygous for C282Y, 12% were compound heterozygous for C282Y/H63D, 1,2% compound heterozygous for C282Y/S65C and 1.2% compound heterozygous for H63D/S65C. No homozygous S65C patient was detected. The allelic frequency for S65C is 2.7% in the investigated Belgian population. Within the 348 patients with C282Y, H63D and/or S65C mutations the frequency for S65D is 4.3%. C282Y and H63D mutations occurred in two times and three times more male than female, respectively. S65C mutation was 6.5 times more detected in male. Clinical data of patients compound

heterozygous for C282Y/S65C are available. Some of them show the HH phenotype. One patient who is compound heterozygous H63D/S65C has the phenotype of the mild HH form. Screening of relatives of this patient with the H63D/S65C mutations revealed that compound heterozygotes H63D/S65C have an increased risk for HH compared to heterozygotes S65C. **Summary/conclusions:** In a population of 550 Belgian patients with suspected iron overload or family history the allelic frequency for S65C is 2.7%. Male are in this patients group with a S65C mutation 6.5 times more represented than women. Our study demonstrates that not only compound heterozygotes for C282Y/S65C can show the mild phenotype of HH but also compound heterozygotes for H63D/S65C.

570 DETERMINANTS OF SERUM FERRITIN IN THE METABOLIC SYNDROME

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Background: Serum ferritin is frequently increased in subjects with one or more of the features of the Metabolic Syndrome (MS), also named the Insulin Resistance Syndrome (IRS). Moreover, a new syndrome has been recently described by French Authors (Moirand R et al., Lancet 1997;349:95-7; Mendler MH et al., Gastroenterology 1999;117: 1155-63), characterized by primary hepatic iron overload (HIO) unlinked to genetic hemochromatosis, in which affected subjects had a high frequency of the IRS features (the insulin-resistance-associated hepatic iron overload or IRHIO). Whether or not the reverse is true, i.e. whether or not there is an increased frequency of iron overload in subjects with the MS-IRS, has not yet been investigated. Similarly, the mechanisms underlying the relationship between serum ferritin and the MS are poorly understood. **Aims:** 1) to determine which component of the MS best correlates with serum ferritin; 2) to evaluate the putative frequency of iron overload in subjects with strictly defined MS, according to the recent consensus criteria of the Adult Treatment Panel III of the NCEP (JAMA 2001;285:2486-97). **Methods:** we studied a total of 877 subjects of both genders. 272 had MS, defined as the presence of at least three of the following features: obesity (BMI >27 Kg/m²), fasting glucose ≥ 110 mg/dl or established diabetes mellitus, high triglycerides (≥ 150 mg/dl), low HDL-cholesterol (<40 mg/dl in males; <50 mg/dl in females), hypertension. 605 subjects without MS were used as controls. Besides the above mentioned parameters, in all subjects we assessed fasting insulin (Insulin Resistance was calculated following the Homeostasis Model Assessment-HOMA), and the presence of the C282Y mutation in the HFE gene. We also measured high sensitivity C-reactive protein (HSCRP), since an important association between this sensitive marker of inflammation and the MS has been recently highlighted (Festa A et al., Diabetes 2002;51:1131-7; Ridker PM et al., Circulation 2003;107:391-7). Using serum ferritin levels as a surrogate marker, putative iron overload was defined as ferritin levels ≥ 350 μ g/l in males and ≥ 250 μ g/l in females. **Results:** serum ferritin was higher in MS subjects than in controls (125.7 μ g/l versus 96.5 μ g/l; $P < 0.001$). Serum ferritin increased linearly with the increasing number of MS features ($P < 0.001$ by ANOVA). In a series of logistic regression models (using hyperferritinemia as dependent variable) or linear regression model (using log-transformed ferritin values), HSCRP in the upper quintile (OR 2.19, 95% CI 1.08 to 4.44, $P = 0.03$) and fasting glucose ≥ 110 mg/dl (OR 2.12, 95% CI 1.34 to 3.34, $P = 0.001$) emerged as the only significant and independent predictors of ferritin values. In spite of these associations, the prevalence of even a mild iron overload (defined as above) was not significantly different between MS subjects and controls (9.2% versus 7.8%, $P < 0.48$ by χ^2). **Conclusions:** among the classical features of the MS, high glucose levels appear as the best predictor of serum ferritin. Data on HSCRP suggest that subclinical inflammation independently contribute to further increase serum ferritin in MS. Insulin resistance, defined following recent consensus criteria, does not appear sufficient by itself to induce a clinically relevant iron overload.

IMMUNOPHENOTYPING / GENE THERAPY

571 BLOOD DENDRITIC CELLS IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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Dendritic cells (DCs) are responsible for the initiation and regulation of immune responses. Myeloid DCs (MDC) and plasmacytoid DCs (PDC) can promote polarisation of naive T cells. Thus, blood DCs might play a key role in promoting antitumour immunity. In this study, we investigated the status of circulating DCs in the blood of 25 acute lymphoblastic leukaemia (ALL) patients. There was a significant reduction in the proportions of both MDC and PDC subsets among B lineage ALL patients. In contrast, DC subsets from T-ALL patients were quantitatively comparable to normal values. After culture in vitro with GM-CSF and TNF-alpha or with Flt3L, the CD34+ stem cell fraction from B-ALL could not give rise to significant numbers of CD1a+ MDCs, or PDCs. The maintenance of blood MDCs in T-ALL correlated with the level of circulating CD14+ monocytes. Furthermore, CD14+ monocytes isolated from T-ALL and cultured with GM-CSF and IL-4 could give rise to genuine functional monocyte-derived DCs, capable to secrete IL-12p70 after maturation with CD40L. PDCs in T-ALL were also functional in response to viral stimulation, with secretion of significant amounts of IFN-alpha following exposure to HSV, further confirming that blood DC compartments are quantitatively and functionally preserved in T-ALL, but not in B-ALL. Dendritopoiesis in B-ALL is significantly affected by the leukemic process, but likely not in T-ALL, in line with data placing the malignant transformation in B-ALL closer to, if not in the pluripotential stem cell. This is also in accordance with data showing the existence of a common multipotential B/NK/DC progenitor clones within adult bone marrow stem cells.

These findings emphasize the need for development of methods to restore DC functions in B-ALL. Restoration or enhancement of DC-related immunocompetence would result in stronger long-term leukemic immune control.

572 PERIPHERAL BLOOD IMMUNOPHENOTYPING BY FLOW CYTOMETRY IN LOW GRADE MYELODYSPLASTIC SYNDROMES

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BACKGROUND: Lower-grade categories of myelodysplastic syndromes (LG-MDS) as defined by WHO criteria (refractory anemia [RA], refractory anemia with ringed sideroblasts [RARS], refractory cytopenia with multilineage dysplasia [RCMD], refractory cytopenia with multilineage dysplasia with ringed sideroblasts [RCMD-RS] and unclassified myelodysplastic syndrome [MDS-U]) are often difficult to identify because of doubtful morphologic dyspoiesis, low count of blast cells and the absence of consistent clonality markers when karyotypic studies are unsuccessful. Recently, bone marrow flow-cytometric analysis has proved to provide usefulness in these cases but its value in peripheral blood (PB) remains undefined. Here we search for the utility of examining mature cells from PB by multiparametric flow cytometry (MFC) in LG-MDS.

AIMS: To evaluate sensitivity of MFC and to define practical patterns of immunophenotypic abnormalities by means of a brief antibody panel in PB from LG-MDS patients (pt).

METHODS: Thirty-one pt (16 f, 15 m, age 15-88) diagnosed with MDS (11 RA, 3 RARS, 13 RCMD, 1 RCMD-RS and 3 MDS-U) on bone marrow aspirates were examined for phenotypic anomalies. No pt showed PB blast cells. Studies were performed in whole lysed PB using 3-color direct MFC. Samples were stained with the following monoclonal antibodies: CD2, CD10, CD11b, CD11c, CD13, CD16, CD33, CD34, CD56, CD65 and CD66b. Data were acquired on a FACScan and analyzed by Paint-a-Gate software. Cell populations were identified on dotplots depicting CD45 vs. SSC. Findings were recorded and compared with healthy controls.

RESULTS: All but 2 pt (29 of 31, 93.5%) showed staining abnormalities in PB neutrophils (PMN) and/or monocytes (Mo). Both pt were RARS so that only 1 of 3 RARS pt (33.3%) had anomalies. Alterations found

were mainly complex ranging from 1 to 6 (median = 3). Only 2 pt had single anomalies. Fourteen cases were abnormal for PMN, 3 for Mo and 12 for both PMN and Mo. Presence of increased CD34+ cells was found in 4 pt (12.9%). The main phenotypic anomalies recognized were: faint/absent CD10 expression on PMN (17 pt, 54.8%), strong expression of CD56 on Mo (10 pt, 32.3%), decreased CD11b expression on PMN (9 pt, 29%), decreased CD33 expression on PMN (9 pt, 29%), decreased expression of CD65 on PMN (8 pt, 25.8%) and CD2 overexpression on Mo (5 pt, 5.1%). A significant positive correlation was found between dim CD10 staining on PMN and RCMD (ANOVA $p = 0.03$).

CONCLUSIONS: Most MDS disclose abnormal immunophenotypic patterns in PB as assessed by MFC. Providing this procedure is rapid and non-invasive, it could be helpful in diagnosing those cases with inconclusive morphology and cytogenetics.

573 HUMAN CD40 LIGAND GENE TRANSFER INTO CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) B-CELLS VIA EBV-BASED VECTORS

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BACKGROUND/AIMS: B-CLL is the most frequent adult leukemia in the western world and is characterized by the accumulation of CD5+ monoclonal B cells in the blood, bone marrow, and lymphoid tissues. CLL B-cells are unable to properly act as antigen-presenting cells (APC) due to the lack of expression of costimulatory molecules which are necessary for induction of a T cell response. In addition, activated T cells of patients with CLL fail to express detectable amounts of CD40 ligand (CD40L). It has been demonstrated before by Wierda et al. that adenoviral transfer of murine CD40L into B-CLL cells induces the expression of a variety of immune coreceptors, including CD80 (B7-1) and CD86 (B7-2), on both transduced and bystander leukemia cells. This restores the capacity of CLL cells to stimulate autologous T cells (1).

Instead of using the murine CD40L as a therapeutic gene we chose the human homolog (hCD40L) and used a gene therapy system based on Epstein-Barr virus (EBV), a gamma-herpesvirus which naturally infects human B-cells.

METHODS/RESULTS: Gene transfer of hCD40L into B-CLL cells requires the production of modified EBV particles carrying the gene encoding for hCD40L. For this purpose we constructed a packaging cell line (HEK293-V) harboring a recombinant EBV helper genome. This EBV mutant lacks its packaging sequences (TR), but supports the packaging of EBV-based gene vectors in trans (2). For safety reasons, we further knocked out BZLF1, which induces the lytic cycle of EBV, as well as the genes encoding for proteins that are important for immortalization of primary B-cells in vitro. These include EBNA2, LMP1, and the EBNA3 family.

Our therapeutic gene vector, carrying the human CD40L, encompasses only a few cis-acting elements of wild-type EBV that are necessary for the amplification of episomal vector DNA during the lytic cycle of EBV (oriLyt) and for packaging of this DNA into viral particles (TR). Instead of cloning an antibiotic resistance gene onto the vector, we took advantage of the E. coli supF gene system. This also includes the construction of a modified E. coli strain that carries the amber amp and amber tet resistance genes for propagation of the gene vector DNA.

Infection experiments with Raji cells showed that unwanted recombination between the EBV helper virus genome and our therapeutic vector is minimized. In addition, incubation of CLL B-cells with low MOIs (0.5 to 1) of the recombinant EBV particles resulted in efficient transduction of the cells and hCD40L expression. As expected, upregulation of costimulatory molecules such as CD80, CD86, and CD54 was observed.

SUMMARY: We could establish a safe and helper-free EBV packaging cell line that enables us to package EBV-based therapeutic gene vectors for the efficient infection and genetic modification of B-cells as e. g. B-CLL cells.

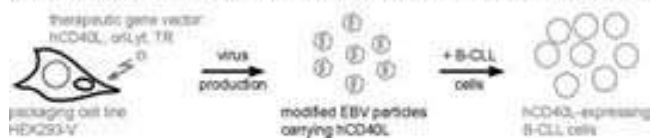
(1) Wierda et al. (2000) Blood 96, 2917-2924

(2) Delecluse et al. (1999) Proc. Natl. Acad. Sci. USA 96, 5188-5193

Establishment of EBV helper cell line



Generation of EBV particles containing hCD40L-encoding gene vector



Caption 1:

574 MONITORING FLT3 ANTIGEN EXPRESSION IN ACUTE LEUKAEMIA USING FLOW CYTOMETRY

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Background: FLT3 is a member of the class III receptor tyrosine kinase family of transmembrane glycoproteins. The FLT3 receptor is activated by its specific ligand resulting in tyrosine phosphorylation. In normal bone marrow FLT3 expression is restricted to immature progenitors, including CD34-positive cells. High level expression of FLT3 in acute myeloid (AML) and lymphoblastic (ALL) leukaemias has been demonstrated by RNA and DNA techniques. This evidence suggests that FLT3 and its ligand may play a significant role in the proliferation of leukaemic blasts cells.

Aims: i). To investigate whether flow cytometry can detect increased FLT3 expression in AML and ALL. ii). To determine if the technique provides comparable results to those found using PCR and blotting techniques and ultimately to provide evidence as to whether FLT3 can play a role in the diagnosis and classification of acute leukaemia.

Methods: The surface expression of FLT3 was measured in 110 bone marrow or peripheral blood samples of patients with AML or ALL using a phycoerythrin-labelled monoclonal IgG1 antibody (Coulter-Immunotech). 10ul of the antibody was added to samples containing 4×10^4 /l lymphocytes. The red cells were lysed using Immunoprep (Coulter-Immunotech) and fluorescence measured using a Coulter XL4-MCL flow cytometer.

Results: Positive results (ie. $\geq 20\%$) were demonstrated in 82% (47/57) of AML samples (in all FAB subtypes) and in 81% (34/42) of B-lineage ALL samples, in all three subtypes. The Mean Fluorescence Intensity (MFI) was similar in the two groups and no difference was found between adult samples (82%) and those from children (80%). In contrast to these high levels of FLT3 expression, all 8 T-ALL samples tested were negative with markedly reduced MFI's. In addition, the diagnostic utility of FLT3 expression was shown in three cases of biphenotypic acute leukaemia. One case with myeloid/B-lineage positivity was FLT3+ as expected. The second case with myeloid/T-lineage markers was negative for FLT3 expression. The third case was morphologically AML (FAB M0, cytogenetics failed) with CD33+ but MP0-, CD13-, CD117- and expressing T-lineage markers (score of 3) CD2, CD5 and CD7 being positive, as well as Tdt. However, the T-lineage specific marker cytoplasmic CD3 was negative. Only FLT3 positivity allowed a definitive diagnosis of AML to be made.

Conclusion: These findings further support the evidence that the overexpression of the FLT3 receptor antigen plays a role in the proliferation of malignant clones in acute leukaemias. FLT3 should therefore be included in the acute leukaemia diagnostic immunophenotyping panel. This would be particularly necessary in cases where T-lineage disease needs to be confirmed or excluded.

575 FLOW CYTOMETRIC ANALYSIS OF THE TCRVBETA REPERTOIRE IN PATIENTS WITH SUSPECT T-CELL PROLIFERATIONS

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BACKGROUND - AIM: In contrast to the easy diagnosis by the use of Flow Cytometry (FC) in mature B-cell malignancies, the diagnosis of

T-cell lymphoproliferative disorders is hampered by the lack of special surface markers. Until now, peripheral blood (PB) samples taken from patients with suspect T-cell proliferation were investigated with Polymerase Chain Reaction (PCR) analysis of TCRbeta or TCRgamma genes in order to assess clonality. However, the molecular techniques are cumbersome and not available to many laboratories. On the other hand, recently TCRVbeta repertoire analysis by FC became usable for clonality detection in T-cell expansions. The aim of this study was the evaluation of FC analysis of the TCRVbeta repertoire for the study of T-cell lymphocytosis in comparison to the PCR analysis of TCR gene rearrangements.

METHODS: PB samples of 65 patients with newly diagnosed absolute T-cell lymphocytosis (either CD4 or CD8) were analysed by FC. A group of 24 fluorochrome conjugated Vbeta specific monoclonal antibodies (Beckman-Coulter) covering about 70% of the whole TCRVbeta repertoire were used. A conjugation trick (combined use of two colours) allows the simultaneous analysis of three Vbeta regions in the same tube, so that the whole analysis can be performed in 8 tubes only. FC analysis was 'gated' either in CD4 or in CD8 T-cells. In parallel the same samples were tested for T cell clonality by PCR analysis of TCRgamma gene rearrangements.

RESULTS: FC analysis of the PB samples taken from our 65 patients showed : a) In 16 cases a high increase of a single Vbeta gene 'family' indicating clonality, confirmed by PCR analysis in all cases. b) In 7 cases a large decrease of the majority of Vbeta gene 'families' considered indicative of clonality concerning an undetectable Vbeta 'family'. Clonality was confirmed in 5 out of the 7 cases. In the rest of our patients (42) no clonality was detected either by FC or PCR analysis.

CONCLUSIONS: It seems that the presented FC method can effectively detect clonality in T-cell expansions. It represents a relatively non time consuming, easy and cheap tool in the diagnosis of T-cell lymphoproliferative disorders, which can be supplemented by molecular techniques in questionable cases (patients with large 'fractions' of Vbeta negative cells in FC analysis and patients with small increases of particular 'families').

576 KIR ASSAY AND ITS CLINICAL IMPLICATIONS IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA USING FLOW CYTOMETRY

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Background: Natural killer(NK) cells are innate immune lymphocytes that ignited clinical studies adopting their ability to attack tumor cells without specific antigen recognition. Clinical transplantation according to killer cell immunoglobulin-like receptor(KIR) differences, HLA-C is predominantly involved in the inhibitory and activating regulation of human NK cells, have shown some remarkable pilot studies to treat high-risk acute leukemic patients, so far.

Aims: For the purpose of assessment of KIR in the transplant setting by flow cytometry.

Methods: Twenty six adult patients with acute myeloid leukemia who were planning to receive allogeneic hematopoietic stem cell transplantation(HSCT) from their sibling donors were enrolled. Two kind of monoclonal antibodies representing subgroup specific KIR were used. That is, according to the reports, CD158b for the group 1 HLA-C antigens including HLA-Cw1, -Cw3, -Cw7, -Cw8 and CD158a for the group 2 including those of HLA-Cw2, -Cw4, -Cw5, -Cw6. Other immunophenotypes including CD3, CD16, CD56, and NKT were also investigated. All patients in this study were in complete remission. Blood sampling from the donors were done just before transplant and just before the conditioning in patients. Data of HLA serotyping in each transplant pair and of flow cytometry were compared.

Results: By this analysis, we found the trend of predominant expression of the group 1 HLA-C antigens in Korean patients. Of note, some patients who showed a balance of the level of expressions between the CD158a and the CD158b revealed the frequency predilection of acute GVHD. However, there has not been noted a clear correlation between the balance and the severity of GVHD. According to our data, NKT cell expression was also another factor of which might be involved in the transplant-related complications.

Conclusion: We suggest that the level of expressions of KIR and NK subsets in the sibling allogeneic HSCT may, at least in part, be one of responsible factors to cause GVHD. Further investigations with more patients are needed to verify the role of KIR balance in the future.

577 QUANTITATIVE ANALYSIS OF EXPRESSION OF CD34, GROWTH FACTORS RECEPTORS, SDF-1- α - AND ADHESION MOLECULE CD49D ON CD34+ PERIPHERAL BLOOD CELLS AFTER MOBILIZATION WITH CYTOTOXIC DRUGS AND/OR G-CSF
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Introduction: The populations of CD34+ peripheral blood cells mobilized with cytotoxic drugs and/or growth factors are characterized by particular phenotype and the success of CD34+ cells transplantation depends also on expression of their adhesion molecules and receptors of growth factors.

Aim of this study was quantitative analysis of expression of growth factors receptors and adhesion molecules: CD34 (sialomucin and stem/progenitor cell marker) and CD49d (chain α of integrin β 1) on peripheral blood CD34+ cells mobilized with cytotoxic drugs and G-CSF in patients with B-lymphoproliferative diseases and assessment of their importance for bone marrow homing.

Materials and methods: Quantitative analysis of growth factors receptors-c-Kit receptor (CD117), GM-CSF (CD116), G-CSF (CD114), SDF-1 α (CD184) and adhesion molecules-CD34 and CD49d was performed using flow cytometry (FACS Calibur Becton Dickinson) and monoclonal antibodies on CD34+ cells collected from 25 patients. The expression of molecules was determined in form of quantitative measurements presented as Antibody Bound per cell (ABC). Control experiments were performed on 10 donors of stem cells.

Results: The percentage of CD34+ cells in the patients was significantly higher in comparison to healthy donors (mean values: 4.1 /WBC vs. 0.7 /WBC; $p < 0.001$). The quantitative analysis presenting by the number of binding places on the surface of stem/progenitor cells showed that the expression of CD34 and CD49d was the most even (SD=11% of mean values; ABC=9927 and ABC=5446 respectively). The expression of growth factors receptors was different: slight individual differences were observed in expression of receptor for SCF-CD117 (SD=14% of mean value; ABC=5228) but the values of ABC were much more different in case of CD116, CD114 and SDF-1 α (CD184) (SD=20-30%). The positive correlation was observed between the ABC of CD34 and CD117 on CD34+ cells ($p < 0.05$). There were not significant differences of ABC values for CD34, CD117 and CD49d on CD34+ cells in the patients and healthy donors of stem cells ($p > 0.05$).

Conclusion: Our results could indicate that the stage of maturity and/or the functions of CD34+ peripheral blood cells are different after mobilization with cytotoxic drugs and/or G-CSF. The estimating of expression of SDF-1 α on CD34+ cells may be useful in particular patients because of its individual differences in expression and importance for bone marrow homing.

578 TRANSFECTION OF IVT MRNA OF TRAIL AND TUMOR ANTIGENS (SART-1 AND WT-1) INTO DENDRITIC CELLS

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Enhancing the cytotoxic ability of dendritic cells (DCs) for efficient ingesting tumor cell components and presenting tumor antigen is one direction of tumor immunotherapy using DCs without manipulation of specific antigen presentation in vitro. On the other hand, establishing an efficient method to load DCs with tumor antigens is important for the development of DC-based tumor immunotherapy using DCs manipulated for presenting specific tumor antigen in vitro. For the investigation of both directions, we adopted the method of mRNA transfection into DCs. In order to enhance the cytotoxic ability of DCs, we explored the feasibility of in vitro transcribed (IVT) TRAIL mRNA transfection into DCs. In order to load tumor antigens on DCs, transfection of IVT tumor antigen mRNA into DCs was tried, and the generation of anti-tumor antigen CTL was performed by priming lymphocytes with mRNA transfected DCs. Human Cord blood (CB)-CD34+cell-derived DCs and PB CD14+ cell or adherent cell-derived DCs were used as the target cells for transfection of IVT mRNA of EGFP, TRAIL, and tumor antigens such as SART-1/WT-1 by electroporation using a single square wave pulse. Autologous lymphocytes were co-cultured with the DCs transfected with SART-1

or WT-1 mRNA at a stimulator to responder ratio of 1:10. The bulk co-cultures were stimulated repeatedly 3-4 times with the same mRNA transfected DCs at the interval of 4-7 days. IL-2 and IL-7 were added every 4-7 days to the co-culture plates. One week after the final priming of lymphocytes with mRNA transfected DCs, antigen specific CTL activity was evaluated by 51Chromium release assay. The DCs, which were transfected with IVT EGFP or TRAIL mRNA and cultured for 1-5 days, were demonstrated to express both cytoplasmic protein (EGFP) and trans-membrane protein (TRAIL) by flow cytometry analysis. The autologous lymphocytes, which were primed with DCs transfected with IVT SART-1 or WT-1 mRNA, were demonstrated to be capable of injuring the cell membranes of SART-1 or WT-1 expressing cell lines and DCs transfected with the same IVT mRNA. However, the primed lymphocytes were demonstrated not to be cytotoxic to the DCs without transfection of the same mRNA. The present study demonstrated that trans-membrane protein such as TRAIL could be expressed in DCs by the IVT mRNA as well as cytoplasmic protein like EGFP, which revealed the possibility of enhancing the cytotoxic ability of DCs by transfecting IVT TRAIL mRNA. In addition, the DCs transfected with IVT SART-1 or WT-1 mRNA were suggested to be applicable in DC-based immunotherapy for the patients with SART-1 expressing squamous cell carcinoma and WT-1 positive tumor including hematological malignancies like leukemia.

579 COMPARED ANALYSIS OF DENDRITIC CELLS AND THEIR SUBSETS IN THE LATE POSTTRANSPLANT PERIOD BETWEEN PATIENTS UNDERGOING ALLO-SCT AND THEIR RESPECTIVE SIBLING DONORS

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Dendritic cells and their subsets play an essential role in the allogeneic stem cell transplant setting (allo-SCT). To date, it has yet to be ascertained if, in the long term period after an allo-SCT transplant, dendritic cell numbers and their subsets in patients are similar or not to that of their respective donors. For this purpose we have evaluated dendritic cells by flow cytometry in 40 patients undergoing HLA-matched sibling Allo-SCT and we have compared it to that of their respective 40 donors. In 11 cases, patients were under treatment for active chronic GVHD. Samples were simultaneously obtained from both patient and donor. In all cases time from engraftment had exceeded one year (mean SD: 928 490 days after the transplant). Complete chimerism in BM was confirmed in all cases by PCR. Peripheral blood samples were stained with the following single monoclonal antibody (McAb) combination: CD3/CD14/CD56/CD19-FITC/CD16-PE/HLADR-PerCP/CD33-APC. This single measure is able to differentiate total percentage of dendritic cells and three different subsets: a) CD16+ subset, b) Myeloid subset, and c) Lymphoplasmacytoid subset.

Our results showed that the percentage of dendritic cells was similar in both patients and donors (0.870.4% vs 0.790.3%, respectively). Regarding dendritic cell subpopulations, we did not find significant differences in the myeloid subpopulation, but the lymphoplasmacytoid subset was significantly decreased (13.78 vs 20.310; $p < 0.001$) and the CD16+ subset significantly increased (72.211 vs 61.113; $p < 0.001$) in the patient group.

When the presence of active chronic GVHD was evaluated, we did not find significant differences in this subgroup of patients. In summary, our results show that in the long term period after Allo-SCT, dendritic cells are similar in patients compared to that of their respective donors, although, interestingly, the lymphoplasmacytoid subset is significantly decreased.

580 SUCCESSFUL RETROVIRAL GENE TRANSFER IN STEM CELLS EXPANDED WITH FIBROBLAST GROWTH FACTOR-1 (FGF-1) AS A SINGLE CYTOKINE

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Background Hematopoietic stem cells (HSC) are defined as clonogenic cells competent for both self-renewal and multilineage differentiation.

An efficient method for gene transfer into HSC is required to study the stem cell biology as well as for gene therapy. In most of the experimental protocols for this purpose retrovirus-mediated gene transfer methods have been used in which the primary requirement is good infection efficiency. Nevertheless, there are still unresolved problems in mouse bone marrow transplantation (BMT) models using retroviral gene transfer. Thus, efforts have been made to raise the infection efficiency by improving the culture conditions, administration of cytotoxic drugs to the donor mice, using various cytokine cocktails, etc. Here we demonstrate a new efficient retroviral infection method for HSC after expansion with FGF-1 as a single cytokine.

Methods Unfractionated BM cells from one B6Ly5.1 mouse were cultured in two 6-well plates at a density of 5×10^6 cells/well in serum-free medium containing FGF-1 for 3 weeks. Both floating and attached cells from one of the wells were transferred into six 1.5cm wells pre-coated with retromectin and infected with virus supernatant from _MP34 cells stably transduced with pMY/GFP retrovirus. After 3 days infection, we sorted the GFP+ fraction and transplanted two B6Ly5.2 sublethally irradiated mice using cells from one 1.5cm well.

Results In each 1.5cm well, average percentage of GFP+ sorted cells was 50-75% (approximately 2×10^5 cells/well). Therefore, the number of cells transplanted to individual mice was 1×10^5 cells in average. We found that all recipient mice survived, while all irradiated control mice died. We evaluated the chimerism of GFP+ cells, which were derived from the expansion culture, in the peripheral blood sample from each mouse. Eight weeks after the transplant, the number of granulocytes, B and T cells in the recipients gradually increased. Evaluated chimerism of GFP+ cells was 85-90% for myeloid, 48-56% for B and 61-81% for T cells. Eight weeks after the transplant, chimerism of GFP+ cells in bone marrow was 5.4-90%, spleen 8-85% and thymus 32-96%. To further investigate the in vivo expansion ability of the FGF-1-expanded and virus-transduced cells, we performed secondary transplantation using GFP+ cells from the recipient bone marrow two months after the primary transplantation. Two months later, the percentage of donor-derived cells in the secondary recipient mice was: 46-72% for granulocytes, 42-50% for B and 18-22% T cells.

Conclusions Here we demonstrate that only a 1/144th part of BM [cells from a single donor mouse is sufficient to have successful engraftment of myeloid, B and T cells for at least 4 months in serial transplantation. We are now evaluating the longer-term engraftment. This is the first method showing successful retroviral infection and transplantation of long-term (up to 3 weeks) in vitro-cultured stem cells. Moreover, the number of initial BM cells required to achieve engraftment from the gene-transduced cells is the smallest among all methods described in the literature up to now. We believe that this method will help improve the use of retroviruses in gene therapy and be of great benefit for its future application.

581 RE-EXPRESSION OF P15 (INK4B) GENE IN KG1A CELL LINE INDUCED BY IN VITRO INHIBITION OF DNA METHYLTRANSFERASES AND HISTONE DEACETYLASES

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The silencing of tumor suppressor genes can occur by both genetic and epigenetic changes. The epigenetic modifications usually involve aberrant methylation of cytosines in their promoter region and histone deacetylation. Treatment with 5-aza-2-deoxycytidine (DB), a potent inhibitor of DNA methylation, can reactivate the genes with aberrant methylation. However, the full chemotherapeutic potential of DB may only be realized when it is used in combination with other agents that also activate gene expression such as histone deacetylase inhibitors (HDACi). Histone acetylation facilitates the binding of transcription factors to the promoter region. Trichostatin A (TSA) is a potent inhibitor of HDAC and can induce differentiation of leukemic cell lines. The tumor suppressor gene p15ink4b is frequently found hypermethylated and it has been implicated in the development of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). We investigated the in vitro antineoplastic activity of DB and TSA at human myeloid leukemic cell line Kg1a in which p15ink4b gene expression is suppressed due to DNA hypermethylation. Kg1a cells were placed at a density of 0.5×10^6 cells/mL with DB and TSA at different concentrations either alone or in combination. After 4 days, the biological characteristics of Kg1a cells untreated or treated with both agents were investigated and analyzed using morphology, real-time quantitative methylation PCR (RQ-MSP) of p15ink4b gene, real-time quantitative expression PCR (RQ-RT-PCR) of p15ink4b gene, cell cycle analysis, western blot of acetylated histones H3 and H4 and surface markers analysis. The quantity of methylated gene copy after DB treatment showed an evidence demethylation (2.9×10^4 -copies/ml vs 6.4×10^3 -copies/ml) that correlates with a weak re-expression of p15ink4b gene. These effects were accompanied by cell cycle arrest in G2/M phase and time-dependent growth inhibition. The morphological analysis revealed an increase of the number of multinucleated cells. Increase of acetylation degree in histones H3 and H4 was observed with TSA alone although it was able neither to demethylate p15ink4b gene promoter nor to reactivate its expression. Although the acetylated status of histones H3 and H4 did not change in combined cultures, a higher re-expression of the gene was observed in association with a decrease in DNA methylation status (2.9×10^4 -copies/ml vs 4.4×10^3 -copies/ml). In conclusion, an enhancement of the in vitro antineoplastic activity of DB by TSA on human myeloid leukemic cell line was observed. Thus, synergistic activity of DNA demethylation and histone deacetylase inhibition induces re-expression of p15 ink4b gene in Kg1a cell line.

This work was supported in part by Grant for Research Groups No. 03/225 from Generalitat Valenciana and by contract No. QLG1-CT-2001-01935 from the Fifth Framework Program of the European Commission.

Simultaneous Sessions

MOLECULAR CYTOGENETICS IN MULTIPLE MYELOMA & CHRONIC LYMPHOCYTIC LEUKEMIA

582 PATHOGENETIC RELEVANCE AND PROGNOSTIC VALUE OF GENOMIC IMBALANCES IN CLONAL PLASMA CELL DISORDERS
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Introduction: Various recurring chromosomal aberrations have been identified in MGUS and multiple myeloma (MM). Illegitimate IgH (14q32) rearrangements, detectable in 50-60% of MM tumors by FISH, are thought to represent early and immortalizing genetic events arising during B-cell ontogenesis. In tumors lacking 14q-translocations (t14q), other (yet unidentified) chromosomal abnormalities could be of importance. Deletion of chromosome arm 13q (13q-) is one of the most valuable independent prognostic markers known in MM today. In contrast, the prognostic significance (as well as the pathogenetic relevance) of other genomic imbalances are still unknown. Recently, we have demonstrated that trisomies of chromosome arms 1q, 9q, and 11q (+1q, +9q, +11q) are among the most common aberrations in MM, pointing to an important role of these chromosomal gains in the biology of clonal plasma cell disorders. **Aim:** To evaluate the significance of +1q, +9q, and +11q by 1. exploring the distribution of the most frequent genomic changes (including +1q, +9q, +11q) in MGUS (n=40) and MM (n=108) 2. comparing the incidences of chromosomal abnormalities in MGUS and MM (n>300), thus distinguishing primary and secondary genetic changes, and 3. determining the prognostic relevance of the most recurring imbalances in MM. **Material and Methods:** Bone marrow aspirates from pts. with MGUS and MM were obtained during routine diagnostic procedures. Positive selection of plasma cells using immunomagnetic beads (CD138) was applied to samples with a low percentage of plasma cells. Tri-color FISH and DNA probes were used as previously described. **Results:** In our series of 108 pts., +9q and +11q as well as 13q- and +1q were strongly correlated (p<.001). The incidence of t14q was 89% in pts. with no 9q/11q extra copy, 56% in pts. with either +9q or +11q, and only 23% in pts. with +9q/+11q (p<.001). The incidence of 13q- in these three groups was 63%, 36%, and 23% (p<.001). The incidence of +9q and +11q in MGUS (MM, n>300) was 39% (48%) and 27% (42%). **Conclusions:** 1. Despite the generally high complexity of genomic changes in MM, there is a non-random distribution of chromosomal abnormalities in this disease 2. MM with concurrent +9q/+11q rarely exhibit t14q, pointing to a novel, translocation-independent (extra copy-regulated) pathogenetic pathway 3. A significant proportion of pts. with MGUS exhibit +9q and +11q, supporting the hypothesis that early chromosomal imbalances with consecutive dysregulation of relevant genes may substitute 14q-translocation 4. In MM with concurrent +9q/+11q, a low incidence of 13q- was found, suggesting a more favorable prognosis of these tumors 5. Correlation of clinical and molecular cytogenetic data is currently under way and will be presented at the meeting. Supported by grants from the Deutsche Krebshilfe (70-2899-Li I) and the Wilhelm Sander-Stiftung (No. 2002.098.1) to P.L.

583 CK2 SERINE-THREONINE KINASE CONTROLS MULTIPLE MYELOMA CELLS SURVIVAL AND PROLIFERATION AND ITS SELECTIVE BLOCK LEADS TO GROWTH ARREST, APOPTOSIS AND ALTERED NFkB SIGNALING

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Background: Multiple myeloma (MM) cells growth and survival largely depend on the aberrant activation of several signaling pathways triggered by external stimuli. CK2 (casein kinase II) is a ubiquitous serine-threonine kinase whose activity is elevated in proliferating and transformed cells

and whose role in oncogenic transformation, apoptosis and cell cycle progression has recently become matter of intense research. Due to its functional connection with signaling molecules pivotal for plasma cell survival, such as those implicated in the TNFalpha/NFkB, IGF1/PI3K/AKT and Wnt/beta-catenin pathways, CK2 is likely to play a fundamental role in MM cells biology.

Aims: we investigated the role of CK2 in MM plasmacells survival and cell cycle progression, in the regulation of the Ikb/NFkB dependent pathway and in the modulation of MM cells sensitivity to chemotherapeutics.

Methods: CK2 protein levels and enzymatic activity were analyzed in MM cell lines and highly purified CD138+ plasma cells from 5 MM patients. We hampered CK2 function by using novel compounds that selectively inhibit CK2 activity and afterwards analyzed MM cell survival, apoptosis and cell cycle progression. After CK2 block, we checked Ikb protein levels at basal and TNFalpha stimulated conditions and NFkB DNA binding by EMSA and transcriptional activity by luciferase assay.

Results: CK2 was over-expressed in MM cell lines and plasma cells from patients, as compared to control resting lymphocytes; its activity seems to be significantly up-regulated by TNFalpha; ablation of CK2 catalytic activity with selective agents determined a dose dependent cytotoxic effect as judged from increased apoptosis and cell cycle arrest in G2-M, and cooperated with the toxic effect of chemotherapeutics

Basal and TNFalpha dependent Ikb degradation, as well as NFkB p50/p105 nuclear shuttling and NFkB transcriptional activity upon TNFalpha stimulation, were impaired by CK2 block in MM cells. A partial nuclear co-localization of the catalytic alpha subunit of CK2 and p50/p105 was observed by confocal microscopy in MM cells. **Conclusions:** CK2 is a kinase pivotal for survival and proliferation of MM cells and its selective blockage is strongly cytotoxic to malignant plasma cells. CK2 regulates Ikb protein levels and NFkB transcriptional activity, this latter effect being possibly mediated through physical association with NFkB transcription factors. Our findings suggest that the CK2 inhibition could be exploited as a novel therapeutic approach for MM.

584 ARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION REVEALS A DNA LOSS AT 14Q32.33 AS A NEW PROGNOSTIC MARKER IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Array-based comparative genomic hybridization (array-CGH) is a relatively new technology that is designed to rapidly screen the entire genome for unbalanced genetic aberrations. Chronic lymphocytic leukemia (CLL) is a clinical and molecular heterogeneous disease. Specific chromosomal imbalances including deletions in 13q, 17p, 11q and trisomy of chromosome 12 are important independent predictors of disease progression and survival and make CLL a good model to study array-CGH sensitivity.

Aims: To assess the utility of array-CGH to identify known as well as previously unidentified genetic abnormalities in leukemic cells, using CLL as a model.

Method: Genomic DNA from both the tumor and normal reference cells are labeled with different fluorescent dyes and then cohybridized to arrays containing Bacteria Artificial Chromosome (BAC) clones. Variations in DNA sequence copy number for each BAC clone can be assessed by relative fluorescence signal intensities, in a single hybridization. FISH was used to confirm array-CGH data.

Results: Samples from 21 previously untreated patients with CLL were analyzed by array-CGH. Array-CGH detected the known genetic abnormalities with prognostic significance in CLL but also revealed other recurrent chromosomal imbalances, most commonly a novel area of genomic loss at 14q32.33. FISH was used to confirm the presence of recurrent genetic abnormalities. Correlating the array-CGH findings with patient outcome data suggested at least one novel cryptic deletion, which appears to have a high correlation with patient prognosis. In a further analysis, a group of previously untreated patients with isolated deletion at chromosome 13q14, shown that an additional deletion at 14q32.33 was associated with an increased likelihood of requirement for treatment (p=0.02), confirming the hypothesis that this deletion was associated with poor prognosis.

Conclusions: These studies demonstrate the complementary nature of array-CGH and FISH to identify genetic abnormalities in cancer cells.

Particularly, in the subgroup of patients, characterized by FISH to have a 13q14 deletion as a sole aberration, array-CGH demonstrated that a supplementary deletion involving the 14q32.33 locus was characteristic for patients with worse outcome. While less sensitive than FISH, the strength of array-CGH lies in the ability of this genome wide screen to detect previously unidentified chromosomal imbalances, which are likely to yield additional prognostic markers and further insight into cancer pathophysiology

585 FREQUENCY AND PROGNOSTIC RELEVANCE OF T(4;14) IN PREVIOUSLY UNTREATED MULTIPLE MYELOMA (MM) PATIENTS RECEIVING EITHER SINGLE OR DOUBLE AUTOTRANSPLANTS

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Background:

In patients with MM a number of recurrent translocations involving chromosome 14 at band q32 have been recently identified, the most common being the t(11;14) and the t(4;14). Both these chromosomal abnormalities are closely associated with particular presenting features of the disease and may help to identify patients at different risk of death. In particular, the t(11;14) predicts for good prognosis, whereas the t(4;14) has been reported to be an unfavorable prognostic feature. The t(4;14) has been described almost exclusively in MM patients, although its exact role in the pathogenesis of the disease has not fully elucidated. The translocation affects at least two potential oncogenes, MMSET on der(4) and Fibroblast Growth Factor Receptor 3 (FGFR3) on der(14); the role of two additional genes (TACC3 and LETM1) located near the breakpoint region on chromosome 4 has not yet been evaluated.

Aims:

In the present study we investigated the frequency and prognostic relevance of the t(4;14) in a series of 63 patients with de novo MM, who were randomized to receive either a single (Tx-1) or a double (Tx-2) autotransplant as primary therapy for their disease.

Methods:

For this purpose we analyzed 1) the presence of t(4;14) by RT-PCR of the hybrid transcript between MMSET and the IgH locus; 2) the overexpression of FGFR3 by Real-time RT-PCR; 3) the frequency of potentially activating point mutations in the FGFR3 translocated coding region, by direct sequencing of RT-PCR products; 4) the relationship between t(4;14), response to high-dose therapy and outcome of autotransplant(s).

Results:

Overall, the t(4;14) was detected in 17/63 patients (27%), a value slightly higher than that reported by others. 13/17 patients had both MMSET/IgH fusion gene and FGFR3 overexpression, while 4 patients had MMSET/IgH but did not overexpress FGFR3. This finding further confirms the possible discrepancy between MMSET/IgH positivity and FGFR3 overexpression. Comparison between t(4;14)+ and t(4;14)- patients revealed that both groups were well balanced with respect to the most common presenting features of MM. In 36 patients, for whom material was available, FISH analysis for the 13q deletion and/or monosomy was performed. Results showed that t(4;14)+ patients were more likely to carry also del(13) than t(4;14)- patients (46% vs. 29%, respectively). Among patients who attained stringently defined complete remission following either Tx-1 or Tx-2, t(4;14)- patients were 35%, as opposed to 6% of t(4;14)+ patients (p=0.05, intention to treat). With a median follow-up of 26 months, no difference in overall (OS) and event free survival (EFS) was disclosed between t(4; 14)- and t(4;14)+ patients.

Conclusion;

In summary, 27% of our MM patients carried the t(4;14). In this cohort of homogeneously treated patients, the t(4;14) predicted for lower response to high-dose therapy. Longer follow-up is required to assess the influence of this abnormalities on OS and EFS. In a subgroup of six patients carrying t(4;14), point mutations were detected in the FGFR3 coding region, thus suggesting a possible constitutive FGFR3 activation.

Work supported by COFIN2002 and 2003, A.I.R.C., ATENEO 60%, FIRB 2001, 'Hairshow' A.I.L., 'Fondazione del Monte di Bologna e Ravenna', Fondazione Carisbo.

586 ASSOCIATION BETWEEN IMMUNOPHENOTYPE AND FLUORESCENT IN-SITU HYBRIDIZATION (FISH) ANALYSIS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: FISH analysis has allowed the identification of different B-cell chronic lymphocytic leukemia (CLL) subtypes with distinctive clinical and prognostic features. Cases exhibiting isolated deletion at 13q14 represent the most indolent form of CLL and are characterized by a 'typical' phenotype. However, except for isolated markers, no clear associations have been defined between immunophenotyping and FISH results.

Aim: To search for immunophenotypic differences among CLL patients (pt) with trisomy 12, 11q22-23/ATM deletion and 17p/TP53 deletion with respect to 13q14 deletion pts, taken as a control group.

Patients and methods: Fifty-nine patients (36 M;24 F) diagnosed with B-cell CLL according WHO recommendations were assessed for immunophenotyping by multiparametric direct flow-cytometry and for genotyping by FISH analysis in EDTA-anticoagulated peripheral blood samples. Antigens studied were CD22, CD24, CD18, CD5, CD19, FMC7, CD79b, CD25, CD11c, CD20, CD38, CD23, CD43, CD48 and surface light-chain Ig. Percentage of cells positive for each marker and intensity of MoAb expression as median fluorescent intensity (MFI) value were recorded. Fluorescent probes (Vysis Inc,IL,USA) were used for chromosomes 12 (CEP 12, p11.1-q11) and 13 (LSI D13S319), p53 gene (LSIp53) and ATM gene (LSI ATM (11q23)) and the evaluation was performed on a fluorescence microscope (Nikon, Japan). In each case, a minimum of 200 nuclei were evaluated.

Results: Thirty-seven pts presented chromosomal anomalies, either isolated or combined: trisomy 12 (14 pts, 37.8%), 11q22-23/ATM deletion (7 pts, 18.9%), 17p/TP53 deletion (4 pts, 10.8%) and 13q14 deletion (20 pts, 54.1%). In the remaining 22 pts, no anomalies were found with the probes used. The control group with isolated 13q14 were 15 pts (40.5%). No phenotypic differences could be found between the isolated 13q14 deletion group and those patients lacking FISH abnormalities. In contrast, trisomy 12 cases displayed a far different phenotype from isolated 13q14 deletion, showing a significantly higher percentage of CD18 (p=0.0001), FMC7 (p=0.01) and CD79B (p=0.003) and lower percentage of CD23 (p=0.04), CD25 (p=.001), CD43 (p=0.02) and CD5 (p=001). In addition, trisomy 12 pts depicted brighter CD20 (p=0.01), CD24 (p=0.01) and CD48 (p=0.04) MFI values than isolated 13q14 deletion. CD38 positivity was only correlated with 11q22-23/ATM deletion (p=0.04). In addition, 11q22-23/ATM group disclosed a lower percentage of CD25 (p=0.03) and CD11c (p=0.001) when compared to the control group. No definitive conclusions could be drawn from 17p/TP53 deletion due to the small number of patients.

Conclusions:

1. In our series, certain immunophenotypic features are associated to chromosomal anomalies in CLL patients: trisomy 12 pt, with or without other concurrent chromosomal alterations, display a different phenotype in relation to isolated 13q14 deletion, while 11q22-23/ATM deletion cases are more likely to show CD38 positivity.
2. The stronger positivity of CD20 in trisomy 12 patients could be a factor to consider in the therapeutic management of these patients, since anti-CD20 monoclonal antibody could add additional benefits to standard chemotherapy.

AML: THERAPY

587 INTENSIVE CONSOLIDATION VERSUS ORAL MAINTENANCE THERAPY IN PATIENTS >60 YEARS WITH ACUTE MYELOID LEUKEMIA: RESULTS OF THE RANDOMIZED AML HD98B TRIAL OF THE AMLSG ULM

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To date, it is unknown how many cycles of postremission therapy are needed for optimal treatment in older patients with acute myeloid leukemia. Between April 1998 and September 2002 we conducted a multicenter treatment trial with a first randomization for the addition of ATRA in induction and first consolidation therapy and a second randomization between a second intensive consolidation and oral maintenance for one year. The induction and consolidation therapy consisted of two cycles of ICE (idarubicin 12mg/m i.v. days 1+3, etoposide 100mg/m i.v. days 1+3, cytarabine 100mg/m cont. i.v. day 1-5) and one cycle of HAM (cytarabine 500mg/m 12h i.v. day 1-3, mitoxantrone 10mg/m day 2+3). Patients were randomized for the addition of ATRA (45mg/m day 3-5, 15mg/m day 6-28) to each of these chemotherapy cycles. The second randomization was performed for further postremission therapy with one intensive second consolidation cycle IEiv (idarubicin 12mg/m days 1+3, etoposide 100mg/m days 1-5) versus a 12 monthly courses of outpatient maintenance therapy IEpo (idarubicin 5mg p.o. days 1+4+7+10+13, etoposide 100mg p.o. days 1+13). This report focused on the second randomization of this trial. Ninety patients in complete remission were randomized between IEiv (n=45) and IEpo (n=45). The median age of the patients at second randomization was 66 years (range 61 to 79 years). The characteristics of these patients such as age, WBC, LDH, bone marrow and peripheral blood blast count, preceding MDS, cytogenetic risk according MRC classification at diagnosis and first randomization were well balanced between the two treatment arms. In the IEpo arm 39 patients received the assigned treatment (median amount of cycles 4; range 1 to 12), 4 patients received IEiv and 2 patients relapsed before start of IEpo. The main cause of premature termination of oral maintenance therapy was relapse in 34 patients. In the IEiv arm all patients received the assigned treatment. One patient died due to severe infection during chemotherapy induced cytopenia. The relapse free survival and overall survival after second randomization were significantly superior in the IEiv-arm compared to the IEpo-arm (p=0.01 and p=0.009, respectively). The median survival after second randomization was 22.3 months in the IEiv-arm and 12.7 months in the IEpo-arm. In conclusion second intensive consolidation with IEiv is superior to maintenance therapy with IEpo.

588 RANDOMISED COMPARISON OF FLA VERSUS ADE, WITH OR WITHOUT G-CSF AND WITH OR WITHOUT ATRA, FOR HIGH-RISK AML: RESULTS OF THE MRC AML-HR TRIAL.

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Regimens containing fludarabine and high-dose Ara-C (FLA), sometimes with the growth factor G-CSF (FLAG), have been widely used for the treatment of high-risk acute myeloid leukaemia (AML), and it has also been proposed that all-trans retinoic acid (ATRA) might have a role. There is, however, no randomised evidence to support the use of these regimens. Thus, the MRC undertook a 2x2x2 factorial design randomised trial comparing: 1) FLA with a standard Ara-C, daunorubicin and etoposide (ADE) regimen; 2) G-CSF versus no G-CSF; and 3) ATRA versus no ATRA. Two courses of chemotherapy were scheduled; G-CSF was given at a dose of 0.5 MU/kg/day from the first day of each course until the neutrophil count was >0.5x10⁹/l or for a maximum of 28 days; ATRA was given at a dose of 45 mg/m²/day from the first day of chemotherapy for up to 90 days. Patients were eligible if they had one of the following forms of high-risk AML: i) relapse from 1st complete

remission (CR), ii) resistant disease (>15% blasts in bone marrow) after one course of induction therapy, iii) refractory disease (i.e. failure to achieve CR) after two or more courses of induction, iv) adverse genetics (-5, -7, del(5q), abnormal 3q, complex karyotype) at diagnosis with patients being entered after one course of induction chemotherapy. The main endpoints were CR rate, disease-free survival (DFS) and overall survival (OS). Between December 1998 and December 2003, 405 patients were entered from 94 hospitals; 188 in 1st relapse, 143 with resistant disease, 13 with refractory disease and 61 with adverse genetics; median age 53 years (range 16-78). It was not compulsory to undertake all three randomisations and 250, 356 and 362 patients were entered into the FLA v. ADE, G-CSF v. no G-CSF and ATRA v. no ATRA comparisons respectively. There were no significant differences in the main endpoints for any of the comparisons (see table DFS and OS are at 2 years). For OS, the hazard ratios with 95% confidence intervals were: FLA 1.26 (0.91-1.74), G-CSF 1.06 (0.81-1.40), ATRA 1.21 (0.92-1.58). Thus, all three experimental arms were non-significantly worse than the control arms. There were no substantial differences in toxicity or supportive care requirements between regimens. Outcome (CR rate and 2-year OS respectively) by disease category was: relapse 58%/26%; resistant/refractory disease 57%/29%; adverse genetics 84%/35%. This is the first reported randomised comparison of FLA(G) with a standard regimen in high-risk AML, and the largest reported trial in this high-risk patient group. It provides no evidence that FLA, with or without G-CSF or ATRA, is more effective than ADE alone, and highlights the dangers of non-randomised reports that have suggested that FLA-based regimens are the optimum treatment for high-risk AML.

Endpoint	FLA v. ADE			G-CSF v. no G-CSF			ATRA v. no ATRA		
	FLA	ADE	p	G-CSF	None	p	ATRA	None	p
CR (%)	63	65	0.9	58	62	0.5	60	62	0.8
DFS (%)	27	30	0.5	35	33	0.5	32	35	0.6
OS (%)	23	35	0.2	30	29	0.6	26	32	0.2

Caption 1:

589 A COMPARISON OF LOW DOSE ARA-C AND HYDROXYUREA WITH AND WITHOUT ALL RETINOIC ACID IN ACUTE MYELOID LEUKAEMIA PATIENTS NOT CONSIDERED FIT FOR CHEMOTHERAPY: RESULTS FROM NCRI LRF AML14 TRIAL

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As part to the UK NCRI AML14 Trial older patients with de novo or secondary AML or high risk MDS could be allocated to a non-intensive treatment option. They were randomised to receive Hydroxyurea to control WBC with supportive care or low dose (LD) Ara-C 20mg bd for 10 days at 4 week intervals. In addition patients were randomised to receive All-trans-retinoic acid (ATRA) 45mg/m² daily for 60 days. One hundred and ninety-nine patients were randomised to HU vs Ara-C and 204 to ATRA vs No ATRA. One hundred and eight patients had de novo AML, 53 secondary AML and 28 high risk MDS: 129 had a WHO performance score of <2; 155 patients were >65 years. The randomisations were balanced with respect to age; sex; disease type, WBC and performance score. Complete Remission (CR) was achieved in 1 of 92 patients allocated to HU, and 15 of 94 patients allocated to Ara-C (p=0.0003). There was no difference in CR whether or not patients received ATRA. Overall survival was significantly superior in the low dose Ara-C arm (p=0.0004). The median survival for LD Ara-C was 124 days versus 71 days for HU and 85 days for patients who received ATRA versus 93 for those who received no ATRA. There were no differences between the arms after course 1 in the mean WBC recovery, mean neutrophil count recovery but the platelet count recovery was superior in the Ara-C arm after course 2. There was no difference between HU and Ara-C in requirement for RBC and platelet transfusion, days on antibiotics or mean number of days in hospital. Platelet support was greater in the recipients of ATRA after course 1 (10 units vs 6 units: p=0.04). The conclusion from this study is that Low Dose Ara-C is superior to Hydroxyurea in patients designated to receive a non-intensive approach to treatment. The addition of ATRA does not influence outcome.

590 VACCINATION WITH WT1 INDUCES HIGH FREQUENCY MEMORY AND EFFECTOR T CELLS IN PERIPHERAL BLOOD AND BONE MARROW ASSOCIATED WITH COMPLETE REMISSION OF RECURRENT AML

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Background: The transcription factor Wilms tumor protein 1 (WT1) is an interesting antigen for use in vaccination and T cell therapy in myeloid leukemias. WT1 is strongly expressed in the majority of myeloid leukemic blasts and is a key molecule for blast proliferation.

Aims : A phase I/II study has been initiated in our institution in patients with acute myeloid leukemia to analyze the immunogenicity and toxicity of WT1 126-134 peptide vaccination in combination with the adjuvants GM-CSF and keyhole limpet hemocyanin.

Methods: We report here on the first 4 patients who have completed vaccination and have received a total of 15, 12, 5, and 5 vaccination cycles, respectively.

Results: While, following chemotherapy, patients 1 and 2 both had 5-10% blasts in bone marrow when vaccination was initiated, patient 3 and 4 had 90% and 70% blasts, respectively. Induction of high frequency T cell responses was detected in 3 of 4 patients with up to 0.92%, 0.43%, and 0.42% in peripheral blood and up to 0.8% in bone marrow by tetramer analysis. Detailed phenotypical analysis in patient 1 showed that WT1-specific peripheral blood T cells were almost exclusively CD45RA+CCR7-granzymeB+, and directly produced IFN γ in response to WT1 peptide, resembling cytotoxic effector T cells, while in the bone marrow both WT1-specific effector and CD45RA-CCR7- effector memory T cells were found. Patient 3 and 4 had progressive disease after 5 vaccinations. Patient 1 who had progressed during the first 4 weeks of vaccination with an increase of blasts to 30% in bone marrow was induced into complete remission after 6 vaccinations which remained for 12 months. Patient 2 is in continuous remission for currently 17 months. WT1 transcripts in bone marrow and peripheral blood were quantitated by PCR to monitor residual disease. In accordance with the clinical course in patients 1 and 2 we observed an approximately 100-fold, and 50-fold reduction, respectively, of WT1 transcripts following vaccination. No side effects as those typically seen with GM-CSF were noted.

Summary/Conclusion: Taken together, these findings underline the efficacy of the vaccine, inducing high frequency WT1-specific effector and memory T cells in peripheral blood and bone marrow associated with complete remission of leukemia in the absence of hematological or renal toxicity.

591 ROLE OF AUTOTRANSPLANTATION AS LATE CONSOLIDATION THERAPY IN ADULTS WITH STANDARD RISK ACUTE MYELOID LEUKEMIA AGED $\leq$ 60 YEARS: RESULTS OF A PROSPECTIVE RANDOMIZED MULTICENTER TRIAL

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Background: Karyotype and response to the first induction cycle are among the most important risk factors in AML. We initiated a risk-adapted multicenter trial for AML patients up to 60 years, in which therapy was stratified according to these factors.

Aims: The aims of this trial were to define the prognostic impact of a risk-adapted treatment strategy and to optimize late consolidation treatment for standard risk patients by a randomized comparison of high dose araC (HDaraC) and autologous peripheral stem cell transplantation (autoPBSCT). **Methods:** 374 patients with de novo (n=286) or secondary (n=88) AML were included. Patients with t(8;21) or inv(16) positive AML (CBF-leukemias) or normal karyotype and good response (GR) to induction I (up to 5% blasts in day 15 BM) were considered standard risk (SR), all others as high risk (HR). Patients with t(15;17) were excluded. The first chemotherapy cycle consisted of standard dose araC, idarubicine and

etoposide (IVA-I). Patients with GR to IVA-I continued with IVA-II on day 21 as double induction. In patients with bad response (>5% day 15 BM blasts), the second cycle consisted of either IVA-II or FLAG/Ida. Double induction was followed by an early consolidation with intermediate dose araC. As late consolidation, SR patients were randomized between HDaraC (12 x 3g/m)/daunorubicine (3 x 30mg/m) and autoPBSCT with PBSC mobilized after early consolidation except SR patients with normal karyotype and an HLA-matched sibling who were allografted.

Results: 185 patients were classified as SR and 174 as HR. In 15 patients, risk assessment was not possible. 90% of the SR achieved CR in contrast to 59% of the HR patients (overall CR rate 75%) and 2% of the SR patients died during induction. After 48 months, overall survival was significantly better for SR patients (52%) than for HR patients (25%). Within the SR risk group, the actuarial relapse free survival at three years was 58% for 17 patients with inv(16), 52% for 21 patients with t(8;21), and 41% for 131 patients with normal karyotype (p = 0.33). 43 SR patients (10 with CBF leukemia) were randomized to receive HDaraC and 46 (13 with CBF leukemia) to undergo autoPBSCT with equal distribution of the main characteristics (age, sex, performance status, cytogenetics, de novo or secondary AML). At three years, the actuarial overall survival was 72% for the patients undergoing autotransplantation and 70% for those receiving HDaraC (p=0.91, intent-to-treat analysis). At the time of interim analysis (09/2003), nine patients have died in each group: 4 in CR and 5 from relapse in the HDaraC group, while 9 patients died in relapse in the autotransplantation arm. The overall survival at three years for the SR patients with normal karyotype who were allografted (n = 23) was 80% with no significant difference to HDaraC or autotransplantation.

Conclusion: Late consolidation with HDaraC, autotransplantation and allograft transplantation showed a similar effect on overall survival in standard risk AML patients as defined in our trial while autotransplantation was the least toxic.

LYMPHOMA & OTHER LPD: TREATMENT

592 MONOTHERAPY WITH THE ANTI-CD20 ANTIBODY RITUXIMAB (RITUX) IN PATIENTS WITH POST-TRANSPLANT LYMPHO-PROLIFERATIVE DISEASE (PTLD) RESULTS OF A MULTICENTRE PHASE II STUDY

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Objectives: Organ transplant recipients show a higher risk of developing malignancies and neoplasia due to immunosuppressive therapy. With an incidence of 1-10%, PTLD is a life-threatening post-transplantational disease of B-cell origin. Generally accepted treatment guidelines for PTLD are in the process of establishing. In patients (pts.) with PTLD the use of cytostatic drugs is limited due to the risk of serious infectious complications. Showing a low toxicity profile, treatment with the anti-CD20 monoclonal antibody ritux is a promising alternative approach. **Methods:** We conducted a multicenter phase II trial investigating ritux as monotherapy in 25 pts. with PTLD. Transplanted organs were: kidney (n=7), lung (n=6), heart (n=6), liver (n=5) and kidney/pancreas (n=1). Ritux was given as 1st line therapy in 15, 2nd line in 8 and 3rd line in 2 pts. The treatment consisted of four infusions ritux 375mg/m on days 1, 8, 15 and 22. **Results:** The median age was 49.9 years (23.0-73.1) with a mean follow up time of 26.67 months in August 2003. Histology comprised 18 diffuse large B cell-, 2 marginal zone-, 1 Burkitt lymphoma and 4 polymorphic lymphoproliferations. The therapy was well tolerated, adverse events WHO 1 were limited to two infusion related reactions (pain/fever). The mean overall survival is 38.77 months with 18 pts. still alive. One pt. died from chronic rejection and three due to progressive PTLD. Three pts. had pre-existing history of recurrent, severe infections, and died of sepsis months after ritux treatment, without evidence of PTLD at autopsy. The total response rate is 64%. In total 13 pts. achieved a complete remission (CR) with a mean duration of 29.6 months. The rate of CR did not differ between 1st line (55.3%), and 2nd line (50%) therapy

with ritux. Partial remission was observed in 1 pt., minor remission in 2 pts., no change in 8 pts. and 1 pt. experienced progressive disease. Among the responders, three relapsed, with a mean time to progression of 31.64 months [1.5-43.8]. Conclusions: Monotherapy with rituximab proved to be well tolerated and effective in the treatment of PTLD. Adverse events were rare and of low grade. Furthermore pts. benefit from the short duration of therapy. The monoclonal antibody rituximab is equally effective in 1st line and in 2nd line therapy of PTLD. Due to the rate of relapse combination of therapy with cytotoxic drugs is to be evaluated.

593 THE MINT TRIAL (CHOP-LIKE REGIMENS WITH OR WITHOUT RITUXIMAB FOR YOUNG LOW-RISK PATIENTS WITH DIFFUSE LARGE B-CELL NON-HODKINS LYMPHOMA [DLBCL]): EARLY STOPPING AFTER FIRST INTERIM ANALYSIS

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Background: The addition of rituximab to CHOP-21 significantly improved clinical outcome in elderly patients with DLBCL (Coiffier et al., 2002). However, it remains to be shown, whether rituximab added to CHOP-like chemotherapy also improves results in young low-risk patients. Aims: To compare a CHOP-like chemotherapy with and without rituximab in young patients with low-risk DLBCL. Methods: In a phase III intergroup study with participating cooperative groups from 18 countries, previously untreated patients (18-60 years) with low-risk DLBCL (age-adjusted IPI 0 or 1, stages II-IV and stage I with bulky disease) were randomized to receive 6 cycles of a CHOP-like regimen (CHEMO) or the same chemotherapy plus rituximab 375 mg/m², given on day 1 of the 3-weekly regimens and on days 1, 22, 43, 64, 85 and 106 of the bi-weekly regimens, respectively (R-CHEMO). Radiotherapy (30-40 Gy) was planned to sites of initial bulky disease and/or extranodal involvement. The primary endpoint was time to treatment failure (TTF) with events defined as failure to achieve complete remission, progressive disease, relapse, or death. The trial was powered to show a 10% difference in TTF rate after 3 years. Results: Between 05/2000 and 10/2003 a total of 824 patients were recruited of whom 397 were allocated to receive CHOP-21, 361 to CHOEP-21, 34 to MACOP-B, and 32 to PMitCEBO with or without rituximab. The first formal interim analysis was performed on 326 evaluable patients with confirmed CD20 positive DLBCL (median age 44 years; 76% stage I/II, 24% stage III/IV disease, 31% elevated LDH, 50% bulky disease). Toxicity, incidence of adverse and severe adverse events in the CHEMO and the R-CHEMO arms were not significantly different. Patients randomized to R-CHEMO had a higher complete remission rate (84.7% vs. 66.0%; p=0.0002) and a lower rate of progressive disease (6.3% vs. 17.7%; p=0.0039). Patients in the R-CHEMO arm had a significantly longer TTF (p=0.000038; log rank test) and overall survival (p=0.0057; log rank test) compared to the CHEMO arm. At a median observation time of 15 months, TTF rates were 84% for R-CHEMO and 62% for CHEMO respectively. As the empirical p-value of the log rank test statistics for the primary endpoint TTF was considerably lower than the critical value provided by the alpha-spending function for the interim analysis (pcrit=0.00105), the formal criterion for stopping the trial was met. Based on these results, the independent Data Safety and Monitoring Committee recommended the early termination of the treatment phase of the trial in 12/2003 with 45/824 patients still under chemotherapy. Summary / Conclusion: The results of the MInT trial indicate that the addition of rituximab to 6 cycles of a CHOP-like regimen significantly improves CR rates, TTF and overall survival in young patients with low-risk DLBCL. An update of the interim-analysis population and the corresponding intent-to-treat population will be presented.

594 SEQUENTIAL TREATMENT WITH FLUDARABINE AND SUBCUTANEOUS ALEMTUZUMAB IS ABLE TO PURGE RESIDUAL DISEASE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background:

Although an increasing number of patients with chronic lymphocytic leukemia (CLL) may achieve complete remission (CR) relapse is inevitable, due to re-emergence of the malignant clone. The eradication of minimal residual disease (MRD) is associated with improved remission durability and has great potential in offering the possibility of cure.

Alemtuzumab is the foundation of many eradication-based treatment approaches because of its ability to achieve clinical remissions and to successfully purge MRD.

We investigate the use of subcutaneous (sc) alemtuzumab for the eradication of MRD in the bone marrow of patients in clinical response after fludarabine treatment, as determined by NCI criteria.

Methods:

At least 8 weeks after fludarabine treatment, 30 CLL patients (9 female, 21 male; median age 55 [range 38-64] years) received sc alemtuzumab, three times weekly for 6 weeks, at escalating doses up to 10 mg. MRD was assessed using a consensus polymerase chain reaction methodology to detect the clonality of IgH sequences. Patients obtaining a successful peripheral blood stem cell (PBSC) harvest (CD34 $\geq 2.5 \times 10^9/l$) after either granulocyte colony-stimulating factor (G-CSF) or intermediate-dose Ara-C (800 mg/sqm, every 12 h, for six doses) plus G-CSF were considered eligible for PBSC transplant. Pharmacokinetic parameters were estimated in seven patients. Serum samples of alemtuzumab were assayed by indirect immunofluorescence with target cells (HUT-78, a human T cells line) and antibody concentration was calculated by comparison with a standard curve.

Results:

Post fludarabine responses were: nine CRs, 10 nodular partial responses (PRn) and 11 PR. Post alemtuzumab responses were: 25 CR, 3 PRn, 2 PR. Overall, 53% of patients converted to polyclonal IgH pattern (MR). Of the patients in CR at baseline, 6 achieved MR; eight of the patients in PRn at baseline, improved to CR, four achieving MR. Of the eleven patients in PR at baseline, nine improved (one PRn, eight CR) and six achieved MR. Fifteen out of 16 patients successfully mobilized PBSC. Ten patients have been transplanted: three, age > 60 y, were conditioned with L-PAM 180 mg/sqm, 7 patients received CTX+TBI as conditioning regimen. Median time to PMN $\geq 500/l$ and PLT $\geq 20.000/l$ was respectively 9 (range 8-10) and 11 days (range 9-12). At a median follow-up of 19 months (range 6-24) disease progression was observed in 5 patients, none of the transplanted patients showed a disease progression after a median follow up of 7 months (range 1-24). Subcutaneous alemtuzumab was well tolerated; fifteen patients (50%) developed cytomegalovirus (CMV) reactivation, CMV disease was prevented by prompt treatment with oral ganciclovir. Alemtuzumab serum concentrations measured at second week after the start of therapy, just before the next dose (Ctough), were relatively stable: Ctough = 0.790.6 g/ml.

Conclusions:

Sequential treatment with fludarabine and alemtuzumab is feasible, effective and safe. Eighty-seven percent of patients improved to CR or PRn and alemtuzumab was able to eliminate residual disease in 53% of patients. Sequential treatment did not compromise PBSC mobilization, allowing patients to proceed to autologous transplantation.

595 A PHASE II STUDY OF PROTEASOME INHIBITOR BORTEZOMIB IN RELAPSED OR REFRACTORY INDOLENT OR AGGRESSIVE B-CELL NON-HODKINS LYMPHOMA

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Background:

Bortezomib (VELCADE®, formerly known as PS-341, Millennium Pharmaceuticals, Inc., Cambridge, MA) is a novel small molecule, which is a potent and selective but reversible inhibitor of the proteasome. Proteasome inhibition results in the disruption of a variety of pathways and checkpoints leading to cellular apoptosis.

Purpose:

The purpose of this phase II study was to establish response rate, time to progression and toxicity of bortezomib in patients with indolent or aggressive B-cell non-Hodgkins lymphoma.

Patients and Methods:

Based on preclinical data, which showed promising activity in mantle cell lymphoma (MCL), we had designed our study in two groups. Patients with relapsed or refractory MCL (Group A) or other B-cell lymphomas (Group B) were eligible regardless of number of prior therapies. Patients received bortezomib 1.5 mg/m² I.V. push on days 1, 4, 8 and 11 of a 21-

day cycle. Restaging was done every 2 cycles. Patients were treated for 6 cycles unless removed from study for toxicity or progression.

Results:

Fifty-one patients were enrolled in this study. Group A included 29 mantle cell lymphoma patients and group B included 22 patients: 12 with diffuse large cell lymphoma, 4 with follicular lymphoma, 3 with transformed follicular lymphoma, 2 with small lymphocytic lymphoma and 1 with Waldenström macroglobulinemia. The median number of prior therapies was 3 (range 1 to 10) in group A and 4 (range of 2 to 12) in group B. In group A, of 23 patients evaluable for response, there were 5 complete remissions and 6 partial remissions for an overall response rate of 48% (95% CI, 29%-69%). At this time only 2 of the 11 responders have relapsed, all others have had maintained response with a median follow up of 5.6 months and a range of 2 to 16 months. In group B, 19 patients were evaluable for response; there were 1 complete remission in a patient with follicular lymphoma and 2 partial remissions in a patient with diffuse large cell lymphoma and a patient with Waldenström macroglobulinemia. All three responders in this group had failed 3 or 4 prior therapies. The overall response rate in this group was 16% (95% CI, 3%-40%). Grade 3 adverse events included thrombocytopenia (44%), gastrointestinal toxicity (24%), hypotension/fatigue (20%), neutropenia (12%) and neuropathy (6%). Grade 4 toxicity occurred in 14% of patients.

Conclusion: Bortezomib demonstrated a remarkable activity in mantle cell lymphoma in this study and encouraging results in other B-cell lymphomas. Further studies will explore the efficacy of bortezomib in combination with other cytotoxic or biological agents.

596 P-VABEC-CIP REGIMEN IMPROVES SURVIVAL IN ELDERLY PATIENTS WITH DIFFUSE LARGE CELLS LYMPHOMAS. FINAL RESULTS OF AN ITALIAN MULTICENTER RANDOMIZED STUDY

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Background: P-VABEC regimen resulted a safe and active therapy for elderly patients (pts) with diffuse large cells lymphomas (JCO 11- 2363,1993). However in spite of a high rate of complete response (CR) the progression-free survival (PFS) rapidly decreased for a high incidence of progressive disease or early relapses. A phase II pilot study (Leuk.and Lymph. 24-335, 1997) demonstrated the CIP regimen was an active and safe therapy in pts with progressive disease after P-VABEC chemotherapy.

Aims: To evaluate the activity and toxicity of CIP consolidation therapy after P-VABEC versus a standard P-VABEC regimen in a prospective multicenter randomized phase III study.

Methods: From October 1995 to June 2000 we enrolled 214 previously untreated pts with diffuse large cell non Hodgkins lymphoma (NHL) according to REAL classification, median age 70 yrs (range 60-85), stage II-IV. All eligible pts were randomized at diagnosis to receive P-VABEC (arm A) or P-VABEC-CIP (arm B). The 8 weeks P-VABEC regimen was delivered on out-patient basis as previously described. The CIP regimen consisted of: Cis-platinum (40mg/td day 1), Idarubicin (15mg/m² day 8), and Prednisone (40mg/td days 1-4/ 8-11) repeated every 21 days for a total of 3 courses. In the arm B the CIP consolidation therapy was delivered only in pts in CR/PR after P- VABEC. Now 202 patients are evaluable for response, 107 pts randomized for P-VABEC and 95 for P-VABEC-CIP. According to the age-adjusted IPI score 89 pts were considered as Low Risk (IPI 0-1) and 113 as High Risk (IPI 2-3).

Results: At a median follow up of 46 months (range 1-94) the CR rate was 72% and 74% (p=ns), the 5-yrs OS was 42% and 61% (p=0.04) and 5-yrs PFS 39% and 51% (p=0.048) respectively in arm A and arm B. According to the IPI score in the Low Risk the 5-yrs OS was 67% vs 70% (p=ns) and 5-yrs PFS was 53% vs 64% (p=ns) while in the High Risk the 5yrs OS was 25% vs 52% (p=0.008) and 5-yrs PFS was 25% vs 36% (p=ns) for Arm A and Arm B respectively. No severe toxicity has been reported for CIP regimen. Thirteen/202 (13%) toxic deaths related to chemotherapy occurred, all during P-VABEC induction chemotherapy.

Conclusions: CIP consolidation chemotherapy, given on out-patients basis after P- VABEC, resulted a safe and active consolidation chemotherapy and improves overall survival in this group of patients.

597 ANGIOGENESIS IN HODGKINS LYMPHOMA: A MORPHOMETRIC APPROACH IN 286 PATIENTS WITH PROGNOSTIC IMPLICATIONS

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Background: Angiogenesis has been recently implicated in the pathophysiology and course of various hematologic malignancies. Its significance in Hodgkin's lymphoma (HL), however, is largely unknown. Aims: To evaluate various morphometric characteristics of microvessels in lymph node sections of patients with HL at diagnosis and investigate their relationship with baseline clinicopathologic parameters and outcome. Patients and Methods: The following morphometric characteristics of microvessels were evaluated by means of anti-CD34 immunohistochemistry in lymph node sections of 286 patients with HL at diagnosis: Microvessel density (MVD), total vascular area (TVA) and several microvessel caliber (namely area, perimeter, major axis length and minor axis length) and shape-related (shape factor) microvascular parameters. These parameters were quantitated in the region of most intense vascularization using image analysis. Results: An increase in microvessel caliber parameters [namely area (p=0.02), perimeter (p=0.007), major axis length (p=0.006), and minor axis length (p=0.02), all evaluated by Student's t-test] and a decrease in MVD (p=0.008, by one-way ANOVA) was noted with advanced stage. An inverse relationship was similarly recorded between MVD and increasing number of involved anatomic sites (p=0.003, by one-way ANOVA) and increasing LDH levels (Pearson's r=-0.149, p=0.03). In univariate analysis, overall disease-specific survival was adversely affected by MVD [5-year rate 88% vs. 95% for MVD >= 37 vs. <37 (median value), p=0.04] and TVA [5-year rate 87% vs. 96% for TVA >=28000 μm² vs. <28000 μm² (median value), p=0.046], whereas inferior failure-free survival was associated with lower shape factor values corresponding to more flattened vessel sections. The 5-year FFS was 75% vs. 90% for patients with shape factor values <0.62 vs. >= 0.62 (median value, p=0.006). Multivariate analysis disclosed that the extent of angiogenesis (MVD and TVA) [p=0.036, Hazard Ratio (HR) 1.65 (1.03-2.63)], age (p<0.001) and the number of involved anatomic sites (p<0.001) independently affected overall disease-specific survival. Accordingly, failure-free survival was independently linked to the shape of microvessels [shape factor, p=0.02, HR 0.45 (0.23-0.87)] and albumin levels (p<0.001). Conclusions: Our data support the view that angiogenesis provides independent prognostic information in HL, requiring the concomitant evaluation of quantitative and qualitative aspects of microvascular network.

ANEMIA

598 PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS DISPLAY HIGH FREQUENCY OF PERIPHERAL BLOOD NKT CELLS EXPRESSING ACTIVATING ISOFORMS OF INHIBITING SUPERFAMILY RECEPTORS (IRS).

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder of the hematopoietic stem cell (HSC) characterized by an somatic mutation in the PIG-A gene, that determines a deficiency of glycosylphosphatidylinositol (GPI) membrane-linked proteins. It has been suggested that in PNH normal HSC are selectively suppressed by autoreactive T cells, while PNH HSC can escape this suppression and thus survive and expand. The identity of the putative autoreactive T cells and their target are still elusive.

Recently it has been reported that a minor subset of T lymphocytes express at the cell surface KIR and/or CLIR members of the Inhibitory Receptor Superfamily (IRS). These T cells may represent chronically stimulated memory cells expanded during viral infection or autoimmune responses. We have analyzed the surface expression and function of IRS members in T cells of PNH patients. We found that the proportion of KIR+ or CLIR+ cells amongst CD3+ cells was consistently higher

in PNH patients than in healthy donors. In addition, both the ratio CD3+KIR+/CD3-KIR+ and CD3-CLIR+/CD3-CLIR+ was always >1 in PNH patients, whereas this ratio was <1 in healthy donors. A minor fraction of CD3+IRS+ T cells express TCR gd (mainly Vd2, as expected) and the remaining large fraction of these cells was TCR ab+. CD3+IRS+ cells from PNH patients appear to have a Vb chain repertoire similar to that found in healthy donors. On the other hand, CD3+IRS+ T cells from PNH patients, unlike those from healthy donors, were characterized by a powerful cytolytic activity against murine mastocytoma P815 cells in a re-directed killing assay using anti-CD3 monoclonal antibodies (mAbs). Anti-KIR or anti-CLIR mAbs triggered cytotoxicity of these T cells, indicating that IRS expressed in ex-vivo isolated lymphocytes belong to the activating type. This notion was further supported by the functional analysis of a panel of T-cell clones isolated from 3 different PNH donors; indeed, in the large majority of these clones the engagement of either KIR or CLIR elicited activation of lysis of P815. By contrast, CD3+IRS+ T cells from healthy donors expressed IRS of the inhibiting type. In addition, the ligation of IRS in CD3+IRS+ T-cell clones of PNH patients induced a strong production of TNF-alpha and Interferon-gamma. Taken together these findings suggest that this population of CD3+ T cells expressing the activating isoforms of IRS that are increased in PNH patients may include auto-reactive cells involved in the pathogenesis of this disease.

599 RITUXIMAB IN CHRONIC COLD AGGLUTININ DISEASE: A PROSPECTIVE STUDY OF 20 PATIENTS

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Background: Chronic cold agglutinin disease (CAD) is an acquired autoimmune haemolytic anaemia, characterised by clonal B cell proliferation with production of a monoclonal IgM antibody of kappa type. Previous treatments have included alkylating agents, interferon and corticosteroids, but none of these have been convincing. Several reports, mainly including a few patients, have demonstrated promising results after treatment with the chimeric monoclonal anti-CD20 antibody Rituximab. Aims: We performed a phase II multicentre trial to investigate the effect of Rituximab in primary CAD and CAD secondary to malignant B cell lymphomas. Methods: Overall 20 patients with CAD were included in the study from October 2002 till April 2003. Baseline characteristics: M/F 5/15, mean age 75 years (55-87), dependent/independent of erythrocyte transfusion 11/9, 13 cases had primary CAD, 3 cases had secondary CAD (1 small lymphocytic lymphoma, 2 immunocytoma) and in 3 cases a bone marrow biopsy was suspected for being a lymphoproliferative disease. 1 patient developed rectal cancer 8 weeks after inclusion and was therefore excluded from the study. All patients were treated with Rituximab 375 mg/sqm at day 1, 8, 15 and 22. The follow-up period was planned to be 1 year. Criteria for response: complete response was defined as normalised Haemoglobin level and absence of all biochemical and clinical signs of CAD; major response was defined as stable increase of Haemoglobin levels >1.0 g/dl, independence of erythrocyte transfusion, improvement of CAD-related symptoms or a 50% reduction in elevated serum IgM; minor response was defined as a stable Haemoglobin level without need of erythrocyte transfusion and improvement of CAD-related symptoms. Non-responders did not fulfil any of the response criteria. Results: 14 patients were been followed for 1 year; 4 patients were followed for 16, 23, 27 and 36 weeks where they were excluded from further follow-up because of complications not related to CAD. 1 patient is still in the study. 15 out of 19 patients (79%) obtained a response to the treatment: 3 patients had a complete response, 8 patients had a major response and 4 patients had a minor response. 4 patients were non-responders. Mean time to response was 2.8 months (1 to 6 months). Relapse was seen in 9 patients with a mean time to relapse 9.5 months (6 to 12 months) after inclusion. There were no serious Rituximab-related side effects. Conclusion: Our study indicates that treatment with Rituximab is an effective therapy in patients with CAD. Only a few patients will obtain complete remission, but the majority of patients will have clinical benefit such as no need of erythrocyte transfusion, reduced haemolytic activity, a stable or increasing Haemoglobin level and improvement in CAD-related symptoms. However, most patients will probably relapse after months.

600 ACTIVATED T-LYMPHOCYTES WITH MYELOSUPPRESSIVE PROPERTIES IN THE PERIPHERAL BLOOD (PB) AND BONE MARROW (BM) MICROENVIRONMENT OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA (CIN)

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Background: We have shown that CIN patients have common pathogenetic features with certain BM failure syndromes, i.e. aplastic anaemia and MDS. Specifically, the patients display increased Fas-mediated apoptosis of the CD34+/CD33+ cells and increased TNF α , IFN γ and FasL (FasL) production in the BM microenvironment.

Aims: To discern between a primary BM progenitor cell and microenvironment abnormality in CIN and identify the type of cells producing the inhibitors of haemopoiesis.

Methods: We studied 75 CIN patients and 47 healthy controls. For the discrimination between a primary progenitor and stromal cell abnormality we used crossover long-term BM cultures (LTBMC) in which purified CD34+ cells from CIN patients and controls were inoculated onto irradiated normal LTBMC stromas and vice versa, i.e. normal CD34+ cells were inoculated onto irradiated CIN and normal stromas. For the identification of the IFN γ - and FasL-producing cells we performed RT-PCR in CD3+ and CD14+ cell extracts sorted from the BM mononuclear (BMMC) and LTBMC adherent cell fraction. Two-colour flow-cytometry was used for the analysis of PB and BM lymphocytes regarding the expression of surface (CD38, CD69, CD25, HLA-DR, CD95) and intracellular (IFN γ) activation markers. To test the inhibitory effect of patient BM T-cells on the progenitor cell growth we performed crossover short-term cultures in which patient or normal CD34+ cells were co-cultured with normal or patient CD3+ cells. RT-PCR and flow cytometry (TCR-V β repertoire) were used to exclude BM T-cell monoclonality.

Results: In LTBMCs, patient CD34+ cells displayed defective clonogenic potential onto normal stromas compared to controls (P<.05) reflecting probably the increased proportion of apoptotic progenitors previously reported in CIN. A marked defect was observed in the capacity of patients stroma to support normal haemopoiesis compared to normal stromas (P<.001) suggesting a primary defect in the BM microenvironment in CIN. RT-PCR showed that the IFN γ - and FasL-producing cells were exclusively the CD3+ but not the CD14+ cells in both the BMMC and the adherent cell fraction, suggesting the presence of activated T-cells in patients BM. Flow-cytometry showed increased proportion of BM CD3+ cells in patients compared to controls (P<.01) and increased expression of HLA-DR, CD25, CD38, CD69, CD95, within the BM (P<.05, P<.05, P<.001, P<.05, P<.001, respectively) and PB (P<.01, P<.01, P<.01, P<.01, P<.05, respectively) CD3+ cells. Increased intracellular IFN γ expression was found in the BM (P<.05) but not the PB CD3+ cells of the patients compared to controls upon activation. This finding coupled with the normal PB CD3+ cells suggests that activated T-cells preferentially accumulate in patients BM. RT-PCR and flow-cytometry did not reveal monoclonal T-cell populations. In the co-culture experiments a significant inhibition of autologous and normal CD34+ cell growth by patient CD3+ cells was demonstrated suggesting a myelosuppressive effect of T-cells in CIN.

Conclusions: Activated T-lymphocytes may have a role in the pathogenesis of myelosuppression in CIN. Whether the BM CD34+/CD33+ progenitors represent the targets of an autoimmune process associated with the HLA-DRB1*1302 predisposition or the innocent 'bystanders' of a local immune dysregulation in the BM microenvironment remains to be clarified.

601 IMPAIRED ERYTHROPOIESIS IN PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA (CIN)

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Background: We have previously shown that patients with CIN have significantly reduced haemoglobin (Hgb) levels compared to age- and sex-matched healthy volunteers. We have reported that 15.5% of these patients have anaemia on the basis of previously established criteria.

Aims: To investigate erythropoiesis in CIN patients and probe the mechanism possibly involved in the pathogenesis of anaemia seen in these patients.

Methods: Forty-two CIN patients and 43 control subjects have been studied. The frequency of erythroid-burst forming units (BFU-E) was

evaluated with short-term bone marrow (BM) cultures of BM mononuclear cells (BMMCs) and immunomagnetically sorted CD34+ cells. The number of CD34+/CD71+, CD36+/GlycophorinA(GlycoA)+ and CD36-/GlycoA+ cells, corresponding to sequential stages of erythroid development, was evaluated by flow-cytometry. The percentage of apoptotic cells within the CD34+/CD71+, CD36+/GlycoA+ and CD36-/GlycoA+ cell compartments was evaluated by flow-cytometry using 7-amino-actinomycin-D (7AAD) stain. Erythropoietin (EPO) was measured in serum and supernatants of long-term BM cultures (LTBMCs) using ELISA. Moreover, a semi-quantitative RT-PCR was performed to detect Epo mRNA expression in adherent cells derived from LTBMCs and flow-cytometry for the evaluation of EPO-receptor (EPO-R) expression on the purified GlycoA+ cells.

Results: The number of BFU-E obtained by BMMCs and CD34+ cells (18351177(SD) and 200187, respectively) were significantly lower in CIN patients compared to controls (41772039 and 308200, respectively) ($P<0.001$ and $P<0.05$, respectively). CIN patients had significantly lower numbers of CD34+/CD71+ erythroid progenitor cells and CD36+/GlycoA+ early erythroid precursor cells, compared to controls (0.490.32 and 21.716.95, respectively, versus 0.890.61 and 27.279.33, respectively) ($P=0.0018$ and $P=0.0094$, respectively). Patient CD34+/CD71+ and CD36-/GlycoA+ cells contained significantly higher proportion of apoptotic cells (23.06%15.83% and 26.82%22.72%, respectively) compared to controls (15.02%6.09% and 19.66%11.21%, respectively) ($P<0.05$ and $P<0.05$, respectively). In contrast, no statistically significant difference was found between patients and controls in the percentage of apoptotic cells detected in the CD36-/GlycoA+ cell compartment (3.56%4.56% and 2.56%2.05%, respectively). Epo was not detected in the supernatants of LTBMCs from patients or controls. No significant difference was found in Epo mRNA expression in cell extracts derived from patient and control LTBMCM adherent layers. EPOR expression however, was significantly lower in patient GlycoA+ cells compared to controls ($P<0.05$). Serum Epo levels were significantly lower in the group of patients, compared to the reference group, which consisted of healthy individuals and subjects with iron deficiency anaemia (9.866.22 mU/ml and 21.6330.76 mU/ml, respectively) ($P=0.0079$). This may be attributed to the higher Hgb levels in patients, compared to the reference group (13.151.03 g/dl and 12.542.25 g/dl respectively) ($P=0.110$). Epo production was considered as appropriate for the Hgb values in our patients [mean observed/predicted InEPO (O/P ratio) in the patients (0.990.26) was within the 95% confidence limits of the reference group]. **Conclusion:** CIN patients display low numbers of erythroid progenitor and early precursor possibly due to increased apoptosis in these cell compartments. Since Epo production is normal in our patients we speculate that EPO-R downregulation on the BM erythroid cells mediated, at least, by the previously described IFN γ overproduction in CIN BM, may be the main pathogenetic mechanism.

602 PRODAPTIN-CD59, A MEMBRANE-TARGETED RECOMBINANT CD59, COATS PNH RED CELLS IN VITRO AND MURINE RED CELLS IN VIVO PROTECTING BOTH FROM HUMAN COMPLEMENT MEDIATED LYSIS

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is characterised by intravascular hemolysis and venous thrombosis. The hemolysis and thrombosis result from increased complement sensitivity of PNH cells because they lack complement regulatory molecules, particularly CD59. CD59 deficiency from PNH red cells can be complete (type III cells) or partial (type II cells). Patients with only type II cells do not suffer from hemolysis indicating that low levels of CD59 control terminal complement activity. The absence of CD59 renders PNH platelets susceptible to complement, resulting in the release of procoagulant vesicles.

Aims: Replacing CD59 on PNH cells may be sufficient to prevent complement-mediated hemolysis and possibly thrombosis. GPI-linked structures are difficult to produce in bulk by recombinant technology. APT3111 (Prodaptin-CD59) is a recombinant human CD59 expressed in *E.coli*, which has been modified using site-specific attachment of a membrane-interactive acylated, charged peptide instead of the GPI anchor of endogenous CD59 (ProdaptinTM technology).

Methods: Hams and complement lysis assays were used to demonstrate protection from hemolysis with Prodaptin-CD59. Whole blood from 8 PNH

patients, all with large PNH Type III red cell populations, was incubated with Prodaptin-CD59, at various concentrations and incubation times. CD59 bound to erythrocytes was assessed by flow cytometry. In order to assess Prodaptin-CD59 in vivo, groups of CD1 mice were treated with various concentrations of Prodaptin-CD59 by intravenous injection. Blood was drawn at various time-points between 15 minutes to 48h after injection, exposed to human complement and assessed for CD59 by flow cytometry. **Results:** Prodaptin-CD59 is effective in complement lysis assays and results in a significant reduction in hemolysis compared to unmodified recombinant sCD59. Prodaptin-CD59 has comparable activity to native CD59. PNH erythrocytes were coated with CD59 to levels similar to type II cells at concentrations of Prodaptin-CD59 ~100nM in all patients. Normal levels of CD59 were achieved at concentrations >1000nM. PNH type III cells were completely coated by CD59 after 1 minute, and still expressed CD59 after 3 days storage at 4 C and at 37 C. Flow cytometry for CD66, CD16 and CD59 demonstrated coating of PNH leucocytes by Prodaptin-CD59. In vivo injection of Prodaptin-CD59 protected murine red cells from complement for up to 6 hours at 10mg/kg and for up to 24 hours at 50mg/kg when compared to control mice given vehicle alone ($p=0.002$). CD59 was detectable by flow cytometry at 24 hours to levels equivalent to Type II cells. No toxicity was observed.

Summary: We report that Prodaptin-CD59, a membrane-targeted recombinant human CD59, is effective in coating PNH erythrocytes in vitro to levels that are sufficient to inhibit complement-mediated lysis. The attachment of Prodaptin-CD59 to the red cell membrane is very rapid and remains attached for at least 3 days in vitro. In vivo data demonstrates that intravenous injection of Prodaptin-CD59 coats mouse erythrocytes with CD59 and protects them from lysis by human complement. Prodaptin-CD59 represents a potential novel therapeutic approach for patients with PNH and merits further investigation.

603 INDEPENDENT RISK FACTORS FOR ANEMIA IN PATIENTS WITH LYMPHOMA/MYELOMA ARE DEFINED BY LOGISTIC REGRESSION MODELING: RESULTS FROM THE EUROPEAN CANCER ANAEMIA SURVEY (ECAS)

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Background: Anemia has been shown to adversely affect patient outcomes and quality of life (QOL) in patients with malignant diseases. [1,2]

Aims: Identification of baseline characteristics in patients with hematologic malignancies that correlate significantly with anemia development would optimize patient care and allow effective anemia management.

Methods: The patient population from the large ECAS survey [3] included 2,316 (15.1%) patients with lymphoma/myeloma of which 679 patients were not anemic at enrollment, not receiving anemia treatment, and received chemotherapy (CT) during the study period of 6 months. These patients served as a basis to develop odds ratios (OR) that could be used to predict anemia in patients with these malignancies. Potential predictors for anemia were limited to data available at enrollment and included tumor characteristics, patient demographics, laboratory parameters, and CT agents. Potential predictors were re-coded to be dichotomous, which allowed evaluation of Mantel-Haenszel unadjusted OR and 95% confidence intervals (CI). Dichotomized variables were entered into a logistic regression equation to simultaneously evaluate the predictors and calculate Adjusted OR (AOR).

Results: Low initial Hb, persistent/recurrent disease, being female, and intent to treat with platinum CT were the 4 variables that significantly ($P<0.03$) predicted anemia. As shown in the table, AOR (the increase in odds of becoming anemic when the 4 variables are considered simultaneously) for these variables indicates that females with initial Hb <12.7 g/dL and males with initial Hb <13.3 g/dL have 4 times the risk to become anemic (AOR 4.2). Hb <12.7 g/dL for females and <13.3 g/dL for males is the within-gender lowest 40th percentile hemoglobin. Having persistent/recurrent disease increases the risk of becoming anemic by approximately 1 times (AOR 1.5). Being female increases the risk for anemia almost 3 times (AOR 2.8); intent to treat with platinum CT increases the risk for anemia greater than 5 times (AOR 5.5). The relatively small difference between OR and AOR indicates that each predictor has a good independent predictive power. Using these predictors, the clinician can estimate the relative risk for anemia for a patient with lymphoma/myeloma. The presence of multiple risk factors

would signal that careful observation is important to identify declining Hb levels before patient QOL and possibly treatment outcomes are affected.

Conclusions: Logistic regression analysis resulted in a useful guide to alert the clinician to the greater risk of anemia development in patients with lymphoma/myeloma, and help identify patients who are likely to become anemic. Proactive anemia management could then optimize overall patient care.

[1] Caro J, et al. Cancer 2001;91:2214-2221.

[2] Crawford J, et al. Cancer 2002;95:888-895.

[3] Ludwig H, et al. Blood 2002;100: 234a.

Predictor	OR	95% CI	P-Value	AOR	95% CI	P-Value
HB <13.3 (males) or <12.7 (females) g/dL at enrollment	3.8	2.76 – 5.29	.0000	4.2	2.99 – 5.95	.0000
Persistent/recurrent disease	1.8	1.31 – 2.54	.0000	1.5	1.05 – 2.16	.0276
Female gender	2.4	1.71 – 3.29	.0000	2.8	1.99 – 4.07	.0000
Treatments with Platinum CT	4.0	1.72 – 9.41	.0006	5.5	2.24 – 13.54	.0002

Caption 1: Logistic Regression Odds Ratio: Anemia Development

MOLECULAR BIOLOGY

604 INDUCTION OF GENOMIC INSTABILITY BY V(D)J-MEDIATED TRANSPOSITION OF SIGNAL JOINTS

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Background:

Lymphoid neoplasms are tightly associated with a wide range of chromosomal aberrations. Many of these lead to proto-oncogene deregulation due to mistakes in the V(D)J recombination process. While signal joints (SJ), the by-products of V(D)J recombination, have been generally believed to be 'harmless' and non-relevant in the dynamics of V(D)J recombination, our detailed in vivo analysis of translocation breakpoints in T-ALL revealed, that they, in fact, are not inert, and can engage in secondary V(D)J recombination.

Aims:

As this SJ reactivity, rather than chromosomal translocation, constitutes in some cases the true initiating step of oncogenic activation, we wanted to test V(D)J-mediated transposition as an additional source of genomic instability in leukemia and lymphoma.

Methods:

Extra-chromosomal recombination and competition substrate assays were used to measure the rates of SJ reactivity in cis. Because the large majority of SJ are normally present on excised episomal products, we used modified recombination substrates, PCR, and primer extension to analyze the capacity and rates of a SJ to undergo V(D)J recombination with a standard coding-end RSS (CER) target in trans.

Results:

We find that 1) each of the 2 RSS of a given SJ (SJR) is able to engage in further cycles of V(D)J recombination with a CER; and 2) the rate of recombination of a SJR is similar to that of a CER. In cis, the frequency of a D-beta CER was 32% vs 68% for the D-beta SJR. In case of D-delta, it was 64% for CER vs 36% for SJR. The trans-V(D)J recombination, leading to the transposition of the episomal circle into the target, yielded efficiencies of 50%:50% for CER and SJR, respectively, for both D-beta and D-delta experiments. Additionally, we tested the capacity of a genuine D-delta/J-delta SJ to target a cryptic site such as the LMO2 pseudo-RSS involved in the T-ALL associated t(11;14)(p13;q11) V(D)J-mediated translocation. Again, our results show similar rates of V(D)J-mediated transposition, 50%, for the D-delta/J-delta SJR into the cryptic RSS compared to an authentic RSS.

Conclusions:

Retained on the chromosome following rare rearrangements by inversion or chromosomal translocations, SJ constitute highly unstable genomic elements with potential oncogenic properties. Located on excised episomal circles, SJ are able to undergo V(D)J-mediated transposition back into the genome. Here, V(D)J-mediated transposition of SJ is not limited to IG/TCR loci, but can target the estimated 10 million cryptic sites present randomly in the genome. Considering the large number of SJ produced daily, and the absence of chromatin accessibility constraints on episomal products, this highly efficient V(D)J-mediated transposition of SJ should represent a major risk of chromosomal alteration, an event difficult to identify by classical cytogenetics and PCR methods. Thus, V(D)J-mediated transposition might in fact constitute a yet unrecognized source of genomic instability and cancer.

605 CODING JOINT FORMATION IS INHIBITED IN V(D)J-MEDIATED TRANSLOCATIONS FROM HEALTHY INDIVIDUALS

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Background:

In T-ALL, at least nine different proto-oncogenes are activated, many of which as a result of V(D)J-mediated translocation. Such translocations generate a coding joint (CJ) on one, and a signal joint (SJ) on the other derivative chromosome. Surprisingly, we recently observed that SJ from LMO2/TCRdelta t(11;14)(p13;q11) and TAL2/TCRbeta t(7;9)(q34;q32) translocations can be detected at low frequency in up to 50% thymus samples from healthy children. This raised the question of the fate of the cells carrying such translocations. Do such cells already over-express the associated proto-oncogene, are they susceptible to/undergoing neoplastic transformation and proliferation? Most importantly, are those children at risk to develop leukemia?

Aims:

To assess the fate of cells carrying t(11;14) and t(7;9) in healthy individuals, we sought to determine intra-thymic clonal proliferation. Because CJ display extensive modifications of the coding segments, these unique molecular signatures represent ideal clonotypic markers.

Methods:

Fluctuation PCR, ex vivo trans-recombination substrate assays, PCR, and primer extension were used to detect and analyze clones and formation of junctions.

Results:

Unexpectedly, while SJ are readily observed, no corresponding CJ can be detected in matched samples.

This contrasts with leukemic cells from T-ALL patients and with trans-TCRdelta/beta rearrangements in healthy individuals, which systematically display both SJ and CJ. Using ex vivo trans-recombination substrate assays, we demonstrate that this inhibition is due to the lack of stabilization of the coding ends in the V(D)J post-cleavage synaptic complex. While LMO2/D-delta SJ and J-beta/D-delta SJ were formed with equal efficiency, there was an 8-fold difference in CJ formation.

Conclusions:

Our data suggest the presence of a 'surveillance mechanism' acting by post-cleavage inhibition of trans-CJ formation so that unresolved breaks would trigger apoptosis pathways. Only rare 'leaky' cells which succeeded to form a CJ would proceed towards the malignant transformation process. Importantly, individuals carrying the SJ marker but not the CJ would not stand at risk for development of T-ALL.

606 TELOMERE LENGTH CORRELATES WITH HISTOPATOGENESIS ACCORDING TO THE GERMINAL CENTER IN MATURE B-CELL LYMPHOPROLIFERATIVE DISORDERS

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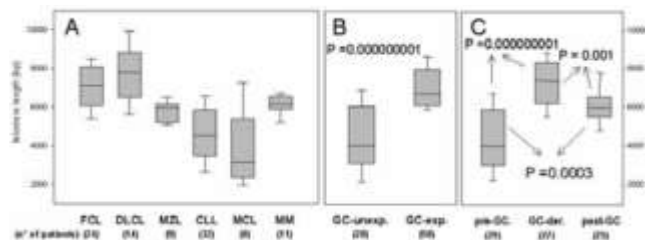
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Telomeres are repeated DNA sequences that play a critical role in maintaining chromosomal stability in both healthy and cancer cells. Telomeres usually shorten at each mitotic division up to a critical phase known as senescence. This limits the number of mitoses that can be performed by somatic cells. In order to divide indefinitely, germinal

cells, cancer cells and few subtypes of healthy somatic cells must avoid telomere shortening. This is achieved by means of an RNA-containing enzyme known as telomerase. Unlike most cell subtypes, normal B-cells have highly variable levels of telomerase activity (TA) according to their differentiation phase. In particular GC-inexperienced B cells have no TA while GC cells have very high levels. Aim of this study was to investigate TL in a large panel of MBCLD to verify whether it might be influenced by TA of the cell originating the tumor or whether telomeres are uniformly short as happens in most non-lymphoid tumors. To address this issue, 112 MBCLD samples containing at least 80% tumor cells were evaluated for TL and histological origin in relationship to the GC. Our panel included 24 FL, 14 DLCL, 4 BL, 9 MZL, 8 MCL, 32 CLL and 21 MM. TL analysis was performed by Southern blot using a chemiluminescence-based assay. TL was calculated using the Kodak Digital Science TM 1D Image Analysis Software. GC-status has been evaluated by analyzing the V heavy chain (VH) gene sequence. Samples were classified as follows: a 2% deviation from any germ line IgH discriminated GC-experienced and GC-inexperienced tumors. GC-experienced tumors were then subdivided as GC-derived or post GC-derived according to presence or absence of the ongoing somatic mutation process assessed by cloning. In our patient sample, median TL was 6014 bp (range 1896-10304 bp). TL in tumor samples did not correlate with patient age, sex and treatment. TL according to Histology and GC-experience has been represented in figure 1. Our results indicate the following: 1) TL in MBCLD is highly heterogeneous and dependent on the histopathogenesis of the disorder; 2) GC-derived tumors are characterized by long telomeres, suggesting that no significant telomere erosion occurs during GC-derived lymphomagenesis; 3) tumors arising from GC-inexperienced (MCL and unmutated CLL) cells have very short nearly sub-critical telomeres and are thus ideal candidates for treatment with telomerase inhibitors such as BIBR1532 (Boehringer Ingelheim Pharma); 4) Finally since TL is related to GC status, it would be interesting to verify whether it might be used as a surrogate prognostic indicator in neoplasms such as CLL in which GC-status is regarded as an important predictor for outcome.



Caption 1:

607 THE SMALL OLIGOMERIZATION DOMAIN OF GEPHYRIN CONVERTS MLL TO AN ONCOGENE

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The MLL gene forms chimaeric fusions with a diverse set of partner genes as a consequence of chromosome translocations in leukaemia. In several fusion partners, a transcriptional activation domain appears to be essential for conferring leukaemogenic capacity on MLL. Other fusion partners, however, lack such domains. MLL-Gephyrin was cloned from AML with t(11;14)(q23;q24). Gephyrin (GPHN) is a neuronal receptor assembly protein which links membrane-associated receptor molecules to cytoskeletal microfilaments. Exon 14 of GPHN encodes a 15 amino acid domain with homology to the C-terminal repeat motifs of microtubule-associated proteins tau and MAP2. These motifs promote tubulin polymerization and self-oligomerization. We used MLL-GPHN to elucidate functional requirements of MLL fusion partners.

To study the transforming properties of MLL-GPHN and various mutants of this chimaeric gene, we retrovirally transduced primary murine haematopoietic progenitors and assessed their growth properties in vitro and in vivo.

We found that MLL-GPHN transforms haematopoietic progenitors, despite the lack of transcriptional activity. The transduction of MLL-GPHN into haematopoietic progenitor cells followed by transfer in vivo resulted in myeloid and lymphoid lineage leukaemias in mice, suggesting that MLL-GPHN can target multipotent progenitors. The small 15 amino-acid tubulin-binding domain of GPHN encoded by exon 14 was both necessary and sufficient for leukaemogenic activity in vitro and in vivo.

We demonstrated that this domain confers oligomerization capacity on MLL protein suggesting that such activity may contribute critically to leukaemogenesis.

These results and other recent data highlight acquisition of oligomerization encoding sequences as a likely important mechanism for the oncogenic conversion of the MLL gene by some of its leukaemia associated partner genes.

608 THE PEPTIDYL PROLYL CIS/TRANS ISOMERASE PIN1 INTERACTS WITH ETV6 AND CONVERTS ETV6 FROM A TRANSCRIPTIONAL REPRESSOR TO A TRANSCRIPTIONAL ACTIVATOR

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ETV6 (ETS variant gene 6, TEL) is a member of the ETS family of transcription factors containing an N-terminal pointed domain and a C-terminal ETS DNA binding domain. ETV6 is involved in many chromosomal translocations forming different fusion genes in both myeloid and lymphoid leukemias as well as in some solid tumors. There is also evidence that ETV6 is a tumor suppressor gene. ETV6 is known as a transcriptional repressor and interacts with several transcriptional corepressors (mSIN3, SMRT and N-CoR) as well as with the polycomb group gene L(3)MBT. In order to learn more about the normal function of ETV6, a yeast two-hybrid screen was performed with ETV6 as the bait. PIN1 was identified as one of the ETV6 interacting proteins. This interaction was confirmed by GST-Pull down and in a mammalian two hybrid experiment. PIN1 was cloned in a yeast two-hybrid screen as a NIMA interacting protein. PIN1 belongs to peptidyl-prolyl cis/trans isomerase family. It has an N-terminal WW domain which binds phosphorylated Ser/Thr-Pro in the substrate protein and a C-terminal PPlase (Peptidyl Prolyl Isomerase) domain, which isomerizes the phosphorylated Ser/Thr-Pro bonds. It is proposed that PIN1 specifically changes the conformation of its target proteins after they have been phosphorylated. PIN1 has an important role in cell cycle regulation by binding to many different substrates: Cyclin D1, NIMA, Plk1, Wee1, Myt1, NFAT, c-Jun, p53. PIN1 overexpression was found in Alzheimers disease and breast cancer.

Mapping of the ETV6 interacting domain of PIN1 in the yeast system showed that PIN1 and the PIN1 WW domain (aa5-40) can interact with full length ETV6 and with several ETV6 deletion mutants. In contrast, a deletion mutant of ETV6 (aa282-345) was able to interact with PIN1, the PIN1 WW domain (aa5-40) and the PIN1 PPlase (aa41-163) domain. Mapping of the PIN1 interaction domain of ETV6 identified two potential binding sites for PIN1: the pointed domain and the C-terminal half of the central domain of ETV6 (aa282-345). The interaction between PIN1 and ETV6 and the ETV6 pointed domain was also verified in a mammalian two-hybrid experiment. Transient expression of a yellow fluorescent protein (YFP) PIN1 fusion protein in NIH3T3 cells showed that PIN1 is localized in both cytoplasm and nucleus. Simultaneous expression of PIN1-YFP and CyanFP-ETV6 revealed colocalization of both proteins in the nucleus. In reporter gene assays in which ETV6 was expressed as a GAL4-DNA binding domain fusion protein (GAL4DBD-ETV6) coexpression of PIN1-YFP relieved the transcriptional repression exerted by GAL4DBD-ETV6 on a luciferase reporter construct with GAL4 binding site. Using a luciferase reporter construct based on the Stromelysin-1 promoter containing both ETV6 and other ETS protein binding sites, coexpression of CFP-ETV6 and PIN1-YFP resulted in a stronger activation than the expression of PIN1-YFP alone. These findings indicate that the interaction between ETV6 and PIN1 can change the function of ETV6 from a transcriptional repressor into a transcriptional activator and suggest that ETV6 might be regulated in a cell cycle dependent fashion.

STEM CELL TRANSPLANTATION II

609 REDUCED-INTENSITY ALLOGRAFTING FOR CHRONIC MYELOID LEUKAEMIA IN THE FIRST CHRONIC PHASE

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Background and methods. In chronic myeloid leukaemia (CML) in the first chronic phase (CP) the results of imatinib are impressive. However, several major questions remain. We would like to mention another treatment option for CML without detracting from the importance of imatinib in the treatment of CML. The present report summarizes our experience with the treatment of CML in the first CP using reduced-intensity allogeneic stem cell transplantation (reduced-intensity conditioning regimen, RIC). RIC consisted of intensive immunosuppression with intravenously administered fludarabine (30mg/m²/d on days -10 to -5), oral busulfan (4 mg/kg/d administered in 4 daily doses of 1 mg/kg on days -6 and -5), and antihuman T-lymphocyte globulin (ATG) at a dose of 10 mg/kg/d on days -4 to -1. Results. Between July 1998 and January 2004, we carried out transplants on 19 patients with CML in the first CP using this NST regimen. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine A in 15 patients. The remaining 4 patients received additional methotrexate (MTX). Peripheral blood stem cells (n=17) and bone marrow (n=2) from 1 human leukocyte antigen (HLA) fully matched unrelated donor and 18 HLA-identical siblings were used. The median age of our patients was 52 years (44-59). The median number of transplanted CD34⁺ cells and CFU-GM was 8.6 x 10⁶/kg (3.1-16.0), and 85.6 x 10⁴/kg (16.0-166.5), respectively. All patients engrafted. Only two patients experienced oral mucositis. Hepatic veno-occlusive disease was not observed. Mortality at day 100 was zero. Febrile neutropenia was noted in 6 patients. Acute GVHD occurred in 6 patients. Chronic GVHD occurred in 7 patients. On day +60, complete chimerism was achieved in 47% of the patients. After a follow up in 64 months, 4 patients had relapsed (2 hematologic relapses, 2 molecular relapses), and 1 patient had died of severe GVHD. Twelve patients are free of leukemia by quantitative reverse transcriptase polymerase chain reaction.

Conclusion. A high proportion of CML patients treated using RIC appear to be cured as defined by the absence of molecular evidence of disease. From our report it could be concluded that although the results with imatinib look promising, given the continuous improvement in the results of patients undergoing allogeneic transplantation, it is critical in the decision-making process to have extensive information on the patients even in the imatinib era. It could also be concluded, regarding conventional allogeneic transplantation, that our report would support the development of a prospective randomised trial comparing RIC and conventional allogeneic transplantation. RIC provide a safe and well-tolerated therapeutic option for patients with CML in the first CP with a matched donor.

610 CLINICAL OUTCOME AND PROGNOSTIC FACTORS OF HIGH-DOSE CHEMOTHERAPY (HDT) AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN PERIPHERAL T-CELL LYMPHOMA (PTCL)

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Background: PTCL are rare and heterogeneous groups of malignancies with a poor outcome after conventional chemotherapy. Although HDT with ASCT improved prognosis of patients with DLBCL, the role of these procedures in PTCL is still controversial.

Aim: To evaluate the impact of HDCT and ASCT in a prospective cohort of patients seen in a single institution.

Patients and Methods: Between 6/1991 and 9/2003, 48 consecutive patients (median age, 44 years, range 15-68) with PTCL (ALCL_{alk}=21; ALCL_{laik}=4; PTCLU=19; AILD=4) received HDT (n=48) and ASCT (n=32) as front-line (n=36) or salvage therapy (n=12). Sixteen patients did not proceed to autotransplantation because of progression/toxicity (n=14) or refusal (n=1). No harvest of PBPC occurred in one. The majority of patients had adverse features such as advanced stage (73%), B symptoms (71%), poor ECOG-PS (54%), elevated LDH (71%), extranodal site >1(60%), bone marrow involvement (25%), bulky disease (29%) and more than one IPI risk factor was present in 71%. Patients were separated into 3 cohorts. Cohort 1 (n=36) received as front-line HDT: 8 MACOP-B, 1-2 courses of MAD (mitoxantrone 8mg/sqm days 1-3; Ara-C 2g/sqm b.i.d days 1-3; dexametasone 4mg b.i.d. days 1-3). Cohort 2 (n=7) received as front-line treatment high-dose sequential chemotherapy (HDS): 1APO, cyclophosphamide 7g/sqm, methotrexate

8g/sqm (n=2) or Ara-C 2g/sqm b.i.d. for 6 days (n=17), followed by a rescue of peripheral blood progenitor cells (PBPC), and etoposide 2g/sqm. Cohort 3 was represented by 12 patients in first relapse after MACOP-B who received the same treatment as cohort 2. Autotransplant conditioning regimen consisted in BEAM in 29 and mitoxantrone (60mg/sqm) and melphalan (180mg/sqm) in the other 2 patients.

Results: All 31 transplanted patients engrafted. By intention to treat analysis the complete response rate was 75% in up-front and 42% in salvage group. After a median follow up of 23 months (range,1-124), the estimated probability of OS, EFS and DFS at 5-year was 56%, 41% and 65%. By univariate analysis 5-year EFS varied according to the cohorts (cohort 2, 89% vs cohort 1, 49% and cohort 3, 17%; P=0.001) and subtype (ALCL_{alk} 67% vs non-ALCL_{alk} 16%; P= 0.006). By Cox multivariate analysis only non-ALCL_{alk} and IPI risk factors>1 predicted a poor EFS. **Conclusions:** Our data confirm that HDT and ASCT were effective like front-line or as salvage treatment in patients with ALCL_{alk}, while the other subtypes of PTCL did significantly worse. In non ALCL_{alk} patients the high rate of failures would indicate that the best results after ASCT were obtained in those patients who received induction therapy with HDS. Novel approaches are required for PTCL patients with non ALCL_{alk} histology and IPI adverse prognostic features.

611 EFFECT OF AGE AND FAILED AUTOLOGOUS TRANSPLANT ON TREATMENT-RELATED MORTALITY AND GVHD IN 110 PATIENTS TREATED WITH REDUCE-INTENSITY CONDITIONING AND ALLOGRAFT FOR ADVANCED HEMATOLOGICAL MALIGNANCIES

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Background: High-dose chemoradiotherapy followed by allogeneic stem cell transplantation (SCT) is associated with a relevant incidence of regimen-related toxicities that increase with advancing age. Myeloablative SCT in heavily pretreated patients, especially after a failed autograft has a non-relapse mortality ranging between 40% to 70%, according to the literature. Recently new transplant strategies with reduced intensity conditioning (RIC) have been developed, making the allografting procedure feasible also in elderly and heavily pretreated patients. However until recently few studies have identified the risk factors that could influence the outcome of RIC transplants.

Aims: We have evaluated if age older than 55 years and a failed autologous transplantation could have an impact on transplant-related mortality (TRM) and graft versus host disease (GVHD) occurrence.

Methods: One hundred and ten allogeneic SCTs from HLA-identical sibling donors were analyzed: all patients received the same conditioning (Thiotepa 10mg/Kg, Fludarabine 60 mg/ms and Cyclophosphamide 60 mg/kg and) and GVHD prophylaxis (cyclosporin 2mg/kg and short course methotrexate). Patients were divided in two cohorts according to the age: 62 patients were younger and 48 older than 55 years (range 20-69). The two groups had similar pretransplant characteristics in terms of: sex, mismatch donor/recipient, previous autografting, disease status at transplant, type of disease (lymphoid vs myeloid), stem cell source and number of previous lines of chemotherapy (<2 vs ≥2).

Results: Median follow-up was 765 days for younger patients and 628 days for the elderly group (p=0.1). The incidence of acute and chronic GVHD was not significantly different in the two cohorts. At a median follow-up of 2 yrs, the 4-year TRM was 14% for both groups; OS was 52% for younger and 59% for older patients. On univariate and multivariate analysis, no pre-transplantation risk factors had statistical significant impact on TRM. Prior failed autografting did not influence the probability of developing GVHD. The occurrence of grade III-IV aGVHD was associated with a significantly worse TRM in both younger (p=0.004) and elderly patients (p=0.04) while extensive cGVHD was a predictor for worse TRM only in older patients (p=0.004). Finally, a strong association between favourable outcome and the onset of limited cGVHD was found in both cohorts. In fact, 4-year OS for younger patients with limited

cGVHD was 80% vs 33% for patients with extensive cGVHD ($p=0.37$), and 100% vs 33%, respectively for the elderly group ($p=0.004$).

Summary: In conclusion our results indicate that: 1) RIC can be safely applied to patients over 50 years; 2) a previously failed autograft is not associated with a higher TRM; 3) achieving limited cGVHD confers a significant survival advantage in both the cohorts.

612 IMATINIB INDUCES SUSTAINED MOLECULAR REMISSIONS IN POST-TRANSPLANT MOLECULAR RELAPSE OF PH+ ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ALL)

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Background: Detection of minimal residual disease (MRD) after stem cell transplantation (SCT) for Ph+ALL indicates impending hematologic relapse. In a multicenter phase II trial we evaluated the safety and efficacy of MRD-triggered post-transplant intervention with Imatinib. Patients: Twenty-seven patients (14 male, 13 female) aged 16-63 (med. 48) yrs. in whom Bcr-Abl transcripts were detected a median of 3.6 (range 0.5-18) mo. after allogeneic ($n=24$) or autologous ($n=3$) SCT received Imatinib at an initial dose of 400mg starting 4.4 (1-19) mo. after SCT. Eighteen patients had received Imatinib as first-line ($n=9$) or salvage therapy ($n=9$) prior to SCT. At study entry 11 pts. had evidence of acute or chronic GvHD.

Results: MRD-negativity (CRmol, defined as undetectable Bcr/Abl transcripts by both quantitative and nested RT-PCR) was achieved in 14/27 patients (52%) within a median of 1.5 (0.9-3.7) mo. After 15.6 (2-31) mo. follow-up, only 2 of these 14 patients (14%) have relapsed, both after Imatinib was discontinued. In contrast, 12 of 13 patients (92%) in whom Bcr-Abl transcripts were still detectable after 3 mo. of Imatinib relapsed. Median time to progression (TTP) in the CRmol group is 28.6 mo. compared with 3 mo. in patients who remained PCR-positive ($p<0.0001$). DFS and OS in the CRmol group were 3426% and 75 22% at 31 mo. compared to 7.77.4% and 23.113% at 13 mo. in MRD non-responders, respectively ($p<0.0001$ and $p=0.0002$). All complete molecular responses occurred at an Imatinib dose of 400mg, dose escalation to 600 mg/d ($n=4$) or 800 mg/d ($n=1$) did not result in conversion to PCR-negativity. Observed side effects were mild to moderate nausea, emesis, periorbital edema and elevated liver enzymes. No grade IV hematologic toxicity was observed. No worsening of pre-existing GvHD and no de novo GvHD occurred.

Conclusion: In patients with Ph+ALL who are MRD-positive after SCT, the molecular response to a 2 month trial of Imatinib discriminates between patients almost certain to relapse and those likely to experience sustained molecular remissions. Early assessment of bcr/abl transcripts by highly sensitive rPCR has profound therapeutic implications by identifying a subset of patients requiring treatment in addition to - or other than Imatinib. In good risk patients, the primary goal is defining the optimal duration of Imatinib therapy.

613 BUSULFAN SYSTEMIC EXPOSURE IN ADULTS OBTAINED AFTER ORAL VS. INTRAVENOUS ADMINISTRATION: DEMONSTRATION OF DOSING EQUIVALENCE BASED ON COMPARISON OF SYSTEMIC EXPOSURE (AUC) VALUES

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Background: Busulfan (Bu) and cyclophosphamide (Cy) (BuCy2) is a commonly used conditioning regimen for patients with hematological

malignancies undergoing stem cell transplantation. A major drawback to the use of oral Bu is unpredictable and erratic absorption, which results in wide inter- and inpatient variation in plasma levels, reported to likely increase the risk of hepatic venoocclusive disease (HVOD) from overdosing and the risk of relapse from underdosing.

Aim: Studies with intravenous (IV) Bu have demonstrated a high degree of inpatient (dose-to-dose) consistency in AUC and pharmacokinetics (PK).

Methods: In a study comparing 30 patients who received oral BuCy2 (Bu dose of 1.0 mg/kg every 6 hr for 16 doses) with 61 patients who received IV BuCy2 (Bu dose of 0.8 mg/kg over 2 hr every 6 hr for 16 doses), the incidence of HVOD (20% vs 5%), HVOD-related mortality (20% vs 3%) and overall 100-day mortality (33% vs 13%) was significantly higher in the oral BuCy2 group (Kashyap, et al. Biol Blood Marrow Transplant [BBMT]. 2002;8:493-500). Studies have suggested that IV Bu at a dose of 0.8 mg/kg does not provide equivalent systemic exposure (SE) as that provided by oral Bu at a dose of 1.0 mg/kg, thereby explaining the differences in outcomes (BBMT 2003;9:282); however, this suggestion has been contested (BBMT 2000;6:548, idem 2003;9:722).

Results: We report here dosing and PK (AUC) data on oral and IV Bu from the patient groups reported by Kashyap, et al. All of the IV BU patients ($n = 61$) received 16 doses as prescribed, and valid dose-1 PK information was obtained from all of the patients. There was 1 complication with infusion at dose 9 (IV pump malfunction). Twenty-six (43%) patients had emesis with IV Bu, but this did not affect their prescribed dosing, require re-dosing, or affect their PK data. Among the oral BU patients ($n = 30$), 24 (80%) patients received 16 doses of oral Bu, and 1-dose PK values of 27 (90%) patients were evaluated. Emesis was reported in 11 (37%) oral BU patients; therefore, PK values of 3 of 11 patients were not evaluated. One patient refused dose 16. The oral and IV BuCy2 patients were compared with respect to first dose AUC. The mean dose 1 AUC of oral Bu ($n = 27$) was 1102 411 Mol.min; the dose 1 AUC of IV Bu was 1106 318 Mol.min ($P = NS$). AUC at steady state for IV Bu ($n = 59$) was 1167 228 Mol.min, demonstrating reliable and predictable dosing and SE. There was no consistent follow-up PK at steady state for patients administered oral Bu; 6 patients had PK and 2 of the 6 patients were not evaluated due to emesis with dosing and/or redosing.

Summary/Conclusion: These findings demonstrate that the reduction in HVOD and mortality observed in patients receiving IV BuCy2 as compared with those receiving oral BuCy2 cannot be explained by differences in the dose of Bu since systemic exposure was equivalent.

ACUTE LYMPHOBLASTIC LEUKEMIA

614 INDIVIDUAL OUTCOME OF ADULT PATIENTS WITH COMMONLY DEFINED STANDARD RISK B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA CAN BE PREDICTED BY GENE EXPRESSION PROFILING.

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In adult patients with B-precursor acute lymphoblastic leukemia (ALL) the standard risk group is defined as having no common risk factors such as peripheral white blood cell count $>30,000 /L$, aberrant cytogenetics ($t(4;11)$ or $t(9;22)$), pro B-ALL or time to first complete response (CR) more than 4 weeks. Unfortunately, at present no prognostic factors are available to predict relapse risk in these patients, which is 50 % in adult standard B-precursor ALL. In our patients cohort, after identical treatment according to the German multicenter protocol for adult patients with ALL (GMALL 06/99), 55 % of standard risk patients have achieved a continuous CR (CCR, median observation period: 30 months) whereas 45 % of CR patients have a relapse (REL) after a median time of 11 months. To assign patients at the time of initial diagnosis to either the CCR or the REL group, we have performed gene expression profiling (HG-U133A, Affymetrix, Santa Clara, CA) of bone marrow blasts from 90 patients with standard risk B-precursor ALL at the first time of diagnosis. Data from 66 evaluable patients were analyzed using the GeneSpring software version 4.2 (Silicon Genetics, San Carlos, CA). For class membership prediction we have utilized data from 11 CCR and 9 REL patients as a training-set. Forty-eight genes were selected to be highly predictive for the CCR group. The accuracy of prediction within the training-set was 95 % (one mis-classification). Using the predictive 48 genes for classification of the bone marrow samples with regard to the individual disease risk,

by supervised hierarchical clustering of all of the data sets we were able to create two main experiment clusters corresponding to the CCR or REL group, respectively. There were 5 mis-classifications of samples (4 samples from the REL group were clustered within the CCR group, one CCR sample was clustered within the REL group). Eight samples formed a separate cluster different from the main clusters whereas 3 samples were not to be classified by this method. To demonstrate the specificity of the predictive genes, in addition single sample prediction was performed using expression of the 48 predictive genes. In the CCR group 31 out of 34 samples could be predicted correctly (specificity 100 %), the remaining 3 samples were not predicted (corresponding to a sensitivity of 91 %). Within the REL group the power of predictive genes was less resulting in the correct prediction of 15 out of 32 REL patients (specificity of 55 %, sensitivity of 47 %). In conclusion, this is the first gene expression study showing that it is possible at the first time of diagnosis to discover a specific genetic signature associated with CCR patients with 'standard-risk' B-precursor ALL. This genetic signature can be used to predict the clinical outcome in single patients with high accuracy.

615 EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ON THE OUTCOME OF BURKITT'S LYMPHOMA AND LEUKEMIA IN ADULT HIV INFECTED PATIENTS TREATED IN THE PETHEMA ALL3/97 PROTOCOL

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Background: From 1997 to 2001, 18 HIV infected patients with Burkitt's lymphoma (BL) and mature B-cell acute lymphoblastic leukemia (ALL3) were included prospectively in a specific Burkitt protocol (PETHEMA ALL3/97). While age and leukemic disease were identified as adverse prognostic factors, HIV status was not.

Aims: Patients were analyzed with an extended follow-up to: a) confirm that HIV positive patients maintained a prognosis comparable to non-HIV infected patients and b) to assess the impact of HAART treatment on outcome.

Methods: Treatment protocol pre-phase with cyclophosphamide (CPM) and prednisone (PDN), followed by induction cycle A (iphosphamide, VCR, dexamethasone -DXM-, high-dose MTX, ARA-C and VM-26). Five alternating consolidation cycles every 3 weeks, 3 cycles B (VCR, HD-MTX, CPM, DXM and doxorubicin), and 2 cycles A (approximate total duration of therapy 18 weeks). CNS intrathecal prophylaxis consisted of MTX+ARA-C+DXM given in each cycle for a total of 6 doses.

Results: Nineteen HIV positive patients, 16 males, median age 41 (range 23-65) were treated. Nine of them were never treated with HAART, 5 were under HAART treatment before diagnosis and 5 started HAART at the time of diagnosis. Median follow-up was 30 months (range 8-73). Twelve patients (63%) achieved CR (7 of them HAART responders), 46% (95%CI 25-68) were alive at 3 years and 70% (95%CI 43-96) of patients achieving CR remained alive and in first CR at 3 years. CR rates, OS and LFS were not significantly different from those of the 40 HIV negative patients of the same age range treated in the same protocol. Seven of 17 evaluable patients achieved immunological and virological response to HAART. In this subgroup the CR rate was 86%, all patients achieving CR remain alive after a median follow up of 42 months and 86% (95%CI 62-99) remain alive at 3 years versus 27% (95%CI 0-53) in the group of HAART non-responders (p=0.04).

Conclusions: The PETHEMA ALL3/97 protocol in HIV positive patients yield results comparable to those obtained in immunocompetent patients. Response to HAART treatment may emerge as a significant prognostic factor in HIV positive individuals with Burkitt's lymphoma or leukemia.

Supported in part with Grant P-EF-03 from Jose Carreras Leukemia Foundation.

616 MONITORING OF MRD LEVELS UNRAVELS A GREAT HETEROGENEITY WITHIN T(12;21)-POSITIVE CHILDHOOD ALL PATIENTS

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Background: the TEL/AML1 fusion, resulting from the t(12;21) translocation, is the most common gene rearrangement in childhood ALL, occurring in about 25% of cases. Conflicting results have been reported on its association with a favourable prognosis and its incidence in relapsing ALL patients. In the AIEOP ALL 95, the EFS at 5 and 8 years confirms TEL-AML as an independent prognostic factor. However, we also observed the occurrence of early (4.0%) and late relapses (10.0%) within TEL-AML1 positive patients. Moreover, in a prospective cohort of 184 relapsing patients, we observed a 15.2% incidence of TEL-AML1 positive cases (23.5% of late and 8.1% of early relapses). Aim: to investigate the heterogeneity within t(12;21) positive childhood ALL patients as assessed by Immunoglobulin and T-cell Receptor minimal residual disease (MRD) analyses. Methods: Ig/TcR gene rearrangements and t(12;21) were analyzed in 847 patients eligible and evaluable to the ongoing AIEOP-BFM ALL2000 clinical protocol, which consider the MRD assessment at day 33 and day 78 as a main feature for risk stratification. In total, 641 patients non-T-ALL and negative for t(4;11) and t(9;22) were successfully analyzed. Results: Within the ongoing protocol, 52.2% of TEL/AML1 were stratified into the MRD-Standard Risk (SR) group, and 45.2% into the MRD-Intermediate Risk (IR) group; interestingly, 3 cases (2.6%) were stratified into the MRD-High Risk (HR) group. In other words, 30.6% of MRD-SR, 20.6% of MRD-IR, and 3/34 (8.8%) of MRD-HR patients were t(12;21) positive. Considering the final stratification (based on MRD and clinical features), 6/75 (8.0%) of final HR patients were t(12;21) positive; while 32.3% of SR and 23.9% of IR were positive. Within MRD-IR patients without clinical HR features, 51 cases were TEL/AML1 positive, with different MRD levels at TP1 and TP2. Briefly, 51.0% of them were MRD-positive <10⁻⁴ at TP1 and negative at TP2, and 14 (27.4%) were positive ≥10⁻⁴ at TP1. Conclusion: a remarkable heterogeneity was observed within t(12;21) patients, in term of response to the frontline treatment protocol; in particular, a proportion of HR patients can be TEL/AML1 positive. As a consequence, caution should be taken in the efforts to reduce treatment for t(12;21) positive patients, at least within BFM-based ALL protocols.

617 THE BCR/ABL ISOFORMS AFFECT THE PRESENTING FEATURES AND TREATMENT OUTCOME OF PATIENTS WITH PH-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA: SEVEN-YEAR UPDATE OF THE GIMEMA 0496 TRIAL..

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Acute lymphoblastic leukemia (ALL) with BCR/ABL fusion gene represents 20-30% of adult ALL. This is a leukemic subtype usually considered having an unfavorable prognosis. However, patient with Ph-positive ALL may present heterogeneous as for clinico-biological features and response rate to aggressive chemotherapies. In particular, due to the different localizations of the cluster region through the BCR gene and the different splicing sites, the majority of cases translate the 190-kd protein, while a sizeable portion of them have the 210-kd fusion protein either alone or associated with the 190-kd protein. To evaluate whether these genetic differences may result in distinct clinico-biological features and treatment outcome, we analyzed 101 consecutive adult patients with Ph-positive acute lymphoblastic leukemia (ALL) enrolled in the Italian GIMEMA 0496 trial from October 1996 to July 2000. At

diagnosis, patient mean age and WBC count were 41,7 years (range: 15-60) and $29.3 \times 10^9/L$ (range: 0.8-371), respectively. CD10 antigen was expressed in 91/98 cases (93%) while CD34 was detected in 91/96 cases (95%). Co-expression of CD13 and/or CD33 myeloid antigens was demonstrated in 42/94 cases (45%). A p190 BCR/ABL-positive ALL was demonstrated in 59 cases (58.5%), while a p210 w/wt p190 BCR/ABL positive ALL was demonstrated in 42/101 (41.5%). The comparison analysis between clinico-biological features and BCR/ABL isoforms showed that WBC count, CD33 and CD34 antigens were significantly associated with type of transcripts. In fact, WBC count $> 16 \times 10^9/L$, CD34 and CD33 expression were 73% vs 54% ($p = .05$), 87% vs 100% ($p = .009$) and 23% vs 43% ($p = .03$) in the p210 w/wt p190 and p190 groups, respectively. Therapeutic response was evaluated in 92 patients. After an induction treatment which includes PDN, VCR, AASE and high dose DNR, CR was achieved in 62/92 (67.4%) patients, while 16/92 (17.4%) were resistant and 14/92 (15.2%) died for therapy related complications. Therapeutic responses to induction treatment were similar in the two BCR/ABL isoform groups. Fifty-nine patients (54 CR and 5 res.) underwent to intensive re-induction treatment with high dose Ara-C and Mitoxantrone with or without Fludarabina. Finally a BMT consolidation was performed in 36 persistently complete responder patients (allogeneic BMT 20 pts.; autologous BMT 16 pts.). At seven years the cumulative OS and DFS survival rates were 23% and 20%, respectively. The univariate analysis showed that the p190 isoform resulted as the only prognostic factor significantly affecting OS and DFS survival rates (25% vs 3% [$p = .008$] and 27% vs 0% [$p = .02$], respectively). In conclusion, these results confirmed that, after intensive chemotherapy programs with hematopoietic stem cells transplantations, the prognosis for BCR/ABL positive ALL remains very poor. However, for the first time, we showed that, respect to p190-positive cases, p210-positive ALLs, have a significantly worse prognosis with no patients surviving without disease at 2,5 years from diagnosis.

618 INTEGRATED ANALYSIS OF GENE EXPRESSION AND SINGLE NUCLEOTIDE POLYMORPHISMS: IDENTIFICATION OF A DISTINCTIVE GENE EXPRESSION SIGNATURE ASSOCIATED WITH LOH ON CHROMOSOME 9P IN ADULT ALL

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Background:

In acute lymphocytic leukemia (ALL), previous studies have demonstrated that specific genetic rearrangements are associated with distinctive gene expression signatures defined by high-density oligonucleotide microarrays. Characteristic expression signatures associated with ALL1/AF4, E2A/PBX1, BCR/ABL and TEL/AML1 rearrangements have provided insights into the mechanisms of malignant transformation and are also likely to improve the identification and classification of leukemia cells with these abnormalities. Nonetheless, many cases of ALL do not carry known molecular abnormalities and do not exhibit unique gene expression profiles. In these cases, the mechanisms of malignant transformation remain unknown.

Aims

Our goal was to develop an integrated genetic approach to discover new mechanisms of malignant transformation in ALL and to define the gene expression signatures associated with these genetic abnormalities.

Methods

Gene expression profiles were determined in leukemia cells from 95 adult patients with pre-B ALL using Affymetrix U95Av2 GeneChips. Within this group, high density SNP analysis was performed on 16 samples of normal hematopoietic cells and tumor cells from the same patients using Affymetrix GeneChip Mapping 10K Arrays. Patients were enrolled in the Italian protocol GIMEMA 0496.

Results

Paired SNP analysis of normal and ALL DNA revealed the presence of LOH in a portion of chromosome 9p (9p13.3 to 9p24.3) in 4 of 16 cases (25%) (Figure 1). This deletion was not detected using conventional cytogenetic methods. This region includes CDKN2A and CDKN2B, MTAP and IFN genes. Analysis of gene expression in these cases revealed increased expression of a small set of 9 genes and reduced expression of a larger set of 60 genes. Using this gene set we examined a larger series of 40 adult ALL cases without previously defined

molecular rearrangements. This analysis identified 8 cases (20%) with a similar pattern of gene expression. These results were confirmed using a multidimensional scaling approach. These 69 genes associated with 9p deletion were subsequently included in the analysis of all 95 cases of adult pre-B ALL. Clustering of all samples based on expression of this expanded gene set confirmed that 9p deletion was associated with a characteristic signature that was distinct from the expression profiles associated with other molecular rearrangements. Furthermore, we applied this set of genes to microarray data from pediatric cohorts and identified 2% of cases with a similar gene expression signature.

Summary

These results demonstrate that integrated analysis of gene expression combined with SNP-based comparison of leukemia cells and normal cells in the same individuals can identify previously unknown groups of tumors with distinct genetic profiles. This event appears to be more frequent in adults than in children (20% vs 2%). Further studies can now be undertaken to better define the genetic mechanisms of malignant transformation in these leukemias.

AML: MOLECULAR BIOLOGY

619 ACUTE MYELOID LEUKEMIA WITH DELETION 9Q IS ASSOCIATED WITH CEBPA LOSS-OF-FUNCTION MUTATIONS

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Background: The transcription factor CCAAT/enhancer binding protein $\square\square$ (C/EBP α) plays an important role in the development of mature neutrophils. Two common types of mutations in the CEBPA gene encoding C/EBP α have been identified in approximately 10% of adults with acute myeloid leukemia (AML): N-terminal, dominant-negative frameshift mutations that result in loss of C/EBP α function and C-terminal in-frame mutations that result in C/EBP α proteins with decreased DNA-binding potential. Previous studies concluded that mutant CEBPA predicts favorable outcome in younger AML patients with intermediate-risk karyotypes or normal cytogenetics. Aims and methods: To assess the prevalence of CEBPA mutations in specific cytogenetic subgroups, mutational analysis of the CEBPA gene was performed in 125 acute myeloid leukemia (AML) patients. Results: No CEBPA mutations were detected in 77 patients with t(8;21), inv(16)/t(16;16), t(15;17), or balanced translocations with breakpoints in band 11q23. In 58 patients with various noncomplex karyotypic abnormalities, CEBPA mutations were present in 8 (14%). Surprisingly, 5 of the 8 patients with del(9q) as the sole aberration or in combination with a single additional abnormality other than t(8;21) had CEBPA mutations associated with loss of C/EBP α function. Consequently, 41 additional del(9q) cases were analyzed; 9 had CEBPA loss-of-function mutations. The overall prevalence of CEBPA loss-of-function mutations in cases with del(9q) within a noncomplex karyotype was 41% (14 of 34 patients), whereas none of the patients who had a del(9q) within a complex karyotype ($n = 7$), in combination with a t(8;21) ($n = 10$), or together with a t(15;17) ($n = 1$) demonstrated mutant CEBPA. Analysis of associated mutations indicated that alterations of the FLT3, MLL, and NRAS genes are not common cooperating events in the pathogenesis of del(9q) AML with inactivating CEBPA mutations. Summary and conclusions: This is the first study to show that AML with del(9q) is strongly associated with CEBPA loss-of-function mutations. The coincidence of del(9q) with inactivating CEBPA mutations and the fact that del(9q) is a common secondary cytogenetic abnormality in t(8;21)-positive AML, that is characterized by specific downregulation of CEBPA, raise the possibility that loss of a critical segment of 9q, most likely in 9q22, and disruption of C/EBP α function cooperate in the pathogenesis of these leukemias. Further refinement of the commonly deleted segment of 9q using high-resolution techniques is necessary to identify the critical gene(s) involved and their role in normal hematopoiesis and leukemogenesis. A collaborative intergroup study has been initiated to define whether the relatively good prognosis associated with del(9q) is related to the presence of a CEBPA mutation.

620 ONCOGENIC ROLE OF THE P110 -Delta-ISOFORM OF PI3K IN PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA

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AMLs constitute an heterogeneous group of hematopoietic stem cell disorders of rather bad prognosis. Abnormal activation of signaling pathways including STAT, MAPK and NF- κ B have been described in AMLs and could constitute new targets for therapy. In this study, we focused on the PI3K/AKT and mTOR/P70S6K activity in primary blast cells purified from the bone marrow of patients with primary (n = 51) or secondary (n = 10) AMLs. Patients with de novo AMLs were issued from the AML2001 French Multicenter Trial directed by the GOELAMS. The FAB classification was the following : M0 (n = 6), M1 (n = 10) M2 (n = 21), M4 (n = 10), M4eo (n = 2), M5 (n = 10), unknown AML (n = 2). In 57% of these patients, a constitutive activation of PI3K/AKT was detected in blast cells by western blot analysis showing AKT and FOXO3a phosphorylation, and confirmed by immunofluorescence confocal microscopy. In 93% of PI3K+ patients, ERK1/2 proteins were also activated and most of these patients had constitutive GAB1/2 phosphorylation. However, we did not find any correlation between PI3K activation and FLT3 gene mutations. Moreover, PTEN and SHIP-1 expression was normal in all tested PI3K+ patients. Spontaneous proliferation of PI3K+ samples was more important than that of PI3K- (p=0.001) and LY294002 inhibited the proliferation and induced dose-dependent apoptosis of blast cells. The mTOR/P70S6K pathway was also activated downstream of PI3K and the mTOR inhibitor rapamycin inhibited proliferation. We determined the expression of the α , β and δ isoforms of the catalytic sub-unit of PI3K (p110) in leukemic cells and found that p110 δ was the only constantly expressed isoform. In 5 PI3K+ patients, IC87114 (Icos corporation), a specific p110 δ inhibitor compound used at 10M totally suppressed AKT and FOXO3a phosphorylation. IC87114 inhibited proliferation of leukemic cells whereas it did not induced apoptosis. Interestingly, this molecule did not inhibit the proliferation of CD34+ cord blood cells used as controls. Overall, our results report, a/ a constitutive activation of PI3K in 57% of AML, b/ a coactivation of the ERK1/2 pathway in 93% of PI3K+ patients, c/ no correlation between FLT3 gene mutations and PI3K positivity, d/ no implication of PTEN or SHIP-1 phosphatases in PI3K activation, e/ a major role of the p110 δ isoform of PI3K in leukemic cell proliferation, f/ a potential therapeutic value of rapamycin and IC87114 in future clinical trials.

621 GENE EXPRESSION PROFILING BASED IDENTIFICATION OF NEW SUBCLASSES IN ADULT ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) encompasses a large number of morphologically similar, but at the molecular level quite distinct variants. Recurrent cytogenetic aberrations have been shown to constitute markers of diagnostic and prognostic value. However, the current classification system for AML does not fully reflect the biological and clinical heterogeneity of the disease and treatment stratification is still difficult, especially for patients with a normal karyotype.

Aims: Today gene expression profiling based on DNA microarray technology allows us to measure mRNA levels across thousands of genes and helps us to capture the molecular variation underlying the biological and clinical heterogeneity of hematologic malignancies. Therefore, we analyzed a large AML sample set using cDNA microarrays to further characterize AML at the molecular level.

Methods: We profiled gene expression in a large series (n=116) of clinically- and cytogenetically-annotated samples of adult AML patients using cDNA microarrays representing approximately 26,000 different human genes. Both supervised and unsupervised methods were used to analyze the gene expression data, identifying biologically and clinically significant patterns of gene expression, as well as candidate target genes involved in leukemogenesis.

Results: By unsupervised hierarchical clustering, we observed that while samples with t(15;17) clustered together, samples with t(8;21) and inv(16) each separated primarily into two different subgroups.

Since the primary translocation events themselves are not sufficient for leukemogenesis, distinct patterns of gene expression found within each of these cytogenetic groups may suggest alternative cooperating mutations and deregulated pathways leading to transformation. For example, one t(8;21) subgroup displayed high expression levels of SKI and IL6ST, which encode proteins involved in TGFB1 and JAK-STAT/ RAS-ERK signaling pathways, respectively.

Unsupervised hierarchical clustering and principal component analysis also stratified most samples with normal karyotype into one of two different subgroups based on distinct patterns of gene expression. These normal karyotype-subgroups were characterized by distinct biology [FLT3 aberrations were more prevalent in one group (P=0.005, chi-square test), and FAB M4/M5 morphologic subtypes were more highly represented in the other group (P=0.013, chi-square test)] and clinical behavior [statistically significant difference in overall survival (P=0.009, log rank test)]. In addition, genes differentially expressed between these subgroups provide insight into distinct pathways for the molecular pathogenesis of AML with normal karyotype. For example, the poor outcome subgroup was characterized by high-level expression of GATA2, DNMT3A, and DNMT3B. The transcriptional regulator GATA2 is required for NOTCH1 signaling-induced inhibition of hematopoietic differentiation, and high-level expression of DNMT3A and DNMT3B suggests a potential role for aberrant methylation patterns in the pathogenesis of this subtype.

Summary/Conclusion: Our findings provide numerous biologic insights into the pathogenesis of AML and support the utility of gene expression profiling for improved molecular classification and disease management in adult AML.

622 INSERTIONS GENERATING THE 5AML1/3ETO GENE IN ACUTE MYELOID LEUKEMIA CASES SHOW VARIABLE BREAKPOINTS

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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 AML1 and ETO genes generating a 5AML1/3ETO transcriptionally active fusion gene on derivative chromosome 8 but some cases with ins(21;8) and ins(8;21) have been observed. However, a detailed breakpoints characterization of the insertion events has never been reported in literature.

Aims. We describe 6 among 82 (7.3%) AML cases characterized by an insertion event responsible for the AML1/ETO fusion. Using FISH experiments with appropriate Bacterial Artificial Chromosome (BAC) and P1 Artificial Chromosome (PAC) probes we were able to perform a detailed molecular cytogenetic characterization of 1 case with ins(8;21) and 5 with ins(21;8).

Methods. The 6 cases were individuated during screening of 82 AML patients bearing the AML1/ETO rearrangement, detected by RT-PCR. Bone marrow samples were studied by conventional cytogenetic analysis after 24 or 48 hours culture. Selected BAC and PAC clones were used to disclose rearrangements involving the ETO and AML1 genes. In detail, the identification of the ETO locus was obtained by using a mixture of BACs RP11-11808 and RP11-777J24, completely encompassing the gene; AML1 gene was detected by PAC RP5-1107L6.

Results. Five cases showed a functional fusion gene on the der(21) instead of the der(8) chromosome as a consequence of ins(21;8). The insertion size was established in all cases and turned out to be very heterogeneous, ranging from a minimum of 2.4 Mb to a maximum of 44 Mb. In one case conventional cytogenetics revealed a t(8;21)(q22;q22) instead of ins(21;8)(q22;q11q22) as a large chromosome 8 region was inserted on der(21). Sequences encompassing the breakpoints on chromosome 8 were compared each other and with the region including AML1 gene on chromosome 21, using the Genalyzer software. No significant similarity was observed.

Conclusions. Overall analysis of the 18 AML cases bearing ins(21;8) or ins(8;21) (12 reported in literature and the 6 analyzed in the present study) suggests that (i) this kind of rearrangement seems not to be associated with a subset of patients with common features in terms of prognosis; (ii) the insertions are not linked to the presence of additional

cytogenetic rearrangements; (iii) the crucial role that 5AML1/3ETO fusion gene plays in leukemogenesis does not appear to depend on breakpoint location and insertion size.

623 CYTOTOXIC POTENTIAL AND ANTI-LEUKEMIC EFFECT OF NATURAL KILLER CELLS FROM AML PATIENTS AGAINST AUTOLOGOUS LEUKEMIC BLASTS IN VITRO AND IN VIVO AFTER ADOPTIVE TRANSFER TO NOD/SCID MICE

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Natural killer (NK) cells participate in the graft-versus-leukemia effect following transplantation of allogeneic hematopoietic stem cells. The goal of this work is to evaluate the ability of NK cells to recognize and kill autologous leukemia cells and to exploit their therapeutic potential in immunotherapy.

We characterized phenotype and function of NK cells from patients with newly diagnosed or relapsed acute myeloid leukemia (AML; n=18). The content of CD56+CD3- NK cells in AML was significantly reduced as compared to healthy donors (2.30.5% vs 7.51.5%). We generated NK cell lines by repeated stimulation of CD56+CD3- cells in the presence of cytokines enhancing growth and cytotoxicity of NK cells. 1800600 fold expansion of AML-NK cells over 2 weeks was achieved. Upon culturing with IL-2, IL-15 or IL-21, surface expression of the activatory receptor NKG2D was increased (2.7 fold; MFI ratio), while the natural cytotoxicity receptor (NCR) NKP46, CD94 and the inhibitory receptors CD158a/b and NKB1 remained at initial levels. AML-NK cells were highly cytolytic against the HLA-class I-deficient human cell line K562, as demonstrated in vitro by 51Cr-release assay. The cytolytic activity of AML-NK cells against autologous blasts in vitro was in general below 10%, but HLA class I-blocking enhanced the killing responses to 70%. To examine the potential of AML-NK cells to recognize and lyse autologous blasts in vivo, we inoculated NOD/SCID mice with primary human AML. 8 to 10 weeks after transplantation, blast content was measured by bone marrow aspiration. Engraftment varied between 3 to 61% (human cells per total BM) and mice received a single i.v. dose of 3-5x10⁶ of autologous AML-NK cells in combination with IL-2 and IL-15. Mice were sacrificed and analysed 1 week after NK cell infusion. In untreated controls (n=4) the content of human leukemic blasts increased 1.5-7 fold (up to 55%). In contrast, in all mice treated with autologous AML-NK cells (n=8), a 2-12 fold reduction of tumor load to low levels (1.5 13%) was found. This effect may be NKG2D mediated since AML blasts recovered from NOD/SCID mice expressed increased levels of ULBP1, the ligand for the NKG2D receptor, and ULBP1 upregulation upon growth factor treatment in cultured AML blasts correlated with a higher in vitro killing by autologous NK cells. We conclude that AML-NK cells which were expanded and activated in vitro in the presence of cytokines are capable of recognizing and killing autologous AML blasts upon adoptive transfer to NOD/SCID mice. Our results suggest that cellular immunotherapy with autologous NK cells may be effective against leukemia relapse in AML patients who are not eligible for allogeneic stem cell transplantation.

624 ACUTE MYELOID LEUKEMIA: GROSS GENETIC INSTABILITY IS ASSOCIATED WITH CRITICAL TELOMERE SHORTENING AND REACTIVATION OF TELOMERASE

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Telomeres, the ends of linear chromosomes, have a critical role in protection against chromosome end-to-end fusion. Telomeres shorten in every cell division due to the end replication problem. Telomerase is a reverse transcriptase that adds telomeric DNA repeats to the ultimate chromosome end. In vitro models of long-term fibroblast cultures have identified two sequential mortality stages, senescence (M1) and crisis (M2). Senescence can be bypassed by loss of p53 or Rb function, whereas escape from crisis can only be achieved by activating a telomere maintenance mechanism, mostly telomerase reactivation. Cells that bypass senescence (M1) did not reactivate telomerase, resulting in further telomere shortening to a critical telomere length upon reaching crisis (M2). In these models, critical telomere shortening induces extensive genetic instability, most likely via chromosome end-to-end fusions. Dicentric chromosomes lead to anaphase breakage-fusion-bridges resulting in multiple chromosomal aberrations. To investigate whether similar mechanisms may be involved in the development of genetic instability in human cancer, we studied telomere length and telomerase activity level in acute myeloid leukemia (AML) patients. AML is a good model

for these studies since distinct subgroups of AML are characterized by either normal cytogenetics, a single translocation or inversion, e.g. t(8;21) and inv(16), or by a complex karyotype with multiple chromosomal aberrations. The groups were age-matched. Telomere length was studied in metaphase arrested leukemic cells using quantitative fluorescence in situ hybridization (QFISH) using a telomere-specific probe. Subsequently, metaphase spreads were hybridized with a leukemia-specific probe to confirm leukemic origin of each metaphase. Telomeres are significantly shorter in AML samples with a complex karyotype compared to AML samples with normal cytogenetics or a reciprocal translocation or inversion (meanSEM=161.7 AFU, n=12 versus 294.3 AFU, n=17; p=0.015). Interestingly, telomerase activity level is significantly higher in AML samples with a complex karyotype, compared to AML samples with a reciprocal translocation or inversion (meanSEM=33095, n=11 versus 7021, n=13; p=0.02). The lower telomerase activity in AML cells with a translocation or inversion is comparable with telomerase activity level in normal bone marrow. Our observations suggest that, consistent with previous in vitro models in fibroblasts, critical telomere shortening may have a role in the development of genetic instability in human AML. Critically short telomeres in association with high levels of telomerase activity suggest that AML cells with a complex karyotype have bypassed crisis (M2). The longer telomeres and low levels of telomerase activity in AML cells with a reciprocal translocation or inversion suggest that they originate from an earlier stage, preceding crisis. Consequently, telomere length modulation may have a role in cancer prevention.

TRANSFUSION MEDICINE & IMMUNOHEMATOLOGY

625 EARLY DETECTION AND RAPID ISOLATION OF ACUTE LYMPHOBLASTIC LEUKEMIA-REACTIVE DONOR T CELLS FOR ADOPTIVE TRANSFER USING THE INTERFERON-GAMMA SECRETION ASSAY

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Treatment of patients with relapsed acute lymphoblastic leukemia (ALL) after allo-SCT with infusions of donor lymphocytes (DLI) is unsuccessful, probably due to limited antigen presenting (APC) function of ALL blasts. Using a serumfree medium we were able to culture primary ALL blasts in-vitro. Due to their lack of expression of costimulatory molecules these ALL cell lines are no appropriate stimulator cells for the induction of specific T cell responses. Therefore, we modified these ALL cells into more professional APCs by culturing the cells in the presence of CpG and IL-4 and subsequent CD40 crosslinking, resulting in upregulation of CD40, CD80, and CD86, as well as adhesion molecules. These ALL-APCs were used as stimulator cells to generate specific T cell lines. As responder cells CD14 depleted PBMC from HLA-matched donors were used. We explored the possibilities of early detection and rapid isolation of leukemia-reactive T cells based on IFN-gamma secretion. In pilot studies we observed that non-synchronized production of IFN-gamma by the T cells at days 1-3 of primary anti-ALL immune responses hampered the isolation of large numbers of leukemia-reactive T cells in the induction phase of the response. Therefore, we explored the possibilities to synchronize the IFN-gamma production. At days 7 and 11 following initial stimulation, low dose (10IU/mL) IL-2 was added to the culture, which was sufficient for survival, but did not induce excessive proliferation. At day 14 when the T cells had re-entered a quiescent state, the cells were specifically restimulated with ALL-APCs. At 16 hours after restimulation the IFN-gamma producing T cells were stained using the IFN-gamma secretion assay. Within the fixed 45 minutes of the IFN-gamma secretion assay 1-7% of the cells secreted IFN-gamma as compared to only 0.1-0.5% in the induction phase of the responses, and <0.1% in the responder control cultured with 10IU/mL IL-2. The IFN-gamma producing T cells from multiple patient-donor combinations were isolated by FACS sorting or MACS isolation. Using a newly developed flowcytometry-based cytotoxicity assay in which the unmodified ALL cells were labeled with CFSE we observed that exposure to the IFN-gamma positive selected T cell fractions added at low effector/target (E/T) ratios (3/1-0.1/1) resulted in survival of only 0-5% (n=6) of unmodified ALL cells, whereas similar exposure to the negative T cell fractions resulted in 30-95% (n=6) survival of the ALL cells. All IFN-gamma positive T cells expressed the activation markers CD25 and HLA-DR. If we isolated the leukemia-reactive T cells based on their expression of the activation

marker CD25, all T cells capable of killing the leukemia were in the CD25 positive fraction. However, higher E/T ratios were needed (>3/1) to achieve lysis of the total leukemic cell population, suggesting that the frequency of leukemia-reactive T cells within the IFN-gamma isolated fraction was much higher compared to the fraction isolated on basis of CD25 expression. In conclusion, the IFN-gamma secretion method was found to be a feasible method for the early detection and rapid isolation of ALL-reactive T cells capable of specific recognition and elimination of the leukemic cells.

626 REPRODUCIBLE, CLINICAL GRADE GENERATION OF HLA-MATCHED DONOR DERIVED CYTOTOXIC T CELLS AGAINST AML AND ALL BLASTS

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Background: During the past years several in vitro-protocols have been described aimed to manipulate donor T cells in order to improve graft versus leukemia (GvL) while diminishing graft versus host disease (GvHD). However only few adoptive immunotherapy trials have been reported so far, due to the complexity of such approaches.

Aims: We evaluated the possibility of generating, expanding and characterising donor derived cytotoxic T cells (CTL) directed toward different types of AML and ALL leukaemia using a rapid, highly reproducible and efficient clinical grade in vitro method.

Methods and Results: After 5 days of in vitro culture with of Stem Span (Life Technologies, Vancouver, Canada) culture medium, GM-CSF (100 ng/ml, Schering Plough), IL-4 (50 ng/ml, Euro-Clone, U.K.) and calcium ionophore (100 ng/ml, Sigma Aldrich, Italy), we were able to differentiate all samples of AML (N13) and B precursor ALL (N23) into leukemia derived-antigen presenting cells (LD-APC). No differentiation was possible when T-ALL (N3) were studied. LD-APC showed a mature APC phenotype (CD40+, CD83+, CD86+ and CD80+), became able to uptake dextran-FITC and made cord blood derived naive T cells proliferate. 5 ALL and 4 AML derived APC successfully generated anti leukemia CTLs from HLA-matched identical sibling (N5) or unrelated donors (N3) and one haploidentical sibling after two repeated round of stimulation. After 14 days of in-vitro culture in the presence of IL-2 (120 U/ml, Proleukin), overall growing cells were found in a median of 73% (range 55-99) of initially plated wells. T cell receptor (TCR) cytofluorimetric analysis of both adult and cord blood derived CTL showed a skewing from a complete repertoire to an oligo-clonal /clonal pattern. The phenotype was CD3+ CD4+ in 53%, CD3+CD8+ in 10%, and a mixed population of CD4+ and CD8+ the remaining wells, all sharing an effector memory T cell profile (CD45 RO+, CCR7-, CD62L+, CD27+, CD11a + and CD28+/-). Cells with similar phenotype were pulled and further cultured for additional 14 days in flasks obtaining a maximal expansion of 150-fold increase. CTL were cytotoxic against patient leukemic blasts (median 18% at a E: T ratio of 10:1 after 4 hours) and not against autologous PHA blasts. They were perforine negative, granzyme positive (median expression 70%) and that they were able to produce IFN gamma upon aspecific stimulation (median expression 34%). As a comparison LD-APC from 4 ALL patients were used to generate leukemia reactive CTL from cord blood naive T cells. Over culture naive T cells changed their phenotype into effector memory cells, became perforine, granzyme and IFN- γ positive and more interestingly showed a higher cytotoxicity against leukemia blasts as compared to adult CTL (median 45% at E: T ratio 10:1). These experiments were performed under GMP conditions so to provide a protocol for further scaling up.

Conclusion: These results demonstrated the feasibility to generate highly cytotoxic, leukemia reactive T cell lines in the majority of AML and ALL patients and provide the biological background to design clinical protocols of adoptive immunotherapy in patients with leukemia relapse after allogeneic transplantation.

627 IMMATURE PLATELET FRACTION MEASUREMENT: A FUTURE GUIDE TO PLATELET TRANSFUSION REQUIREMENT AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background:

At present there is no routine laboratory parameter to predict platelet regeneration after high dose chemotherapy or transplantation. A new automated method to reliably quantitate reticulated platelets, expressed as the immature platelet fraction (IPF), has been developed utilising the XE-2100 (Sysmex). The IPF is identified by flow cytometry using a

polymethine dye in the reticulocyte channel.

Aim:

To evaluate a new rapid method for the analysis of the IPF using the Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan), a widely used automated haematology analyser, and to establish the predictive value of the IPF in determining platelet recovery following chemotherapy or haematopoietic transplantation

Method:

151 patients from several different clinical groups, in which platelet production or destruction might be abnormal, were studied. 28 had received high dose chemotherapy or undergone haematopoietic transplantation and followed serially during treatment.

Results:

Reproducibility and stability results over 48 hours were acceptable. The IPF normal range was established in adults as 1.1-6.1%, mean 3.4% and 2 standard deviation (SD) 2.3%. The IPF% is raised in several disease states. The most significant increases are found in patients with untreated ITP, n = 12, mean 22.3%, range 9.2-33.1% and active TTP, n = 5, mean 17.2%, range 11.2-30.9%. Following these patients during treatment demonstrated that as the platelet count recovered the IPF% fell. The recovery phase of thrombocytopenia in most chemotherapy, n=13, and stem cell/bone marrow transplant patients, n=15, was preceded by a rise in IPF% several days prior to platelet recovery. In particular, patients undergoing autologous transplantation (n=8) using peripherally collected stem cells have a very characteristic IPF% motif one day prior to platelet recovery. Recognising this motif as an early marker of platelet regeneration, in the absence of infection or bleeding, could avoid the need for prophylactic platelet transfusion.

Conclusion:

A parameter that can predict the timing of platelet recovery after chemotherapy or transplant would guide prophylactic platelet transfusion and provide the potential to reduce the use of platelet concentrates in these patients, thus minimising donor exposure, infection risk and allowing substantial financial savings. These results show the IPF% has this potential and should become a standard parameter in the clinical evaluation of the thrombocytopenic patient.

628 INTERCEPT PLATELETS EXHIBIT IMMUNOLOGIC REFRACTORINESS COMPARABLE TO CONVENTIONAL PLATELETS

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Background: INTERCEPT platelets (plt) are prepared using Helinx® technology to inactivate a broad spectrum of viruses, bacteria, protozoa, and leukocytes.

Aims: A randomized, double blind, controlled, parallel group Phase 3 trial was conducted using a prototype processing set to evaluate the hemostatic efficacy and safety of INTERCEPT plt (IP) compared to conventional plt (CP) collected on the Amicus® Separator. Patients (pts) with severe thrombocytopenia requiring multiple plt transfusions (tx) were enrolled.

Methods: Amicus plt were process leukoreduced (residual WBC content 5×10^6). Eligibility criteria included absence of clinical refractoriness and negative lymphocytotoxic antibody (LCA) test (<20% panel reactive cells) at study entry. LCA was assayed at baseline and end of study for all pts and weekly for pts with clinical refractoriness (2 consecutive corrected count increments (CCI) < 5,000). Specific anti-plt antibodies (ab) were measured for pts with clinical refractoriness, at baseline and post-onset of refractoriness. Ab to potential amotosalen neoantigens were measured by ELISA at baseline and end of study for all pts. Immunologic refractoriness was defined as a positive LCA, plt ab, or ab to amotosalen neoantigens.

Results: 645 pts (318 IP and 327 CP) received 4719 plt tx (2678 IP and 2041 CP) over a 28-day tx period. 54% of pts had exposure to plt products, and 77% to RBCs prior to study entry. At least 1 episode of clinical refractoriness was reported in 68/318 (21%) IP and 23/327 (7%) CP (p<0.01). No ab to amotosalen neoantigens were detected. IP resulted in lower plt doses and lower plt count increment, but comparable hemostasis. Immunologic refractoriness was comparable between treatment groups (Table).

Conclusions: More tx in the IP group had CCI <5000. However, comparable numbers of pts in both groups were persistently refractory

until study completion, experienced grade 2 bleeding, and had immunologic refractoriness. INTERCEPT Platelets were comparable to conventional platelets in managing bleeding and were not associated with a higher rate of immunologic refractoriness.

Observations in Refractory Patients	P (n=68)	CP (n=23)	p-Value
Pts with prior plt/RBC exposure (%)	74	70	0.79
Txn with plt dose < 3.0×10 ¹¹ (%)	19	12	<0.01
Mean no. plt tx given prior to onset refractoriness	5.1	5.5	0.87
Txn with 1-hr CCI < 5000 (%)	42	34	0.01
Single episode of two 1-hr CCI < 5000 (% pts)	57	65	0.63
Persistently refractory until end of study (% pts)	5.9	8.7	0.64
Mean duration of refractoriness (days)	5.0	4.0	0.19
Pts with grade 2 bleeding (%)	76	87	0.38
Pts with pos. LCA (%)	15	39	0.02
Pts with anti-plt ab (%)	12	10	1.00
Pts with pos. LCA and/or anti-plt ab and/or ab to amotosalen neoantigens (%)	22	43	0.06

Caption 1:

630 PEGYLATION OF HUMAN RED BLOOD CELLS INDUCES SUSCEPTIBILITY TO COMPLEMENT MEDIATED LYSIS

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Introduction: Surface modification of human red blood cells (RBC) with mPEG (PEGylation) can transform AB/D positive RBC to an O negative phenotype RBC that also are negative for minor antigens. Pegylation shows promise as a method to generate RBC compatible with all blood recipients, independent of blood group (Universal RBC). In PEG RBC preparation, equivalent antigen coverage can be obtained with different sizes of mPEG. However longer PEG molecules achieve efficient antigen (Ag) coverage with smaller RBC surface modification. Use of a 20kDa mPEG results in minimal surface modification (2 million copies/cell), while much larger surface modification (9 million copies/cell) is required for similar Ag masking by of the 5kDa mPEG homolog. We studied the in vitro compatibility of different PEG RBC preparations with sera of mismatched, matched and autologous phenotypes as a sensitive model for in vitro compatibility for recipients with different blood groups.

Methods: Active PEGs of different sizes were prepared. Sera were collected, and stored as aliquots at -80 °C until used. Standard methodology was followed to achieve complement (C) inactivation. Commercial (IBGRL) monoclonal FITC anti-CD 55/59 antibodies (Ab) and MTS Cards (Ortho) were used to study Ab binding and C mediated lysis and agglutination.

Results: PEG RBC prepared with a 5kDa mPEG lysed completely in a C-mediated reaction when incubated with matched or even autologous sera. The same RBC were stable in the absence of serum complement and lysis was inhibited by chelators and eliminated when the C-enzymes were heat inactivated. 5kDa PEG RBC prepared with increasing surface modification and antigen coverage, became more susceptible to C-mediated lysis, indicating that reaction of PEG with an RBC component induced lysis. Reduction in C-mediated lysis of PEG RBC was observed when larger (10 or 20 kDa) mPEGs were used. 20kDa mPEG RBC resulted in least lysis when exposed to different sera. RBC receptors (CD55/59) important for control of C molecules on RBC surface were found to be completely masked in PEG RBC prepared with 5kDa mPEG. Coverage of CD55 and CD59 was reduced in PEG RBC prepared with 20kDa. The mechanism of C-mediated lysis for the PEG RBC appears to be similar to the mechanism observed for red cells of patients with paroxysmal nocturnal hemoglobinuria (PNH), because the protection offered by CD 59 and CD 55 is inhibited.

Conclusions: Modification of RBC with high copy numbers of mPEG generated PNH-like RBC susceptible to C-mediated lysis. Susceptibility to C-mediated lysis was minimized by the use of large mPEG molecules that allowed efficient Ag coverage with minimal surface modification.

STEM CELLS & IMMUNOTHERAPY

631 MOBILIZED PERIPHERAL BLOOD AND CORD BLOOD: TWO OTHER REAL SOURCES OF MESENCHYMAL STEM CELLS

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Introduction: Mesenchymal Stem Cells (MSC) represent an attractive therapeutic tool due to their plasticity into various tissues such as muscle, adipose tissue, bone, cartilage, nervous tissue and their potential capacity to accelerate hematopoietic recovery after chemotherapy. MSC can be isolated from bone marrow, adipose and foetal tissues, but their isolation from Mobilized Peripheral Blood (MPB) and Cord Blood (CB) remains controversial. Aims: In this study, we investigated MPB and CB as sources of MSC compared to Bone Marrow (BM).

Methods: MPB-MSC and CB-MSC have been isolated from CB (n=5) and MPB (n=5) collected from volunteers donors after informed consent. The first culture was performed in alpha-MEM medium supplemented with FCS and conditioned medium from BM-MSC obtained after the second passage. MSC derived from MPB or CB were identified by their expression of mesenchymal markers (SH2, SH3) and hematopoietic markers (CD14, CD34, CD45 and CD62-E). MPB-MSC and CB-MSC were also tested for their capacity to generate CFU-F (Fibroblast colony-forming units) and to differentiate into adipocytes, osteocytes, chondrocytes and neural/glial cells after specific induction. Flow cytometry, western blots and RT-PCR were used to confirm their differentiation.

Results: During the first culture of MPB or CB, the cell population is composed of osteoblasts, osteoclasts, macrophages and hematopoietic cells. After several passages, the cells were found to be 95.73.3% SH3 and 96.12.9% SH2 for MPB-MSC and 85.310.1% SH3 and 78.511% SH2 for CB-MSC. Cells obtained in these conditions were able to differentiate into adipocytes, osteocytes, chondrocytes and neural/glial cells. Indeed, 2-3 weeks after specific inductions, lipid vacuoles, calcium deposits and chondrogenic matrix were stained with Oil Red O, Von Kossa and toluidine blue respectively. Neural and glial cells were obtained 10 days after induction and were positive to neural and/or glial proteins such as Nestin, Tuj-1, MAP-2, TH and GFAP.

Conclusions: The expression of specific mesenchymal markers (SH2 and SH3) by MSC derived from MPB and CB and their capacity to differentiate into various cell lineages confirm their presence in both sources. These results suggest that MSC are present in MPB or CB and may have a major clinical importance. They could be attractive targets for cellular or gene therapies.

632 IDENTIFICATION OF RPS4Y AS A HLA-B*5201-RESTRICTED H-Y ANTIGEN OVEREXPRESSED BY PROLIFERATING B AND T CELLS

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Background: Despite the progress in understanding mechanisms of the Graft versus Leukemia (GvL) effect of allogeneic stem cell transplantation and donor lymphocyte infusion the antigenic basis of GvL remains largely unknown.

Aim: We report an H-Y antigen which is a target of a CTL response directed against lymphoblasts.

Methods and Results: The CD8+ CTL clone YKIII.8 was isolated from a T cell line obtained by repeated stimulations of T cells of a female donor with CML cells and EBV-LCL of her HLA-identical brother. YKIII.8 reacted specifically against EBV-LCL of the patient (EBVp), but not donor (EBVd) origin, both in an IFN-gamma assay (693 vs. 0 pg/ml) and the 51Cr release assay (84 vs. <5% lysis). Reactivity against EBVp was completely blocked by an anti-HLA-B/C antiserum. When a selection of 24 EBV-LCLs was transduced with the two HLA-B alleles of the patient, HLA-B*3508 and HLA-B*5201, cloned into a retroviral pMX vector, 10 EBV-LCLs (all derived from male patients) were recognized by YKIII.8 after transduction with the pMX-HLA-B*5201/EGFP. Therefore, we concluded that YKIII.8 recognizes an H-Y antigen presented on HLA-B*5201. To identify the molecular nature of the antigen a cDNA library derived from EBVp cells was cloned into the pCEP4 vector. Pools of 50 individual clones were transfected into B52+ 293-EBNA-B7 cell line. Transfected cells were screened for their ability to induce IFN-gamma secretion by YKIII.8. After subcloning of positive pools five individual clones were isolated. Electroporation of EBVd with these cDNAs led to their recognition by YKIII.8. Sequencing of those cDNA inserts showed that all positive clones contained the 931 bp full-length cDNA of the male

isoform of 40S ribosomal protein S4. By generation of truncated RPS4Y mini-genes and peptide loading experiments we found that YKIII.8 clone recognizes TIRYPDPVI peptide of RPS4Y protein at minimal concentration of 10⁻⁷ M. We found that YKIII.8 recognizes HLA-B*5201+ male CD40-activated B cells, EBV-transformed B cells, PHA-activated T cell blasts but neither CD14+ cells nor resting B and T cells. In a separate experiment we found that male B52+ fibroblasts induced only a weak response of YKIII.8 (24 pg/ml IFN-gamma vs. 2306 pg/ml on EBVp). Western blot analysis showed that hematopoietic cell subsets recognized by YKIII.8 overexpress RPS4 protein in comparison with non-recognized cell subsets. We found increased level of RPS4 expression in 3 of 3 multiple myeloma, 3 of 3 ALL and 2 of 3 AML samples in comparison to normal PBMC. 2 B52+ male multiple myeloma cell lines were found to be susceptible to lysis by YKIII.8.

Conclusion: We have identified RPS4Y as an H-Y antigen that is able to elicit a CTL response. Due to a low avidity of YKIII.8 and overexpression of RPS4 in proliferating lymphocytes, this response is directed specifically against lymphoblasts. It makes RPS4Y a new attractive target for the immunotherapy of hematological malignancies. A transfer of YKIII.8 TCR into HLA-B*52+ donor T cells would selectively enhance the GvL effect of donor lymphocyte infusion.

633 EXPANSION OF HUMAN UMBILICAL CORD BLOOD-DERIVED CD34+ STEM/PROGENITOR CELLS TO TREAT MYOCARDIAL INFARCTION

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The contribution of human umbilical cord blood (CB)-derived Stem/Progenitor cells (S/P) in post myocardial infarction (MI) healing and regeneration was examined. S/P cells were isolated from CB of newborns by the Miltenyi Biotecs magnetic cell separation device and expanded ex-vivo. Expanded CD34+ cell numbers increased by 316 fold and early progenitor (CD34+CD38-) cells increased by 409149 fold. An MI model was established in athymic nude rats by permanent ligation of the left anterior descending coronary artery. Ex-vivo expanded S/P cells (1x10⁶ cells/rat) or saline (control) were injected at the scar tissue one week post MI. Four weeks after cell therapy, the hearts were harvested and examined for the presence of human-donor cells in recipient hearts by fluorescent in situ hybridization (FISH) using deoxyribonucleic acid probes specific for human X and Y chromosomes or immunostaining for HLA-DR. HLA-DR immunostaining revealed that S/P cells engrafted and colonized the infarcted myocardium. Positive HLA-DR staining and X/Y signals were observed in vessel walls and cardiomyocytes, suggesting differentiation or fusion of S/P donor cells into vasculature or myogenic cells. Control hearts were negative for both HLA-DR and human sex chromosomes. Staining for smooth-muscle alpha-actin detected a higher density (vessels/mm²) of capillaries and arterioles in the expanded S/P cell-treated vs. control hearts (125 ± 15 vs. 44 ± 15/mm², p=0.02). Serial echocardiographic studies before and 4 weeks post cell therapy revealed that ex-vivo expanded S/P cells prevented LV dilatation accompanied by progressive LV dysfunction. Conclusion: Injection of S/P cells into the post MI myocardial scar tissue results in engraftment, neovascularization and in prevention of LV dilatation and myocardial dysfunction. The use of ex-vivo expanded SP/progenitor cells in cardiac healing and regeneration will be tested in an upcoming clinical trial.

634 PGP (MDR1)-DEPENDENT RESISTANCE OF MALIGNANT CELLS TOWARDS NATURAL KILLER (NK) CELLS IS REVERSED BY IL-2 AND KT-5720: IMPLICATIONS ON GRAFT-VERSUS-TUMOR (GVT) EFFECTS IN ADOPTIVE IMMUNOTHERAPY

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BACKGROUND: Intrinsic and acquired multidrug resistance (MDR) is a major obstacle for curative chemotherapy as a treatment of cancer patients. The multidrug transporter, P-glycoprotein (Pgp) that is encoded by the MDR1 gene and over-expressed in different malignancies, reduces the accumulation and decreases intra-tumoral retention of various drugs below therapeutic threshold, thereby limiting tumor kill. A major strategy in treating cancer patients with solid tumors and hematological malignancies is adoptive-immunotherapy using donor lymphocytes infusion (DLI). Subsequent T-cell-mediated GVT effects

play an important role in the eradication of residual malignant disease after allogeneic stem-cell transplantation. However, resistance of tumors to effector, T-cell-mediated killing and DLI failures were correlated with high-expression level of Pgp in malignant cells of a significant number of patients. Conversely, Pgp-activity in the effector cells was previously found essential for high-GVT effects, probably via cytokines-directed transport. AIMS: 1) Investigating the relationship between Pgp expression and activity levels in malignant, target cells and their cytolytic response to effector NK-cells; 2) Studying the relationship between the level of GVT effects and the allele polymorphism C3435T of MDR1 that was previously correlated with phenotypic variations in Pgp expression levels (high-, intermediate- or low-expression level of the CC, CT or TT genotypes, respectively); 3) Identifying means to increase the GVT effect towards tumors showing Pgp-dependent resistance to immunotherapy. METHODS: Human NK (CD56+, CD3-) cells were separated from healthy donors and their killing effects against sets of Pgp-expressing and non-expressing cell-variants (of various leukemia and carcinoma cell lines) were measured in the absence or presence of IL-2 and the Pgp-inhibitor KT-5720. Target cells and effector cells from various donors were analyzed for MDR1 expression levels and C3435T polymorphism. RESULTS: 1) All tested, Pgp over-expressing cell-variants were found significantly less sensitive to NK-cells than their parental, Pgp non-expressing cell lines. In these cell lines, a direct correlation was found between the CC-genotype of MDR1, high-expression level of MDR1 and resistance towards NK-cells. However, malignant cells carrying the TT-genotype of MDR1 were found sensitive towards NK-cells with low MDR1 expression level; 2) The cytolytic effect of NK-cells from donor carrying the CC-genotype of MDR1 was approximately 100-fold higher than from TT-genotype donor; 3) All tested Pgp-overexpressing target cells were more resistant to resting-NK cells than to IL-2-activated NK-cells; 3) Addition of IL-2 to resting-NK cells during the 4 hours of the cytotoxicity assay increased, significantly, the killing of target cell lines. Surprisingly, the effect of IL-2 was larger on Pgp-high expressing- than on Pgp-non expressing-target cells; 4) The Pgp-inhibitor, KT-5720, increased the killing of Pgp-overexpressing, target cells by a factor of 5-13. CONCLUSIONS: 1) The GVT effects of DLI might be elevated if the donor carries the CC genotype of MDR1 gene; 2) Short pre-incubation of donor lymphocytes with IL-2 may also increase the GVT effect of DLI, possibly by elevation of their binding to adhesion molecules on target cells; 3) Since we previously found that KT-5720 reverses Pgp-activity in vitro and in vivo, it might be also useful for elevating GVT effects in adoptive-immunotherapy.

635 A NEW ROAD TO T CELL DIVERSIFICATION

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Background: Classic reports on lymphocyte development hold that B and T cells have distinct functions and originate independently. Since these reports use tissue tumor transplants as a model for T cell responses, they would infer that rejection of tissue tumor grafts depends predominantly on T cells and hence only on the thymus. Our recent findings however challenge this concept. We and others have recently found that T cell diversity depends not only on the interaction of T cell precursors with thymic epithelial cells but also on other cells, the identity of which is not known. Here we report that B cells contribute to T cell diversification. Aims: This study aims to determine the role of B cells, normally present on the thymus, on T cell development and in the generation of TCR diversity. Methods: We compared TCR repertoire diversity in B-cell deficient and B-cell proficient mice and in B cell deficient mice following adoptive transfer of B cells or immunoglobulin injections. TCR repertoire diversity was measured by a novel method based on hybridization of specific RNA on a gene chip platform. Results: T cell receptor Vb diversity of thymocytes in mice that lack B cells and immunoglobulin was reduced to 6 x 10² from wild type values of 1.1 x 10⁸; in mice with oligoclonal B cells T cell receptor Vb diversity of thymocytes was 0.01% of wild type. Adoptive transfer of diverse B cells or administration of polyclonal immunoglobulin into mice that lack B cells increased thymocyte diversity, 8 and 7 fold, respectively, while adoptive transfer of monoclonal B cells or monoclonal immunoglobulin did not. Conclusions: Here we report that B cells and polyclonal immunoglobulin drive generation of T cell repertoire diversity. These findings reveal a heretofore unrecognized and vital function of B cells and immunoglobulin for generation of T cell diversity and suggest a potential approach to immune reconstitution.

CHRONIC LYMPHOCYTIC LEUKEMIA: BIOLOGY ZAP

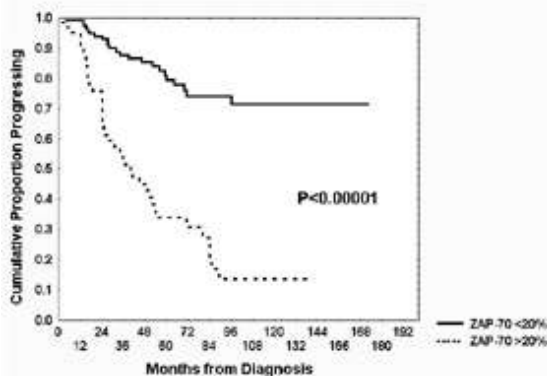
636 ZAP-70 PROTEIN TYROSINE KINASE IS AN INDEPENDENT MARKER OF DISEASE PROGRESSION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (B-CLL) can be classified into one of two subgroups based upon the mutational status of immunoglobulin variable (IgVH) genes. Patients (pts) with B-CLL cells that express non-mutated genes have an aggressive clinical course when compared with B-CLL pts that express IgVH genes with somatic mutations. Gene expression profiling analysis revealed that restricted sets of genes were able to discriminate between the non-mutated and mutated type clones. The gene encoding zeta-associated protein 70 (ZAP-70), a tyrosine kinase required for T-cell receptor signalling, normally expressed in T cells and natural killer cells, was expressed in 100% patients with non-mutated but only 10% of those with mutated IgVH genes. Moreover, recent data indicated that CD38 positive B-CLLs are characterized by unmutated VH genes and poor outcome. These findings led us that the expression of ZAP-70 or CD38 could predict not only IgVH mutational status, but mainly prognosis in B-CLL. We investigated 183 pts, median age 65 years (range 37-84), 89 males and 94 females. With regard to modified Rai stages, 64 patients had a low stage, 116 an intermediate stage and 3 a high stage. ZAP-70 protein and CD38 antigen were determined on mononuclear cells by a multicolor flow cytometric method, using an anti-ZAP-70 (2F3.2, Upstate Biotechnology) and an anti-CD38 monoclonal antibody (Becton Dickinson), respectively. A cut-off value of 20% was used both for ZAP-70 and CD38. ZAP-70+ and CD38+ B-CLL patients were 63/183 (34.4%) and 33/183 (18%), respectively. In 77 B-CLL patients we determined IgVH mutational status and samples in which fewer than 2 percent of base pairs differed from those of the consensus sequence were considered unmutated (19/77, 24.7%). There was a significant correlation between IgVH mutations and ZAP-70: 16/19 (84%) unmutated pts had ZAP-70>20% and 49/58 (84%) mutated pts had ZAP-70<20% (P=0.00001). On the contrary, 7/19 (37%) unmutated pts had CD38>20% or 48/58 (83%) mutated pts had CD38<20% (P= 0.1, not significant). There were significant correlations between ZAP-70 values and soluble CD23 levels (r=0.32, P=0.000019) or CD38 percentages (r=0.19, P=0.009). Fifty-eight pts were treated with fludarabine and a lower significant complete remission rate (36% vs 63%, P=0.006) was found within ZAP-70+ pts. Moreover, a shorter progression-free survival (PFS) was observed in ZAP-70+ pts (14% vs 71% at 10 years; P<0.00001) and in CD38+ pts (16% vs 56% at 10 years; P=0.00004). Also a shorter overall survival (OS) was found in ZAP-70+ pts (80% vs 96% at 10 years; P=0.019) and in CD38+ pts (78% vs 92% at 10 years; P=0.035). With regard to PFS, combining ZAP-70 and CD38 expression, the subset [ZAP-70+CD38+] showed the worse outcome. In multivariate analysis, with regard to PFS, ZAP-70 resulted to be more significant (P=0.0002) than age (P=0.03) or Rai modified stage (P=0.004). In conclusion, the overexpression of ZAP-70, performed by flow cytometry, was associated with IgVH non-mutated status and predicted a rapid progression and a poor survival. The analyses of the receptor signalling capacity might provide for a more efficient prognostic indicator than clinical stage or other clinico-biological features.



Caption 1: Progression-free survival by ZAP-70 in B-CLL

637 CXCL12 INDUCES ACTIN POLYMERISATION AND SUSTAINED ERK ACTIVATION IN B-CLL CELLS EXPRESSING ZAP-70

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Background: B-CLL patients with an unmutated immunoglobulin heavy chain gene (IgVH) status have a poorer prognosis than those with somatically mutated variable region heavy chain genes. The protein tyrosine kinase, ZAP-70, is normally expressed by T and NK cell sub-types, but in B-CLL B cells its expression correlates strongly with mutational status and patient prognosis. It is not yet clear if ZAP-70 is simply a marker of poor prognosis or actually contributes to pathogenesis. The chemokine CXCL12/ SDF-1 α induces B-CLL B cell chemotaxis and protects them from apoptosis in vitro via ligation of its receptor CXCR4. It is currently not understood how CXCL12 protects B-CLL cells from apoptosis. In T lymphocytes stimulation with CXCL12 results in prolonged activation of extracellular signal-regulated kinases (ERK) ERK1 and ERK2, a property unique to this chemokine. This occurs through ligation of CXCR4, and requires both ZAP-70 and the Src homology 2 domain-containing protein of 76kDa (SLP-76).

AIM: To determine whether the expression of ZAP-70 in CLL B cells has an effect on responses to CXCR4.

Methods: Expression of CXCR4 and ZAP-70 were determined by flow cytometry and the activation of ERK and the presence of SLP-76 were determined by western blotting. Polymerisation of cytoskeletal actin, an early indicator of chemotaxis in response to the chemokine CXCR4, was measured by flow cytometry.

Results: Previously described significantly higher expression of surface CXCR4 on B-CLL B cells was confirmed compared to healthy controls (p<0.005). CXCL12 induction of actin polymerisation and ERK activity in B-CLL B cells were also found. Actin polymerisation was not influenced by ZAP 70 status. However, prolonged ERK activation occurred in a sub-population of patients and did correlate with expression of ZAP-70 and SLP-76. In CLL B cells expressing ZAP-70, prolonged ERK activation was inhibited by pre-incubation with a blocking anti-CXCR4 antibody and pertussis toxin, but not by the PI3 kinase inhibitor, wortmannin.

Summary: The association of expression of ZAP-70 with increased B-cell receptor (BCR) signalling in CLL(1) indicates that ZAP-70 may contribute to CLL pathogenesis. This study suggests that ZAP-70 may be involved in signalling pathways induced by CXCL12, which mediates B-CLL B cell survival and chemotaxis. Together these findings suggest that ZAP-70 in CLL B cells may provide links to a variety of downstream signalling pathways, which are absent in ZAP-70-ve cells and could confer a survival advantage.

1 Chen L, Widhopf G, Huynh L et al. Blood 2002; 100: 4609-4614

638 ZAP-70 PROTEIN EXPRESSION IS NEGATIVE, AND THE GENE PROMOTER REGION IS METHYLATED, IN MOST CASES OF MCL AND SLVL, AND DOES NOT CORRELATE WITH IGVH GENE MUTATIONAL STATUS

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Background: CLL prognosis is closely linked to IgVH gene mutational status, survival being shorter in unmutated cases. cDNA microarrays have identified mRNAs which are differentially expressed between the two mutational sub-sets, particularly ZAP-70, which codes for a tyrosine kinase normally expressed in TNK cells but not mature B cells. ZAP-70 protein expression shows strong, but not complete, correlation with unmutated IgVH genes, and methylation of the promoter region of the gene is found in non-expressers. Aims: To investigate ZAP-70 methylation and expression in two other lymphoproliferative disorders where IgVH gene mutational status is known to be heterogeneous: Mantle cell lymphoma (MCL) and splenic lymphoma with villous lymphocytes (SLVL).

Methods: 37 SLVL and 23 MCL cases diagnosed on standard clinical, morphological, immunophenotypic, histological and cytogenetic criteria. ZAP-70 protein was measured by 2 colour flow cytometry, using the monoclonal antibody 2F3.2 (Upstate), and CD2-PE to delineate T&NK cells. This antibody was used on tissue sections (lymph nodes and spleens) where available. IgVH gene mutational status was determined as previously described, criterion for unmutated cases being 98-100% homology to the germline. Analysis of the methylation status of the CpG island overlapping exon 1 of ZAP-70 was carried out by Southern blotting and by sequence analysis following DNA bisulphite treatment and PCR. In cases with >25% T cells, samples were sorted prior to methylation determination.

Results: Of 37 SLVL patients, 10(27%) had unmutated IgVHgenes ZAP-70 protein expression was positive in 2 patients and negative in 8. 27 SLVL patients (73%) had mutated IgVHgenes; ZAP-70 was positive in one and negative in 26. Of 23 MCL patients, 11(48%) had unmutated IgVHgenes; ZAP-70 was positive in one patient and negative in 10. 12 MCL patients (52%) had mutated IgVH genes; ZAP-70 was positive in one patient and negative in 11. Immunohistochemistry for ZAP-70 was available in 7 SLVL and 7 MCL cases and correlated with negative flow values in all. ZAP-70 promoter methylation status was available in 14 SLVL cases and was methylated in all, including one case which expressed ZAP-70 protein. Of 13 MCL cases, the promoter region was methylated in 12, all of which were negative for ZAP-70 protein, and showed a mixed pattern in one case which expressed ZAP-70 protein.

Summary/Conclusions: In 60 cases of SLVL and MCL, 35% of which had unmutated IgVHgenes, ZAP-70 protein expression was negative in 55(92%) and showed no correlation with IgVHgene mutational status. In CLL, both ZAP-70 positivity and low mutational load are poor prognostic factors and these are strongly correlated, but whether there is a mechanism which links them, or whether ZAP-70 is simply an aberrant tumour marker or a reflection of B cell maturation, is not known. Such a mechanism appears not to hold in SLVL or MCL, two other lymphoproliferative disorders with heterogeneous IgVH gene mutational status. The correlation between ZAP-70 protein non-expression and promoter region methylation, however, is similar to that in CLL and normal B and T cells.

639 ZAP-70 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKAEMIA: CORRELATION WITH CLINICAL AND BIOLOGICAL FEATURES

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Background. The expression of the tyrosine kinase ZAP-70 in chronic lymphocytic leukaemia (CLL) cells has been proposed as a surrogate marker for the mutational status of immunoglobulin VH genes. Recent reports suggest that ZAP-70 overexpression is an independent prognostic factor for short progression-free and overall survival. There is little information about its correlation with genetic abnormalities.

Aims. To evaluate prospectively ZAP-70 expression in a series of CLL patients and to correlate this with clinical stage, lymphocyte morphology, CD38 expression and chromosomal abnormalities detected by FISH analysis.

Methods. ZAP-70 expression was evaluated using four-colour flow cytometry (as described by Crespo et al, NEJM 2003; 348:1764-75) with the following monoclonal antibodies (anti-ZAP, clone 2F3.2, Upstate; CD3 PE, CD56 PE, CD19 PerCP-Cy5.5 and CD5 APC, BD Biosciences). Statistical analysis using Fishers exact test was performed.

Results. 142 CLL patients were evaluated. Less than 1/3 were ZAP-70 positive (+) (cut-off > 20% of CD5/CD19+ cells). ZAP-70 positivity was associated with higher M:F ratio (see Table), prevalence of stage B/C at diagnosis, CLL with >10% prolymphocytes (CLL/PL) or atypical morphology, CD38 positivity (regardless of the cut-off point), trisomy12, del6q21 or no detectable abnormalities. ZAP-70 negative (-) cases had more often del13q14. There was no difference in the frequency of del11q23 or p53 abnormalities between ZAP-70+ and ZAP-70- cases. Of 142 patients, 83 patients (stage A progressive, B or C) were entered in the LRF CLL4 trial. Among these, 29 (35%) were ZAP-70+ and 54 (65%) ZAP-70-. Median time to progression from diagnosis was 12 months for ZAP-70+ and 21 months for ZAP-70- cases.

Conclusions. We document a significant correlation between ZAP-70 overexpression and other adverse prognostic factors (such as CD38 and advanced stage) and provide new data concerning its association with cytogenetic abnormalities detected by FISH in CLL.

	ZAP-70 POS	ZAP-70 NEG	Fisher's exact test (p values)
Number of cases	38 (27%)	104 (73%)	
M:F ratio	3.75	2.3	
Stage A	32%	62%	p = 0.002
Stage B/C	68%	38%	
CLL/PL	42%	10%	
atypical morph			
CD38+ (≥ 7%)	32 (86%)	58 (56%)	p = 0.001
del13q14 (sole)	0/14 (0%)	9/27 (34%)	p = 0.017
trisomy 12	6/19 (31%)	7/56 (12%)	p = 0.058
del11q22-23	3/16 (19%)	7/29 (24%)	p = 1.00
del17p13 (p53)	3/20 (15%)	4/47 (8%)	p = 0.41
del6q21	3/14 (21%)	2/29 (7%)	p = 0.30
no abnormalities	5/14 (36%)	2/27 (7%)	p = 0.035

Caption 1:

Publication Only

640 EARLY SEVERE PULMONARY COMPLICATIONS IN 90 PEDIATRIC PATIENTS UNDERGOING ALLOGENIC STEM CELL TRANSPLANTATION

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OBJECTIVE: We analyzed the early severe pulmonary complications (sPC) defined as any case of lung injury with breath insufficiency (with exception of fluid overload) within first 100 days after allogenic stem cell transplantation in a group of pediatric patients.

MATERIAL AND METHODS: Between January 1994 and June 2003, ninety patients underwent allogenic stem cell transplantation (SCT) at Russian Institute for Pediatric Hematology. Median age of the patients was 10.2 years (range 1-19 years), M/F ratio was 1,1/1. Forty-five patients were with hematological malignancies (30 ANLL, 8 CML, 7-ALL) and 45 with non-malignant diseases (30 severe aplastic anemia, 6 anemia Fanconi, 5 SCID, 4 lymphohistiocytosis). All but two patients had normal lung function tests, measured before SCT. Patients were grafted with G-CSF mobilized PBSC in 33 cases (HLA-matched related donor (MRD) 29, HLA-matched unrelated donor (MUD) 2, haploidentical 2), or bone marrow (BM) in 57 cases (HLA-MRD BM - 54, MUD-BM 1, haploidentical BM 2). The median number of infused CD 34+ cells in the BM and PBSC group were 4.7x106 cells/kg (range 0.5-15x106) and 8.8x106 cells/kg (range 3-25x106) respectively. Forty-one patients with malignant disease received Bu-containing condition regimens prior to SCT, 4 TBI-based regimens. In the group of non-malignant disease twenty-one patients received Bu-containing regimens, and 24 pts received reduced intensity condition regimen (Cph + ATG). Median time to achieve neutrophils >0.5x109/L was 17 days (range 11-35 days) in the PBSC group and 20 days (range 13-31) in the BM group of patients.

RESULTS: Nine patients (10%) showed clinically relevant sPC at median + 29 day after stem cell infusion (range 1-87 days). Four cases were acute respiratory distress syndrome (ARDS) attributable to engraftment syndrome, with occurrence at median + 8 day after SCT (range 3-17 days): all patients were treated with methylprednisolone 10-20 mg/kg body weight. Two deaths were linked to ARDS, one of them was patient with previsions lung impairment due to the primary disease. Five cases were of infectious origin: three cases of invasive lung mycoses (1 proven Candida sepsis, 2 proven Aspergillus sepsis) on median + 58 day after SCT (range 22-87 days); all patients died in spite of the antifungal treatment. Two cases were successfully treated interstitial pneumonia (1 Pneumocystis, 1 - RSV associated). In multivariable analysis we identified rinse of neutrophils counts as the only significant variable predicting for the early sPC ($p=0.01$). Delayed engraftment and aGVHD was highly expected for the occurrence of the sPC, associated with infection ($p<0.01$).

641 TREATMENT WITH FORMALINE (WATER SOLUTION OF FORMALDEHYDE) FOR REFRACTORY HEMORRHAGIC CYSTITIS IN CHILDREN AFTER STEM CELL TRANSPLANTATION

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BACKGROUND: Hemorrhagic cystitis (HC) is a rare but severe complication of stem cell transplantation (SCT). It occurs in about 7 to 35% of transplanted patients and its incidence increases with the age of the patients, depends on the conditioning regimen (cyclophosphamide, busulphan, TBI), type of SCT (auto/allo) and presence of acute GVHD. Many therapeutic modalities have been investigated to treat HC, but a few were shown to be effective.

OBJECTIVE: In the present study, we assessed the clinical effect of formaline treatment for intractable HC (grade IV according to the WHO classification) in three transplanted children. Characteristics of patients resume in the Table A. Between 2000-2003 in our Bone Marrow Transplantation Unit three children (median age of 10.7 years, 2 males and 1 female) who underwent a SCT (two allogenic and one autologous), on the +32 mean day from stem cells infusion, showed symptoms of dysuria, severe bladder spasms and macroscopic hematuria with clots formation and acute obstructive urine retention (pts 3). One patient with B-NHL was conditioned with BuVpCy, one patient with ALL - with

BuThiotCy while the third with chronic myeloid leukemia received BuCy and antithymocyte globulin (ATG). All patients required repeated red blood cell and platelets transfusions due to HC, suffered from severe pain syndrome requiring narcotics. All therapeutic attempts, including cystoscopy, continuous bladder irrigation, instillation with 1-3 % AgNO₃, hyperbaric oxygenation (pts 2) and systemic ganciclovir (pts 1 with PCR positive for CMV in blood/urine), had no positive effect. Finally under general anesthesia and after insertion of cystostomic tube, 30 ml 10% formaline solution was instilled in the bladder with the 15 min exposure time. Just after bladder was irrigated with 500 ml isotonic solution.

RESULTS: All children showed improvement of cystitis and decreased transfusion request on the 2nd day after formaline instillation, with a complete recovery of symptoms by the 10th day. The therapy was well tolerated, the only adverse event was the pain at the cystostomic tube site. There were no urologic complications seen during the follow-up period. One patient (pts 2) is still alive, disease-free, at 24 months after SCT; one patient (pts 1) died of CMV pneumonia, in complete hematological remission on 9 months after SCT, one patient (pts 3) was relapsed and died 12 months after SCT.

CONCLUSIONS: The 10% formaline is an effective alternative treatment for the refractory hemorrhagic cystitis after stem cell transplantation. We consider that an earlier application of this method ought to be auspicial.

Patient's characteristic	Patient 1	Patient 2	Patient 3
Primary disease	CML	B-NHL	ALL
Age (y)	17	6	10
Sex	M	M	F
Type of SCT	alloMUD	auto	alloMRD
Condition regimen	BuCy + ATG	BuVpCy	BuThiotCy
GVHD	Yes (gr2)/ no	-	Yes (gr 2)/ no
acute/chronic			
DFS (months)	+9 (died)	+24 (w.a.)	+12 (died)

Caption 1: Table A

642 OUTCOME OF SPLENECTOMY AND RELEVANCE OF EARLY AND LATE INFECTION COMPLICATIONS AFTER SURGERY IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA.

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INTRODUCTION. In this retrospective study we revised the data regarding 50 patients (pts) with Idiopathic Thrombocytopenic Purpura (ITP) unresponsive to steroids or other conventional therapies who underwent laparoscopic (LS) or open (OS) splenectomy. We would like underline the incidence of early and late infections after splenectomy for ITP.

PATIENTS AND METHODS. From June 1990 to June 2001, 289 cases of ITP were diagnosed at our Department and in 17% of them (50/289) a splenectomy has been performed; 29 out of 50 pts were female and 21 males with 27 years as median age at diagnosis; the median age at splenectomy was 34 years (range 14-85). All pts were relapsed or refractory to first line therapy that was standard or HD of steroids in the majority of cases (steroids alone 30/50, steroids/immunosuppressive therapy 2/50, steroids plus immunoglobulins 15/50, other in 3/50). The median platelet count before splenectomy was 30.000/mm³ (range 6-416.000). 31/50 (62%) pts had received anti pneumococcus and anti

meningococcus vaccination. Definition of Response after Splenectomy: Complete Remission (CR) was defined as platelet count >150.000/mmc lasting for 3 months or longer without treatment, Partial Response (PR) as platelet count between 50 to 150.000/mmc for the same period of time.

RESULTS. 39/50 (78%) of pts received a LS while 11/50 (22%) an OS. LS was completed in 37/39 (95%) pts with a conversion rate (OS) of 5%. Operative and postoperative complications occurred in four (8%) pts; no deaths were attributed to the procedure. A response (CR plus PR) was obtained in 48/50 (96%) of pts, only 2/50 (4%) were not responders (PLT count after splenectomy lower than 50.000/mmc). Of responsive pts 30/48 (62%) were in continuous CR after a mean follow up of 64 mths and 6/48 (13%) were in PR after a median follow-up of 79 mths; 12/48 pts (25%) relapsed after a mean disease free of 13 mths. The OS at 73 mths was 97% and the probability of Relapse free survival at 48 mths was 71%. The probability of CR at 96 mths was 52%. Early infections complications (within the first 60 days from surgery) occurred in 4/50 (8%) pts (1 HZ, 2 pneumonia, 1 abdominal abscess) and a FUO occurred in 3/50 (6%) of cases; 5/7 of these pts are older than 50 yrs, 6/7 had received a prolonged immunosuppressive therapy and 5/7 pts had been vaccinated before surgery. The mortality infection related was 0%. No significant late infection complications have been recorded.

CONCLUSIONS. 1) Splenectomy is a safe and effective option for ITP unresponsive to steroids or other therapies. 2) The early infection complications can be possible in a small number of cases (less than 15%) and their outcome is favorable. 3) We have not seen significant late infection complications and largest studies are necessary to revised the role of vaccination before splenectomy in ITP in order to decide if it could be reserved for a subset of cases (older pts or those with longer immunosuppressive therapy or concomitant disease).

643 LIPOSOMAL DAUNORUBICIN PLUS ARACYTIN FOR RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA CAN BE A GOOD REINDUCTION CHEMOTHERAPY BEFORE MATCHED UNRELATED BONE MARROW TRANSPLANTATION.

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INTRODUCTION. The relapsed ALL have a poor prognosis and only 10% of these pts are alive at five years from relapse. So would be very important to explore new protocols of reinduction chemotherapy in order to perform a subsequent allogenic transplant in a condition of remission. Here we resume our experience in this setting using Liposomal Daunorubicin (Daunoxome-DNX) and Aracytin (Ara-C) as reinduction chemotherapy before Matched Unrelated Bone Marrow Transplantation (MUD-BMT).

PATIENTS AND METHODS. 10 relapsed ALL pts (8 B phenotype, 2 T phenotype), 5 males and 5 females, median age 28 yrs (range 17-48); 7/10 pts were at first relapse and 3/10 at second or subsequent relapse. All pts (10/10) had one or more adverse prognostic factors at diagnosis and all of them had been treated with antineoplastic with a median cumulative dose of 233 mg/m² (range 55-336). All pts had received a reinduction chemotherapy before MUD-BMT with DNX 80 (6/10) or 100 (4/10) mg/m², days 1-3 plus Ara-C 2 g/m², days 1-5.

RESULTS. Eight pts (80%) achieved a complete remission (CR) and 1/10 (10%) a partial remission (PR) for an overall response rate of 90%; one pt was resistant to reinduction therapy. DNX plus Ara-C has been well tolerated without cardiotoxicity. All pts in CR or PR obtained a fast hematologic recovery. MUD transplant was performed after a median of 3 mths (range 1-5) from DNX based reinduction chemotherapy. At the time of transplant 7/10 (70%) pts were still in complete remission, 2 were relapsed, 1 was resistant. All pts received Total Body Irradiation plus cyclophosphamide as conditioning regimen and immunosuppression with cyclosporin and metotrexate. 10/10 (100%) pts achieved a CR after transplant. The median follow-up from transplant was 10 mths (range 3-41); 4/10 pts (40%) are alive and 6/10 (60%) are death: 3/6 for TRM in a condition of CR (acute GVHD in 3 pts and infection in 1 pt) and 3/6 died for relapsed leukemia. The OS from diagnosis was 50% at 38 mths. The OS from MUD-BMT was 50% at 9 mths. The RFS after MUD-BMT was 65% at 6 mths.

CONCLUSIONS. These data, taking into account the small number of cases, would confirm that: a) despite the adverse prognosis of relapsed ALL pts, MUD-BMT can rescue a part of them; b) TRM is still the most important problem and needs to be reduced; c) DNX and Ara-C might be a good and well tolerated reinduction chemotherapy for poor-risk ALL that allows to reach the transplantation procedure in a condition of remission

644 MOLECULAR-GENETIC POLYMORPHISM INTERLEUKIN-6, TUMOR NECROSIS FACTOR- α , β AND GLUTATHIONE-S-TRANSFERASE M1 GENES IN PATIENT WITH CHRONIC LYMPHATIC LEUKEMIA

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The linkage of polymorphism '174G C of the interleukin 6 (IL-6) gene promoter region, polymorphism '308G A of the tumor necrosis factor α (TNF- α) gene promoter region, polymorphism +252A→G of the tumor necrosis factor β (TNF- β) gene, polymorphism of GSTM1 gene predisposed to the development of chronic lymphatic leukemia (CLL) has been studied. The study comprised 104 patients with CLL, living in the Republic of Bashkortostan, including 54 patients with CLL non progressive course and 50 patients with CLL progressive course. The control group consisted of 101 subjects similar in sex and age to the patients studied. It has been shown that the polymorph variant 174G→C of the IL-6 gene is not associated with predisposition to CLL development. The genotype of the TNF- α locus 308G→A early in the disease course of the patients with CLL would help isolate a group genetically predisposed to a more severe course of the disease. While studying the TNF- β gene polymorphism it has been established that LT*22 genotype having the TNF- β gene mutations in the homozygous state, was two times more frequent in patients with CLL than in the control group. The study of genotype combinations by TNF- α and TNF- β loci demonstrates that variants of TNF*22/LT*22 and TNF*12/LT*11 with frequency about two fold greater in the progressive course than in the non progressive form of the disease, appeared to the prognostic markers for revealing subjects predisposed to CLL more severe progressive course. A trend to higher frequency of the GSTM1 gene deletion in CLL severe course may point to prognostic value of the present index regarding unfavorable development of the disease.

645 AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) AS POST-REMISSION THERAPY FOR ADULT ACUTE LYMPHOID LEUKEMIA IN FIRST OR LATER COMPLETE REMISSION.

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INTRODUCTION. The current therapies for ALL result in a high complete remission (CR) rates (80-90%) but only 25-30% of patients (pts) achieve a long-term Disease Free Survival (DFS) that is the most important challenge in ALL. The role of Autologous Bone Marrow Transplantation (ABMT) and its optimal timing in adult ALL pts is still a matter of debate. We report herein a single centre experience about this procedure.

DESIGN AND METHODS: From 1992 to 2003, 24 consecutive ALL pts, without an HLA-identical sibling donor, received ABMT as consolidation therapy after remission. Pts median age was 27 years (range 15-58), 16 were males and 8 females. ALL B 17/24 and T 7/24, Standard-risk (SR) 10/24 (42 %) and High risk (HR) 14/24 (58 %). There were 5 Ph+ ALL. The median time from diagnosis to ABMT was 10 months (range 2-15). The disease status at ABMT was: 1CR in 18/24 (75%) pts, > 2 CR in 6/24 (25%) pts. Hematopoietic stem cells source was peripheral blood in 14 pts (58 %) and bone marrow in 10 (42 %). In 19/24 (79%) cases the harvest was done after priming with granulocyte colony-stimulating factor (G-CSF). Conditioning regimen: busulfan + cyclophosphamide in 23/24 (96%) cases and melphalan + TBI in 1/24 (4%) cases. The mean of CD34 positive unpurged cells reinfused was 2,82.9x10⁶/Kg.

RESULTS: With a median follow-up of 66 (6-130) months, 15 (62 %) pts are alive and in continuous complete remission and 9 (38 %) pts are death after a relapse that occurred at a median time of 10 (2,2-59) months after ABMT. The probability of OS and DFS of the whole population were 65% and 58%, respectively, at a median time from transplant of 60 months. There was a statistically significant difference in OS (P 0,001 by the log-rank test and P 0,004 by the Wilcoxon test) and DFS (P 0,0016 by the log-rank test and P 0,009 by the Wilcoxon test) comparing ALL pts transplanted in first CR with those in > 1 CR. No transplant related mortality (TRM) was recorded; transplant related complications were not important and all pts obtained a fast granulocyte recovery. No secondary MDS or malignancies were observed during the post-transplant follow-up.

CONCLUSIONS: Our experience, with the caution of the small number of cases, would confirm that ABMT might be a good, well tolerated and effective option in adult ALL pts without an HLA-sibling donor particularly for those who received ABMT in first CR. In this setting (ALL pts in 1 CR without donor) randomised and multicenter studies that compare the

efficacy of ABMT maintenance therapy versus maintenance therapy alone are needed to define the best therapeutic program after induction/consolidation treatments.

647 THROMBOSIS IN MULTIPLE MYELOMA PATIENTS

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Thrombosis is a frequent complication in cancer patients receiving chemotherapy. Mauricio Zangari's group at Arkansas University, reported an increased thrombotic risk in some multiple myeloma patients receiving thalidomide associated first line chemotherapy. The present study compared the thrombotic risk in two populations of multiple myeloma patients who were treated with or without this antiangiogenic drug. From 1987 to 2004 where included in this study 41 patients with multiple myeloma. Their ages ranged from 37 to 88 years old. 24 women and 17 men. The Durie-Salmon stage where IIIA in 35 cases and IIIB in the other 6. IgG type in 30 patients and IgA type in 11 patients. 20 of them received melphalan 10 mg/daily plus prednisone 40 mg/ daily during 4 days, as a sequential therapy, without thalidomide (group A). 21 patients received thalidomide 200-400 mg/daly as oral maintenance therapy associated with dexametasone 20 mg/daily, 4 days (group B). The occurrence of thrombotic events in this population is expressed in table 1.

One group A patient had an antithrombin deficiency associated with nephrotic syndrome, but without thrombosis. This results are by now not confirming in our initial experience a higher incidence of thrombotic events in group B, thalidomide group ($p>0.05$). In the study of Zangari et al., the association of doxorubicin / thalidomide in combination therapy as part of the Total Therapy II (TTII) for newly diagnosed multiple myeloma the authors found that low dose warfarin failed to decrease the risk of deep vein thrombosis. They recommended the employment of enoxaparin 40 mg/ sc qd during the induction phase of TTII+thalidomide to abrogate the thrombogenic effect of thalidomide. In the case of our therapeutical approach, the preventive usage of anticoagulant therapy seems to be not necessary, unless other associated prothrombotic conditions where present.

Thrombosis in multiple myeloma

Group	A	B	(p)
DVT	10%	14,3%	>0,05
PE	5%	4,7%	>0,05

DVT: deep vein thrombosis, PE: pulmonary embolism

Caption 1: Table 1

648 IMATINIB MESYLATE IN VARIOUS PHASES OF CHROIC MYELOID LEUKEMIA IN 174 PATIENTS: A SINGLE INSTITUTE EXPERIENCE

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Background: Imatinib mesylate has become a standard therapy for all newly diagnosed Philadelphia positive CML patients. In addition to the international collaborative trial data individual institute data also have its importance as newer information in terms of response and side effects may emerge.

Aim: to study the response to imatinib mesylate in various phases of Ph+ CML in a single institute.

Patients & Methods: Imatinib was administered to 174 patients with various phases of Ph+ CML in a single institute. Patient characteristics-M:F::122:52, age median 38 yrs (4-79 yrs), chronic phase 97 (55.7%),

accelerated phase 47 (27%) and blastic phase 30 (17.3%). Ninety-nine patients received prior interferon alpha therapy (including 41 with ara-C). Most (92%) adult patients received imatinib 400 mg daily (300-600 mg) and pediatric patients 100-300 mg daily.

Cytogenetic responses were evaluated by conventional method and FISH analysis in cases where metaphases were absent or variant Ph+ chromosomes were suspected.

Results: Complete hematologic response (CHR) and major cytogenetic response (MCyR) were achieved in 91.8% and 50.5% (complete 30.9% CCyR) patients respectively. In a cohort of patients (#24) with early stage (<1-yr) disease CHR and MCyR were noted in 100% and 62.5% respectively and CCyR 41.7%. In AP patients (400-800 mg daily) CHR and MCyR occurred in 55.3% and 21.3% (CCyR in 6.4%) respectively. In BP patients (400-800 mg daily) CHR and MCyR occurred in 36.7% and 23.3% (CCyR in 13.3%). Side effects were not much different from the ones reported in the literature. However, generalized hypopigmentation of the skin was noted in 73% of patients and significant weight gain in 38%. This skin changes appear unique among patients of Indian subcontinent and remained sustained on continued therapy. A few patients also developed hyperpigmentation of the skin over the maxillary area, similar to chloasma. With a median follow-up of 20 months 94% CP patients are alive while considerably inferior survival was noted in AP and BP patients.

Conclusion: In this single arm study of imatinib in various phases of CML show effectiveness of the drug as in other studies. Generalized hypopigmentation of skin in majority of patients has remained a sustained phenomenon in our cohort of patients.

649 HIGH DOSE CYCLOPHOSPHAMIDE, ETOPOSIDE PHOSPHATE AND MELPHALAN WITH PERIPHERAL BLOOD STEM CELL SUPPORT - AN EFFECTIVE CONDITIONING REGIMEN FOR RELAPSED NON-HODGKINS LYMPHOMAS

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Background:

The prognosis for patients with relapsed aggressive Non Hodgkin's lymphomas treated with conventional salvage chemotherapy remains poor. High dose chemotherapy with peripheral blood stem cell support has been shown to be superior to conventional chemotherapy in randomized controlled clinical trials.

Aims:

We attempted to design a chemotherapy regimen that could be administered over the course of a single day, enabling us to reinfuse non cryopreserved stem cells to the patient.

Methods:

Between August 1999 and July 2003, twenty five patients underwent high dose chemotherapy followed by autologous peripheral blood stem cell transplantation. All had recurrent disease following initial chemotherapy. Pretransplant chemotherapy consisted of a standard DHAP regimen for 2-4 cycles. Only patients exhibiting at least a partial response to DHAP proceeded to high dose chemotherapy. Nineteen patients were male and 6 female. Patients ranged in age from 16 to 61 years. Twenty one patients had intermediate / high grade tumours according to the Working Formulation and 4 low grade tumours. Peripheral blood stem cells were mobilized using filgrastim alone and stored in a refrigerator. Conditioning chemotherapy consisted of cyclophosphamide 5000mg/m² over 4 hours, etoposide phosphate 1500mg/m² over 4 hours and melphalan 140mg/m² over 1 hour with mesna uroprotection all given on day -2. Unmanipulated non cryopreserved PBSC's were reinfused on day 0. Patients were managed in single rooms with standard antimicrobial and blood product support.

Results:

Four patients died as a result of transplant related complications - 3 due to sepsis / ARDS and one due to a cardiomyopathy, possibly related to cyclophosphamide. Seventeen patients (68%) remain in ongoing complete remission. Median duration of complete remission has not yet been reached. Four patients experienced progression of disease, of which 2 have died.

Conclusion:

The combination of high dose cyclophosphamide, etoposide phosphate and melphalan with peripheral blood stem cell support results in durable remissions in a high percentage of patients with relapsed Non Hodgkin's lymphomas. The relatively high transplant related mortality is of concern and patients need to be carefully selected for such a treatment regimen.

650 SUCCESSFUL TREATMENT OF RESISTANT THROMBOTIC THROMBOCYTOPENIC PURPURA WITH RITUXIMAB AND CYCLOPHOSPHAMIDE

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Thrombotic thrombocytopenic purpura (TTP) is an uncommon acquired disease in adults, especially young women, characterized by fever, neurological manifestations, micro angiopathic hemolytic anemia (MAHA) and renal dysfunction. Plasmapheresis has increased survival from 10% to more than 90%. Currently, there is no standard treatment for TTP, which is resistant to plasmapheresis and high-dose steroids. We report a young woman with severe resistant chronic relapsing TTP. The patient relapsed, showing severe neurological manifestations and sepsis. Intensive treatment with plasmapheresis and high-dose steroids reversed the neurological manifestations, but she was completely dependent on plasmapheresis on an almost daily basis. In an attempt to bring the patient to complete remission, she was successfully treated with rituximab and cyclophosphamide. She is now more than two months disease free. Summary: This novel treatment modality for TTP is described only by a handful of case reports. Clearly a well-controlled clinical trial is needed to assess this treatment.

651 CONGENITAL NEUTROPENIA AND PRIMARY IMMUNODEFICIENCY DISORDERS: A SURVEY OF 26 IRANIAN PATIENTS

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Background: Congenital neutropenia is characterized by decrease in the absolute number of circulating neutrophils and increased susceptibility to infections. Although neutropenia may occur in any of the primary immunodeficiency disorders, hereditary neutropenia may only be seen in some of the above-mentioned.

Aims: The current study was performed in order to determine the clinical and laboratory findings of congenital neutropenia in Iranian patients with primary immunodeficiency.

Methods: The patients' records of 26 patients with the mean age of 11.04 ± 5.44 years -who had been referred to Childrens Medical Center, a referral center for immunodeficiency disorders- during a 22-year period (1981-2003) were reviewed. Primary immunodeficiency disorders of patients, investigated in this study, were as follows: Cyclic neutropenia (8 patients), Shwachman-Diamond syndrome (7 patients), Kostmann syndrome (6 patients), and Chediak-Higashi syndrome (5 patients).

Results: Laboratory analysis revealed that the mean of absolute neutrophil count was 398.2 ± 259.3 cells/mm³, at the time of first visit (range: 74-1152/mm³). Twenty-one patients showed severe, 4 moderate and 1 mild neutropenia. Meanwhile, 16 out of these patients had leukopenia, 7 anemia, 2 thrombocytopenia, and only one patient had monocytosis. The most common presenting complaints in these patients were oral ulcer (34.6%), otitis (15.4%), pneumonia (15.4%), diarrhea (11.5%), cutaneous abscess (7.7%), and oral candidiasis (7.7%). The median age for the onset of disease and diagnosis age were 7.5 months (1 month - 10 years) and 33.0 months (5 months - 11 years), respectively. During the mean follow-up period of 7.585.15 years, respiratory infections were developed in 92.3%, and oral manifestations have been involved in 76.9% of patients. The most common infections, in descending order of frequency, were as follow: otitis media, abscesses, pneumonia, oral ulcers, acute diarrhea, cutaneous infections, oral candidiasis, and periodontitis. Other less frequent infections were sinusitis, cystitis, conjunctivitis, meningitis, and osteomyelitis. Non-specific symptoms like hepatomegaly and splenomegaly were also detected in 38.5% and 3.8% of patients, respectively. Two patients died because of recurrent infections.

Summary/Conclusions: Persistent or severe infections always raise a suspicion, which deserves further evaluation for detecting an underlying immune deficiency syndrome and congenital neutropenia, as a delay in diagnosis may result in a serious organ damage or even death of the patient.

652 TH1 AND TH2 CYTOKINE PRODUCTION BY T-CELL FROM ACUTE MYELOID LEUKEMIA (AML) PATIENTS.

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CD4+ 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. In order to assess the influence of T-cell-response polarization on the course of the disease intracellular cytokines (IFN- γ , IL-4) were investigated in T-cells of peripheral blood in AML patients.

Methods. Th1 and Th2 cytokine production was detected in CD3+CD8- (consider as CD4) and CD3+CD8+ lymphocytes from 13 AML patients by flow cytometry analysis. Lymphocytes were isolated from the whole blood by Ficoll-Hypaque density centrifugation, activated with PMA (phorbol12-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, CD4, CD8 antigens and intracellular IFN γ , IL-4. Samples from 13 AML patients were collected at diagnosis (n=7), during remission (n=3), and at relapse (n=3). Seven healthy donors constituted the control group.

Results. There were CD3 77,56,4%, CD4 48,78,5%, CD8 17,24,5%, CD3+CD8+IFN- γ 38,86,6%, CD3+CD8-IFN- γ 12,61,8%, CD3+CD8+IL-4 7,33,6%, CD3+CD8-IL-4 6,81,4% in 7 healthy donors and there were 25,411%, 11,94,5%, 11,110,4%, 33,415,8%, 26,412,7%, 3,51,6%, 3,41,3% in 7 AML patients at diagnosis respectively. There were 60,522,4%, 31,615,2%, 24,713,5%, 46,912%, 27,77,2%, 63,8%, 7,13% in 13 AML patients at remission respectively and there were 41,424,8%, 18,99%, 20,916,2%, 46,611,4%, 18,94,8%, 14,91,9%, 14,41,2% in 3 AML at relapse respectively.

The percentages of IFN- γ producing CD3+CD8+ cells did not differ much in AML patients and in donors. The percentages of IFN- γ -producing CD3+CD8- T cells in AML patients were similar at diagnosis and in remission and exceeded such counts in healthy donors (p<0,0001, p<0,02). The amount of IFN- γ -producing CD3+CD8-cells decreased at relapse. Simultaneously prior to relapse the increase of IL-4 producing cells, both CD3+CD8+ and CD3+CD8-, was observed (p<0,01, p<0,0001). Additionally, it was registered, that IL-4-producing CD3+CD8-cells were increased prior to molecular relapse (PML-RAR α) in Acute promyelocytic leukemia (APL) patients (n=6).

Conclusion. The activation of pro-inflammatory cytokine response (Th1) was detected in AML patients at diagnosis and during remission. Th2 cytokine IL-4 increasement was registered before AML relapse and molecular APL relapse. The results provide the evidence of an altered cytokine secretion by T-cell subsets in AML patients at different time points of acute leukemia treatment.

653 ESTIMATING THE COST OF HAEMORRHAGE DURING CHEMOTHERAPY-INDUCED THROMBOCYTOPENIA IN SPAIN

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Background:

Major haemorrhage during chemotherapy-induced thrombocytopenia is a serious clinical problem with an incidence of between 2.1 and 10.2%. Platelet transfusions are the current gold standard of controlling bleeding due to thrombocytopenia associated with the treatment of haematological malignancies and solid tumours. However, concerns such as availability, cost and refractoriness, suggest the need to identify alternative strategies to reduce the demand for platelets.

Aims:

A study was undertaken in Spain to estimate the value of direct health care resources such as blood product components, in-patient stay and ICU care, used to prevent or treat haemorrhage due to chemotherapy-induced thrombocytopenia from the perspective of the third party payer.

Methods:

A thorough literature review was supplemented by a number of direct interviews with haematology and oncology specialists. Information on estimated amounts of health care resources attributable to bleeds due to chemotherapy-induced thrombocytopenia per cycle was obtained. Country specific unit costs, expressed in 2003 values, were derived from a national health care cost database. Simple models were constructed and populated with the available information and estimates

of the attributable costs according to treatment philosophy, underlying condition, bleed severity and location were estimated.

Results:

For moderate and severe bleeds (WHO type 3 or 4), blood product use and other health care resource utilisation vary according to bleed location and severity. The costs for GI bleeds were found to range between 2,690 and 30,106, the main economic drivers being the length of stay in the ICU (1 to 14 days at an average cost of 1,147 per day) and the number of units of platelets (2 to 40 with an average cost of 243 per unit based on an estimated distribution of 70:30 of platelet type (random donor to HLA matched)) according to the bleed severity (varying from moderate to severe). For pulmonary and IC bleeds mean ICU length of stay would be two days. Estimated costs of blood products, hospital stay and ICU ranged between 3,541 and 11,421 for pulmonary and IC bleeds.

Summary/conclusions:

The high associated costs in certain subgroups of patients (especially haematological malignancies with severe GI bleeds) and the significant number of patients refractory to platelet transfusions (15-40% of patients who receive multiple platelet transfusions over time) indicate that current management may be suboptimal and the need to identify alternatives. Results from studies of pharmacological alternatives currently available and new interventions under consideration need to demonstrate their impact on securing haemostasis and on reducing the demand for platelet transfusions and other health care resources.

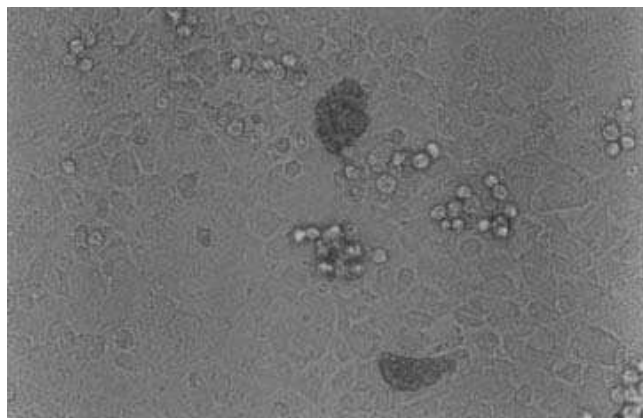
654 MOUSE LUNG CONDITIONED MEDIUM INDUCE ERYTHROPOIESIS PROCESS IN MOUSE LONG TERM BONE MARROW CULTURES.

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Dexter type mouse long term bone marrow culture (LTBMC) is a model for the study of myelopoiesis. The erythropoiesis process has no activity in this model of hematopoiesis. Mouse lung conditioned medium (MLCM), which is a source of myeloid growth factors, has been applied in myeloid progenitor assay.

In this study, various doses of MLCM were added to long term bone marrow cultures. Number of cells in adherent and nonadherent layer of cultures were counted weekly and also morphologic appearance of mature cells which were produced in nonadherent layer were analyzed. Results indicated that in the presence of MLCM, erythropoiesis was activated and mature red blood cells were appeared in nonadherent layer of cultures (fig-1). In unperturbed LTBMCs, erythropoiesis were absent but neutrophils and macrophages were produced.

There are some hematopoietic growth factors and cytokines in MLCM that they have direct or indirect effect on hematopoietic cells. MLCM don't stimulate erythroid colony formation in soft agar assay. MLCM induce proliferation and differentiation of GM-CFU's to new macrophages in long term bone marrow cultures. Macrophages as bone marrow stromal cells have important role in erythropoiesis. Activation of erythropoiesis process in treated LTBMCs may be mediated by macrophages.



Caption 1: LTBMC in the presence of MLCM, Week 3

655 THE POTENTIAL RISK OF MICROFIBRILLAR COLLAGEN IN CONJUNCTION WITH CELL SAVER DEVICES

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Background:

The authors encountered individual clinical cases with in whom microfibrillar collagen hemostat (MCH) was used during open-heart surgery in conjunction with blood saving devices who developed perioperative brain injury. The possibility emerged that these events may have been caused by embolization of microfibrillar collagen passing through micropore-filters.

Aim:

The issue was examined in vitro and in animal experiments performed on New Zealand rabbits. We found that:

Methods/Results:

1. Injection of MCH into the circulation may causes embolization of the substance to parenchymal organs and, if administered in sufficient amounts, causes the animals death.
2. MCH may pass unimpeded through blood-saving systems and pump-oxygenators.
3. Addition of low-porosity filters to such systems prevents the passage of only 93.3% 0.2% of MCH particles, and also that the filtrate maintains platelet aggregability.

Conclusions:

For the above reasons, we recommend that blood contaminated with MCH should not be returned into the patients circulation.

657 PLASMATIC ANGIOGENIC PROFILE IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS

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BACKGROUND: Angiogenesis is an established indispensable process in the development and metastasis of solid tumors. The significance of angiogenesis in the pathogenesis and progression of haematological malignancies is still to be elucidated. Research data have recently been accumulated about its role in myeloproliferative disorders (MPD). Vascular Endothelial Growth Factor (VEGF) and Hepatocyte Growth Factor (HGF) are among the most potent angiogenic promoters and their elevated plasmatic levels serve as indirect markers for increased angiogenic activity. **AIMS:** The aims of our study were: (I) to measure their levels in plasma samples in patients with MPD and to establish an angiogenic profile; (II) to analyze the correlation between their levels and some markers reflecting the tumor burden. **METHODS:** Plasma levels of VEGF and HGF were determined by ELISA in 41 patients with MPD: chronic myeloid leukemia (CML n=18), Agnogenic myeloid metaplasia (MMM n=7), polycythaemia vera (PV n=9), essential thrombocythaemia (ET n=7) as well as in 21 healthy individuals as a control group. **RESULTS:** All of the patients with MPD showed significantly higher levels of VEGF and HGF comparing to the control group (with p ranging from 0.01 to 0.001). The highest VEGF levels were determined in patients with ET (1048 pg/ml), followed by CML (701 pg/ml), PV (495 pg/ml), MMM (126 pg/ml) and control group (49 pg/ml); the HGF levels were as follows: CML (5046 pg/ml), MMM (4595 pg/ml), PV (3621 pg/ml), ET (2504 pg/ml) and the control group (761 pg/ml). We found a significant correlation between HGF and the leukocyte count ($r=0.75$, $p<0.001$), LDH ($r=0.72$, $p<0.001$), splenomegaly ($r=0.53$, $p<0.05$), blast percentage in the peripheral blood ($r=0.683$, $p<0.01$). A significant correlation was found between VEGF and platelets ($r=0.549$, $p<0.01$) in all groups of patients, and with leucocytes in CML ($r=0.588$, $p<0.05$). **SUMMARY:** Our data indicate that MPD are highly associated with elevated plasmatic levels of VEGF and HGF, which corroborates the thesis of their angiogenic dependency. According to our results, HGF could serve as a reliable marker reflecting the tumor burden as well as the activity of the disease. Such an angiogenic profile could be of benefit for clinical use as a diagnostic and prognostic method to follow-up patients with MPD. Likewise, with the initiation of angio-suppressive therapy in clinical trials, the clinicians will be faced up with the necessity of following its effectiveness through reliable and easily accessible markers reflecting the angiogenic activity.

658 TOSOH-G7 HbA2, HbF AND HbS RESULTS CORRELATE SIGNIFICANTLY WITH IN HOUSE GOLD STANDARD KONTRON HPLC

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The Tosoh-G7 with beta-thalassaemia mode is an automated high performance liquid chromatography(HPLC) system that utilizes the principle of high resolution nonporous cation-exchange chromatography column system for the fractionation and quantification of normal haemoglobin and haemoglobinopathy.

Patient samples(n=250) requested for haemoglobinopathy screen were analysed on the Tosoh-G7 and the results were compared with in-house gold standard Kontron HPLC system(Central Quality Assurance Scheme 2002 Gold Star award winner for HbA2 results). The correlation of HbA2, HbF and HbS levels between the two HPLC systems were assessed using the Mann-Whitney U test and Spearman's Rank correlation method. Data is presented as median(%) and inter-quartile range.

This study demonstrated that HbA2 levels for Tosoh-G7(2.50[2.20-2.70]) and Kontron(2.40[2.20-2.61]) correlated significantly ($r=0.979, p<0.001$). HbF levels for Tosoh-G7 (0.75[0.60-3.40]) and Kontron(0.80[0.60-1.00]) also correlated significantly($r=0.650, p<0.001$). In addition, HbS(n=30) levels for Tosoh-G7(40.0[34.0-42.0]) and Kontron(39.0[33.0-40.0]) showed a significant correlation($r=0.994, p<0.001$).

Tosoh-G7 Intra-assay C.V. was 2.5% for HbA2 of 2.60%, 2.9% for HbA2 of 6.10% and 2.3% for HbS of 40.0%.

The number of haemoglobinopathies detected by Tosoh-G7 paralleled with the Kontron HPLC. This study demonstrates that there is a strong correlation between Tosoh-G7 and Kontron HPLC for HbA2 and HbS levels.

This study indicates that Tosoh-G7 with beta thalassaemia mode is reliable and can be used as an effective HPLC system for routine haemoglobinopathy screening. Tosoh-G7 with beta thalassaemia mode is easy to use without the need of any sample pretreatment(unless it is a neonatal specimen) and user friendly HPLC system. This study demonstrates that the Tosoh-G7 HPLC system haemoglobinopathy results correlate with the in house Kontron HPLC system suggesting that Tosoh-G7 HPLC with beta-thalassaemia mode could be utilized in a diagnostic haematology laboratory for screening of haemoglobinopathies, reporting of HbA2 and HbS level.

659 THE USE OF SERUM FERRITIN AND SERUM TRANSFERRIN RECEPTORS FOR THE ESTIMATION OF THE MARROW IRON STORES IN A GROUP OF PATIENTS WITH ANEMIA, SUFFERING FROM INFECTION, CANCER AND CHRONIC DISEASE

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Background:Bone marrow aspiration is a useful estimation method of the iron deficiency that provides information on the macrophage storage iron and the iron that is aggregated in the cytoplasm of the erythroid precursors.This invasive method is painful, lacks of acceptability from the part of the patients and is expensive.Serum ferritin measurement is a non-invasive procedure for the estimation of iron stores and has become well established.Conditions such as hypothyroidism and ascorbate deficiency may decrease serum ferritin independently of iron stores.Also a number of disorders such as infections, chronic diseases and tumors may increase the serum ferritin that is an acute phase reactant.A reliable non-invasive procedure is the measurement of serum transferrin receptors (sTfr). STfr are high in iron deficiency and can be used as an indicator of tissue iron stores.

Aims:application of non-invasive methods for the assessment of the marrow iron stores.Methods: We studied 45 patients, 24 women and 21 men aged from 60 to 85 years old (M=69,15).Of the patients studied 18 had infectious disease, 14 had chronic disease and 13 had cancer.Bone marrow from each patient was stained using the Prussian blue method.We measured iron granules within macrophages and graded as 0+ if no granules were observed,1+ if small granules were seen with only oil-immersion lens,2+ if few smallgranules were visible with low-power lens,3+ if numerous small granules were seen in all marrow particles,4+ if large granules were seen in small clumps, 5+ if dense large clumps were seen and 6+ if very large deposits were seen. At the same time we estimated the percentage % of erythroblasts containing iron granules.Serum ferritin and sTfr were measured by AIA pack method of AIA 600 II analyzer. The statistical method used was non parametric Spearman's correlation.Results:The correlation of the erythroblasts containing siderotic granules and the sTfr is highly significant ($p=0,01$ Spearman's 2-tailed).Also the correlation between the macrophage iron storage and the sTfr is highly significant

($p=0,01$ Spearman's 2-tailed).The correlation between the macrophage iron storage and serum ferritin is significant ($p=0,05$ Spearman's 2-tailed). There is no correlation between the erythroblasts containing siderotic granules and serum ferritin.Conclusion: STfr is a highly correlated non-invasive method of estimating the body iron status for a cluster of disorders of patients, in which serum ferritin method doesn't work, eliminating the need of bone marrow aspiration.

660 EFFECT OF ERYTHROPOIETIN, INTERLEUKINS 3 AND 6, AND MACROPHAGE COLONY STIMULATING FACTOR ON HEMOGLOBIN SYNTHESIS IN SICKLE CELL ANEMIA

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Background: Sick cell anemia is characterized by significant morbidity and early mortality. Determination of physiologic factors that interact to regulate fetal hemoglobin synthesis is important for the design of strategies to alter globin chain production.

Aim: This study was conducted to assess serum erythropoietin level, IL-3, IL-6, GM-CSF in SCA children during steady state and their relation to fetal hemoglobin in them. The study was also aimed to assess the hypothesis that different set of regulatory might be required to maintain the level of increased hematopoiesis in HFSS and LFSS patients.

Subjects and Methods: 30 children with homozygous SCA in the steady state were involved in the study. Their age ranged from 2-10 years (mean 5.352.55 yr.). They were divided according to their level of HbF into two groups : 15 patients with HbF >9gm/dl(HFSS) and 15 patients with HbF<9 gm/dl (LFSS).15 apparently healthy age and sex matched children served as a control group. All children had complete blood count, estimation of fetal hemoglobin, and serum level of erythropoietin, IL-3, IL-6, GM-CSF .

Results: SCA patients had significantly increased levels of EPO, IL-3, IL-6,and GM-CSF than controls. HFSS group had significantly higher levels of IL-3 (69.316.1 pg/ml vs 10.4 11.3pg/ml) and LFSS group had significantly higher levels of GM-CSF (28.121.3 pg/ml vs 4.736.58 pg/ml) and significantly higher erythropoietin levels(9140.8 iu/l vs 24.96.08iu/l). There was no statistical difference in the level of IL-6 between the two groups.

Conclusion: SCA children exhibit differences in the capacity of their mononuclear and endothelial cells to produce cytokines and hematopoietic growth factors according to their peripheral HbF level and GM-CSF and IL-3 are differentially involved in the control of SCA depending on the level of hematopoietic stress. Elevated levels of cytokines is a characteristic of SCA even in the steady state and it is indicative of ongoing micro-vascular adhesion and severity of the degree of anemia.

661 INVASIVE PULMONARY ASPERGILLOSIS (IPA) IN IMMUNOCOMPROMISED CHILDREN: 15-YEAR SURVEY OF CASES FROM A SINGLE INSTITUTION

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Background: The diagnostic difficulties, significant morbidity and high mortality rate of IPA in immunocompromised hosts, as well as the toxicity and cost of treatment still challenge physicians caring for patients with this disease. Nevertheless, early diagnosis and aggressive antifungal management may improve outcome. In addition, reliable control of the infection is a prerequisite for providing curative treatment for the underlying disorder. This in turn could improve the overall prognosis.

Aims: To review our experience in the diagnosis and management of IPA in immunocompromised pediatric patients over a 15-yr period. Patients & Methods: IPA cases diagnosed and managed at the Dept. of Pediatrics, Regional Hospital, Ceske Budejovice, Czech Republic from 1.3.88 to 30.6.03 were reviewed and analyzed. The diagnosis was based on a constellation of clinical, radiological & mycological findings. Management consisted of amphotericin B (AMB), voriconazole, itraconazole and, if deemed necessary, surgery. Survival data were updated to 31.1.04. Retrospectively, the cases were classified using modified EORTC-IFICG/ NIAID-MSG criteria. Results: Among the 85 children & adolescents receiving intensive chemotherapy and/or immunosuppressive treatment for a hematologic malignancy or benign disorder during the 15-yr period from 1988 to 2003, six (5M, 1F) had IPA, i.e. 7% of all pts & 2.8% of

all episodes of febrile neutropenia. Four pts had ALL or AHL, three of whom in first relapse, and one each RAEB-T or severe HLH following EBV infection. Risk factors lasting a median of >3 to 5 wks included severe neutropenia as well as use of steroids & antibiotics. By a modified scheme of the EORTC-IFICG/NIAID-MSG consensus proposal, IPA can be considered proven in three cases, and probable, suspected or possible in one case each. *Aspergillus fumigatus* was found in four pts, and both *Aspergillus fumigatus* & *Aspergillus niger* in an additional one. AMB was given to all pts to a median total dose of 107.1 mg/kg (range: 15.5-325.75) over a median of 41 days (range: 10-83). Three pts received voriconazole as follow-on/alternative treatment or secondary prophylaxis for 66, 78 & 261 days. Itraconazole was used for primary or secondary prophylaxis in four pts 9 & 17 days or long-term, and therapeutically for 12 days in one. Both azoles were given as recommended by the manufacturer and were well tolerated. Surgery was performed in a therapeutic and/or diagnostic intent on two pts with a negative result in both. One boy died of acute necrotizing pancreatitis possibly induced by L-asparaginase with evidence of aspergillosis while in CR of ALL. A girl with relapsed AHL died of disseminated aspergillosis before achieving CR. The remaining four pts could resume the scheduled treatment including two allogeneic grafts for the underlying disease. They are alive & well 99, 62, 10 & 9 months since the diagnosis of IPA. Conclusions: Early diagnosis coupled with the prompt initiation of effective antifungal therapy can increase the cure rate of IPA. Provided the infection is reliably controlled, management of the underlying disorder including allogeneic HSCT may become feasible, thus improving the overall prognosis.

662 ACUTE MYELOGENOUS LEUKAEMIA TREATMENT IN PATIENTS OVER 60 YEARS OF AGE. COMPARISON OF PALLIATIVE AND CURATIVE THERAPY

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Introduction: Acute myelogenous leukemia (AML) is usually a disease of elderly patients, in whom the treatment is very difficult.

Goals and methods: We have performed a retrospective analysis including all consecutive patients without any selection over 60 years of age who were treated with AML in our center from 1998 till 2000. The goal of this analysis was to compare not only survival period in patients undergoing curative, palliative, and symptomatic treatment, but we have also attempted to compare their quality of life (measured by the total time they spent in a hospital after their diagnosis was determined). We have also determined the results of treatment in relation to cytogenetics, leukocytosis and whether their AML was of primary or secondary nature. Results: We have analyzed data from 69 patients (37 males and 32 females) who were diagnosed with AML (excluding AML M3). Intensive induction therapy was administered to 28 patients (median age 65 years), palliative therapy was administered to 39 patients (median age 69 years), and symptomatic treatment was administered to 11 patients (median age 73 years). Median survival from diagnosis in the curative therapy group was 4.8 months (0.03 - 46). First induction therapy reached complete remission (CR) in 32 % of patients. There are still two living patients in CR. Only one patient from our group underwent allogeneic transplant. This patient is alive disease free. Total survival period in patients who received palliative therapy was 2.7 months (0.06 - 9.5) and in patients who received symptomatic therapy it was 0.2 months (0 - 2). The difference between patients who received curative vs. palliative therapy is on the margin of statistical significance ($p = 0.05$). Patients receiving curative therapy spent in a hospital 70% (median, 11% - 100%) of their life after their diagnosis was set, and patients receiving palliative treatment 64% (median, 16% - 100%) of their life after diagnosis. Statistic analysis revealed no significant difference. Conclusion: Elderly patients with AML should receive intensive curative therapy especially in cases of disease with good prognosis. Without any selection of patients the prognosis of elderly patients with AML is poorer than published.

663 ORAL COBALAMIN THERAPY FOR THE TREATMENT OF PATIENTS WITH PERNICIOUS ANEMIA

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BACKGROUND: Standard treatment for cobalamin (vitamin B12) deficiency involves regular intramuscular cobalamin administration. It has been suggested that oral cobalamin therapy may be an effective therapy for treating patients who have pernicious anemia.

SUBJECTS AND METHODS: We studied in an open study 10 patients with well-documented cobalamin deficiency related to pernicious anemia who daily received 1000 g of oral crystalline cyanocobalamin for at least 3 months. Complete blood counts and serum cobalamin levels were determined at baseline and after 3 months of treatment.

RESULTS: After 3 months of treatment, serum cobalamin levels were increased in all 9 patients in whom it was measured (mean increase of 117.4 pg/mL; $P < 0.0000003$ compared with baseline). Eight patients had increased hemoglobin levels (mean increase of 2.45 g/dL; $P < 0.01$). All 10 patients had decreased mean erythrocyte cell volume (mean decrease of 10.4 fL; $P < 0.003$). Three patients experienced clinical improvements.

CONCLUSIONS: Our findings suggest that crystalline cyanocobalamin given orally may be an effective treatment for pernicious anemia.

664 COMPARISON OF NESTED-PCR AND COMMERCIAL ASSAYS FOR THE DETECTION OF HEPATITIS B VIRUS (HBV) CONTAMINATION OF BLOOD AND BLOOD PRODUCTS

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Background : Contaminations of Hepatitis B virus are detected usually by the detection of hepatitis B surface antigen (HBsAg) in the blood and blood products. Aims : This study aimed to evaluate the diagnostic performance of a reliable tool for discriminating 'window period' donations. Methods : Recently, our laboratory have developed an rapid and highly sensitivity detection system (Nested PCR) for screening HBV in blood and blood products. In this study describes a comparative analysis on the performances of three assays each based on a different methodology. Result : The first object was to develop a sensitive nested-PCR for the HBV DNA screening via PCR and applied to clinical specimens comparing with Hybrid-capture, PCR-ELISA. In order to validate detection limit and specificity, a diluted series of the WHO HBV international standard (97/746) were tested, and the detection limit was calculated to be 32.5 IU/ml and HBV DNA negative plasma pools showed negative. Both hybrid-capture and PCR-ELISA showed 92%, 90% positive rate for ELISA confirmed positive samples separately. The second object was to apply the method for the HBV DNA detection from blood products. With a spiking of HBV [WHO HBV international standard (97/746)] to albumin, immunoglobulins and coagulation factors, the nested-PCR method could detect up to 65 IU/ml. And, Hybrid-capture and PCR-ELISA methods could detect 16 ~ 50 IU/ml. Conclusion : As a results, it was concluded that, Hybrid-capture and PCR-ELISA assay were slightly more sensitive than nested-PCR for the detection of HBV DNA and may be used for the detection of HBV DNA. Although considerable progress has been made, nested PCR of quality control in blood and blood products remains an ongoing challenge.

665 RE-INDUCTION WITH 3+7 REGIMEN OR HIGH-DOSE CYTARABINE FOR PATIENTS WITH AML WHO FAIL FIRST INDUCTION WITH 3+7 REGIMEN

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Background: About 20% of newly diagnosed patients with AML fail to enter morphological remission following a standard 3+7 regimen of anthracycline and cytarabine. The common practice is to use the same regimen for re-induction. However, a higher dose of an effective chemotherapy such as high-dose cytarabine might be able to achieve a more meaningful remission as shown by the Australian and German studies.

Aim: To study the impact of leukemia cell kill following a repeat 3+7 induction (anthracycline and standard-dose cytarabine) or high-dose cytarabine (HiDAC) in de novo acute myeloid leukemia (AML) patients who have failed first induction with 3+7 regimen.

Patients & Methods: We analyzed the outcome in 30 de novo AML patients (between March 1997 and January 2003) who failed first induction therapy. Patients received (non randomized) either a repeat 3+7 regimen (daunorubicin 60/m sq/dx3 or idarubicin 12 mg/m sq/d x 3 + cytarabine 100 mg/m sq/d civ x 7) or HiDAC (3 G/m sq/12 hour x 6 doses on days 1,3 & 5) for re-induction. Complete responders proceeded to receive post-remission therapy with either 2 cycles of HiDAC or an allogeneic HSCT.

Results: The complete response (CR) was 40% in the repeat 3+7 regimen group and 65% in the HiDAC group (overall 80%, CR+PR) ($p =$ not significant). The toxicity and requirement for supportive care in either

regimen was not different. Following post-remission therapy, the median survival was 9.3 weeks and 37 weeks respectively.

Conclusion: The results suggest that HiDAC might be a better alternative for re-induction to a repeat 3+7 regimen in resistant AML.

666 HEMATOLOGICAL ABNORMALITIES IN LEPROSY

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Background: Leprosy is a chronic ancient disease, maybe the oldest disease known to man. It is caused by *Mycobacterium leprae* and affects primarily peripheral nerves. Damage of internal organs in this disease has been reported, including infiltration of bone marrow by *M. leprae*.

Aims: The goal of this work is to describe hematological abnormalities seen in patients with leprosy.

Methods: It has been retrospectively analyzed the medical record of all leprosy patients seen in the Walter Cantidio University Hospital, from Fortaleza City, Brazil, between January 1976 and December 2002. Statistical analysis was done by the program Epi-Info 6.04d, vs. 2001. The data are expressed in mean standard deviation.

Results: In the period of this research it has been attended 547 patients with confirmed diagnosis of leprosy of which 461 had no co-morbidity and were included in the study. We analyzed the full blood count of these patients in order to detect any abnormality. The types of leprosy, according to WHO classification, in our sample, were: tuberculoid (28%), borderline tuberculoid (11%), mid-borderline (4%), borderline lepromatous (8%), lepromatous (30%) and indeterminate (18%). The ages ranged from 4 to 87 years (mean of 18 39 years). It was 217 (47%) male and 244 (53%) female. The mean red cell count was 4,200,000 700,000/mm³, mean hemoglobin was 12.9 7.3mg/dL and mean hematocrit was 37 5%, with MCV of 88 9fL. Anemia (hemoglobin <12mg/dL) was seen in 130 cases (28%). The mean white cell count was 7,712 2,775/mm³, with neutrophils of 58 10% and lymphocytes of 27 8%. Leucopenia (<4000/mm³) was found in 10 cases (2%) and leucocytosis (>12000/mm³) in 24 cases (5.2%). The mean platelet count was 251,000 8,000/mm³. Thrombocytopenia (<150,000/mm³) was seen in 9 cases (1.9%). Pancytopenia was not found in any of these cases.

Summary/Conclusion: Important hematological abnormalities can be found in leprosy. The causes of these changes are still unknown. The dissemination of hematogenously spread *M. leprae* is an important mechanism of internal organ involvement in this disease. Anemia could be attributable to the chronic course of the disease (anemia of chronic disorders), but could also be due to bone marrow infiltration by *M. leprae*. Miliary lepromas and acid-fast bacilli have already been found in leprosy patients. The exact etiology of the disorders presented here is unknown. The patients had no other disease besides leprosy, so that the hematological abnormalities detected can be attributable to leprosy or to another medical condition still not diagnosed in some cases. Doctors who take care of leprosy patients should always look for hematological abnormalities in this population, because this is a multi-system disease that can affect not only the peripheral nerves but also internal organs, including the hematological system.

667 CYTOKINE PRODUCTION BY NORMAL HUMAN BONE

MARROW ERITHROID CELLS

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Background: As shown earlier, human fetal liver erythroid nuclear cells (ENC) with Glycophorine A (GI A) and erythroblast antigen (AG-EB) membrane markers produce cytokines.

The aim: We investigated cytokine production by the bone marrow Glycophorine A + ENC of healthy individuals in vitro as a starting point for delineating the physiologic role of linear-derived cytokines in steady state immunohematopoiesis.

Methods: The bone marrow biopsy specimens from 15 healthy individuals were used for positive selection of GI A+ ENC. Erythroid cells were isolated with the help of specific antibodies to GI A by panning procedure. Controls of purity were performed using the smears staining of hemoglobin by benzidine. ENC (106 cell/ml) were cultivated for 24 hours. Cytokine concentration in the condition medium was measured by multiplex cytokine array assay (BioPlex, BioRad, USA) using a Human 17-plex panel.

Results: Spontaneous production of IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12(p70), IL-13, IL-17, TNF-alpha, IFN-gamma, G-CSF, GM-CSF,

MCP-1, MIP-1beta by bone marrow ENC in vitro was found. The level of IL-5 and IL-7 was in the limit of methods error.

Conclusion: Normal bone marrow Glycophorine A + ENC are active constitutive producers of the number of hemo- and immunoregulatory cytokine. These data suggest that marrow erythroid progenitor are probably involved in autocrine/paracrine influence as well as local/distant regulation of immunohematopoiesis.

668 THE PLANT LOW MOLECULAR WEIGHT HEPARIN-LIKE ANTICOAGULANT PREVENT VENOUS THROMBOEMBOLISM

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Background. Low molecular weight heparin of animal origin has been reported to be an effective antithrombotic agent which does not prolong time of coagulation (Leclers et al.,1996;Bauer et al.,2001). Low molecular weight heparin from plant (peony roots) enhances the anticoagulant activity in vitro and in vivo (Lyapina et al.,1999-2002). The more of action by which heparin exerts their antithrombotic effect is not fully understood.

Aims. The purpose of the present study was to determine in vivo the prevention of venous thromboembolism in animal models by purified plant heparin (PH).

Methods. Electrophoretic, chromatographic and spectral analyses of PH were conducted. Thrombogenicity testing of albino rats (250-300 g) using the Wessler stasis venous model (model 1) and model generation of thrombin in bloodstream by intravenous injection of thromboplastin (model 2) were conducted. The PH in dose 1 mg/kg was introduced peroral for 8-10 days before using the model 1 and model 2. The antithrombotic effect of PH was measured by reduction of formation of fibrin clot and following thrombformation.

Results. It was determined by using model 1 reduction thromb weight by PH action. The weight of thrombs in this case formed only 15-25 per cent in contrast to control (100 per cent at NaCl action instead of PH). By using model 2 (generation of thrombin) PH preparation increased the survival of the animals from 95 to 100 per cent in contrast to control (survival from 0 to 15 per cent by introduction of NaCl).

Conclusion. Thus low molecular weight heparin-like anticoagulant from peony can serve as perspective antithrombotic remedy.

669 THE PROGNOSTIC VALUE OF CYTOGENETIC EXAMINATION IN ACUTE MYELOID LEUKEMIA PATIENTS IN COMPLETE

CYTOLOGICAL REMISSION

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Background: It is known that cytogenetic analysis has diagnostic and prognostic value in acute myeloid leukemia (AML) patients. South West Oncology Group (SWOG) differentiates three groups of cytogenetics, that influence on prognosis and disease course in AML. The part of patients with abnormal karyotype at diagnosis achieves cytological complete remission (CR). Not many studies estimate the cytogenetics in patients in cytological CR.

Aims: To asses the prognostic value of cytogenetic examination in acute myeloid leukemia patients in cytological CR, who have had abnormal karyotypes at diagnosis.

Material and methods: We studied 16 AML patients aged 20-69 (median 46) with cytogenetic abnormalities at diagnosis, who achieved complete CR after one course of induction chemotherapy and were still in CR after second course of consolidation. The diagnosis was established according to FAB classification. The peripheral blood morphology, bone marrow cytological, cytogenetical examination and immunophenotyping by flow cytometry were examined at diagnosis. The GTG and CBG chromosome banding was performed and chromosomal abnormalities were described according to ISCN (1995).

All patients received standard chemotherapy, consisting of 1 induction course followed by 2 consolidation courses. Second cytogenetic examination was done after two consolidation courses, in cytological CR. As a cytogenetic remission we considered normal karyotype in all examined metaphases.

Results: All the patients achieved complete CR after one induction chemotherapy.

Twelve patients (75%) obtained cytogenetic remission and 4 patients (25%) not.

Among 4 patients without cytogenetic remission - 3 relapsed after 5-25 months and died. One patient with maintaining abnormal cytogenetics (but detected in 2 metaphases only - 2.4% of all analyzed metaphases) (inv. 16(p13;q22)) is still in CR.

Among 12 patients with normal karyotype in cytological CR, 2 relapsed (17%) after 4 and 9 months. One of them died. The second one achieved cytological CR2. The remaining 10 patients are still alive in CR. Disease free survival was significantly longer in the group of patients with normal karyotype in second examination ($p=0.04719$). Overall survival was significantly higher in the group of the patients who achieved cytogenetic remission ($p=0.0443$). Considering our patients karyotypes according to SWOG we qualified them in 3 prognostic groups: favorable in 4 cases, unfavorable in 9 and intermediate cytogenetic abnormalities in 3 cases. Among 9 patients with unfavorable cytogenetic abnormalities 7 patients (78%) had cytogenetic remission (6 of them have maintained in CR up to day, one relapsed and died). Two patients (22%) did not achieve cytogenetic remission, relapsed and died.

Conclusions: The assessment of cytogenetic remission at the moment of cytological CR in AML patients with abnormal karyotype at diagnosis may have significant value for reevaluation of prognosis and influence on further therapeutic decisions.

Cytogenetic remission even in patients with unfavorable cytogenetic abnormalities according to SWOG has good prognostic value.

670 TREATMENT OF REFRACTORY IDIOPATHIC AUTOIMMUNE HAEMOLYTIC ANAEMIA WITH RITUXIMAB

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Background: Glucocorticoids are the mainstay of treatment for patients with idiopathic autoimmune haemolytic anaemia (AIHA) due to warm-reacting autoantibodies. If haemolysis cannot be controlled by a low dose of steroids, then splenectomy is indicated. Immunosuppressive therapy with cytotoxic drugs is reserved for those patients who fail to respond to glucocorticoids and splenectomy. Innovative approaches are needed for those rare patients that do not respond to conventional immunosuppressive therapy. Rituximab is a monoclonal antibody against CD20 antigens on B cells. It has been introduced recently for the treatment of some autoimmune disorders. Favourable responses to rituximab have been described in idiopathic chronic cold agglutinin disease. There are rare reports on its efficacy in adult refractory idiopathic AIHA due to warm-reacting antibodies.

Patient: We are presenting a report on a 66-year-old man, admitted to our hospital in November 1989. Laboratory data were consistent with the picture of an idiopathic AIHA. An improvement of anaemia was achieved by a treatment with glucocorticoids. In the subsequent years the anaemia had worsened especially during winter periods. At those times an improvement was achieved through a cyclophosphamide therapy with a partial resolution of hemolysis. In December 2002 the anaemia worsened again. The treatment with cyclophosphamide was unsuccessful as there was no haematological improvement during the following 3 months and the patient required multiple transfusions. He later received four weekly doses of rituximab at 375 mg/m². Red cell transfusions were stopped after the first rituximab infusion. The treatment was well tolerated. At the last follow-up, 11 months after the treatment with rituximab the patient was still in a remission, without anaemia and without laboratory signs of hemolysis.

Conclusions: Treatment with rituximab appears promising for idiopathic refractory AIHA.

671 IMMUNOHISTOCHEMISTRY DISCLOSES C-KIT EXPRESSION IN DESMOID TUMORS AS A POTENTIAL TARGET FOR SPECIFIC MOLECULAR THERAPY WITH THE THYROSINE KINASE INHIBITOR STI-571

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Background: Desmoid tumors (DT) are slowly growing invasive tumors, at high risk of relapse after surgical treatment. Conventional chemotherapy, radiotherapy, hormone-therapy or anti-inflammatory treatment demonstrated only transitory effectiveness, indicating the necessity of evaluating new therapeutic strategies such as molecular therapy with the tyrosine kinase inhibitor STI 571.

Aims: The purpose of this study was to determine the frequency of c-Kit staining in DT and to assess the clinical response rate to treatment with STI 571.

Methods: In patients diagnose with DT immunohistochemical staining for c-kit expression was performed and correlated with the response to treatment with STI 571.

Results: Immunohistochemical staining of 6 DT biopsies from different sites demonstrated 5 out of 6 (83%) to be positive with different intensities of c-kit expression. 2 of 2 pts (100%) responded to treatment, one pt had to abandon treatment due to liver damage that occurred under STI 571. The intensity of c-kit expression was correlated with the treatment response.

Conclusions: We conclude that c-kit expression was present in a relatively large subgroup of DTs and that STI 571 may offer a new therapeutic strategy to treat this tumor entity.

672 D-DIMERS IN PLASMA AND URINE IN PATIENTS WITH KIDNEY AND URINARY TRACT DISEASES.

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Background: D-Dimers are fragments of the fibrinogen molecule that are produced during the degradation process and in these cases circulate in the plasma and serum. The levels in the plasma indicate the rate of fibrinogen or fibrin degradation. They are found increased in all cases where the fibrinolytic mechanism is activated, i.e. essential fibrinolysis, intravascular coagulation, massive tissue destruction, sepsis, extended malignancies.

In malignancies the mechanism of fibrinolysis may be activated because of: a. agents that are produced by the tumor, b. microorganisms that invade the tumor, c. secondary intravascular coagulation, d. inflammatory processes around the tumor.

Aims: We studied the D-Dimers as a marker of possible activation of fibrinolytic mechanism in the plasma and locally in cases of bladder tumors and compared the levels with those of patients with pyelonephritis, lithiasis and normal.

Methods: Thirty-four patients with bladder tumors (group A: 32 males, 2 females), 4 with lithiasis (group B: 3 males, 1 female), 7 with pyelonephritis (group C: 3 males, 4 females) and 7 normal individuals (control group: 5 males, 2 females) were included in the study. D-Dimers were measured in the plasma and 3 hours urine specimen. Immune nephelometric assay (Biomerieux, France) was used.

Results: In group A D-Dimers were found increased in urine compared with controls ($p<0.001$) but in plasma there was no difference with the controls ($p=0.2$). In group C the D-Dimers were increased both in urine and plasma compared with the control group (<0.001 and $p<0.005$). There was no difference in urine D-Dimers between group A and C ($p=0.5$) but in plasma D-Dimers were significantly increased in group C compared to group A ($p<0.001$). In group B urine D-Dimers were significantly increased compared to the controls ($p<0.001$) but significantly lower compared to group A and C ($p<0.001$ for both). In plasma the D-Dimers in group B were similar to controls ($p=0.07$).

Conclusion: The increased levels of D-Dimers in urine suggest activation of fibrinolytic mechanism locally and may be used as differential and follow-up marker in these patients.

673 TRANSIENT PML-RARA-POSITIVITY IN COMPLETE REMISSION OF ACUTE PROMYELOCYTIC LEUKEMIA

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An identification of acute promyelocytic leukemia (APL) patients who are at high relapse risk is a very hard task. In this light minimal residual disease (MRD) assessment by sequential RT-PCR analysis has been shown to be the most valuable prognostic tool.

A longitudinal RT-PCR monitoring of APL is conducting from 1990s. As a result of these studies the terms molecular remission and molecular relapse were created. Molecular remission means a negativization of the PCR test after treatment and subsequent long-term absence of hybrid transcript (PCR-negativity). Molecular relapse means a recurrence of the PML-RARa transcript i.e. conversion from PCR-negativity to PCR-positivity with following persistence of PCR-positivity. As a rule, a molecular relapse precedes to hematological one for a few months.

It is noteworthy that PCR-positivity can be transient and cannot obviously proceed to overt relapse.

Our experience shows that transient PCR-positivity in complete remission (CR) of APL is not exclusively rare event. This phenomenon is not completely clear and needs to be studied.

We performed serial nested RT-PCR assays during 7-42 months of APL CR with 2-3 months intervals using BIOMED-1 recommendations. PML-RARa transcript was detected in one or two subsequent samples of 5 out of 26 patients studied during CR. No treatment intensification was performed after PCR-positive test. Three of the patients are in CR yet (9+, 22+ and 23+ months after positive test). The fourth patient showed transient PCR-positivity twice: in one sample at the 7th month of CR and one more sample after 9 months interval of PCR-negativity. The fifth patient has demonstrated PCR-positivity at the 8th month after the treatment beginning and after interruption of maintenance treatment due to viral hepatitis. In the patient molecular remission recovered after treatment prolongation. After next 18 months the transcript was newly detected and one month later a hematological relapse was diagnosed. No approach exists yet to distinguish a transient PCR-positivity from molecular relapse but first of them needs treatment intensification to avoid hematological relapse. Detection of PML-RARa transcript in two subsequent samples cannot be used as an absolute relapse predictor. To resolve this problem additional randomized studies with large multicenter patient series using quantitative RT-PCR are needed.

674 EARLY INTERVENTION WITH EPOETIN ALFA DURING PLATINUM-BASED CHEMOTHERAPY

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Background: Epoetin alfa (EPO) is effective in treating moderate to severe chemotherapy-associated anemia (CAA). Further research is required to clarify its role in treating mild CAA.

Aims: This multicenter randomized trial assessed the effect of EPO on hemoglobin (Hb) level, transfusion requirements, and quality of life (QOL) in patients with mild CAA.

Methods: Patients with Hb ≤ 12.1 g/dL scheduled to receive 2 to 6 more cycles of platinum-based chemotherapy for a solid malignancy were randomized (2:1) to EPO 10,000 U three times weekly (TIW) or best supportive care (BSC). If after 4 weeks of EPO therapy Hb was < 12.1 g/dL and Hb increase was < 1 g/dL, the EPO dose was increased to 20,000 U TIW. Functional Assessment of Cancer Therapy-Anemia (FACT-An) and Cancer Linear Analog Scale (CLAS) scores after 2 cycles of chemotherapy (QOL-2) and at study completion (4 weeks after the last cycle of chemotherapy; QOL-end) were compared with QOL at baseline.

Results: A total of 315 patients (211 EPO and 104 BSC) were enrolled. There were no statistically significant differences between groups in gender, age, and tumor type. The mean study period was 97 days for EPO and 102 days for BSC. Median baseline Hb was similar between groups (10.7 g/dL and 10.8 g/dL, respectively). Hematologic response was significantly higher in the EPO group. The EPO group, compared with the BSC group, had a significantly ($P < .05$) greater percentage of correctors (Hb > 12.1 g/dL and not transfused in previous 28 days) [67% vs 30%], responders (Hb increase > 2 g/dL and not transfused in previous 28 days) [69% vs 31%], and hematopoietic responders (corrector or responder) [76% vs 45%]. The percentage of patients transfused during the study was significantly lower in the EPO group than in the BSC group (37% vs 65%; $P < .05$). QOL was comparable between groups after 2 cycles of chemotherapy (QOL-2), but both FACT-An and CLAS scores were significantly higher in the EPO group at QOL-end. The mean change in QOL (standard deviation) is presented in the table.

Conclusions: EPO therapy for mild anemia results in significant increases in QOL and Hb and reduces transfusion requirements in patients receiving platinum-based chemotherapy.

QOL Scale	EPO	BSC
CLAS QOL-2 (mm)		
Energy	2.4 $\pm$ 23.6	0.2 $\pm$ 24.6
Activity	1.0 $\pm$ 21.9	2.5 $\pm$ 22.5
Overall QOL	0.2 $\pm$ 22.7	-0.8 $\pm$ 21.7
CLAS QOL-end (mm)		
Energy*	6.2 $\pm$ 25.3†	-6.9 $\pm$ 29.6
Activity*	4.4 $\pm$ 26.8†	-5.3 $\pm$ 26.0
Overall QOL*	5.3 $\pm$ 24.1†	-7.3 $\pm$ 27.6
FACT-An (points)		
QOL-2	1.6 $\pm$ 23.9	1.6 $\pm$ 23.6
QOL-end†	4.0 $\pm$ 26.8†	-3.7 $\pm$ 24.4

QOL, quality of life; SD, standard deviation; EPO, epoetin alfa; BSC, best supportive care; CLAS, Cancer Linear Analog Scale; FACT-An, Functional Assessment of Cancer Therapy-Anemia.

* $P < .05$ for EPO vs BSC.

† $P < .05$ for increase in QOL-end score vs baseline.

Caption 1:

675 EFFECTS OF ANAGRELIDE ON PLATELET FACTOR 4 AND VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA : A PRELIMINARY REPORT

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Thrombocytosis and platelet hyperaggregability are characteristic features of essential thrombocythemia (ET) and are closely linked to thrombotic complications which represent major causes of morbidity and mortality. In consequence, thrombocythemia control needs cytoreductive and antiaggregant treatment with alkylating agents, hydroxyurea (HU), interferon- α (IFN- α) and aspirin (ASA), indobufen (IND), ticlopidine (TIC), dipyridamole (DYP), respectively. Anagrelide (A), a new oral imidazoquinazoline compound, was shown to reduce the platelet count and inhibit platelet aggregation in a dose-dependent fashion by interfering with megakaryocyte maturation and through an inhibition of platelet cyclic adenosine monophosphate (cAMP) phosphodiesterase and enzyme phospholipase A2. Experimental and clinical studies have demonstrated that platelet-decreasing doses have no effect on platelet aggregation. Therefore, we conducted a study consisting of 11 patients (6 males and 5 females, mean age 54 years) affected by ET diagnosed according to PVSG criteria. All patients were on antiaggregants either ASA (3 patients) or IND (5 patients) and IND+DYP (3 patients). Their mean duration of disease was 4.2 years. After a median time from diagnosis of 24 months all patients were started on cytoreductive therapy either HU (4 patients) or IFN- α (3 patients) and A (4 patients). HU was used at doses ranging between 1 and 1.5 g/day. The dosage of IFN- α was 3 MIU, 3 days/week. A was initially administered in dose of 0.5 mg/day, with increases of 0.5 mg/day every 7 days until the platelets began to drop below $500 \times 10^9/L$ and with a average maintenance dosage of 1.5 mg/day. Platelet count, platelet factor 4 (PF4), as a marker of platelet activation, and vascular endothelial growth factor (VEGF), as a cytokine released by activated platelet, were determined before cytoreduction and to complete response defined as platelets $< 500 \times 10^9/L$. PF4 and VEGF were measured by ELISA. Before treatment all patients had marked platelet count ($1061203 \times 10^9/L$) and higher PF4 and VEGFPLT (13466 IU/ml and 1.607 pg/ml, respectively) than controls (2.11 IU/ml and 0.2008 pg/ml, respectively) ($p < .0001$ and $p < .0001$, respectively). After a median time of 3 months from cytoreduction all patients had platelets $< 500 \times 10^9/L$ ($41456 \times 10^9/L$), whereas PF4 and VEGFPLT were elevated (12570 IU/ml and 2.719 pg/ml) in the HU- and IFN- α -treated patients and normal in the A-treated patients (4.209 IU/ml and 0.403 pg/ml, respectively) and there was a significant difference ($p = 0.008$ and $p = 0.054$, respectively). These measurements were repeated in another sample collected after 1 or 2 months and showed concordance for PF4 and VEGF concentrations. No correlation there was between PF4 and platelets, whereas a positive correlation we found between PF4 and VEGF ($p = 0.044$). These preliminary data might suggest that A at doses administered in daily practice may have anti-aggregating effect. In light of this encouraging information A may represent a very attractive first line therapy in ET.

676 INCIDENCE AND CLINICAL CHARACTERISTICS OF CHRONIC MYELOID LEUKEMIAS IN UZBEKISTAN: 10 YEAR RETROSPECTIVE STUDY

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Background: Chronic myeloid leukemia (CML) is a malignant stem cell disease in which the BCR/ABL oncogene is responsible for enhanced survival of leukemic cells. The aim of study was to analyze epidemiological and initial clinical characteristics of 303 patients with CML, primary diagnosed and treated at our Institute of Hematology since Jan 1990 through Jan 2000. Results: There were 139 males with a median age of 51yrs (range 17-88) and 164 females with a median age of 48 yrs (23-81). In common structure of main type of leukemia in Uzbekistan CML consists about 20-25%. The rural population was affected more than urban, the high risk of development CML was in cotton growing areas. CML were revealed frequently among women, male: female ratio was 1: 1.2. Distribution of CML among ethnic group was in the following way: in native Uzbek population it consisted 27%, in Kazakh population-29%, in Slavonic population 20%, in Korean population-24%. The chronic phase CML ($< 15\%$ blasts or basophiles in the peripheral blood or bone marrow) was diagnosed in 61(21%) patients, the acceleration phase CML ($> 15\%$ but $< 30\%$ blasts in peripheral blood or bone marrow, or $> 30\%$ blasts plus

promyelocytes in peripheral blood or bone marrow, or >20% basophiles in peripheral blood or bone marrow) was in 187(62%) and blast crisis (>30% blasts in the peripheral blood or bone marrow) was in 51(17%) respectively. Clinical features were characterized in 291(96%) cases by splenomegaly, in 269(89%) cases by abdominal pain, in 169(56%) cases by ossalgia, in 175(58%) by asthenia. Hemorrhagic syndrome was revealed in 57(19%) patients, anemia in 181(60%) patients, median hemoglobin value was 97.4±2.6 g/l, median RBC count ' 3.6±0.1x10¹²/l, median WBC count ' 148.2±12x10⁹/l and median platelet count ' 244±1.2x10⁹/l. Conclusion: This retrospective study revealed correlation between incidence and age, sex, race distribution, unfavorable ecological exposure, and will be useful in the creation of the national register of haemoblastosis. Further studies in potential therapeutic strategy in CML are warranted.

677 VYGON CATHETER IN 302 CANCER PATIENTS: COSTS, SAFETY, AND COMPLICATIONS.

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Background: In cancer patients (pts), the use of central venous catheters (CVC) has markedly increased during the past decades; CVC facilitates chemotherapy, transfusions, parenteral nutrition, and blood sampling. Several types of CVC have been introduced in clinical practice; they are classified as partially or totally implantable. These catheters are often positioned with a surgical approach, and are usually very expensive. Since February 2000, to minimize costs and reduce the invasiveness of the insertion procedures, we used a tunnelized catheter (Vygon) with no dracon cuff and antireflux valve. Vygon is less expensive and can be easily inserted under fluoroscopic or ultrasonographic guidance, and removed by all clinicians.

Aim : To examine costs, safety, as well as early and late complication rates of this device for long-term chemotherapy delivered in a large cohort of pts.

Methods: Vygon catheters were placed on the right side under local anaesthesia in the outpatient angiographic room. The average costs per patients for Vygon positioning was \$ 250.

Between February 2000 to October 2003, 458 Vygon were placed in 302 pts (166 male, 136 female) resulting in a total of 59580 days in situ (mean, 130 days, range 2-598). Median age of pts was 51 years (range 19-78). One-hundred eight-nine (62.5 %) had haematological malignancies and the remaining 113 (37.4%) were affected by solid tumors; 177 pts required venous access for high-dose chemotherapy (HDT) followed by peripheral-blood stem-cell transplantation (PBSCT). The devices still in situ at the end of follow-up were 32 (6.9%). Results: We did not observe any catheter-related deaths in this series. Early complications included six pneumothoraces (1.3% of devices; no tube-thoracostomies were applied), and twelve hematomas (2.6%), (overall early complications in 18 patients). No case required an early revision of the implant for malfunction of the catheter due to a narrowing of the lumen or primary dislocation.

Late complications included 13 cases (2.8%, 0.21 episodes/1000 days of use) of venous thrombosis, and 12 cases (2.6% of devices, 0.20 episodes/1000 days of use) of catheter-related bacteraemias. The infections were caused by coagulase-negative *Staphylococcus aureus* (8 cases), *Pseudomonas* (three cases), *Escherichia Coli* (one case); Vygon removal was necessary in four of 13 cases. There were also two cases (0.4%, 0.03 episodes/1000 days of use) of catheter rupture and embolization. Overall, 68 (15.9 %, 1.1 episodes/1000 days of use) CVCs were prematurely removed for accidental loss.

The total cost per patient, consisting of the costs of purchase and implantation, treatment of early and late complications, and maintenance of the device, was US\$ 535.

Conclusion: Vygon catheters do not seem inferior to other commonly-used peripheral devices in terms of early and late complications. Weighed against the low cost of the device, the higher risk of accidental loss makes Vygon-catheters more suitable for short-term chemotherapy, such as HDT followed by PBSCT.

678 LEVELS OF INTERLEUKINS AS A RESPONSE PREDICTOR FOR THE IMMUNOSUPPRESSIVE THERAPY IN PATIENTS WITH APLASTIC ANEMIA

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Background: Aplastic anemia is one of bone marrow failure disorders and either immunosuppressive therapy (IST) or allogeneic hematopoietic stem cell transplantation can recover hematopoiesis. The response rate is range between 30% and 60% to the conventional IST. Further, there has been no specific marker of better response to the IST which we can confer before, during, or even after therapy.

Aims: We investigated to see the evidence for which factor is useful as a predictive marker of response to the IST in patients with aplastic anemia.

Methods: Serum levels of tumor necrosis factor-alpha, interferon gamma, interleukin-2, interleukin-4, interleukin-6, interleukin-10 were analyzed. Antithymocyte globulin (ATG) was administered for 5 days in combination with methylprednisolone in addition to short-term G-CSF. Cyclosporin A was administered for at least 6 months. Total number of patients were 38. Hematologic response was evaluated 2-6 months after the IST. FACS analyses were done at each time point of pre-ATG and post-ATG.

Results: Lower level expressions of interleukin-10 and interleukin-6 at day 7 post-ATG were closely related to the favorable clinical responses. Also, higher level expressions of interleukin-4 and interleukin-6 pre-ATG showed the same responses.

Conclusions: In this study, we could detect some of abnormal or different cytokines expression profiles in the point of clinical responses to IST in patients with aplastic anemia. Further sequential monitoring of various cytokines is needed to verify the complicated influential network in the future.

679 MOLECULAR BIOLOGIC DIFFERENCES BETWEEN YOUNG AND ELDERLY ACUTE MYELOGENOUS LEUKEMIAS ON THE BASIS OF RESPONSES TO INDUCTION CHEMOTHERAPY

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Background: Elderly AML patients have been excluded from the chemotherapeutic trial due to various poor clinical conditions. We have indulged in searching the way to achieve maximal treatment effect within the limit of minimal toxicity using the most appropriate chemotherapy. Our interest is in this question: Is there any molecular biological differences in leukemic cells between younger and older AML patients?

Aims: We investigated the clinical responses along with focusing on some of apoptotic responses during the induction chemotherapy.

Methods: As one of molecular searches for the possible explanation of unresponsiveness to treatment, level of Fas and FLIP expressions concomitantly with apoptotic responses during the induction chemotherapy was evaluated. Seventy six patients including 42 of young age and 34 of old age were enrolled. Among them, 64 patients agreed to receive an induction chemotherapy. All samples were obtained at initial diagnosis before therapy and at day 7 of chemotherapy from all patients accepted to either way of treatment for young (less than 50 years) or old age (above 50 years) groups. Leukemic blasts were enriched by density gradient method and assays were performed with either fresh cells or after cryopreservation and thawing. Apoptosis was assessed by Annexin V/7-AAD binding method. Western blotting and RT-PCR of Fas and FLIP mRNA from leukemic cells which were collected from bone marrow or peripheral blood samples were assessed.

Results: CR was achieved in 48 patients (75%), specifically 64% for old age group and 81% for young age group. Generally, CR was strongly correlated with higher proapoptosis/apoptosis by flow cytometry, while not for 16 cases of IR (p < 0.01). There were no age-group specific differences in the level of expressions of Fas, FLIP according to clinical response, that is, CR vs IR. We discovered various intracellular FLIP levels or extracellular Fas expressions during chemotherapy, but those were not well correlated with clinical response at all.

Conclusion: Although we could not find definite difference between the young and the old age groups, this monitoring strategy might be feasible to get the prognostic clue if we get large sample numbers and it is necessary to further expand our study to address any role of specific apoptotic molecules during the time course of treatment.

680 IRON DEFICIENCY AND IRON OVERLOAD IN AN ADULT POPULATION OF CATALONIA

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Background: Some epidemiological studies have shown that iron overload may be more frequent than iron deficiency.

Aims: The objective of this study was to ascertain the frequency of iron deficiency and iron overload in an adult Catalan population

Patients and methods: Multiple iron measures, including transferrin saturation (TS) and serum ferritin (SF) were calculated in a representative sample of 1296 adults.

Results: The prevalence of iron deficiency was 5,6% (IC95%: 4,4 a 6,9) (SF< 12 microg/L) and 9,3% (IC95%: 7,7 a 10,9) had an iron overload (SF> 300 microg/L in men and SF> 200 microg/L in women). Iron deficiency was especially frequent in women < 50 years (14,8%; IC95%: 11,4 a 18,1), while in men of the same age it was 1,1% (IC95%: 0,1 a 2,1), but there was 11,7% (IC95%: 8,7 a 14,7: homes) of iron overload. In the population over 50 years there was an iron deficiency of 0,9% (IC95%: 0,0 a 1,8), and an iron overload of 15,1% (IC95%: 11,7 a 18,4). A 1,6% (IC95%: 0,9-2,3) of all population and a 3,9% (1,4 A 6,4) of men > 50 years had a SF > 500 microg/L.

Conclusions: Iron overload is more prevalent than iron deficiency in Catalonia, particularly in men and people over 50 years. Causes and effects of the disorder should be investigated in order to carry out corrective measures in the future.

681 QUANTITATIVE MORPHOLOGICAL PARAMETERS OF ERYTHROBLASTS ARE USEFUL FOR DIFFERENTIAL DIAGNOSIS IN HEMATOLOGIC DISEASES

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Background: Dyserythropoiesis is an important criterion for the diagnosis of myelodysplastic syndromes (MDS). Its evaluation is routinely done by subjective examination of May-Grünwald-Giemsa stained bone marrow (BM) smears.

Aims: to study the utility of several morphometric and granulometric parameters of erythroblast nuclei in order to objectivate the diagnosis and make differential diagnosis between MDS and non-clonal disorders.

Material and Methods: bone marrow smears of 27 controls, 40 MDS patients, and 22 cases of megaloblastic anemia were included in the study. Diagnosis of MDS was based on FAB criteria. Gray-scale transformed digitalized images of erythroblast nuclei were analysed for nuclear area, perimeter, longest chord. Optical density was also determined. Granulometry was applied using gray level height of the basins as a filter parameter. The number of residues and their mean area was registered for each gray level.

Results: Area, longest cord, perimeter, form factor, mean transversal diameter, as well as the mean optical density and its standard deviation discriminated well between erythroblasts of the 3 groups. These parameters could not distinguish erythroblasts of patients with refractory anemia (RA) from those with refractory anemia with ring sideroblasts (RARS), but both were different from refractory anemia with excess of blasts (RAEB) and megaloblastic anemia. These both were similar. However, applying granulometric features, significant differences between the groups could be seen for nearly all mean area levels and for the lower levels of the number of residues. Granulometry was not able to differentiate between erythroblasts of RA and RARS patients. Standard morphometric variables could not distinguish between RAEB and AM. Yet, the area of granulometric residues (levels 20-40) distinguished clearly between both entities.

Conclusion: the combination of usual morphometric parameters and granulometric features are very useful to distinguish normal BM, deficiency anemia and several types of MDS. Supported by FAPESP and CNPq.

682 BIOLOGICAL AND CLINICAL FEATURES OF T-BIPHENOTYPIC ACUTE LEUKEMIA: REPORT FROM A SINGLE CENTRE

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BACKGROUND. Various reports have shown that 20 to 30% of patients with acute leukemia have a simultaneous expression of myeloid and lymphoid markers on blast cells. This cases are now known as

biphenotypic acute leukemias (BAL). Adult T-biphenotypic leukemia is rare and its biological and clinical characteristics are poorly defined .

AIMS AND METHODS. We have reviewed our file of acute leukemias, both lymphoblastic and myeloid, to sort the BAL and, among them, the cases co-expressing T-lymphoid and myeloid markers.

RESULTS. In the last ten years, we have diagnosed 280 acute leukemias, 202 myeloid (LAM) and 78 lymphoblastic (ALL). Using the criteria recently proposed by the EGIL, 26 (9%) patients had a BAL. In accordance with the published experiences, the most common BAL phenotype consisted of co-expression of myeloid and B-lymphoid markers, but three of these patients (11.5%) had a T-BAL.

Two of the patients were women. Median age at diagnosis was 58 years (range: 26-60). According to the FAB classification, all the patients had a type 2 ALL, with a score of at least 2 for both T-lymphoid and myeloid markers. Cytogenetic analysis was available for two patients. Both showed a deletion of chromosomes 7 and 12, plus other abnormalities; no Ph+ metaphases were documented. When karyotype was missing, BCR-ABL transcripts were not detected by molecular biology.

Two patients (#2 and #3) received and ALL-designed induction therapy (vincristine, idarubicin prednisone and asparaginase). One patient died during induction due to hemolytic-uremic syndrome and pneumonia. The second patient (#3) achieved a complete remission (CR) that was consolidated with two courses of therapy (high-dose cytarabine, then vincristine, methotrexate, cyclophosphamide and adriamycin). She relapsed after five months, attained a second CR with a salvage therapy (liposomal daunorubicin and high-dose cytarabine) but relapsed again after four months and died of cerebral haemorrhage.

Patient #1 displayed an undifferentiated morphology, with two blast populations. He received an AML-like course with idarubicin and low-dose cytarabine. Day +14 bone marrow was still completely blastic, but with more distinctive lymphoid features. He therefore was switched to our ALL protocol, attaining a CR and subsequently underwent an autologous bone marrow transplant. He is actually alive in CR nine years after transplant.

CONCLUSIONS. Our small experience partially confirms the findings of a work recently published. Our patients were morphologically classifiable as L2 and presented superficial adenopathies, but none had mediastinal involvement. None of our patient was Ph+ or BCR-ABL+, but two cases displayed an unfavourable cytogenetics, with deletions of chromosome 7. In a previous report, four out of six patients with T-BAL had a complex karyotype and none was Ph-positive.

Treatment of BAL remains controversial and these forms have generally a bad prognosis. We observed a good response when a mixed or 'sequential' AML/ALL induction therapy was used, possibly followed by intensification with transplant. When an ALL-type course was used alone, a CR was obtained but it lasted for a short period, and also a salvage therapy was only transiently effective.

684 CHRONIC LYMPHOCYTIC LEUKEMIA: CORRELATION AMONG CYTOMETRY, MORPHOLOGY AND MOLECULAR ANALYSIS: PRELIMINARY RESULTS

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A correlation among morphology, immunophenotyping profile and cytogenetic abnormalities in chronic lymphocytic leukemia (CLL) has already been reported (Krober et al, Blood 2002;100:1410).A strong association has been found between trisomy 12 and increased percentage of atypical lymphocytes and/or prolymphocytes in CLL (Matutes et al, Br J Haem 1996;92:382; Lanza et al, Leuk Res 1992, 16:639). Aim of this study is to evaluate cytogenetic predictivity of automated analysis of peripheral blood and optical microscope evaluation in patients with CLL.

Thirteen B-CLL patients were divided into two groups:

- group A: N5 patients (3F, 2M), median age 64 (61-69), at observational stage 0/A;
- group B: N8 patients (4F, 4M), median age 66 (41-73), at any stage different from 0/A.

The cytometric, morphological, interphase fluorescence in situ hybridization (FISH) and immunoglobuline heavy chain variable region (VH) mutation status analysis were independently conducted in both groups.

FISH was used to detect 11q-, +12, 13q-, 17p- and t(11;14) in interphase nuclei from 13 patients. Nine patients (69,2%) had a FISH anomaly: 4 patients showed 13q-x1, 2 patients 13q-x2, one patient +12, one

patient 17p-. Nobody showed 11q-, while one patient carried a complex karyotype: 13q- and 17p- in 70% of nuclei, t(11;14) in 50% of nuclei. The VH mutation status was evaluated in 11 patients, 7 resulting mutated, 4 unmutated. Six out of seven mutated patients showed 13q-. Among unmutated patients, one has 17p-, one +12, one the complex karyotype. All unmutated patients were in group B. The morphological and cytometric features in 12 cases showed some differences among groups of patients with similar cytogenetic abnormalities.

Three patients with normal karyotype and one with 17q- in 12% of nuclei showed a population of small mature B lymphocytes, corresponding to a little spike of large unstained cells (LUC) in the ADVIA cytogram. Six patients showed a double population of small and medium sized lymphocytes with mature chromatin but a more abundant cytoplasm, represented in the ADVIA cytogram by a more high spike of LUC and the flag atypical lymphocytes from + to ++. 5 of these patients had 13q-. The patient with +12 showed, as expected, the atypical morphology associated to this cytogenetic anomaly, as well as a very high spike of LUC and the+++ flag for atypical lymphocytes. The patient with a complex karyotype showed a lymphocyte population with large cytoplasmic protrusions and rare lymphoid elements with dispersed chromatin. The ADVIA cytogram showed a very high spike of LUC and the presence of two flags for atypical lymphocytes (+++) and blasts (+). The immunophenotype study confirmed some abnormal features in these lymphocytes, such as bright SIg positivity and expression of CD11c, CD62L, FMC7 together with CD5 and CD20.

These data suggest a relationship between morphological and cytochemical features and a corresponding cytogenetic profile. A more extensive study is needed to confirm these data.

685 IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDHOOD - CLINICAL PRESENTATION AND MANAGEMENT

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Acute childhood Immune Thrombocytopenic Purpura (ITP) is a common pediatric disease characterised by sudden onset of profound, isolated thrombocytopenia in an otherwise healthy child.

The purpose of the present study was to review the clinical course of all children with ITP administered over a five-year period (from January 1998 to December 2002) to the Pediatric Clinic in Skopje which is the only institution that provides tertiary care and copes with this problem in our country. Ninetyeight cases were identified, indicating an incidence of 4,1 per 100000 children under 15 years. The sex ratio (female/male) was 1,3:1. There was a peak in occurrence of childhood ITP during spring (32,6%) and a nadir in autumn (15,3%). Sixtytwo of the patients were at the age between 2-10 years. Fiftytwo percent presented with cutaneous signs only, and 39% had plus mild mucous membrane bleeding a few weeks following an upper respiratory track infection or other infection. Physical examination was normal other than petechiae and bruising, just 8,1% had serious bleeding symptoms: nosebleeds requiring nasal packing and increased menstrual bleeding. No patient suffered an ICH or severe bleeding requiring transfusion. The mean platelet count on admission was 21X10(9)/L, lowest count 3X10(9)/L. Bone marrow aspiration was performed in all cases. Almost all (93,8%) received glucocorticosteroids (GS), just 3% (infants aged 2-4 months) received intravenous immunoglobulins (IVIG) and 3% were not treated. Side effects were noted in 5% of the children treated with GS. All the children benefit from the therapy. Very high percent of the children in our study treated with GS is due to the limited economic capabilities of the country.

We can conclude that ITP is a benign disease with practically very low percent of serious bleedings.

686 RECOMBINANT ACTIVATED FACTOR VII FOR TREATMENT OF ACQUIRED HAEMOPHILIA-CASE REPORT

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Background: Acquired haemophilia is a very rare coagulation disorder caused by factor VIII autoantibodies. The recombinant human F VIIa is a therapy of choice in acquired haemophilia.

Case report: A 53-year old woman was admitted urgently to the hospital because of spontaneous appearance of haematoma in the region of neck and extremities. There was a history of treated bullous pemphigoid two months before this admission, for which she had been receiving

corticosteroid therapy and dapsone. At the time of admission there was a large haematoma of the floor of the mouth and submandibular regions, a large subcutaneous haematoma of left upper arm, and small subcutaneous haematomas of lower extremities. There was a prolonged APTT of 48 s (Ratio 2.18), F VIII C: 0,40 U/ml, and normal other screening test of haemostasis. The patient received the infusions of three doses of fresh frozen plasma. During the first twelve hours of admission there was an enlargement of haematoma on the neck with compression of upper respiratory system and development of inspiratory stridor. The urgent tracheostomy was made which allowed respiratory function, but provoked intensive bleeding from the tracheostomy site and severe posthaemorrhagic anaemia (RBC 1,49 x 10⁹/L, Hb 46 g/L, HCT 0,13 L/L). The diagnosis of acquired haemophilia was made on the basis of haemostasis tests: APTT 48 s (Ratio 2.18), F VIII C: 0,03 U/mL, FVIII antibodies 2.25 Bethesda U, other haemostasis tests were normal (PT, TT, fibrinogene, vWAg, lupus anticoagulant, F IX, XI, XII, antibodies to F IX, XI, XII). The patient received rhVIIa (NovoSeven) 90 mg/kg BW every 2 h 3 doses, then every 4 h two doses first day of therapy and the bleeding was stopped. Treatment was continued in doses of 75 mg /kg BW every 6 h the second day, then 60 mg/kg BW twice daily three days and 40 microgram/kg BW twice daily 7 days. From the first day of therapy the patient received prednisone 2 mg/kg BW and azathioprine 2 mg/kg BW during ten days without obvious effect, and on the 11th day of admission got Cyclophosphamide 1000 mg + Vincristine 2mg followed by two plasmapheresis. Control coagulation investigation on the 16th day of admission shown normalization of all results. The patient was discharged with recommendation for tapering prednisone + azathioprine treatment. The repeated coagulation tests after discharge were normal. Conclusion: The rhVIIa treatment was successful in controlling life threatening bleeding with no adverse effects in the case of acquired haemophilia.

687 CONTRAST-MEDIA INDUCED HEMORHEOLOGICAL ALTERATIONS IN NIGERIAN INTRAVENOUS PYELOGRAPHY (IVP) PATIENTS.

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BACKGROUND: Intravenous pyelography is the injection of a radio-opaque liquid (contrast media) into the bloodstream which is first excreted by the kidney, opacifies the renal parenchyma and later the collecting system. This provides important structural information and also reveals the presence or absence of excretory function. There has been several reports of renal blood flow reduction due to contrast media injection. There is paucity of information about possible haemorheological changes in these patients.

AIM: This study therefore is to ascertain the possible effects of the media on the flow properties of blood.

MATERIALS AND METHODS: Ten selected patients among those going in for IVP who consented, were studied. Their blood samples were collected before (Pre-IVP), during the procedure (Pro-IVP) and after the procedure (Post-IVP) for analysis of packed cell volume(PCV), whole blood viscosity (WBV), plasma viscosity (PV), plasma fibrinogen concentration (PFC) and euglobulin lysis time using Standard methods.

RESULTS: There were no statistical significant differences in PCV, WBV and PV in both Pre and Post-IVP samples but an enhanced fibrinolysis and a concomitant decrease in PFC in the Pro-IVP samples were observed (P<0.005) respectively while. The Pro and Post-IVP samples compared exhibited a parallel increase in all the parameters except in PCV (P<0.005) respectively.

CONCLUSION: The enhanced fibrinolysis coupled with hypofibrinogenaemia after contrast injections could be an acute risk factors to bleeding while the effect on other flow properties of blood is insignificant provided the contrast is excreted. Therefore, the blood flow reduction earlier observed in these patients could be due to probable vascular response.

688 THE MONOCLONAL ANTIBODY DACLIZUMAB DOWN-MODULATES CD25 EXPRESSION IN ALLOREACTIVE CELLS IMPLICATIONS FOR GVHD TREATMENT

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Daclizumab is a monoclonal antibody directed at the a subunit of the IL-2 receptor, that blocks the interaction between IL2:IL2Ra and is used for the

prevention and treatment of GVHD. Although it is known that Daclizumab destroys alloreactive donor cells, further mechanisms of action are under investigation. The aim of this study was to evaluate the mechanism of action of Daclizumab using as an in vitro model for GVHD a one way mixed lymphocyte reaction between HLA-identical, haploidentical and mismatched donors. The expression of the lymphocyte activation markers CD69, HLA-DR and CD25 (evaluated by flow cytometry), as well as the inhibition of cell proliferation (evaluated by incorporation of FITC-labeled BrdU) were studied at 72 and 168h in cells cultured with 1mg/ml or 10mg/ml of Daclizumab at 37C in a 5% CO₂ atmosphere. Control cultures were not exposed to Daclizumab. The fluorescence-labeled anti CD25 antibody used (M-A251) was specific for an epitope distinct from the one recognized by Daclizumab. After 7 days, lymphocyte proliferation was inhibited by 77.2% (range 20.0 to 86.2%) and 60.4% (range 18.9 to 83.4%) with 1mg/ml and 10mg/ml of Daclizumab, respectively. The extracellular expression of CD25 in CD3+ cells was inhibited by Daclizumab in a time- and dose-independent way. At 1mg/ml, inhibition varied between 38.2% (36.0 to 54.7%) and 55.34% (38.5 to 69.7%) at 72h and 168h, respectively, for mismatched pairs. The corresponding values for haploidentical and identical pairs were 49.4% (37.8 to 60.7%) and 43.2% (16.5 to 47.8%) at 72h and 59.3% (50.8 to 81.2%) and 38.7% (24.9 to 87.9%) at 168h, respectively. The inhibition of the intracellular expression of CD25 was usually slightly higher, being 58.9% (41.9 to 61.5%) and 67.4% (21.4 to 82.3%) with 1mg/ml of Daclizumab at 72h and 168h, respectively, for mismatched pairs. Inhibition of CD25 expression in the CD4+ T cell subset was similar to what was observed in the whole T cell population. Daclizumab inhibited CD69 and HLA-DR expression inconsistently in 5 independent experiments. The results suggest that besides blocking the interaction between IL2:IL2Ra, Daclizumab can down-regulate CD25 expression. This knowledge may impact in the planning of the dose and schedule of Daclizumab infusions but its importance needs further investigation.

689 ENHANCED NEUTROPHIL APOPTOSIS IN NEUTROPENIC PATIENTS WITH HEPATOSPLENIC SCHISTOSOMIASIS : EVIDENCE OF SERUM FAS LIGAND

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Enhanced neutrophil apoptosis has been reported in neutropenic hepatosplenic schistosomiasis. Shortening of neutrophil survival via apoptosis may explain neutropenia that occur in those patients. However, the regulation of neutrophil apoptosis in hepatosplenic schistosomiasis has not been clearly defined. Neutrophils harvested from neutropenic patients with hepatosplenic (HS) schistosomiasis, (n=25), non-neutropenic patients with hepatointestinal (HI) schistosomiasis (n=10), and age-/gender-matched healthy control subjects (n=10) were incubated with autologous serum. Neutrophils apoptosis was quantified by flow cytometry through determination of propidium iodide nuclear staining and confirmed by DNA gel electrophoresis at 0 time (fresh neutrophil), 4h, and 24h culture. Neutrophils from healthy subjects were also incubated with either 10% heterologous normal or neutropenic serum with and without anti-Fas ligand antibody. Fas expression was assessed in fresh neutrophils using flow cytometry. Compared with normal healthy neutrophils, and HI neutrophils, Neutropenic neutrophils demonstrated greater apoptosis in the presence of autologous serum (P<0.01, P<0.05 respectively). Furthermore, compared with normal neutrophils exposed to heterologous normal serum, those exposed to heterologous neutropenic serum exhibited higher apoptosis rates (P<0.01). Moreover, anti-Fas L antibody attenuated the neutropenic serum-induced neutrophil apoptosis in normal neutrophils. Fas expression was significantly higher in neutropenic group as compared to both HI and normal healthy controls (P<0.05). Further, as expression by neutrophils was paralleled by high neutrophil apoptosis. On the other hand, neutrophil apoptosis was not correlated to the size of spleen in neutropenic group. In conclusion the rate of neutrophils apoptosis is accelerated in neutropenic hepatosplenic schistosomiasis. These findings suggest that enhanced neutrophil apoptosis that occurred in neutropenic HS patient is triggered by serum factor which is mostly a Fas ligand.

690 FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB (FCR): AN EFFECTIVE, WELL-TOLERATED COMBINATION WITH HIGH REMISSION RATES FOR CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background:

Fludarabine (F) is now standard first line therapy in CLL. The use of combination therapy increases complete remission (CR) rate as follows: F-35%, F/Cyclophosphamide (FC) - 43% and FC Rituximab (FCR) - 66%. We present a retrospective review of the efficacy and toxicity of FCR in untreated (n=9) and previously treated (n=2) patients with CLL.

Methods:

Eleven patients, (6 M, 5 F) with a median age of 59 (range 38-63) were treated with F (25 mg/m² i.v. days 1-3), C (250 mg/m² i.v. days 1- 3) and Rituximab(R) 375mg/m² on day 1 from cycle 2 onwards, repeated every 28 days for 4- 6 cycles depending on disease response. Disease characteristics pre-treatment were: Binet stages - 4 progressive A, 5 B, 2 C, median WCC 57.6x10⁹/l (20.0-363), platelet count 169x10⁹/l (114-276), Hb 12.5 g/dl (9.6-15.5), b2m 3.71 (1.79-5.6) and LDH 345 (134-1388). CD38 results were available for 8 patients of which 2 were positive (>30% cells CD 38+). Four patients had IgVH mutational analysis, of which 2 were unmutated.

Result:

Seven patients completed 6 cycles and 4 completed only 4/5 cycles for the following reasons; attainment of CR (n=1), patient preference (n=1), prolonged neutropenia (n=1) and planned PBSCT (n=1). Outcome at 6 weeks post treatment was: CR 6 (55%), PR 4 (36%), Nodular PR - 1 (9%) with an overall response rate of 100% (NCIWG criteria). Median follow up is 9 months (range 2-14), with 9 patients maintaining post-treatment disease status. One heavily pre-treated patient in PR post-FCR died of transplant related toxicity. A second patient died 2 months after FCR having achieved PR with massive nodal relapse consistent with Richter transformation. The treatment was clinically well tolerated with neutropenia (<0.5x10⁹/l ANC Grade III-IV) the main toxicity in 18/57 (31%) cycles requiring G-CSF therapy in 13/57(23%) cycles. Peripheral stem cell harvest was successfully carried out in 2/4 patients 3 months after FCR completion.

Conclusion:

The FCR regimen is well tolerated and achieved a CR rate of 55% in a mixed prognosis group of patients, further follow up will be needed to determine long term efficacy.

691 STEM CELLS IN PATIENTS WITH HEMATOLOGIC PATHOLOGY AFTER RADIATION EXPOSURE

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Background. Hematopoietic and immune systems' reconstitution after irradiation depends greatly on the functional abilities of the stem cells. Aims. To study CD34 progenitors in hematologic pathology in radiation exposed. Methods. Flow cytometric study of CD34+ cell subsets in bone marrow and peripheral blood cells was performed in 77 patients, including 47 Chernobyl radiation workers (mean dose 242.0±86.2 mGy) and 20 patients exposed to the natural levels of irradiation. Results. Stable leukopenia 10-17 years after the exposure exhibited the decrease of CD34+ progenitor counts mainly related with CD34+38-subset changes. In hypoplastic anemia the counts of CD34+38-progenitor cells was increased as comparing to control. Counts of lineage-committed lymphoid CD34+HLADR+ and CD34+19+ progenitor cells were not changed, CD34+33+ myelomonocytary cell counts were decreased. G-CSF and erythropoietin in vitro induced elevation of CD34 expression as well as CD33 and HLADR on CD34+cells (p < 0,05) but not CD38 FI changes. In myelodysplasia the prominent elevation of early CD34+ cells was detected in bone marrow and peripheral blood. In leukemia the primitive progenitor cell counts were elevated mainly in patients with proliferation of poorly differentiated cells while in ALLs the CD34+ counts were smaller (M=0,820, SD=0,185) but also exceeding the control figures (p<0,02). For CD34+33+ cells maximal counts were detected in FAB M4 and M5 subvariants, in ALL CD34+33+ progenitors were not detected. In FAB M0-M1 blast cells co-expressed low density CD34 together with high density HLADR and also CD33 and CD38. Summary. Our data indicate the existence of changes at least at several levels of CD34+cells from primitive CD34+33-38-cells to myeloid CD34+HLADR+33+ and lymphoid CD34+HLADR+19+10+/- progenitors.

Comparative analysis of bone marrow and peripheral blood CD34+ cells showed the delayed maturation of the last group.

692 MULTIPLE MYELOMA IN THE ELDERLY: PRESENTING FEATURES AND MANAGEMENT IN A SINGLE INSTITUTION
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Multiple Myeloma (MM) is a malignancy typically occurring in the elderly. About one third of the patients is 70 years older (Kyle, 2003). Many patients are not suitable for intensive chemotherapy and, given the frequent coexistence of other diseases, they may have a dismal outcome. So, the conservative management is a relevant problem, implicating social and economical aspects, because it concerns a great number of patients, whom often presents severe disability and the need to be assisted at home.

We report on the clinical data and the disease outcome of MM patients diagnosed in our institution from 1985 to 2003. There were 118 patients, 75 (63%) out of these were over 65 years old. The median age of the elderly population was 74 (65-90) years. Out of the 75 elderly patients, in 9 (12%), 5 (6.5%), 27 (36%), 17 (22.5%) and 1 (1.5%), the MM isotypes were IgA-k, IgA-l, IgG-k, IgG-l and IgM-k respectively. Nonsecretory MM was recognized in 2 (3%) patients, whereas light-chain MM was present in 14 (18.5%) cases (9 k and 5 l).

At diagnosis, 13 (17.5%), 7 (9%), 1 (1.5%), 45 (60%) and 9 (12%) patients were in IA, IIA, IIB, and IIIA e IIIB Durie e Salmon stages respectively. Anemia was revealed in 58/75 (77%) cases. Out of 75 patients, 13 (17%), 20 (27%) and 25 (33%) presented at diagnosis a hemoglobin level lower than 10, 11 and 12 gr/dl respectively. A serum creatinine level of 2 mg/dL or more was found in 12 (16%) cases. Skeletal abnormalities were revealed by x-ray surveys in 51 (68%) patients. Pain of skeletal origin was referred, at any time of the disease course, by 61 (81%) patients, whom complained of at least one-pain syndromes for a total of 102. The underlying mechanisms were identified as follows: 80 (78%) osteolysis, 5 (5%) artroses, 5 (5%) painful plasmocytomas, 8 (8%) peripheral neuropathies and 4 (4%) postherpetic neuralgia. Antineoplastic treatments, radiotherapy, orthopaedic devices and analgesics were applied as required. Thirteen (17%) patients received no therapy, 46 (62%) were treated with cyclic or continuous melphalan plus steroids, 4 (5%) steroids alone and 12 (16%) other combined chemotherapies. The patients presenting almost one skeletal lesion or with severe osteopenia, received intravenous bisphosphonates (clodronate, pamidronate and, more recently, zoledronate). During the four last four years, fourteen patients were managed by our home care service, receiving supportive and palliative cares. With a median follow-up of 19 (1-112) months, 32/75 (43%) patients were alive. The median duration of survival was 21 (1-112) months. In conclusion, although the therapeutic options in MM are moving toward curative intents, our experience in this setting outlined the central role of a comprehensive approach that may be based on the control of the symptoms and on the careful consideration of the psychological aspects. Moreover, an experienced home care service could allow the patients to stay at own home, given that the quality of life remain in most cases the most important goal.

693 ACTIVITY OF THE KALLIKREIN-KININ SYSTEM IN BLOOD PLASMA OF PATIENTS WITH ATOPIC FORM OF ALLERGIC DISEASE

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The activation of the kallikrein-kinin system has been observed in acute forms allergic reactions. Hageman factor has ability to activate prekallikrein. In this work we estimated activity of kallikrein, content of prekallikrein and the level of XII factor in children with bronchial asthma caused by sensitization to house-dust mites.

Activity of a1 proteolytic inhibitor (a1-PI) and a2 macroglobulin (a2 MG) in the blood plasma of 15 children with bronchial asthma caused by sensitization to Dermatophagoides mites was studied. Blood plasma was taken before, during and after specific immunotherapy with water-saline curative allergens obtained from D.farinae. The allergens were injected in increasing doses till the maximal tolerable dose ensuring the therapeutic effect was achieved. At the acute period bronchial asthma caused by sensitization to house-dust mites activation of the kallikrein-kinin system was found, which correlated with the disease aggravation.

During specific immunotherapy of the patients the activity of a2 MG was markedly increased, in the same time distinct increase in the a1-PI activity was not found. The activity of Hageman factor in the blood plasma of the patients was determined with the use of plasma, deficient in factor XII. In addition, were determined APTT, heparin by USP, fibrinogen content, because these indices of blood plasma are significantly change in during anaphylactic and allergic state. Statistically significant changes in the activity of Hageman factor were observed in the process specific immunotherapy. Prior to specific immunotherapy the values characterizing the activity of Hageman factor were significantly decreased in the patients (42-53%). During hyposensitization activity of Hageman factor increased and by the end of the course of specific immunotherapy reached the normal level (84-96%). Change of others indices of blood plasma were not tell from norma.

In the process of specific immunotherapy with curative allergens activation of the kallikrein-kinin system occurred, which depended on the total concentration of allergen administered.

694 TRANSFERRIN RECEPTOR FROM PLACENTA IS NOT A RELIABLE STANDARD FOR THE DETERMINATION OF SOLUBLE TRANSFERIN RECEPTOR IN HUMAN SERUM

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BACKGROUND. Transferrin receptor (TfR) is a membrane protein that takes part in the transportation of iron from serum into cells. TfR binds with iron loaded transferrin (Tf) and the resulting complex is internalized by cells. The largest pool of TfR is located on maturing erythroid cells which need a lot of iron for haemoglobin synthesis. TfR is truncated from mature red cells and circulates in blood as soluble TfR (sTfR). The level of sTfR in blood is an important parameter for the diagnosis of iron deficiency anemia. TfR from placenta is widely used as a standard in ELISA for sTfR determination.

AIMS. The aim of this work was to study the validity of the use of placental TfR as a standard in ELISA for sTfR determination.

METHODS. Placental TfR, sTfR, and monoclonal anti-TfR antibodies for their determination were from HyTest, Finland. FPLC gel filtration of placental TfR and sTfR was performed using a Superdex 200 column and the AKTA-FPLC system (Amersham Pharmacia Biotech, Sweden).

RESULTS. FPLC gel filtration of sTfR and TfR from placenta showed that sTfR eluted as a protein with a molecular weight of 237 kDa whereas TfR from placenta eluted in a void volume with or without detergent (Tween 20) in the buffer. TfR from placenta, purified sTfR, and serum as a source of sTfR were tested by ELISA sandwich immunoassay using a panel of monoclonal anti-TfR antibodies. It was found that there were several combinations of antibodies forming pairs that make it possible to determine sTfR. The pairs suitable for sTfR determination also detected sTfR in serum. The pairs which did not react with purified sTfR could not be used for sTfR determination in serum. TfR from placenta, on the contrary, could be detected by any combination of antibodies including those in which the same antibodies were used as capture and conjugated ones.

CONCLUSIONS. Our results show that sTfR and placental TfR have different physico-chemical properties that leads to the differences in their immunological properties. It can be explained by the aggregation of placental TfR molecules because of their hydrophobic transmembrane and intracellular parts and/or micelle formation in water solution with or without detergent that leads to multipoint interaction between placental TfR and antibodies. This makes results of sTfR and placental TfR incomparable. It means that placental TfR cannot serve as a reliable protein standard in ELISA method for sTfR determination.

695 SENSITIVITY OF MARKERS THAT DETERMINE IRON NEEDS IN HEMODIALYSIS PATIENTS VALIDATION OF RETICULOCYTE SUBPOPULATIONS

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AIM: To study the sensitivity of Reticulocyte Hemoglobin content (CHr), percentage (%) of hypochromic erythrocytes (%hypoRBC), and Ferritin, as markers that determine iron needs in hemodialysis patients. To examine the probable development of functional iron deficiency and to evaluate the usefulness of low, median and high absorption subpopulations, according to reticulocyte maturation (L-, M-, H-Retic).

MATERIAL-METHODS: 17 hemodialysis patients (11men -6 women) of mean age 62.5 (50-75) were selected. They were discontinued the monthly dose of iron (200mg sucrose iron IV) but continued the individualized, for each one, dose of recombinant Erythropoietin. Per month, patients blood specimens were analysed for total blood count, number (#) and percentage (%) of Reticulocytes (Retic-Technicon H3) and Ferritin (ACS180SE Bayer).Total follow up of patients was 12 months. Data were analyzed with SPSS 11.5 for Windows. For the comparison of mean values of parameters during the study, the Friedman test was applied and for the correlations, the Pearson correlation test. The statistical significance was defined to $p < 0.05$.

RESULTS: The p values of examined parameters were:(table 1)

The falling of Ferritin correlates positively with #Retic, %Retic and CHr ($r = 0.623$, $r = 0.521$,

$r = 0.598$, respectively) but inversely with %hypoRBC ($r = -0.503$) and %H-Retic ($r = -0.414$).The change in H-Retic number correlates negatively with number and %Retic($r = -0.430$ and $r = -0.599$) as well as the number and %L-Retic($r = -0.230$ and $r = -0.906$).

There is strong negative correlation between %H-Retic and %L-Retic($r = -0.904$),%H-Retic and %Retic($r = -0.726$), %M-Retic and %L-Retic($r = -0.991$) but on the contrary, positive correlation between % H-Retic and %M-Retic($r = -0.903$).

CONCLUSIONS: Ferritin proved to be, the most sensitive marker in comparison with CHr and %hypoRBC in determination of iron needs in hemodialysis patients. None of patients developed functional iron deficiency. The detection of changes into Reticulocytes subpopulations, -decrease in L-Retic with simultaneous increase in H- and M-Retic -can be a simple and economic way for estimation of the rate of erythropoiesis and for exclusion of functional iron deficiency, in patients with anemia of chronic disease.

Reference: Bhandari S. Reticulocyte indices in patients with end stage renal disease on hemodialysis. J Nephrol. 1998 Mar-Apr; 11(2):78-82.

Ferritin	<0.001	%L-Retic	0.097
%hypoRBC	0.010	# L-Retic	0.249
CHr	0.451	%H-Retic	0.104
Hct	0.712	#H-Retic	< 0.001
%Retic	0.328	%M-Retic	0.110
# Retic	0.365	#M-Retic	0.407

Caption 1: Table 1

696 EVALUATION OF ERYTHROCYTE (RBC) AND RETICULOCYTE (RETIC) INDICES IN ANEMIA INVESTIGATION

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AIM: To evaluate the RBC and Retic indices in investigation of anemia.
MATERIAL and METHODS: By criterion the value of Hemoglobin, 45 patients (22men, Hgb<13g/dl and 23 women, Hgb<11.8g/dl; mean age 60.5(45-76) were selected. As healthy controls, 59 {33 men, 26 women; mean age 46(37-55)}, without hemoglobinopathy, were used. Hematocrit (Hct),Hgb, RBC Hemoglobin content(CH),Reticulocyte Hemoglobin content(CHr),proportion of hypochromic RBC(%Hypo),Retic and subpopulations of low,mean,high absorption according to Retic maturation (L-,M-,H-Retic ADVIA120), were analysed in blood specimens. The % of Retic was corrected according to Hct (CRI). CHr/CH ratio was also measured. Ferritin (Fer-Chemiluminiscence ACS180Bayer), Transferrin soluble receptors (sTfR-Nephelometry DadeBehring) and ratio of sTfR/logFer (index) were also determined. For the elaboration of results, the SPSS 11.5 program for Windows was used. For the comparison of mean values (MV), Independent Samples T

test was applied. The statistical significance was defined to $p < 0.05$.The correlations were estimated by Pearson correlation.

RESULTS: CHr, CH, %Hypo values between patients/healthy were significantly different ($p < 0.001$). Reversion of CHr/CH ratio (normal value >1) presented only in 7/45(15%) patients. % of L-,M-, H-Retic and CRI values between patients/healthy were significantly different. Positive correlation was observed between M/H-Retic($r = 0.633$), negative correlation between L/M-Retic ($r = -0.949$) and L/H-Retic ($r = -0.844$).CRI and % of L and M+H-Retic in relation to index, are presented in table:
CONCLUSIONS: The study of sensitive markers of early (CHr) and midterm (CH, %Hypo) distress of Hemoglobinization (Iron Restricted Erythropoiesis-IRE) should not be underestimated in anemia investigation, given the low cost and handiness. The usefulness of CHr/CH ratio as early marker of IRE wasn't displayed in this study. There may be required larger number of patients. It is purposeful to determine CRI, that better corresponds to effective erythropoiesis, as well as, M-and H-Retic that express the stress of erythropoietic stimulation.

C.Thomas et al, Clinical Chemistry 2002: 48; 1066-1076.

		Patients			Healthy controls		
		Mean	Min-Max	Std Dev	Mean	Min-Max	Std Dev
<1	CRI	1.02	0.12-2.12	0.48	1.33	0.72-2.6	0.34
	% L-RET	82.37	56.6-91.8	7.40	85.15	71.9-94.5	5.17
	% M- & H-RET	17.63	7.3-43.4	8.25	14.85	4.7-28.2	5.60
1-3	CRI	1.21	0.86-1.58	0.25	1.13	0.80-1.57	0.20
	% L-RET	80.25	47.9-97.2	6.94	88.18	75.5-92.4	4.20
	% M- & H-RET	19.75	12.7-32.1	6.21	13.72	6.90-23.5	4.28
>3	CRI	0.87	0.55-1.30	0.37	none		
	% L-RET	84.72	76.1-92.4	5.95			
	% M- & H-RET	15.28	7.60-23.9	6.42			

Caption 1: Table 1

697 GLOCOSE-6-PHOSPHATE DEHYDROGENASE, IDS AND P55 RNA POLYMORPHISM FREQUENCIES IN IRANIAN POPULATION: RELEVANCE TO X-CHROMOSOME INACTIVATION (XCI) STUDIES

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Background: X-chromosome inactivation has widely been used in female individuals to differentiate reactive hyperplasia from true clonal cell expansion (neoplasia) arising from somatic mutation in a number of hematological disorders. X-inactivation analysis can be performed in the absence of any specific tumor markers, which allows in theory analysis of all human neoplasms. In order to study X-chromosome inactivation in hematological neoplasms, we first proposed to determine the frequency of G6PD, IDS and P55 polymorphisms at the RNA level.

Purpose: To investigate the rate of heterozygosity and frequency of G6PD (nt. 1311 C/T), IDS (nt. 438 C/T) and P55 (nt. 358 G/T) RNA polymorphisms in some Tehrani female patients and normal individuals. **Subjects and Methods:** This study was carried out on DNA from 170 normal females and 35 patients with de novo acute myeloid leukemia (AML) aged 25 to 45 years old using polymerase chain reaction (PCR) and restriction endonuclease analysis.

Results: The frequency of heterozygosity for P55 was found to be 48% (98/205). Forty two percent (86/205) were heterozygous for IDS and only 27% (53/205) of individuals showed polymorphism at nt.1311 C/T for G6PD gene. Some individuals were heterozygous for more than one gene polymorphism.

Conclusion: The rates of heterozygosity obtained for the above gene polymorphisms were similar to the results reported by other investigators. It can be concluded that these polymorphic gene sequences are useful for studying X-chromosome inactivation in hematological disorders in Iranian populations.

698 DRUG SENSITIVITY OF LEUKEMIC CELLS IN CHILDHOOD ACUTE MYELOID LEUKEMIA

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Background: Prognosis of childhood acute lymphoblastic leukemia (ALL) has improved dramatically; survival has reached 70-80%. Children with acute myeloid leukemia (AML) develop a worse prognosis: survival rates reach 40-60% versus 70-80% and refractory to induction therapy occurs in 5-10% versus 1-2% in ALL. Differences in prognosis may reflect differences in cellular drug resistance, which can be studied using a total cell kill assay such as methyl-thiazol-tetrazolium (MTT) assay.

Aims: to compare the drug sensitive profiles of several FAB-type subgroups of AML, and to analyze the differences in cellular resistance to 10 drugs in 33 AML samples versus 110 ALL samples.

Methods: The blast cells sensitivity to chemotherapy was examined in MTT-assay. The material of the study: leukemic cells of bone marrow from 33 children with newly diagnosed AML and from 110 children with newly diagnosed ALL. The LC50 value, the drug concentration needed to kill 50% of the cells, was used as a measure of resistance. For each drug (Cytarabine, Daunorubicine, Doxorubicine, Mitoxantrone, Etoposide, 6-Mercaptopurine, L-Asparaginase, Vincristine, Prednisolone, Dexamethasone) 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 1st rank concentration of the drug lower than minimal value of the scale; the 7th rank concentration of the drug higher than the greatest value. The high sensitivity to a drug - LC50 value from 1 to 4 ranks, the low sensitivity - LC50 value from 5 to 6 ranks, resistance - LC50 value of 7 rank. Ranks scale allowed comparing the drug sensitivity profiles for several FAB-types of AML.

Results: We compared the drug sensitivity profile of following FAB-type subgroups: M2 (10 cases), M3 (5 cases), M5 (9 cases). Numbers in the other FAB-type subgroups were too small for separate analysis. M2 appeared to be high sensitive to Cytarabine only, low sensitive to all others, and did not demonstrate resistance to any drugs. M3 showed the high sensitivity to Cytarabine, Daunorubicine, 6-Mercaptopurine, Vincristine, the low sensitivity to Prednisolone, Doxorubicine, Mitoxantrone and resistance to Dexamethasone. Leukemic cells of M5 subgroup were high sensitive to Cytarabine, Mitoxantrone, Doxorubicine and 6-Mercaptopurine, low sensitive to Etoposide, L-Asparaginase, Vincristine, Prednisolone and Daunorubicine, resistant to Dexamethasone. In comparing of M2- and M5-subgroups we found M5 to be significantly more sensitive to Cytarabine ($P=0.005$), Mitoxantrone ($P=0.01$) and Daunorubicine ($P=0.002$). The AML cells were found to be less sensitive in vitro to most of the drugs than ALL cells. The highest statistic significance was obtained for Prednisolone ($P=0.0008$), Dexamethasone ($P=0.003$) and Etoposide ($P=0.007$). FAB M5 is relatively sensitive in vitro to most drugs usually used in AML treatment, but it is also as sensitive to Vincristine and L-Asparaginase as childhood ALL cells.

Conclusions: The poor prognosis of childhood AML can be attributed, at least partially, to cellular drug resistance. The results of drug sensitivity of AML cells can be used in the rational design of further chemotherapy protocol.

699 PAI-1 LEVELS ARE RELATED TO DURATION OF EPO TREATMENT IN HEMODIALYSIS PATIENTS

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Background: Recombinant human erythropoietin (rHuEPO), an effective agent in the treatment of renal anemia can predispose to thrombotic events, particularly to A-V fistula occlusion in hemodialysis patients (HD pts) (Canadian EPO Study Group, 1990; Viron, 1997 etc.). This can be due to the increased red blood count, the rise in blood viscosity and a direct effect of rHuEPO on some hemostatic parameters. More established risk factors of rHuEPO therapy in pts undergoing regular HD include: the increased platelet count and functions of platelets with shortening of bleeding time, the impairment of fibrinolytic response, a decreased levels of the anticoagulant proteins and the endothelial dysfunction.

Aim: We were interested about the relationship between rHuEPO treatment and changes of molecular markers of hemostasis in HD pts.

Patients and methods: 38 HD pts divided into 2 groups: 1. HD group with EPO (19 pts), 2. HD group without EPO (19 pts). The plasma

levels of PAI-1, tPA, tPA/PAI-1, endothelial and platelet markers were measured before HD and after 4 hours of a single HD session using EIA (enzyme-linked immunoassay). All results were corrected for changes of hematocrit (Hct) during HD.

Results:

EPO group : Plasma PAI-1 levels before and after HD were 21,3 39,8 vs. 18,026,9 ng/ml (n.s.); t-PA levels 7,1 6,6 vs. 9,510,2 ng/ml (n.s.); t-PA/PAI-1 levels 1,740,66 vs. 0,990,31 ng/ml ($p<0,05$) respectively.

HD group without EPO : Plasma PAI-1 levels before and after HD were 48,8 97,9 vs. 36,9 88,2 ng/ml (n.s.); t-PA levels 12,0 21,2 vs. 19,2 41,5 ng/ml ($p<0,0005$); t-PA/PAI-1 levels 1,170,28 vs. 1,150,36 ng/ml (n.s.) respectively.

The interesting finding was an inverse correlation between duration of EPO treatment (years) and PAI-1 levels in EPO group ($r=-0.50385$, $p<0,05$) that was not present in HD group without EPO.

Conclusion: We conclude that already a single HD session led to a potent activation of both fibrinolysis and endothelium in HD pts (especially expressed by the increase of t-PA in HD group without EPO). The interpretation of PAI-1 and t-PA Ag changes during single HD is uncertain to explain the short-term effect of rHuEPO on fibrinolysis. On the other hand our results of the inverse correlation between duration of EPO treatment and PAI-1 plasma levels in the EPO group support a favourable role of the long-term rHuEPO administration towards to fibrinolysis.

700 SOLUBLE TRANSFERRIN RECEPTORS/LOGARITHM OF FERRITIN (STFR/LOGF) FOR DISTINGUISH IRON DEFICIENCY (ID)/IRON DEFICIENCY ANEMIA (IDA) FROM ANEMIA OF CHRONIC DISEASE (ACD) AND THE COMBINED STATE OF ID/ACD

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BACKGROUND. sTfR/logF (index), as referred in bibliography, is useful in distinction between ID/IDA (index >3) and ACD (index <1).

AIM: To evaluate the extent to which the use of sTfR /logF can be helpful in differentiation of ID/IDA from ACD and combined state ID/ACD.

MATERIAL and METHODS: By criterion the value of Hemoglobin (Hgb), 45 patients (22 men, Hgb $<13g/dl$ and 23 women Hgb $<11.8g/dl$; mean age 60.515.5) with renal function within normal, were selected. 59 healthy subjects (33 men, 26 women; mean age 46.10) without hemoglobinopathy were used as control group. Blood specimens were analyzed for : Transferrin soluble receptors (sTfR-Nephelometry Dade Behring), Ferritin (Automated Chemiluminescence ACS180SE Bayer), beta2microglobulin (beta2m-ACS180SE Bayer), as marker of the activated cellular immunity involved in ACD, and Reticulocyte Hemoglobin content (CHR-ADVIA 120) as sensitive marker of iron deficiency. The two groups were categorized according to the index values and were checked up on CHR. Data were evaluated using the SPSS 11.5 program for Windows. For the comparison of the mean values (MV), the Independent Samples T test was applied ($p<0.05$), and for the correlations the Pearson correlation Test.

RESULTS: The control group data corresponded to normal, found in bibliography. Moreover, there was no difference between men and women for beta2m, CHR, sTfR/logF values. MV for Hgb, Ferritin, beta2m and CHR, between patients and controls, were significantly different ($p<0.001$) in contrast to sTfR, sTfR/logF values. 41/45 patients (89%) presented beta2m values, above normal.

According to index the classification was (Table 1):

The index values were:

Healthy: Mean 0.98 (0.24-2.24) SD 0.40. Patients: Mean 1.67 (0.09-11.79) SD 2.53. In 38 patients with elevated beta2m: Mean 1.2 (0.09-7.93) SD 1.61. In 7 patients with normal beta2m: Mean 3.84 (0.51-11.79) SD 4.69.

Healthy CHR value was: 32.1pg (27.6-36.7). Based on CHR value, patients were classified (Table 2):

In patients group with CHR <27.6 the index value where: Mean 2.55 (0.57-11.79) SD 3.31. In patients group with CHR 27.6-36.7: Mean 1.13 (0.09-6.93) SD 1.76.

CONCLUSIONS: Index values <1 alone, are not trustworthy for ACD diagnosis. On the contrary, index values >3 for ID or/and IDA, are. As expected, Acute Response Reaction (APR)-not rarely becoming chronic-expressed by the increase of beta2m, lowers the index MV and transposes the range of values. ID raises index values and further more, affects patients classification. In conclusion, distinction of ACD and ID/ACD based on index must be considered in combination with markers of restricted erythropoiesis.

Reference: Thomas C. and Thomas L. Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency. Clinical Chemistry 48: 71066-71076 (2002).

Index	<0.99	1-3	>3	Total
Patients	32(71%)	8(18%)	5(11%)	45(100%)
Healthy	40(68%)	19(32%)		59(100%)
Total	72	27	5	104

Index	<0.99	1-3	>3	Total
ChR<27.6	9(20%)	6(13%)	3(7%)	18(40%)
ChR 27.6-36.7	20(44%)	2(4%)	2(4%)	24(53%)
ChR>36.7	3(7%)			3(7%)

Caption 1: Table 1 and 2

701 THE TRANSCRIPTION RIBOSOMAL RNA GENES IN LYMPHOCYTES OF INDUSTRIAL WORKERS: EFFECT OF METALS AND HF RADIATION

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The structure, volume and number of nucleoli reflect the transcription ribosomal RNA genes, which correlates with the intensity of cell metabolism in general. Specific features of nucleolar organization in cells of different transcription rates are little understood. However an increased volume of nucleoli Purkinje cells of rat cerebellum during development is evidence in favor of consolidation of chromosomes with Nucleolar Organizer Regions (NOR). In the interphase cells nucleus, the ribosomal RNA genes

are located in the fibrillar centers and in the associated dense fibrillar component of the nucleolus. Some acidic proteins are associated with the fibrillar components and in particular with the highly decondensed chromatin of nucleolus. These proteins are selective stained by silver using the known method for visualizing metaphase and interphase NOR. For this reason, the method can be called Ag-NOR reaction.

We studied ribosomal chromatin in normal human lymphocytes and lymphocytes workers using Ag-NOR reaction and computer image analysis. A few large grains are specific of normal G0-lymphocytes. An increase in number and decrease in size of silver grains in nucleoli accompany active cell proliferation in vivo and in vitro. Different work conditions cause specific Ag-NOR patterns. Our method is high reproduced. A number of people can be tested by this method, which is very important for revealing conditions that could cause an increased risk for employee and for establishing a baseline for future health monitoring. Thus, immediate morphological evaluation of ribosomal RNA genes transcription with Ag-NOR reaction is a useful tool for periodic medical examinations of all workers directly involved in production, or otherwise exposed to contact with harmful industrial compounds.

702 THE MECHANISM OF G-CSF INDUCED CD34+ MOBILIZATION

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Background: It is known, that the level of G-CSF-receptor expression is the highest on mature granulocytes, so these cells can serve as targets for G-CSF action in mobilization of CD34+cells. According to this granulocytes and monocytes can behave as messengers, producing several chemokines and cytokines effective in mobilization of CD34+cells.

Aims: to study the influence of G-CSF on the proliferative activity of CD34+cells; to evaluate the function of IL-8 in G-CSF induced mobilization of CD34+cells; to value the level of G-CSFR and IL-8R on CD34+cells during the treatment of G-CSF.

Methods: Flow cytometry for evaluation of CD34+cells number and measurement of the fraction of CD34+cells, granulocytes, monocytes, expressed receptors to G-CSF and IL-8 (CD114, CDw128type A and CDw128type B) during G-CSF administration; clonogenic cultivation for calculation of CFU-GM number in peripheral blood (PB) and bone marrow (BM); ELISA-assay for the measurement of serum level of IL-8.

Results: We found a significant direct correlation ($P<0.001$) between the number of granulocytes prior to G-CSF administration and the number of CD34+cells mobilized subsequently. It was not seen any increasing of CFU-GM count, as well as CD34+cells, in BM during the administration of G-CSF. The level of CFU-GM in PB demonstrated positive correlation with the count of CD34+cells ($P<0.05$), but fraction of CFU-GM among CD34+cells was not changed. The level of IL-8 in serum appeared to become 6.5 fold greater during G-CSF treatment. There was observed a positive correlation between the increasing rate of IL-8 serum level and CD34+cells number ($P=0.05$), as well as CFU-GM count ($P=0.04$) in PB after mobilization with G-CSF. There was no evidence of the increasing expression of G-CSFR and of IL-8R at CD34+ cells during G-CSF administration.

Conclusion: The number of granulocytes at the moment of administration of G-CSF turned out to be the most important criterion for predicting the efficacy of stem cells mobilization. They may be the main target for G-CSF, releasing other factors, stimulating CD34+cells mobilization. G-CSF does not stimulate the proliferative activity of stem cells; its mobilization effect is equal for the both types of progenitors (CD34+ and CFU-GM cells). CD34+cells cannot be the target for IL-8 - induced mobilization, because they do not express its receptor prior or during G-CSF mobilization. It seems that IL-8 can play the role of the messenger for the G-CSF induced mobilization of bone marrow CD34+cells by stimulating other molecules e.g. MMP enable to cut off progenitors from bone marrow stroma.

703 CLINICAL AND GENETIC ASPECTS IN 29 PATIENTS WITH TYPE I AND III GAUCHER DISEASE- EFFICACY OF ENZYME REPLACEMENT THERAPY IN FIVE PATIENTS

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Background: Gaucher disease (GD), the most prevalent of the genetic lysosomal storage disorders is caused by a severe deficiency of glucocerebrosidase. The accumulation of glucocerebroside in macrophages results in hepatosplenomegaly, cytopenia, bone lesions and variable clinical symptomatology. The most prevalent form of GD is the type I; chronic non-neuronopathic which incidence is 1/100.000-200.000 in the non-Jewish population. The prevalence of the type II (acute neuronopathic) and type III (subacute neuronopathic) type is even smaller. Imiglucerase (Cerezyme), a macrophage-targeted recombinant form of the enzyme has proven to be effective in the treatment of type I and III GD. Aims: to present clinical and genetic aspect of 29 patients (pts.) with GD and our 2-yr experience with 5 pts. on enzyme replacement therapy (ERT) with Cerezyme, obtained through humanitarian aid. Patients and methods: There were 27 pts. with type I and 2 pts. with type III GD. There were 16 female and 13 male, 21 adults (range: 16-74) and 8 children (range: 2-14 yrs); 11 pts. were splenectomized. Anemia, thrombocytopenia and massive splenomegaly (15-32 cm) were present in all 18 unsplenectomized pts; hepatomegaly was present in 29 pts. and bone disease in 20/29pts. (bone crises in 3). Among type III pts., one had supranuclear ophthalmoplegia and the other is without neurological signs yet. The diagnosis was confirmed by evaluation of glucocerebrosidase assay and DNA analysis.

Results: All pts. presented with elevated serum acid phosphatase (median 35 U/L; normal < 9) and chitotriosidase activity rates (median: 6.050 nmol/h/ml; normal < 250). Bone NMR was abnormal in 23/23 pts. Erlenmeyer flask deformity was present in 7, vertebral fractures, osteolyses and aseptic necrosis of femoral head in 3 pts. Two pts. homozygous for N370S mutation and 25 with various mutations in conjunction with N370S (R463H, RecNcil, L444P, D409H, Δ551236-1317) exhibit GD type I; one pt homozygous for L444P and one with L444P in combination with D409H exhibits type III. Five pts. with most progressive disease (3 with type I; 2 with type III) were started on ERT in 2002 (Cerezyme at a dose of 60IU/kg, biweekly for 1.5 yr). After one year of ERT all pts. gained weight (2-10 kg). They also presented a significant hematological improvement, as soon as after 4-6 months of ERT, especially in hemoglobin value (20g/L increase of Hb mean level), while 4 of them reached normal platelet count within the first year. The liver and spleen sizes objectively decreased (approximate volume reduction 10-15% and 30% respectively within the first year). Bone disease did not progress. Acid phosphatase and chitotriosidase activity rates decreased with ERT

and assessment of antibodies remained negative in all five patients. Therapy was well tolerated and quality of life markedly improved.

Conclusions: The most frequent mutations in our pts. series are the most common, pan ethnic N370S and L444P mutations. ERT proved effective and safe. A model for financing ERT for all pts. with GD in the national health care system is needed.

We wish to thank 'Genzyme' for providing ERT and diagnostics.

704 PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY AFTER FLUDARABINE AND POSTTRANSPLANTATION RITUXIMAB THERAPY FOR FOLLICULAR LYMPHOMA

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BACKGROUND: Progressive multifocal leukoencephalopathy (PML) is a rare but fatal demyelinating disease of the brain caused by the JC papovavirus (JCV), affecting mainly to immunocompromised patients. In the last 10 years, an association between PML and the application of fludarabine and posttransplantation rituximab has been discussed. We report here one additional case of PML associated with fludarabine and posttransplantation rituximab in a heavily pretreated patient. **CASE REPORT:** A 51-year-old man had a stage IV follicular (grade 2, WHO) non-Hodgkin's lymphoma, diagnosed in 1999. He was treated with CHOP regimen for 6 cycles achieving a complete clinical remission. Four months later he suffered a generalized relapse. Between October 1999 and March 2000 he received ESHAP regimen for 4 cycles and an unmanipulated autologous peripheral blood stem cells after conditioning with CBV achieving a clinical and molecular remission. However, four months later, a bone marrow and abdominal relapse was documented. This second relapse was treated with a CVP regimen combined with rituximab (375 mg/m²) for 6 cycles, retroperitoneal irradiation, and fludarabine combined with cyclophosphamide (FC) regimen for 4 cycles, achieving again a complete clinical response. Three months later (in September 2002), the patient developed severe neurological symptoms (visual disturbances, limb weakness and behaviour abnormalities) associated with low CD4+ counts (0.2 x 10⁹/L) and magnetic resonance imaging was consistent with PML. Polymerase chain reaction (PCR) in cerebrospinal fluid (CSF) was positive for JCV. No other neurotropic viruses were detected in the CSF. Despite treatment with cidofovir, the patient died two months after the beginning of the neurological symptoms. On post-mortem examination, the histopathologic findings of brain were characteristics of PML. No lymphoma cells could be seen either in the brain or in any other organ. **CONCLUSION:** This case further strengthens the need for an accurate surveillance and reporting of this rare viral infection in patients with lymphoproliferative disorders who have received treatment with fludarabine and rituximab. A diagnosis can be made by CSF PCR examination for JCV in conjunction with compatible symptoms and radiographic findings.

705 JUMPING TRANSLOCATIONS IN A CASE OF FOLLICULAR LYMPHOMA IN REMISSION

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Background.

Jumping translocations (JTs) are unbalanced translocations involving a donor chromosome arm or a chromosome segment that has fused to multiple recipient chromosomes usually at their telomeric site. In leukemias and lymphomas the donor segment (usually 1q) preferentially fuses to the telomere regions of recipient chromosomes. Jts are relatively rare and are associated with poor prognosis. The objective of this study is to present a case of follicular lymphoma in remission that presented JTs. This rare cytogenetic aberration was studied also by SKY, that permitted to define precisely all the chromosomes involved in the translocations. We will discuss our case and compare with those previously reported in other lymphoid tumours.

Patient and methods.

We present a patient diagnosed of follicular lymphoma in November 1992. The patient received 7 cycles of COPP achieving a good response. In 1997 and 1998 the patient suffered a relapse. In October 2002 a third relapse was observed and the patient was treated with FCM for 6 cycles. In January 2004 a cytogenetic and molecular study was performed. Conventional cytogenetic study was carried out on lymphoid cells from 72 hour-bone marrow culture stimulated with TPA. FISH was applied with

SKY (Spectral Karyotyping) probe. PCR study to detect B-cell clonality, MBR and mcr rearrangements of t(14;18) were performed.

Results.

Among 20 metaphases analyzed by conventional cytogenetics, the following abnormal translocations were observed in different cells: t(1;5)(p36;q33), t(1;5)(q32;q33), add(1)(q44), t(1;8)(p34;q13), and t(1;2)(p11;q37). Fifteen cells presented a normal karyotype.

SKY revealed the following aberrations in different metaphases: der(1)t(1;16)(p11;p11), t(2;7), t(2;?), t(3;14)(q29;q11), t(1;2)(p11;q37) and t(2;14)(p11;q11).

PCR at the time of the cytogenetic study did not reveal a MBR nor mcr t(14;18) rearrangement, nor clonality of the B lymphoid population.

Conclusions.

- Our case represents a variant of JT involving preferentially chromosome 1.

- The clinical impact of this rare finding is unknown.

- The abnormal karyotypes could be attributed to the previous treatment that received the patient.

- In reviewing the literature, the presence of JTs is associated with poor prognosis.

- This rare phenomenon may occur with increased frequency in lymphomas.

- SKY technique is very useful to detect chromosomal imbalances.

Acknowledgments. This work has been partially supported by the grants SAF-4947 and GO3/179, CO3/07 and CO3/10 from the Spanish government. We want to thank Carme Melero for their excellent technical assistance.

706 INCIDENCE OF SECOND MALIGNANCY IN B-CELL CHRONIC LYMPHOCYTE LEUKEMIA: RETROSPECTIVE STUDY ON 358

PATIENTS FROM A SINGLE CLINIC

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The incidence of second malignancy was retrospectively analyzed on 358 CLL patients consecutively observed in our clinic between 1990-2003. Second malignancy was found in 46 (12.8%), in 9 of them before CLL diagnosis, in 12 at the same time and in the remaining 25 subsequently. The most frequently involved areas were: skin 19.2%, lung 17.8%, gastrointestinal tract 17.1%, breast 10.1%, pancreas 1.1% and prostate 5.5%.

Richter syndrome occurred in 16.9% of cases in evolution. After excluding cases with second malignancy preceding CLL, 349 patients were accounted.

The ratio sex was: 225 males/124 females with mean age 63.5+/-10 years and with the following stage distribution at diagnosis (according to Binet): 86 in stage A, 110 in stage B and 153 in stage C. At this time of analysis, 168 patients are alive, 139 dead and 42 lost to our observation. Death causes were: 42% progression of CLL, 22.6% infections, 9.8% second malignancies and 25.6% others causes.

In univariate analysis, only stages according to Binet showed a nearly significant relationship ($p=0.051$) with second malignancy occurrence; in fact, 68.8% of second malignancy were diagnosed in stage A cases, as expected because of their longer survival and especially the skin cancers.

The impact of chemotherapy is not relevant. The other types of malignancies who appeared contemporary to CLL diagnosis had an important impact on the evolution of the CLL and they had shortened the survival.

707 GENE FREQUENCIES OF HUMAN PLATELET ANTIGENS HPA -1, -2, -3 AND -5 IN STYRIA AND ESTABLISHMENT OF A PANEL OF HPA-TYPED PLATELET DONORS

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Background:

Immunization against human platelet antigens (HPA) can lead to clinically significant syndromes like fetal (FAIT) or neonatal alloimmune thrombocytopenia (NAIT), posttransfusion purpura (PTP), and refractoriness to platelet (plt) transfusion therapy.

Aims:

The investigation of allele frequencies and genotype distribution of human platelet antigens is important for clinical transfusion practice and enables better prediction of the risk of platelet-specific alloimmunization.

To identify the allele frequencies of HPAs in our region and to establish a panel of HPA homozygous plt donors for the substitution of patients with anti-HPA-alloantibodies we performed genotyping of our plt donors. We additionally screened samples of random blood donors for HPA 1a serologically and performed genotyping for HPA -1, -2, -3 and -5 of negative reacting samples.

Methods:

DNA was extracted from samples of 217 plt donors and HPA-1, -2, -3 and -5 alleles were determined by PCR-SSP (Domino System HPA/Protrans).

Serological screening for HPA-1a of 651 random blood donors was performed by a commercially available HPA-1a typing assay (DiaMed Switzerland).

Results:

The distribution of HPA genotypes (217 plt donors) were as follows: HPA-1 aa 153 (70,5%), HPA-1 ab 57 (26,3%), HPA-1 bb 7 (3,2%). Including the results of the random blood donors (16/651 HPA-1bb) the percentage of HPA-1bb donors in our population was 2.6% (23/868); HPA-2 aa 165 (76%), HPA-2 ab 47 (21,7%), HPA-2 bb 5 (2,3%), HPA-3 aa 82 (37,8%), HPA-3 ab 105 (48,4%), HPA 3 bb 30 (13,8%), HPA 5 aa 182 (83,8%), HPA-5 ab 34 (15,7%) and HPA-5 bb 1 (0,5%).

The allele frequencies of the four HPA systems in our population were 0,84 and 0,16 for HPA-1a and -1b, 0,87 and 0,13 for HPA-2a and -2b, 0,62 and 0,38 for HPA-3a and -3b and 0,92 and 0,08 for HPA-5a and -5b.

Conclusions:

1. The allele frequencies of HPA-1, -2, -3 and -5 detected in our plt donor population are comparable with previous studies in Caucasians.
2. In our laboratory we successfully established a panel of HPA-typed plt donors by PCR-SSP for the substitution of patients with NAIT, PTP and plt refractoriness due to antibodies against human platelet antigens.

708 EFFECTS OF DEFERIPRONE IN IRON CHELATION THERAPY IN PATIENTS WITH THALASSEMIA

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Introduction: Deferiprone (DFP) is an orally active iron-chelation agent that is being evaluated as a treatment in thalassaemic patients and has improved their quality of life. Our aim is to record the long-term safety and effectiveness of iron-chelation therapy with deferiprone in our thalassaemic patients. **Patients and methods:** We studied 17 males and 25 females (n=42), mean age 31 years old (22-57). All the patients received the drug for a mean of 20 months (2-39). 34 of them received DFP as monotherapy (75 mgr/Kgr/day) and 8 received DFP in the same dose in combination with desferioxamine (DFO), (40 mgr/Kgr/day, 2 days/week) by continuous s.c 12 hours infusion. We discontinued treatment with DFP in 5/42 patients, because of agranulocytosis (1 patient), arthritis (1 patient), and gastrointestinal problems (3 patients). Depending on the ferritin levels the patients were divided into 4 groups. Non parametric Wilcoxon Signed Ranks test was used for statistical analysis.

Results: The effects of DFP on serum ferritin in 4 groups of patients are reported in the table. **Conclusions:** Statistical significant decrease was determined in 3 groups of patients with ferritin levels > 1000ng/ml. Our findings support the effectiveness of deferiprone in reducing or stabilizing the body iron load, as assessed by serum ferritin levels in our thalassaemic patients.

709 ETOPOOSIDE AND DOSE DENSE SCHEDULING OF ALFA 2A INTERFERON IN TREATMENT OF RESISTENT CUTANEOUS T-CELL LYMPHOMA (CTCL) STAGES IB-IIB

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Background:

Standard treatment of Cutaneous T-Cell Lymphoma (CTCL) in early MFCG stages (I-II) is alpha-interferon with target dose of 18 MU/week, with DFS 75% at 1 year (Rupoli S. et al, 1999). In advanced MFCG stages (III-IV) alpha-interferon is combined with isotretinoin, skin electron beam therapy, topical nitrogen-mustard, with DFS 27% at 5 years (Duvic M. et al, 2003). In recent years chemotherapy was included in therapy schedules, especially low dose methotrexate (RR 58%, TTF 31 m., Zackheim H, 2003), and as novel agents fludarabine and DCF.

Aims:

The aim of study was to define the place of chemotherapy in patients with MFCG stages IB-IIB, resistant or not-treatable with local treatments and interferon.

Methods:

Dose dense scheduling of alpha- interferon was introduced in attempt to override resistance. Complete therapy was administered as 'in patient' schedule. Alfa 2A interferon dosage was 3 MU/m2/I-XIV days ad etoposide dosage was 120 mg/m2/I, III and V day of the first week. Interval between cycles was four weeks.

Results:

Between III 1991. y. and IX 2003. y., 17 patients (14 evaluable for effects) were treated. M/F 15/2; M 63 y.; MFCG stages: IB 8 pts., IIA 1 pt., IIB 8 pts.; 14/17 patients previously treated with topical corticosteroids, PUVA therapy or chemotherapy (3 pts.: 2 cyclophosphamide, 1 CHOP). 4/17 patients were previously treated with interferon as continuous therapy 6 MU/ alternate days.

Number of cycles delivered: 122, median 7. ORR 8/14 (57%), CRR 3/14 (21%), PRR 5/14 (36%), SDR 3/14 (21%). Median time for reaching response was 4 months. Among previous interferon patients response was: 1 CR, 1 PR and 2 SD. Haematological toxicities were moderate and tolerable: leucopenia gr. III 22 episodes, median duration 2 days; granulopenia gr. III 21 episodes, median duration 3 days; infection gr. II 1 episode; infection gr. III 1 episode. Non-haematological toxicities were sporadic: peripheral neuropathy gr. II 1 episode; peripheral neuropathy gr. III 1 episode.

Number of cardiac events was unexpected: rhythm gr. II 2 episodes; angina pectoris 1 event, myocardial infarction 1 event.

One event of fatal pulmonary embolism was noted. Duration of response: CR 63+, 71+, 129+ months, PR 3,4,6,8 and 17 months.

Duration of SD: 6,8 and 17 months.

Median TTP was 17 months (several patients being salvaged by retreatment).

Median OS was 46 months.

Summary/Conclusions:

Dose dense scheduling of alpha 2A interferon with etoposide is effective treatment in locally not-treatable and even resistant disease, previously treated with conventional doses of interferon.

Haematological toxicity was moderate but number of potentially dangerous cardiac events was unexpectedly high.

710 CYCLIN D1 EXPRESSION IS USEFUL IN DISTNGUISHING MANTLE CELL LYMPHOMA FROM FOLLICULAR AND SMALL LYMPHOCYTIC LYMPHOMA

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Objective. The distinction between mantle cell lymphoma (MCL) and other low-grade B cell lymphoproliferative disorders e.g. follicular lymphoma (FL), small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) is important because MCL has a more aggressive course. MCL is a subentity of malignant lymphomas that is characterized by the chromosomal translocation t(11;14), resulting in the overexpression of cyclin D1. Immunohistochemical detection of cyclin D1 has been shown as highly sensitive and specific marker for MCL. The aim of our study was to evaluate the immunohistochemical expression of cyclin D1 in MCL, FC and SLL/CLL.

Material and Methods. We analyzed 62 NHL cases referred to our institution in the period 1998-2002. There was 21 case with MCL, 20 FL, and 21 with SLL/CLL, with median age 59,24 year, female/male ratio 34/21. Lymph nodes were enlarged in 19 pts with MCL, 13 pts with FL and 15 pts with SLL/CLL. Splenomegaly has been found in 18 pts with MCL, 8 pts with FL and 7 pts with SLL/CLL. Hepatomegaly was detected in 16 pts with MCL, 7 pts with FL and 7 pts with SLL/CLL. Mean Hb value in MCL group was 105,68 g/l, 107,31 g/l in FL and 108,13 in SLL/CLL pts. Thrombocytopenia was found in 10/21 MCL, 5/20 pts with FL and 9/21 pts with SLL/CLL. WBC 48,12x109/l in MCL, 54,23x x109/l in FL and 33,08 x109/l in SLL/CLL. Average LDH in MCL was 702,26 UI/l, 578,00 UI/l in FL and 495,54 UI/l in SLL/CLL. Clinical stages of the disease were distributed as follows: MCL: I- 4 pts, II 3 pts, III 7 pts and IV 7 pts; FL I 4 pts, II 4 pts, III 4 pts, IV 8 pts; SLL/CLL IV CS 21 pts. Bone marrow infiltration was found in 20 pts with MCL(nodular type 12 pts, diffuse 4

pts, interstitial 4 pts), in FL in 14 pts (without infiltration 2 pts, nodular 6 pts, diffuse 3 pts and interstitial 5 pts), and in 13 pts with SLL/CLL (nodular 6 pts, diffuse 4 pts, interstitial 3 pts). Immunohistochemical analysis detected CD79alfa+, CD5+, CD43+, CD19+, bcl-6+(10/21 pts) and CD10-, and CD23- phenotype in majority pts with MCL. In group of FL immunophenotype was: CD79alfa+, CD10+, bcl-2+, bcl-6+ and CD5-, CD43-, CD23-. In SLL/CLL immunophenotype was: CD79alfa+, CD23+, CD5+, bcl-2+ and CD10-, bcl-6-. Cyclin D1 was positive in 18 pts with MCL, weak positive in 2 pts with FL and negative in all pts with SLL/CLL.

Conclusion. Our analysis suggest that histological analysis should be completed with immunophenotyping with panel of monoclonal antibodies and cyclin D1 for better distinction of low grade lymphomas and MCL. In our group of most MCL pts were in IV clinical stage of disease and clinical course was aggressive with poor response to chemotherapy.

711 MUTATION AND METHYLATION ANALYSIS OF P16 GENE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic Lymphocytic Leukemia (CLL) comprises a heterogeneous group of at least 2 types of disease entities characterized by distinctive clinical, immunophenotypical and genetic features. The molecular mechanisms underlying the pathogenesis and the histological transformation of CLL are not well known. INK4A (p16) a cyclin-dependent kinase inhibitor has been considered a tumor-suppressor gene. Inactivation of this gene by homozygous deletions, mutations, and hypermethylation occurs in a variety of human neoplasms.

Aims: The aim of the present study was to determine the frequency of p16 gene deletions and mutations as well as the methylation status of the same gene in CLL patients.

Methods: We examined 34 peripheral blood DNA samples from CLL patients. P16 gene rearrangements were studied by Southern Blotting using a p16 exon 2 probe. Mutational analysis of p16 coding region was performed by Polymerase Chain Reaction Single-Strand Conformation Polymorphism (PCR-SSCP) and DNA sequencing. The methylation status of p16 gene promoter was examined by Methylation-Specific PCR (MSP).

Results: Southern Blot analysis revealed non rearranged bands in all cases examined. PCR-SSCP analysis of p16 gene exon 2 revealed an abnormal migration pattern in 2/34 cases. Sequencing analysis detected the already known polymorphism ALA148 THR in exon2 in both these cases. Other alterations of p16 gene coding region were not identified in any case. Hypermethylation of p16 promoter region was identified in 6 cases (17,6%). Summary/ Conclusion: These results indicate that genetic alterations of p16 gene are rare events in patients with CLL. The clarification of the role of p16 hypermethylation in the pathogenesis and evolution of CLL needs further investigation.

712 COMPLEX KARYOTYPE IN COEXISTENT LIGHT CHAIN DISEASE AND CHRONIC LYMPHOCYTIC LEUKAEMIA, A CASE REPORT

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Background: We report a 48-year old man with concomitant light chain disease and CLL, both diagnosed at presentation. The clinical course of the disease was very aggressive and our patient died one month after diagnosis.

Methods and results: In the peripheral blood an absolute lymphocytosis was present. Cytologic examination of the blood revealed a picture morphologically corresponding with chronic lymphocytic leukaemia. By screening rare plasma cells could be observed. Investigation of the bone marrow resulted in a prominent plasma cell population (70%) with clear aberrant morphology. Immunophenotyping confirmed the CLL (CD5+, CD19+, CD23+, FMC7-), in blood as well as in bone marrow and demonstrated a kappa monoclonality. In contrast the Bence Jones protein in serum and urine was of the lambda type. The karyotype of the bone marrow was very complex and hypodiploid (41-43,XY) with the following abnormalities: DUP(1)(Q12;Q31), +2DER(2)T(2;8)(P16;Q21) -6, -8, -10, -11, -12, -13, DER(14)T(11;14)(Q13;Q32), -16, -17, +MAR1, +MAR2,

+MAR3. FISH on bone marrow, after cell culture, confirmed the deletions of 13Q14 and 17P13 in 52% of cells and showed abnormalities of 14Q32, consistent with T(11;14), in 61% of cells. FISH on the plasma cells only (separation based on immunostaining for light chains) also showed deletion of chromosome 13Q14 and 17P13 in 51% of the cells, but 14Q32 appeared abnormal only in 5%. In contrast, chromosome 13Q14 and 17P13 were normal in blood, while 14Q32 was rearranged in 14,5% of cells. IgH rearrangements (14Q32) and bcl-1(11Q13) proto-oncogen were analysed by Southern blot. Different IgH rearrangements in blood and in bone marrow were detected. The bcl-1 rearrangement was absent both in blood and in bone marrow. This can be explained by the fact that the breakpoint region of multiple myeloma (330kB) on 11Q13 is much larger than the breakpoint region of lymphoma (120-130 kB) and only this latter short region has been investigated.

Summary: In the literature we found 45 cases of concomitant multiple myeloma and CLL, of which 27 cases were diagnosed simultaneously. In only a few cases a single clone is suggested, however older reports only make use of immunological data and no molecular analysis. In our case a very aberrant karyotype was found. Deletions of 13Q14 and 17P13 were present in bone marrow, but not in blood. This together with the different IgH rearrangements in blood and bone marrow detected by Southern Blot, demonstrate the distinct clonal origin of both lymphoproliferative diseases.

713 SCREENING OF MUTATIONS CAUSING HAEMOPHILIA A AND B IN LATVIAN POPULATION

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In 2001 molecular diagnostics of haemophilia A and B has been started in Latvia by Biomedical Research and Study Centre, University of Latvia in collaboration with National Centre of Haematology, Hospital 'Linezers' and State Children's Hospital. Today most of haemophilia patients in Latvia have been included in a database. Blood samples from patients and their relatives are collected from 43 of haemophilia A and 11 of haemophilia B families. 9 new mutations in haemophilia A and 2 in haemophilia B are found. Intron 22 inversion was detected in 47% of severe haemophilia A patients. 5 female samples with reduced coagulation activity (FVIII:C<60) were analysed by direct gene F8 sequencing and intron 22 inversion PCR detection without the silent x-chromosome test. We have detected no mutations in 3 severe haemophilia A and 1 severe, 1 mild haemophilia B patients tested by direct sequencing and intron 22 inversion PCR test. The common mutation database consisting of phenotype and genotype information of tested haemophilia patients and their relatives has been formed.

714 RISKS AND BENEFITS OF SPLENECTOMY IN PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA

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Background: splenectomy may contribute to improve outcome of the patients with myelofibrosis with myeloid metaplasia (MMM) refractory to therapy, but it is associated with significant perioperative mortality and morbidity. Aim: to evaluate risks benefits ratio of splenectomy in patients with MMM, with particular attention to thrombotic and hemorrhagic complications.

Methods: we retrospectively analysed 14 MMM patients (8M, 6F), who underwent splenectomy between September 1996 and October 2002. The median age at splenectomy was 64 years (range 44-74 y). Median time from diagnosis of MMM to splenectomy was 25 months (range 0-104 m). The patients have been classified according to prognostic Dupriez score in 3 groups: low (4), intermediate (8) and high risk (2), respectively.

Primary indications for splenectomy were: progressive transfusion-dependent anemia (11/14), symptomatic splenomegaly (9/14), severe thrombocytopenia (4/14), portal hypertension (3/14). Only one patient was electively submitted to surgery before allogeneic stem cell transplantation from HLA identical donor family. All patients were subjected to vaccine-prophylaxis and 13/14 patients (92,8%) received anti-thrombotic prophylaxis with low molecular weight heparin (LMWH). Results: evaluation after 6 months revealed: improvement in constitutional symptoms related to splenomegaly (55%), reduction of transfusion dependence (63,6%), increase of platelet count (25%). The main peri- and post-operative complications included infections (8), acute

bleeding (1), thrombosis (2) and extreme thrombocytosis (5). Leukemic transformation occurred in 3 of 14 patients (21.4%). In addition to prophylactic LMWH, 5 patients with rebound thrombocytosis ($PTL > 1.000 \times 10^9/L$) received ASA. Hydroxyurea was administered only for concomitant leucocytosis ($WBC > 30 \times 10^9/L$). Perioperative early mortality (within 30 days) was 21.4% (3/14): all patients died for thrombotic or hemorrhagic events, particularly 2 patients for pulmonary embolism and 1 patient for gastro-intestinal bleeding. Preoperative severe thrombocytopenia ($PTL < 50 \times 10^9/L$) and high Dupriez score seem to be the only predictive variables related to postoperative thrombosis. No correlation has been observed between postoperative thrombosis and thrombocytosis. At present overall survival is 43% (6/14), with a median survival of 15 months after splenectomy (range 15 days -72 months).

Summary/Conclusions: splenectomy seems an effective palliative procedure for patients with advanced MMM, even if it is associated with relevant perioperative mortality (in our group 21.4%) and morbidity. The primary causes of perioperative death include thrombotic or hemorrhagic events. Our results suggest the importance of thrombotic risk factors analysis for preoperative evaluation of patients candidates to splenectomy (Dupriez score, platelet preoperative count); prophylactic LMWH and treatment of thrombocytosis post-surgery is essential to reduce complications and perioperative mortality.

715 CLINICAL OUTCOME AND TREATMENT RESULTS IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: About 60% of acute myeloid leukemia (AML) patients are over 60 years of age. The disease in this age group is less responsive to therapy, and the postchemotherapy aplasia is longer. Treatment related mortality is higher than 20%. The elderly are at higher risk of relapse despite successfully completing intensive consolidation therapy. There is no consensus about the best regimen or the number of treatment cycles for postremission therapy in older adults.

Aims: The aim of our study was to assess the clinical outcome and the treatment results in elderly AML in terms of therapeutic response and survival.

Methods: A retrospective analysis was performed on 106 AML patients aged 60 years or more (median age 67 age), consecutively admitted to a single hematological department during a 12-year period. Five patients with acute promyelocytic leukemia were excluded. The induction treatment was adapted according to the patients performance status. Sixty patients (arm A) received epirubicin 50 mg/m² i.v. on days 1-3 and cytarabine (Ara-C) 100 mg/m² i.v./24 hours on days 1-5; forty-one patients (arm B) received, every 12 hours, etoposide 100 mg i.v. for 3 days, and low doses of Ara-C (10 mg/m²) s.c. for 5 days.

Results: After the first induction course, complete remission (CR) rate was 39.60% (with 40% in arm A and 39.02% in arm B). Partial remission (PR) was achieved in 30.69% of patients (28.33% and 34.14%, respectively). 11.88% of patients (11.66% and 12.19%) were refractory to treatment. The induction mortality rate was 17.82% (20% in arm A and 14.63% in arm B; $p < 0.005$). The median survival of all 101 patients was 21 weeks, and median duration of CR was 8.5 months. Median disease-free survival (DFS) was 19 weeks in arm A and 25 weeks in arm B, with a 2-year survival rate of 15.84% (15% and 17.07%, respectively), and with a 3-year survival rate of 2.97% only. None of the 12 patients treated with palliative measures achieved CR, and their median survival was 2.5 months. The evaluation of the CR rate after two courses was available in 71 out of 101 patients. 54.92% of these patients achieved CR. CR rate, overall survival and DFS did not differ significantly between both analyzed arms. At the time of this analysis 9 patients were alive. The toxicity profile was similar in both groups, and no cardiotoxicity was observed.

Conclusions: Our experience suggests that a high proportion of de novo AML patients over 60 years can be treated with standard chemotherapy having high probability both to achieve CR and to prolong survival. Etoposide may constitute an alternative approach for the treatment of elderly patients with AML when anthracycline could not be used. AML in elderly patients remains a severe and incurable disease.

716 ADULT ACUTE LYMPHOCYTIC LEUKEMIA WITH PHILADELPHIA CHROMOSOME POSITIVE: TREATMENT, RESULTS AND PROGNOSIS

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In July 1997- February 2004 period, in Clinic of Hematology from Craiova were hospitalized a number of 62 cases with acute lymphocytic leukemia (ALL); from these, 19 cases were Philadelphia chromosome positive. These 19 cases were treated with VAD regimen (vincristine+adriablastine+dexamethasone) or cyclophosphamide plus VAD (CVAD regimen).

Results

The 19 Philadelphia chromosome positive ALL patients were older ($p = 0.02$) more likely to have anaemia ($p < 0.02$) and had a higher incidence of peripheral leukocytosis ($p = 0.03$), FAB L2 morphology ($p < 0.01$), common antigens (CALLA) and CD34+ marker positive ($p < 0.02$) than non-Philadelphia chromosome positive ALL patients.

Complete response was noted in 14 patients (73.8%), with median survival and remission duration of 9 and 17 months, respectively. Of the remaining Philadelphia chromosome positive ALL patients, 2 (10.5%) achieved partial response, 1 died as a results of induction therapy and 2 (10.5%) had resistant disease.

Survival and remission data were significantly worse for patients in the Philadelphia chromosome positive ALL group than in any other ALL subset. In all 15 cases treated with VAD regimen, 13 (96%) achieved a complete response compared with 4 cases treated with CVAD regimen in which only 2 cases achieved a complete response (50%).

Conclusion

The patients with Philadelphia chromosome positive ALL have a poor prognosis when they are treated with conventional therapy, with overall complete response rates, shorter remission duration and shorter survival when compared with other ALL patients.

The use of VAD or CVAD rescue regimens in Philadelphia chromosome positive ALL patients was in our cases comparable with cytosine arabinoside high doses who are well known from efficiency in consolidation of induction regiments.

717 EFFECT OF TNF AND TGF ON PROLIFERATIVE ACTIVITY OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECS) IN PATIENTS WITH NON-HODGKIN'S LYMPHOMAS

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Background. Neoangiogenesis is one of the crucial factors of tumor progression and widespread including non-Hodgkin s lymphomas (NHL). This process may be influenced by a network of different mitogens and cytokines which can regulate it in a positive or negative fashion depending on their source and concentration. Tumor necrosis factor (TNF) as well as tumor growth factor beta (TGFβ) are regarded among them but their possible role in NHL angiogenesis isn't yet proved.

The aim of our study was to compare the proliferation of HUVECs after influence of recombinant human TNF (rhTNF) or recombinant human TGFβ (rhTGFβ) and supernatants of primary 24h cultures of mononuclear cells from lymph nodes (MNLN) or spleen (MNS) as well as from peripheral blood (MNPB) and plasma of 48 NHL patients (pts).

Methods. HUVECs were obtained from human umbilical vein by digestion with 0.2% collagenase and were cultivated as described (E.A.Jaffe, 1973). TNF and TGFβ levels were evaluated by bioassays using sensitive L929 and CCL64 cell lines respectively as a target. Both rhTNF and rhTGFβ influence as well as cells supernatants activity concerning HUVECs proliferation were measured by incorporation of 3H-thymidine and estimated by the index of proliferation (IP).

Results. Our investigations have shown that in NHL pts plasma and supernatants of MNPB had slightly diminished proliferative activity on HUVECs (IP=1.360.19 and 1.260.15) in comparison with MNLN and MNS ones (1.660.19 and 1.900.39), whereas TNF concentration in plasma of NHL pts was 6.9-11.5 fold higher (0.940.11 ng/ml) than in cells supernatants ($p < 0.001$ in all cases). In contrast to TNF contents the TGFβ level in plasma of NHL pts was appreciably lower (3.960.41 ng/ml) than in supernatants of MNPB, MNLN, MNS (from 5.820.42 to 6.620.54 ng/ml).

On the other hand we found that lower concentrations of rhTNF (0-1.55 ng/ml) inhibited 3H-thymidine incorporation in HUVECs (IP<1), whereas intermediate rhTNF contents (1.55-13.63 ng/ml) induced a considerable growth of endothelial cells in a dose-dependent manner (IP from 1.0 up to 2.5). However higher levels of rhTNF didn't increase proliferative activity of HUVECs ('plateau phase'). In rhTNF concentrations over 25.0 ng/ml an increasing cytolysis of HUVECs was observed. In contrast to rhTNF we found that lower concentrations of rhTGFβ (<3.6 ng/ml) induced a considerable growth of cells, whereas higher contents of rhTGFβ (>3.6 ng/ml) resulted in progressive cytolysis of HUVECs.

Summary. On the other hand our investigations revealed that rhTGFβ, and rhTNF influenced on proliferation of HUVECs in the different manner: contrary to rhTNF rhTGFβ had a tendency to inhibit proliferation of HUVECs in a dose-dependent manner with increasing of its concentration. Taking into account the revealed levels of both cytokines in biological samples from NHL pts, in contrast to respective concentrations of rhTGFβ, and rhTNF, plasma and cells supernatants stimulated proliferation of HUVECs in all cases. This discrepancy may indicate the presence of a complex network of cytokines and other factors in relationships between lymphoid tumors and vascular endothelium.

718 INFLUENCE OF COMMON INHERITED THROMBOPHILIA POLYMORPHISMS IN THE INCIDENCE OF THROMBOSIS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

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BACKGROUND: Among the major myeloproliferative disorders (MPD) polycythemia vera (PV) and essential thrombocythemia (ET) are reported to be associated with increased thrombotic complications. In addition to well-established acquired risk factors for thrombosis, several genetic risk factors are known to influence thrombotic risk. Among them, the common polymorphisms in the genes of coagulation factors (factor V Leiden [FVL], FII-G20210A) and hyperhomocysteinemia (caused by either acquired or inherited MTHFR-C677T variant being the most common- conditions) represent the prevailing causes for thrombosis. However, the contribution of inherited abnormalities to thrombotic tendency in patients with MPD remains poorly explored.

AIMS AND METHODS: We performed a retrospective study to evaluate the prevalence and clinical significance of three gene variations (FVL, FII-G20210A and MTHFR-C677T) in patients with PV and ET, with and without thrombotic episodes. We enrolled 36 patients (M/F ratio 1.77, mean age 65.02, range 35-85) with a diagnosis of PV (9 patients) and ET (25 patients). Twelve patients (33.33%) had at least one thrombotic episode (deep vein thrombosis, pulmonary embolism, stroke, acute myocardial infarction, peripheral vascular thrombosis, spleen infarctions), which had been objectively verified; 24 patients (66.66%) had no evidence of thrombotic event. FVL and MTHFR-C677T mutations analyzed by PCR-RFLP, and FII-G20210A polymorphism analyzed by allele-specific PCR.

RESULTS: Interestingly, FVL was found in none of 12 thrombotic patients (incidence of 0%) and in 4 of 24 non-thrombotic patients (16.66%). FII-G20210A was found in one of 12 thrombotic patients (8.33%) and in one of 24 non-thrombotic patients (4.16%). MTHFR-C677T (homozygous 677T and heterozygous form) was present in 7 of 12 thrombotic patients (58.33%) and in 13 of 24 non-thrombotic patients (54.16%), respectively.

CONCLUSIONS: Despite the limited number of studied patients, our results suggest that there is no association between the three investigated polymorphisms and the risk of thrombosis in patients with MPD. It is transparent, that acquired defects due to malignant clone (increased viscosity and/or increased number and functionality of platelets) are responsible for the increased prevalence of thrombotic events in patients with PV and ET. These data are in concordance with previous reports, where no effect of genetic predisposition to thrombosis in cancer patients was also established.

719 PROGNOSTIC SIGNIFICANCE OF IRON STATUS INDICES IN HEMODIALYSIS PATIENTS RECEIVING RECOMBINANT HUMAN ERYTHROPOIETIN

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Background: Absolute and functional iron deficiency is the most important cause of erythropoietin (rHuEPO) resistance in common practice. Early detection of iron sufficiency at the level of the erythropoietic cell is necessary to optimize management of anemia in hemodialysis patients receiving rHuEPO. Thus, the evaluation of the prognostic significance of iron status indices is very important to achieve this goal.

Patients and methods: The aim of this study was to evaluate the prognostic value of both traditional as well as newer iron status indices, in uremic patients receiving rHEPO. Sixty chronic hemodialysis patients, 34 males and 26 females with mean age 60.12 years (range 36-80), and dialysis duration 3836 months were enrolled. All patients were on maintenance rHuEPO treatment for at least three months and no iron supplementation was used for the same period of time. All patients received 1gr of IV iron sucrose, 100mg per hemodialysis session for 10 consecutive sessions. Blood samples were examined in week 0 (baseline measurement) and every week for 5 consecutive weeks. The evaluated parameters were: hemoglobin, percentage of reticulocytes(%retics), reticulocyte hemoglobin content (CHr), percentage of hypochromic erythrocytes (%HE), the ratio of reticulocyte mean corpuscular volume and the erythrocyte mean corpuscular volume (MCVr/MCV), percentage of high fluorescence reticulocytes (%HFR), percentage of immature reticulocyte fraction (%IRF), serum ferritin (FER), transferrin saturation (%TSAT), and soluble transferrin receptor (sTfR).

Results: Thirty patients responded to i.v. iron administration (hemoglobin elevation 1g/dl at the end of the study). Serum ferritin, TSAT and sTfR were the only statistically significant indicators of functional iron deficiency. Mean values of baseline ferritin in responders and nonresponders were 101,2112,8ng/ml and 185,6116,8ng/ml respectively (p=0,003). Baseline TSAT was 24,4710,5% and 30,18,2 respectively (p=0,036). Baseline sTfR was 2,030,86mg/l and 1,580,59mg/l in each group respectively (p=0,04). All other evaluated parameters had no statistical significance. Combining serum ferritin and sTfR (cut off values serum ferritin <150ng/ml and sTfR>1,8mg/l) we found high specificity (93,3%) in the prediction of functional iron deficiency.

Conclusions: Our results suggest that baseline sTfR and serum ferritin either alone or in combination are strong indicators of functional iron deficiency and may be used to predict the response to IV iron administration in rHuEPO treated hemodialysis patients.

720 PROTEOME CHANGES IN LEUKEMIC CELLS INDUCED BY PHOTODYNAMIC TREATMENT

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Background: Photodynamic therapy (PDT) is an approach to cancer treatment which is based on the application of a photosensitive compound that is subsequently activated by irradiation with visible light. By energy-transfer processes the oxygen molecules present in tissues are activated and highly reactive singlet oxygen molecules, ¹O₂, are formed. These cause oxidation of cellular compounds (proteins, lipids) leading to the cell death. A variation of PDT is based on the administration of 5-aminolevulinic acid (ALA), the distant precursor of heme in biosynthetic pathway. Due to defective heme biosynthesis in most cancer cell types the compound with excellent photosensitizing properties, protoporphyrin IX, is accumulated in cancer cells. By subsequent irradiation the tumor destruction is achieved.

Aims. The aim of the project was by evaluating global protein profiles of promyelocytic leukemia cell line HL60 and chronic myelogenous leukemia cell line K562 to reveal and identify proteins that were affected (enhanced, suppressed, de-/hyper-phosphorylated) by ALA-PDT and to propose mechanism of ALA-PDT cytotoxicity.

Methods: Employing two-dimensional electrophoresis (2-DE) we examined the effects of ALA-PDT on the proteome of HL60 and K562 cells. The 2-D peptide maps of ALA-induced cells (treatment with 1 mM ALA for 4h) as well as cells subjected to ALA-PDT (irradiation of ALA-induced cells with blue light dose of 18 J/cm²) were obtained and compared by image analysis of silver or phosphorylation stained

gels. Protein spots that differed in the position, density or fluorescence intensity, were subjected to mass spectrometry analysis and identified by bioinformatics means.

Results: PDT based on ALA-treatment leads to the extensive changes in the protein profile of HL60 cells. Among 1350 protein spots recognized on the 2-D peptide map of HL60 cell lysates, expression of thirty proteins was affected by ALA-PDT. Three of the down-regulated proteins, calreticulin, ERp57 and protein disulfide isomerase are Ca²⁺-binding endoplasmic reticulum (ER) chaperones. On the other hand, expression of mitochondrial stress protein HSP60, ribosomal protein S12, triosephosphate isomerase and mitochondrial malate dehydrogenase were increased while the mobility of GDP dissociation inhibitor Rho-GDI 2 was altered by ALA-PDT.

In K562 cells of 89 phosphoprotein spots observed on the 2-D map of the control, 26 changes were found (mostly decrease) following ALA-PDT. Changes in the cytoskeleton organizing proteins (CLP-LIM kinase), stress proteins (HSP90) and transcription regulators, both in their level and phosphorylation state, were observed.

Conclusions: Several cellular pathways are affected in HL60 cells by ALA-PDT mediated cytotoxicity including glycolytic and oxidative phosphorylation functions, DNA-repair processes as well as translation mechanism. The proposed mechanism of ALA-PDT mediated cytotoxicity includes mitochondrial perturbation and perturbation of ER Ca²⁺ stores which can activate Ca²⁺-Mg²⁺-dependent endonucleases. Application of ALA-PDT to K562 cells leads to several proapoptotic events but apoptosis did not proceed to the execution phase. High levels of molecular chaperons are possible explanation for K562 cells resistance to apoptotic execution by ALA-PDT.

Acknowledgment: The work was supported by the grant NL 7681-3 of Internal Grant Agency, Ministry of Health Czech Republic

721 ASSOCIATION STUDIES OF CD28 AND ICOS GENE POLYMORPHISMS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Background. In B-CLL patients not only humoral but also cellular immunity is severely impaired. T cell-specific surface receptors CD28 and 'inducible co-stimulator' (ICOS) are main costimulatory molecules expressed on cell surfaces and provide regulatory signals for T-cell activation. CD28 potentially enhances T-cell functions essential for effective antigen-specific immune responses. Unlike the constitutively expressed CD28, ICOS is induced on the T-cell surface and does not upregulate the production of IL-2, but induces the synthesis of IL-4. Aim. The study was undertaken to evaluate the association between the T19int3C CD28 gene polymorphism, microsatellite (GT)_n polymorphism in intron 4 of the ICOS gene and susceptibility to B-CLL in a Polish population. Methods. One hundred thirteen B-CLL patients and 95 healthy subject were studied. Genomic DNA was isolated from whole frozen blood using the NucleoSpinR Blood kit (MARCHERY-NAGEL, Germany). Allele identification was achieved by PCR amplification. The amplified product for SNP (single-nucleotide polymorphism) loci was purified and minisequenced using the commercial kit SnapShot of 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. The distribution of CD28 T19int3C genotype and allele frequencies did not differ between patients and healthy subject. The frequencies of the allele 270 were significantly increased, and allele 272 significantly decreased in B-CLL compared with healthy controls. Summary. Our results suggest that the CD28 T19int3C gene polymorphism does not contribute to genetic susceptibility to B-CLL while the microsatellite ICOS gene (GT)_n polymorphism in intron 4 appears to confer risk for the development of this disease.

722 CORRELATION OF THROMBOEMBOLIC EVENTS WITH THROMBOPHILIA POLYMORPHISMS IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Thromboembolism is not uncommon in multiple myeloma (MM) but the pathogenesis still remains poorly understood. Patients who are on treatment with dexamethasone, doxorubicin, and thalidomide are in a higher risk of experience such a complication. Factor F Leiden and prothrombin G20201A are the most common congenital risk factors for thrombosis. Furthermore the role of the MTHFR gene mutation in thrombosis, especially in MM patients is still unclear. The aim of our study was to investigate the role of these three genetic factors in the thromboembolic disease, which may present in the course of MM.

Patients and Methods: We studied 40 patients, 18 women and 22 men (aged 29-81, mean 65,92 ± 10,6). All patients suffered from M, 29 IgGκ type, 5 IgGλ type, 2 IgAκ type, 1 IgAλ type, 2 κ light chain and 1 λ light chain MM. Thirty two patients received several chemotherapeutic regimens including doxorubicin and dexamethasone with or without thalidomide and 8 received MP regimen. Fifteen patients (5 in first line and 8 in second line regimens and 2 in maintenance therapy) received thalidomide. The examination of the thrombophilia polymorphisms revealed 16/40 (40%) MTHFR heterozygosity, 4/40 (10%) MTHFR homozygosity, 4/40 (10%) FV Leiden heterozygosity and 1/40 (2,5%) prothrombin G20201A heterozygosity. Two out of 40 patients (5%) experienced a thromboembolic episode during the course of the disease (one with peripheral arterial infarction and one with pulmonary embolism). These patients were homozygous in MTHFR polymorphism and had received a VAD regimen without thalidomide.

Conclusions: The frequency of the three thrombophilia polymorphisms in patients with MM does not defer from that of the general population in Greece. No correlation was observed between the FV Leiden and the prothrombin G20201A polymorphisms with the occurrence of thromboembolic episodes. However, the fact that the two patients who experienced thrombosis were homozygous in MTHFR polymorphisms, may imply that there is a relationship between this polymorphism and thrombosis in MM patients. Further investigation is determined.

723 SERUM CONCENTRATIONS OF SOME AGIOGENETIC FACTORS IN MULTIPLE MYELOMA: VEGF, bFGF IN MMP-9

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Background Angiogenesis is a crucial process in progression of multiple myeloma. Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) are multifunctional cytokines that stimulate angiogenesis and myeloma growth. Matrix metalloproteinase 9 (MMP-9) plays a critical role in osteolytic bone destruction, angiogenesis and invasive growth of myeloma cells. We evaluated serum concentrations of these factors in patients with multiple myeloma.

Methods Levels of active and pro- matrix metalloproteinase 9 (total MMP-9), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) were determined with a commercial quantitative enzyme immunoassay Quantikine (R&D Systems, USA). All of these factors were measured in the serum obtained from peripheral blood of 36 patients affected by multiple myeloma. This series included 12 patients with disease in plateau phase and without treatment, 14 patients on Thalidomide therapy and 10 patients at the beginning of chemotherapy because of active disease.

Results VEGF showed a strong correlation with MMP-9, while VEGF and bFGF did not correlate with each other. Blood platelets correlated with VEGF and MMP-9. The concentration of MMP-9 and VEGF were the highest in group of patients with active disease where the chemotherapy started. The level of bFGF was the lowest in the group devoid of treatment (plateau phase of disease).

Conclusions. Production of the angiogenic factors such as VEGF, bFGF and MMP-9 are increased in multiple myeloma patients. The levels of these factors correlate with the activity of disease.

724 BONE MARROW B AND T LYMPHOCYTES PRESENT SELECTIVE CHANGES IN MYELODYSPLASTIC SYNDROMES
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Background: the role of the immune system in the pathophysiology of myelodysplastic syndromes (MDS) is not well understood. There are evidences of an immune reaction against the abnormal clone. However, the participation of lymphocytes in this clone is controversial.

Aims: to study the BM lymphocyte subsets in newly diagnosed patients with MDS by flow cytometry (FCM), and their relation to the degree of marrow failure and FAB type..

Material and methods: Total lymphocytes as well as their subsets were studied in a 3 color protocol and gated in the SSC/CD45 plot. They were compared with those of normal BM and were also correlated with peripheral blood (PB) counts and CD34+ cells.

Results: Although the total number of BM lymphocytes was not significantly different in MDS and controls, CD3+ lymphocytes were decreased in RA/RARS but increased in RAEB, due to changes in CD3/CD8+ cells. Among PB counts, neutrophils showed a correlation with total lymphocytes ($r = -0.31$), CD3 ($r = -0.36$), CD3/CD8 ($r = -0.43$) and CD3/CD4 ($r = -0.42$). No correlation was found with CD19+ lymphocytes. Platelets showed only a negative correlation with CD3/CD8+ cells ($r = -0.37$). In RA and RARS only peripheral neutrophil count presented this relation. In RAEB, however, this was observed also with platelets and hemoglobin. B-lymphocytes were decreased, more pronouncedly in RAEB.

Conclusion: the changes found in T Lymphocytes are in keeping with an immune reaction against the abnormal clone, more pronounced in RAEB. This affects more pronouncedly the peripheral neutrophil count than hemoglobin and platelets. The changes in B-lymphocytes might be related to the loss of ability of the abnormal stem cell to differentiate for the lymphoid lineage. On the other hand, B-lymphocytes could be inhibited by activated T cells.

Supported by FAPESP and CNPq

725 A NEW ANTIGEN EXPRESSED ON CELLS IN DEATH RECOGNIZED BY MONOCLONAL ANTIBODY

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Background and aims: Monoclonal antibody reactive with normal and leukemic cells in late stages of cell death was prepared in aim to recognize living cells from cells dying by apoptosis or necrosis.

Methods: BALB/c mice were immunized by cells of chronic myeloid leukemia cell line, which contained a large proportion of cells in spontaneous death. Mouse spleen cells from immunized mice were fused with SP 2/O mouse myeloma cell line and cells producing antibody reactive with dying leukemia cells were selected and cloned. One clone (2E12) secreted the desired antibody.

Results: Monoclonal antibody of IgG class reacted with an unknown surface and cytoplasmic antigen of cells, which were morphologically in the late stage of apoptosis. Apoptosis of many leukemic and lymphoma cell lines was induced by camptothecin or by anti-Fas monoclonal antibody 7C11. An increase of cells reactive with antibody 2E12 in immunofluorescence and flow cytometry was clearly detected after 12-48 hours of apoptosis induction.

Conclusions: Antigen recognized by antibody 2E12 is a new unknown marker of cells in late stages of apoptotic and necrotic death.

(supported by grants IGA of the Ministry of Health of the Czech Republic)

726 BCR-ABL/BCR TRANSCRIPT RATIO > 1 AND P21CIP OVEREXPRESSION AS MARKERS OF CML ACCELERATION AND PROGRESSION TO BLAST CRISIS

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Chronic myeloid leukemia (CML) is a typical progressive disease, proceeding from the initial mild chronic phase to the invariably fatal acute phase blast crisis (BC). To be able to prevent this progression by early intervention of intensive therapy, the knowledge of early marker(s) of the disease acceleration is necessary. In our study we searched for such highly predictive molecular markers of disease progression. We investigated CML patients during treatment and focused on 1) changes

in expression of BCR-ABL gene which was proved to play a crucial role in the disease pathogenesis and 2) changes in expression of cell cycle regulators, cyclins D1, D2, D3 and A1 and inhibitor of cyclin dependent kinases p21Cip. We used competitive Q-RT-PCR assays determining BCR-ABL transcript concentration and BCR-ABL/BCR transcript ratio (R), and comparative and real-time RT-PCR for monitoring of cell-cycle regulator expression. Although both BCR-ABL transcript level and R statistically significantly correlated with the response to therapy (Wilcoxon Singed-Rank test, $p < 0.0001$) it was only R that really unambiguously differed in good and bad responders (McNemar Paired Value test, $p < 0.0001$), being < 1 in good and > 1 in poor responders. BCR-ABL transcript levels were atypically low in some non-responders. Thus R values > 1 clearly identified a group of patients at a high risk of disease progression to BC, however, they were not able to indicate the seriousness of the risk for particular individuals. Expression of cyclins D1 and A1 increased with deteriorating quality of response to treatment, resembling the dynamics of BCR-ABL expression, thus bringing no new information. Surprisingly, with the loss of response to therapy and commencement of BC abrupt increase of p21Cip expression was found. This evident deregulation of the cell cycle inhibitor preceded poor outcome in all patients tested. In conclusion, our results indicate that monitoring of BCR-ABL/BCR transcript ratio and p21Cip transcript level during treatment of patients with CML enable to detect the loss of response to therapy and the commencement of progression to BC. BCR-ABL/BCR > 1 and p21Cip overexpression are unambiguously negative prognostic factors.

The study was supported by grant NC/7550-3 IGA MZ CR

727 DETECTION AND QUANTIFICATION OF MINIMAL RESIDUAL DISEASE AFTER BONE MARROW TRANSPLANTATION IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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Background: Chronic Myelogenous Leukemia (CML) is characterized by reciprocal translocation between chromosome 9 and 22 forming a BCR-ABL gene which is a specific marker of disease. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment. The detection of molecular relapse in CML after allogeneic BMT seems to be important, since donor lymphocyte infusions are much more effective when given as early as possible. Karyotyping, Fluorescence in situ Hybridization (FISH), RT-PCR, and Real-time quantitative PCR (RQ-PCR) are well-established molecular techniques used to detect and to confirm the initial diagnosis and to monitor the status of the CML during the course of the disease.

Aims: The aim of the present study was to evaluate and compare different molecular techniques for the detection and quantification of residual CML disease after BMT.

Methods: Molecular follow up was carried out on 56 CML patients after HSCT. Sampling time was 1-48 months post transplant. We simultaneously performed interphase FISH, multiplex RT-PCR, nested-PCR and RQ-PCR to detect BCR-ABL rearrangement at the initial diagnosis, and to compare their feasibilities in terms of MRD monitoring after HSCT. Interphase FISH analysis was performed on 14 new cases according to the standard protocol. Multiplex RT-PCR and nested-PCR were successfully performed on all cases. RQ-PCR was performed on positive samples of PCR (Positive 6 month post-HSCT) for monitoring of MRD on Light-Cycler using Taqman probes.

Results: The primer combinations in multiplex PCR allowed simultaneous detection of all known types of BCR-ABL and BCR transcripts in one reaction. The frequency of the different fusion transcripts was 76% for b3a2, 17.3% for b2a2, 2.6% for 2e1a2 and 4% for rare type fusions. The results showed that FISH and 1st RT-PCR were 93% concordant at diagnostic level; and FISH positivity ranged from 30-65% CML cell in PB samples. In the stable remission levels of MRD were significantly lower, which was hardly detectable by FISH and 1st RT-PCR, but remained detectable in the majority of samples with nested- and RQ-PCR techniques, and they were the method of choice for detecting MRD. Our results demonstrated that during 1-6 months post-BMT only 5% of the patients were PCR negative. During 6-12 months and 1-2 year post-BMT the PCR negativity increased to 52% and 53%, respectively. After 2 years, 75% of the patients achieved PCR negativity and all have remained in CR.

Conclusion: The multiplex RT-PCR allows to detect different types of fusion transcripts and to setup a correct strategy for subsequent MRD monitoring. Interphase FISH is technically more useful at the time of diagnosis due to its rapid execution and accuracy. Nested-PCR is the most sensitive test in detecting residual disease, and it is the method of

choice after BMT. RQ-PCR is the most useful assay for MRD monitoring in CML patients post-BMT, it may indicate the patients at particular risk of relapse.

728 FLUDARABINE, CYTARABINE, IDARUBICIN AND ETOPOSIDE (FLAIE) :A FEASIBLE AND EFFECTIVE TREATMENT FOR DE NOVO AML PATIENTS (<60 YEARS): PRELIMINARY RESULTS OF A PROSPECTIVE UNICENTRIC STUDY

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Background and aims. 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. **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.

Thirteen consecutive patients (8 males, 5 females) with de novo and untreated non M3 AML (FAB M0 2, M1 5, M2 3, M4-M5 3) entered the study between October 2002 and November 2003. The mean age of patients was 36 (range 24-54). Eleven patients had intermediate prognosis cytogenetic alterations; poor prognosis karyotypes have been detected in 2 patients.

Results. Non hematological toxicity of FLAIE was low. The median time to PMN recovery ($> 0.5 \times 10^9 / l$) was 16 days (range 9-19) and $50 \times 10^9 / l$ platelets were reached at a median of 17 days (15-21). During the neutropenic phase Gram+ or Gram- bacteria were isolated from the blood in 5 patients. In addition, fever of unknown significance (FUO) was observed in 8 patients. Pulmonary aspergillosis occurred in 2 patients. Ten (77%) patients were evaluated for response. Three patients died during induction of sepsis (*Pseudomonas aeruginosa*, 1 patient) and gastrointestinal (1) and cerebral (1) haemorrhage and were not evaluated for response. Complete response was achieved in 8/13 patients (62%) whereas 2/13 patients had resistant disease (15%). Three patients underwent allogeneic bone marrow transplantation. After a mean follow up of 9 months, 9 patients are alive, 4 have died (3 during induction, the other of disease progression). One patient has relapsed 5 months after the completion of induction therapy. The other complete responders maintain remission. The median duration of CR is 6 months (range 5-12) and the median survival is 6 months (range 1-14).

Conclusions. The very preliminary natura of this report does not allow to draw any conclusion on the efficacy and the tolerability of this induction regimen. Anyway, compared to our previous experience with the combination of fludarabine, intermediate dosage Ara-C and idarubicin (FLAG-Ide), the addition of etoposide does not seem to increase the antileukemic efficacy.

729 DELAYED PRESENTATION OF AML AFTER ORCHIDECTOMY FOR A TESTICULAR TUMOUR

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In rare cases, AML initially presents in extramedullary myeloid cell tumours (EMCT). The most commonly affected site is the skin (Byrd et al. 1995; McIlwain et al. 2003). AML initially presenting in the testis is very rare with 75% of cases being misdiagnosed (McIlwain et al. 2003). We report on a 52 year old man presenting with testicular swelling and subsequent skin lesion. Original bone marrow investigations revealed no haematological or cytogenetic abnormalities. A diagnosis of B-cell lymphoma was established and orchidectomy was performed. 16 months later chromosome analysis of bone marrow revealed the majority of cells to have trisomy 8 and a balanced t(9;11)(p22;q23) translocation suggestive of myeloid disease. We carried out FISH on formalin-fixed, paraffin-embedded tissue sections to determine whether these abnormalities represented a separate disease or were also present in testis and skin. Use of an alpha satellite CEP 8 DNA probe (Vysis) revealed that 50% of cells of the testis and skin had an additional chromosome 8. An MLL Dual Colour, Break Apart Rearrangement Probe (Vysis) showed that 69% of cells of the testis and 57% of cells of the skin

had abnormal MLL signal patterns. The diagnosis was changed from B-cell lymphoma to AML M5a with chloromas and the patient has achieved complete remission after combination chemotherapy. Future diagnosis of such potentially equivocal cases may benefit from early FISH studies on paraffin sections using a panel of probes specific to AML.

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730 MOLECULAR CHARACTERISTICS OF THE CHRONIC MYELOID LEUKEMIA IN THE PERSONS IRRADIATED AS A RESULT OF THE CHERNOBYL ACCIDENT

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Background. Chronic myeloid leukemia (CML) belongs to diseases characterized by increased risk of developing after exposure to ionizing radiation (IR). The CML incidence rate increased among Hiroshima and Nagasaki atomic bomb survivors, medical diagnostic X-ray workers, patients treated using X-rays. Therefore, the study of CML in the persons irradiated as a result of the Chernobyl NPP accident is of special interest.

Aim. The aim of our work was to give comparative molecular-genetic characteristics of de novo CML developed in the persons following the Chernobyl accident.

Methods. The bcr rearrangements was studied using RT-PCR in 24 CML patients irradiated as a result of the Chernobyl accident (I group) and 35 CML patients without exposure to ionizing radiation (control group).

Results. 58 patients, irrespective of radiation dose exposure in average 25 cSv, had M-bcr mRNA, one – m-bcr. Clinical-hematological data and analysis of breakpoints (b2a2 to b3a2 transcripts ratio) demonstrated prevalent b3a2 in the exposed persons. It was revealed in 19 patients (79.16%) of the first group. In control group b3a2/b2a2 ratio was about 1.0: b3a2 was found in 18 patients (52.94%) and b2a2 in 16 ones (47.06%) ($X^2 = 4.19$; $p < 0.05$). Irrespective of the exposure to ionizing radiation the platelet count was higher in the patients with leukocytosis $< 100 \times 10^9/l$ in b3a2-carriers (402.68 ± 48.98 G/l as compared to 161.73 ± 17.45 G/l in b2a2 carriers; $p < 0.05$).

Conclusion. The present study suggests that formation of the chimeric BCR/ABL gene and its genetic products may play an important role in the development of leukemia in both radiation-induced and de novo CML.

731 IMPACT OF T(14;18)(Q32;Q21) AS GENETIC MARKER IN LYMPHOPROLIFERATIVE DISORDERS. AN EPIDEMIOLOGICAL CASE-CONTROL STUDY

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Background: Genetic markers has been considered as specific to recognised significant steps in the development of oncogenic process. t(14;18)(q32;q21) is present in 85% of patients with follicular non Hodgkin lymphoma(NHL) as well as 30% of patients with diffuse NHL. The consequence of this translocation is the over expression of the BCL-2 protein that have a negative influence in the apoptotic pathway. Nevertheless t(14;18) has been detected in apparently healthy people with an incidence ranging 23 to 50% according to geographic area, demographic patterns and technical procedures. For these reasons real significance of the presence of t(14;18) as genetic marker for NHL is controversial.

Aim: To determine the incidence of t(14;18)(q32;q21) in a representative sample of individuals. **Design:** Observational, transversal case-control study.

Material and Methods: A total of 427 DNA samples were analysed; the source of these were: 100 cord blood samples and peripheral blood in 327 cases: 100 apparently healthy people ranged 18-65 years; 100 apparently healthy over 65 and 127 patients with different lymphoproliferative disorders. Molecular analysis was performed using nested PCR as previously described (Salvakumar et al, 1997) followed by electrophoretical separation in agarose gel. According to case-control study an epidemiological study was performed in 54 cases and 100 controls, stratified by gender and age groups. The inquiry including the

following items: environmental exposure to toxics, alimentary habits, working activity, lifestyle, viral diseases and cancer incidence in relatives. A database file was developed and SPSS packet was used for statistical analysis.

Results: Cord blood samples were negative for t(14;18), for individual aged 18-65 the estimated incidence was 3% increasing to 5% in population aged over 65. In patients population follicular NHL 36%, Diffuse NHL 22% and CLL 17%. Correlation analysis was negative for toxic exposure and positive for the viral infections antecedents, age and familiar history of neoplasia.

Conclusions: Negativity of cord blood sample clearly demonstrate that t(14;18) is an acquired translocation. In spite of the high incidence in lymphoproliferative disorders, the presence of 3-5% of the abnormality in healthy people don't make useful this marker to identify population at risk to NHL and to monitoring minimal residual disease in this setting.

732 EFFECTIVE PROGENITOR CELL MOBILIZATION IN PATIENT WITH MULTIPLE MYELOMA (MM) USING IFOSFAMIDE, ETOPOSIDE AND GRANOCYTE COLONY STIMULATING FACTOR (G-CSF)

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The optimal regimen method for progenitor cell mobilization in MM is unknown. Mobilization is often accomplished using combined cyclophosphamide and G-CSF but the quantity of stem cells is frequently insufficient and this regimen is associated with a significant toxicity and a need for supportive care. We studied the safety and efficacy of mobilization using ifosfamide (4g/m²) combined with etoposide (500mg/m²) followed by G-CSF administered daily until leukaphereses were completed. Twenty nine newly diagnosed MM patients stage II and III according to Durie and Salmonn were mobilized for progenitor cell collection : (14f, 15 m) ; (9 IgA, 13 IgG, 5 light chain, 2 other) ; median age 59,5 (range 42 - 70). The median number of previous chemotherapy regimen was 3 (range 3-4), the most common regimen was VAD (vincristine, adriablastine and dexamethasone). The median time to leukapheresis was 12 days. The median CD34+ cell count prior to the first leukapheresis was 67,47/ul (range 7,63 - 218), a median of 2,4 (range 1-5) leukaphereses was performed. Mobilization was successful in 27 of the 29 patients (93%) who achieved the minimal transplant dose of 2 x 10.6/kg CD34+ cells. The median total number of CD34+ cells collected was 7,22 x 10.6/kg (range 1,79 - 16). There were no major side effects and no patient required admission for febrile neutropenia. All patients successfully mobilized were transplanted after a myeloablative regimen consisting of Alkeran or Alkeran-TBI. The engraftment was rapid and complete : ANC>0.5x10.9/l and platelet>20x10.9/l were attained at day 12 and 13 respectively.

In summary, this study shows that the combination of ifosfamide, etoposide and G-CSF is safe, well tolerated, and effective in progenitor cell mobilization in patients with newly diagnosed MM.

733 IMMUNOLOGICAL CLASSIFICATION OF 32 AML CASES ACCORDING TO GEIL'S PROPOSAL

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Background. Based on the expression of seven antigens (CD13, CD33, CD117, CD7, CD35, CD36 and CD15), GEIL proposed an immunological classification of Acute Myeloblastics Leukemias (AML) in five clinically relevant subsets: MA: CD13+ or CD33+ or CD117+; CD7-; CD35- or CD36-; CD15-; MB: CD13+ or CD33+ or CD117+; CD7+; CD35- or CD36-; CD15-; MC: CD13+ or CD33+ or CD117+; CD7+/-; CD35+ or CD36+; CD15-; MD: CD13+ or CD33+ or CD117+; CD7+/-; CD35+/- or CD36+/-; CD15+; ME: CD13- or CD33- or CD117-; CD7+/-; CD35+/- or CD36+/-; CD15+/- (hematologica, 87, suppl. nr.1, 2002, p.10).

Aims. Between 25.09.2002-12.12.2003, we analysed bone marrow samples from 32 AML cases (21 females, 11 males, median age 36.06/-24.66 years, 7 months-78 years) which were classified according with GEILs proposal. We correlated the immunological subsets with clinical and biological features.

Methods. Flow cytometric immunophenotyping was performed on a FACSCalibur cytometer, after staining whole bone marrow samples with a large panel of monoclonal antibodies: CD2, CD3(cy/s), CD4, CD5, CD7, CD8, CD10, CD13(cy/s), CD14, CD15, CD16, CD19, CD20,

CD22(cy/s), CD33, CD34, CD35, CD36, CD38, CD41, CD45, CD56, CD61, CD64, CD65, CD71, cyCD79a, CD117, CD123, HLA-DR, cyMPO, nTdT, IgM (cy/s); lysis with FACS Lysing solution, Becton Dickinson, permeabilization with IntraPrep Coulter. Blast cells were gated on CD45/SSC and CD45/CD34 plots.

Results. Our cases were classified as: MA - 17 cases, MB - 1 case, MC - 4 cases, MD -10 cases.

We found a significant correlation (p=0.000011) with FAB classification: in MA subset the majority of cases were M1 or M2, in MC and MD, most of the cases were M4 or M5. However, like in GEILs large cohort of patients, no overlap of this stratification with the FAB classification. There was no correlation found with leucocytes count: median was 25.3x109/L in MA subset, 84x109/L in MB, 18.25x109/L in MC and 27.8x109/L in MD. Also, no correlation was found with positivity for CD34 and CD14 or lymphoid markers coexpression. A good correlation (p=0.0032) with CD64 expression was found.

Most of the patients received chemotherapy (DAT, DA, ADE or LAME 91). Only one has submitted to autotransplantation. Half of the patients have died. The incidence of death significantly correlated with classification subsets (p=0.04), but no other correlation was found. Their average survival period was 4.37+/-4.16 months (0-10 months, median=2). The patients alive are in partial or complete remission at 9.81+/-3.94 months (3-16 months, median=10) from diagnosis. The patient submitted to autotransplantation is alive at 16 months from diagnosis. Summary. The results we got by immunological classification of our small series of patients are somehow different from those reported by GEIL.

734 NORMAL PREGNANCY OUTCOME IN CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB AND INTERFERON. A CASE REPORT

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Chronic myeloid leukemia (CML) represents about 10% of leukemias diagnosed during pregnancy. Management of CML patients during pregnancy represents a difficult task with no clearly established standards. Treatment of CML in pregnancy is mandatory, because of high leukocyte and platelet counts, which are known to have adverse effect on pregnancy outcome. Chemotherapy during pregnancy carries a significant risk of teratogenicity, especially during the 1st trimester. Interferon alfa (IFN) inhibits cell proliferation due to its effect on protein synthesis and RNA degradation and among therapeutic agents used in CML treatment, it is unique due to the lack of DNA synthesis inhibition. Neither in vitro mutagenicity nor teratogenicity has been observed in animal studies.

Imatinib (IM) has demonstrated high efficacy as target therapy in CML. Preclinical data showed imatinib to be teratogenic in rats, but not in rabbits and to cause impaired spermatogenesis in rats, dogs and monkeys. Because of the teratogenic effect in rats, effective contraception is recommended before IM therapy in humans in order to prevent pregnancy. In the following case report we describe a case of CML pregnant woman treated with imatinib and later with interferon.

Philadelphia chromosome positive (Ph+) CML in 1st chronic phase was diagnosed in March 2000 in a 34-year-old woman out of clinical trial. She was 68 weeks treated with imatinib, 400mg per day, when pregnancy in 21st week of gestation was diagnosed. The patient decided not to interrupt pregnancy. We immediately stopped imatinib treatment and started application of interferon, 5 million units subcutaneously per day. White blood cells and platelets were maintained within acceptable levels. Four weeks before delivery a new leukocyte increase occurred. During the whole period of pregnancy the BCR-ABL transcript levels were monitored. The patient had no clinical symptoms. A healthy boy was spontaneously delivered during the 37th week of gestation. The newborn transiently showed symptoms of cardiopulmonary distress, which quickly disappeared because of the following intensive care. He had no congenital malformations. The imatinib treatment of the mother was restarted 3 days after the delivery. She did not suckle her child. Seven months following delivery the mother is still in chronic phase, treated with imatinib 400mg per day. The child is in good general condition, with normal growth and development.

Eventhough the referred case of CML woman treated with imatinib and IFN with non complicated pregnancy followed by healthy new born delivery, the pregnancy outcome data should be interpreted with extreme caution. Interferon remains one of the safest agents to be used in CML treatment during pregnancy. Women treated with imatinib should be

advised to use effective contraceptive methods throughout the whole period of treatment. If pregnancy occurs during imatinib treatment, the drug should be immediately stopped. The totally safe agent for CML treatment during pregnancy still remains to be established. Supported by grant NC/7550-3 from Internal Grant Agency of Ministry of Health of the Czech Republic

735 INTERLEUKIN-2 AND LYMPHOKINE-ACTIVATED KILLER CELLS AS IMMUNOTHERAPY BY RECURRENCE OF ACUTE LEUKAEMIA IN CHILDREN

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Background: the outcome of recurrence by leukemia is generally poor. Different treatments have been proposed. In vitro studies suppose that tumor immunology linked therapies might present promising tool for relapse treatment. These include measures to improve immune functions and overcome immunosuppression.

Aim: to assess therapeutic potential, tolerability and safety of interleukin-2 and lymphokine-activated killer cells in children with acute leukemia in recurrence.

Patients and methods: immunotherapeutic modality was comprised of administration of low dose interleukin-2 (IL-2) and lymphokine-activated killer (LAK) cells. Eight patients with acute leukemia (AL) in relapse were enrolled in the study. Patient characteristics: age median 9 years (range 6-13); 5 male, 3 female; recurrence of acute lymphoblastic leukaemia - 5 pts, recurrence of acute myelogenous leukemia - 3 pts. All patients received standard antitumor chemotherapy according to the type of relapse. 0.5x10⁶ IU/m² IL-2 was given on days 1-5 followed by LAK cell transfusion on days 6-7. Cycle was repeated every 3 weeks in first 3 months, then monthly. LAK cells were produced from lymphocytes obtained from healthy unrelated donors by incubation with interleukin-2 in vitro. The dose of LAK varied from 1x10⁸ to 3x10⁹ cells per transfusion. Vaccination using dendritic cells primed with whole tumor antigens was performed additionally in one patient. MTT test was used to assess cellular cytotoxicity.

Results: moderate toxicity was observed by LAK cell transfusions. Fever maximum grade 2 occurred in all patients, hypotension maximum grade 1 - in one patient. No changes in disease status was observed. The increase of mononuclear cells (MNC) cytotoxicity against K562 cell line from 30,2% to 54,2% (median) and against autologous leukemic cells from 24,9% to 30,4% (median) was detected in seven patients after the second cycle of immunotherapy. We also observed an improvement of life quality and decrease in transfusion requirement during the immunotherapy in 3 patients.

Conclusions: low dose IL-2/LAK immunotherapy has no significant toxicity and is feasible in children with recurrence of acute leukaemia. Applied modality results in augmentation of cellular cytotoxicity of blood MNC.

736 THE PROGNOSTIC VALUE OF MAJOR CYTOGENETIC RESPONSE FOR THE CML PATIENTS ON IMATINIB THERAPY

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Background. A new era in CML treatment has started with the involving Glivec (imatinib mezylat, STI 571) into the clinical practice. Due to the Glivec International Patient Assistant Program (GIPAP) supported by Novartis Pharma 386 Ph-positive CML patients have already received Glivec treatment in Russia. The results of the treatment for 157 patients who participated in clinical trial since February till September 2001 were analyzed in December 2003.

Aims. On this moment there is a lack of information about the correlation of survival and major cytogenetic response. The aim was to estimate the survival of the patients with major cytogenetic response.

Patients and methods. 79 CML patients with chronic phase (CP) with previous ineffective interferon-alpha therapy and 78 CML patients with accelerated phase CML (AP) received Glivec treatment. The median age was 44 years, m:f = 84:73, median duration of CML before starting treatment was 42 months (1-156 months). The median time of treatment

(till December 2003) was 30 months. The initial dose for the CML CP patients was 400 mg daily and for CML AP patients the initial dose was 600 mg daily.

Results. Complete hematological remission has been received for 95% and 71% CML patients in CP and AP accordingly. A greater stability of hematological response in CP than in AP was revealed: 93% patients with CML CP are alive and continue treatment versus 77% of patients with CML AP. The unprecedented ability of significant reduction of Ph-positive cells (less than 35% - major cytogenetic response) in bone marrow was found. Major cytogenetic response (MCR) was achieved for 56% and 44% of patients in CP and AP accordingly. MCR is a favorable factor significantly correlated with the growth of two-year survival in CP (97%) and in AP (86%) comparatively with the patients without MCR (86% and 60% accordingly; p<0.05). Complete cytogenetic response (CCR) after 24 months treatment was received for 40% of patients in CP and for 35% of patients in AP. We have defined clinical factors with significant unfavorable influence on the ability of MCR receiving and survival. They are: anemia <110 g/l (p=0.02), splenomegaly >5 sm below the costal level (p=0.03), blasts in peripheral blood >1% (p=0.02). Analyzing the quantitative combination of this factors the probability of MCR and the risk of disease progression can be established. Also we have found a satisfactory tolerability of the first-year Glivec therapy. Besides there was an improving of the therapy tolerability during 2 years.

Conclusion. Glivec therapy in later CP CML and AP CML is able to get a significant reduction of the percent of Ph-positive cells, which is associated with the slowing progression and relapse-free survival. The factors of unfavorable prognosis and the receiving of MCR should be taken into consideration during the developing of the program therapy of CML in order of intensification of the treatment for this group of patients.

737 POINT MUTATIONS OF BCR/ABL GENE IN CML PATIENTS RESISTANT TO IMATINIB THERAPY

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Background: Imatinib is a drug that competitively targets the adenosine 5-thriphosphate (ATP)-binding site of the kinase domain of ABL with high specificity and is used as front line therapy for CML. Patients treated with Imatinib in chronic phase (CP) have 95% of hematological response and 85% of major cytogenetic response ; however, most of patients treated in accelerated phase (AP) or blast crisis (BC) present primary refractoriness or relapse after initial response to treatment. Mutations in the kinase domain of BCR/ABL are the most frequent mechanisms associated to resistance in vivo. Aims: detect mutations in the kinase domain of BCR/ABL in CML patients with primary or secondary resistance to Imatinib. Patients and Methods: we evaluated five patients in blast crisis followed in Hemocentro - Unicap with primary or secondary resistance to Imatinib. After RNA extraction from peripheral blood samples, amplification of the kinase domain of ABL from BCR/ABL was performed, using a semi-nested RT-PCR. For the first PCR, primers PCR1 were used and for the second PCR, PCR2 primers; PCR 1 - BCRF (sense - 5' TGA CCA ACT CGT GTG TGA AAC TC) and ABLKinaseR (anti-sense - 5' TCC ACT TCG TCT GAG ATA CTG GAT T); PCR 2 ABLKinaseF (sense - 5' CGC AAC AAG CCC ACT GTC T) and ABLKinaseR (anti-sense - 5' TCC ACT TCG TCT GAG ATA CTG GAT T). PCR product was submitted to direct automated sequencing and compared with normal sequences of BCR-ABL gene (M14752, GenBank). Results: We found mutations in four of five CML patients in blast crisis, which acquired resistance to Imatinib (E255K, E279K, F359V)(table 1). All patients were in blast crisis, except patient 5 (in second CP) when mutation was detected. Mutation E255K was found in two patients with lymphoid BC. Summary/Conclusions: E279K mutation (patient 1) was not previously described and additional studies are necessary to categorize it. Mutation E255K was found in two patients with lymphoid BC; it is located in P-loop and related to a worse prognosis, while F359V is outside P-loop and related to a longer survival. In fact the patient with this last mutation is still alive and in second CP after had a good response to dose increase. Mutations were not identified in patient 3, the only case with primary resistance, corroborating literature data. Mutations detection may have prognostic implications and help in the development of new drugs and design of new strategies to prevent the emergency of resistant cases.

Patient	Age/ gender	Years from diagnosis	Type of blast crisis	Mutation	Previous treatments	Imatinib treatment (months)	Evolution
1	36M	6	Myeloid	B270K	Hydrea, chemotherapy	18	SR, death (PD)
2	36M	8	Lymphoid	B255K	Hydrea, chemotherapy	11	SR, death (PD)
3	50M	8.7	Myeloid	Not detected	Hydrea, chemotherapy	5	Primary resistance, death (PD); CNS bleeding
4	27F	2.1	Lymphoid	B255K	Hydrea/IFN	3	SR, death (sepsis and aplasia after chemotherapy)
5	33F	28	Myeloid	F350V	Bussulfan, Hydrea	32	Alive, SR 18 months after Imatinib, 2nd CP after dose increase

PD (progressive disease); CNS (central nervous system); SR (secondary resistance)

Caption 1: Clinical data and evolution of CML patients

738 P63 EXPRESSION IS ASSOCIATED WITH THE GERMINAL CENTER MARKERS, BCL-6 AND CD10 IN B CELL LYMPHOMAS.

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Abstract

Background: P63, a family member of p53 related genes, plays a role in regulating epithelial proliferation and differentiation program. In addition, recent studies have indicated that p63 expression was observed in various human epithelial tumors, suggesting a pivotal role of p63 in the growth and development of epithelial tumors. However, the functional role of p63 in hematological malignancies has not yet been elucidated.

Aim: p63 protein is occasionally expressed in the germinal center cells of normal human lymphnode and in tumor cells of B-cell malignancies. Recent DNA microarray analyses of lymphoid malignancies has identified several germinal center markers associated with prognosis, especially for diffuse large cell lymphoma. To investigate the expression pattern of p63 in B-cell malignancies, we evaluated correlation between the expression of p63 and other germinal center markers.

Method: 53 B-cell lymphoma (15 follicular lymphoma, 5 mantle cell lymphoma, 33 diffuse large cell lymphoma) were analyzed by immunohistochemical examination the using antibodies for p63, bcl-6, CD10, SHP-1 and p53.

Results: Among 53 B-cell lymphoma, p63 expression was observed in 11 of the 15 (73%) follicular lymphoma, 13 of the 33 (40%) diffuse large cell lymphoma, but none of the 5 mantle cell lymphoma. In diffuse large cell lymphoma, the expression of p63 and bcl-6 was significantly correlated ($p=0.05$), and this tendency of correlation was also observed between the expression of p63 and CD10 ($p=0.08$), although no reverse correlation was observed between p63 and SHP-1.

Conclusion: p63 is expressed in the majority of follicular lymphoma, but not in mantle cell lymphoma. In diffuse large cell lymphoma, 40 % of the cases expressed p63 correlated with the expression of bcl-6 and CD10. These results suggest that p63 could be an additional germinal center marker of diffuse large cell lymphoma, although further investigation to clarify its clinical significance in B-cell lymphoma is needed.

739 FLUDARABINE, CYCLOPHOSPHAMIDE AND THEIR COMBINATION WITH RITUXIMAB IN THE TREATMENT OF LOW-GRADE NON-HODGKIN'S LYMPHOMAS - THE EFFICACY AND TOXICITY OF THE REGIMEN

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Background: Patients with advanced stages of indolent lymphomas are not currently curable. The treatment strategy that is effective and safe is intensively searched.

Aims: To analyze retrospectively the efficacy and toxicity of a combination of fludarabine and cyclophosphamide (FluCy) alone or with rituximab (FluCy-R) in the treatment of advanced low-grade non-Hodgkin's lymphomas.

Methods: 23 adults, aged 40 to 75 years (median 59 yrs), mainly with small cell lymphocytic lymphoma (SCLL - 11 patients), mantle cell lymphoma (MCL - 10 pts), follicular lymphoma (FL - 1 pts) and one lymphoplasmocytic lymphoma were treated with the FluCy (12 pts) or FluCy-R (11 pts) in the period 2001-2003. 14 patients (60%) were pretreated, nine ones were newly diagnosed. 20/23 pts had stage IV disease. The therapy consisted of 4-6 cycles of i.v. fludarabine 25mg/m² and cyclophosphamide 250mg/m²

on days 1-3. Rituximab (Mabthera ®) 375 mg/m² i.v. was given before chemotherapy on day 1. Cycles were repeated in 4-week intervals. Cotrimoxazol was given as a prophylaxis of Pneumocystis pneumonia.

Results: 17 pts were evaluable for response. Almost all of them have responded (OR 94%), 10/17 achieving a complete remission (CR 59%), 6/17 a partial remission (PR 35%). One patient had a stable disease (SD). Median follow-up is 9 months. One patient in CR has relapsed 8 months after completing the last cycle; one patient in PR and the one with SD have progressed. The rest of patients remains in achieved remissions. Follow-up is too short to define overall and failure-free survival.

All 23 patients were evaluated for toxicity. 108 cycles were administered and evaluated. Prolonged myelosuppression and related infections were the most prevalent toxic effects, non-hematological toxicities (neuro, cardiotoxicity, intravascular hemolysis, allergic reaction) were rare. Hematological toxicity was only moderate; grade III-IV granulocytopenia occurred only at 5/108 cycles (5%), anemia and thrombocytopenia were unimportant. We have noted 23 infectious complications in 12 pts; six infectious episodes were severe with a necessity of hospitalization and parenteral ATB therapy. 14 infectious episodes followed directly after chemotherapy (14/108 cycles complicated - 13%), nine occurred in longer follow-up period. Chemotherapy doses were reduced in 11% of cycles because of leukopenia. Slow granulocyte or platelet count recovery caused delay of 5% cycles. One patient required a discontinuation of the therapy after the second cycle because of persistent leukopenia and one after the 5th cycle because of neurotoxicity. No treatment-related mortality occurred.

Conclusions: Fludarabine + cyclophosphamide is well-tolerated and effective chemotherapeutic regimen for patients with advanced low-grade non-Hodgkin's lymphomas. An addition of rituximab seems to be useful in the treatment of MCL patients not suitable for more aggressive chemotherapy, with a good clinical response and no negative impact on the toxicity. Low occurrence of complications allows a safe outpatient administration. A possibility of finishing the therapy after the fourth cycle to avoid possible late toxicity and especially problems with stem cells stimulation in patients with a very good clinical response needs to be investigated.

740 ANGIOGENESIS IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS: A PILOT STUDY

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Background: Angiogenesis is the development of new blood vessels from preexisting ones.

Its role in the pathogenesis of solid tumors has been well established. Interestingly, its significance in hematological malignancies only started to be investigated in 1990s. Several studies have found increased angiogenesis in acute and chronic leukemias, Non Hodgkin's lymphoma, multiple myeloma and myeloproliferative disorders.

Aim: To assess feasibility of microvessel density measurement and to verify increased angiogenesis in patients with malignant lymphoproliferative disorders.

Methods: Paraffin embedded trephine bone marrow biopsy specimens from patients treated at Hematology Department, Charles University Teaching Hospital were examined. The disease spectrum included Non-Hodgkin's lymphoma (n=4), chronic lymphocytic leukemia (n=5), multiple myeloma (n=4), and Waldenstrom's macroglobulinemia (n=2). Control group consisted of patients treated for non-hematological diseases with no evidence of bone marrow involvement (n=9). Samples were stained with anti - CD31 monoclonal antibody and analyzed in optical microscope. Microvessel density was measured according to 'hot spot' method - all slides were reviewed at low magnification and three areas with the highest accumulation of microvessels ('hot spots') were examined at 400x magnification. Microvessels were counted in three fields for each hot spot. Larger vessels (i.e. arterioles, venules) were excluded. **Results:** Median microvessel density in each field was 6 (range, 0-16) in patients with hematological malignancies, whereas in control group the median MVD was 1(range, 0-3). The difference was statistically significant ($p<0,001$). Megakaryocytes often stained falsely positive with anti - CD31 but could be easily distinguished from microvessels on the basis of magnitude and shape. **Conclusion:** anti - CD31 monoclonal antibody is a good endothelial marker for the assessment of microvessel density in bone marrow trephine biopsies. We got similar results to previous studies suggesting increased angiogenesis in patients with lymphoproliferative diseases. However, large studies are needed to confirm these findings.

741 CONSTRUCTION OF A REAL TIME QUANTITATIVE-PCR FOR DETECTION OF TCRV-gamma- J-gamma- GENE REARRANGEMENT IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Objective: To improve the clinical significance of detection of minimal residual disease(MRD) in acute lymphoblastic leukemia (ALL). **Methods:** A real time quantitative PCR method was constructed for quantitative the clonal TCRVγ-Jγ gene rearrangement in 36 ALL cases. **Results:** The sensitivity of the established real time quantitative PCR were 10-4 level. The amount of TCRVγ-Jγ gene rearrangement in newly diagnosis group, complete remission(CR) group and after hematopoietic stem cell transplantation(HSCT) group were (7.386.65)10-2, (1.02~1.08)10-2 and (3.89~5.65)10-3 level. The amount of TCRV I-Jγ gene rearrangement of ALL patients in newly diagnosis group is higher than that in CR group and HSCT group(P=0.001). The MRD level of ALL patients in CR group is higher than that in HSCT group(P=0.022). MRD can be detected in 6 ALL patients after HSCT, 2 cases with low MRD level(< 110 3) received long disease-free survival. The other 4 cases with high MRD level all suffer relapse within one year. **Conclusion:** The established real time quantitative PCR assay is simple, repaid, sensitive and specificity. The real time quantitative PCR assay to evaluation MRD in the remission period of All cases is more helpful for prognosis prediction.

742 ASPECTS OF PLATELET FUNCTION IN PATIENTS WITH POST VIRUS B HEPATITIS CIRRHOSIS

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Background/Aims: Platelet function disorders commonly develop in patients with cirrhosis of the liver. Our aim was to investigate some parameters that reflect platelet function 'in vivo' and 'in vitro' for this type of patients.

Methods: 24 patients with liver cirrhosis (post hepatitis with virus B) and 20 healthy controls were prospectively studied. The parameters of platelet function assessed included was 'in vivo' bleeding time (Duke method) and 'in vitro' platelet count (PLT), platelet's constants (MPV, PDW, PCT) and platelet aggregation at 10μM epinephrine and 10μM ADP final concentration.

Results: Cirrhotic patients had a significant decreased of platelet aggregation (on both inductors ADP and epinephrine) and a prolonged bleeding time. Platelet count (PLT) was low than 15x10⁹/L in 75% patients and PCT low then 0.100% in 79.16% patients. The values of MPV and PDW were normal range in both groups.

Conclusions: Platelet disorders are common in liver cirrhosis, more significantly on platelet function then platelet 'morphology'. This imbalance could be caused, besides the hypersplenism producing cytopenia, by 'hepatic endotoxemia' and probably by cytokine activation after the chronic inflammation and regeneration after the infection with hepatitis virus B.

743 THE EFFECT OF DEFERIPRONE ON FERRITIN LEVELS AND HEPATIC ENZYMES IN -THALASSEMIC PATIENTS IN LARISA - GREECE. A THREE -YEAR FOLLOW-UP

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BACKGROUND Deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one) is the world's first and only orally active chelating agent extensively used in thalassemia. The use of deferiprone whether as monotherapy or in combination therapy has been very well established over the last decade.

AIMS In our study we evaluated the effect of deferiprone (monotherapy and combination therapy with desferrioxamine) on ferritin and hepatic enzyme (SGOT, SGPT) levels in β-thalassaemic patients in the Thalassemia Unit of the General Hospital of Larissa, (a town of Central Greece with a high carrier frequency of thalassaemia trait) following three years of treatment.

METHODS Sixty-one patients participated in this study. Thirty five of them underwent combination treatment after two years of monotherapy treatment. The therapeutic regimen was deferiprone (Ferriprox®, APOTEX) 75 mg/kg/day for 7 days (monotherapy or combination therapy)

and desferrioxamine (Desferal®, NOVARTIS) 40 mg/kg three times weekly (combination therapy). All studied parameters (ferritin and hepatic enzyme levels) were measured just before the initiation of monotherapy chelation treatment, before combination chelation treatment and at the end of the third year.

RESULTS The results of this study (means ± S.E. of the studied parameters) are presented in table 1. Since there wasn't any statistically significant difference in the values of the studied parameters between the patients that underwent combination treatment after two years of monotherapy with those who continued monotherapy treatment, pooled values were used in the evaluation. There was a discrete statistically significant decrease in the values of ferritin levels after two and three years of chelation treatment compared to values of ferritin prior the initiation of chelation therapy. The noticed decrease of the values of hepatic enzymes concentration became statistically significant at the end of the third year of chelation treatment with the exception of SGOT whose decrease was statistically significant even at the end of the second year of chelation treatment (Table 1)

CONCLUSIONS Our findings are in agreement with the overall general view that deferiprone is an active iron chelator whether it is being used as monotherapy or in combination therapy with desferrioxamine. The most interesting finding was that administration of deferiprone did not increase hepatic enzyme levels; on the contrary, it improved them after three years of therapy whether in combination with desferrioxamine or alone.

	Ferritin (ng/dl)	SGOT (μU)	SGPT (μU)
Initiation of chelation treatment	3.228 ± 272	45 ± 4	57 ± 5
Two years of chelation treatment	2.522 ± 255	29 ± 3	41 ± 4
Three years of chelation treatment	2.321 ± 174	34 ± 2	39 ± 3

Caption 1: Table 1

744 PREVALENCE OF THROMBOPHILIC POLYMORPHISMS IN ISCHEMIC STROKE PATIENTS WITH SICKLE CELL DISEASE FROM SULTANATE OF OMAN.

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Background: Cerebrovascular disease occurs in 10% patients of sickle cell disease. [SCD] The risk factors for ischemic stroke in SCD include transient ischemic attack, recent acute chest syndrome, fall in steady state hemoglobin, an increase in the systolic blood pressure, or hyperhomocysteinemia.

Aim: To study the prevalence of thrombophilic polymorphisms in ischemic stroke patients with sickle cell disease from Sultanate of Oman.

Methods: We studied 15 patients with SCD who developed stroke and had radiological corroboration with MRI, suggestive of cerebral infarction with no other apparent etiology for their stroke. These patients include 7 females and 8 males, with a mean age (±SD) of 21(±7) years, [range 10-39 years] and were evaluated for 8 common thrombophilic markers. Protein C,[PC] Protein S, [PS] Anti-thrombin III and activated protein C resistance were estimated in the coagulation laboratory, whereas, Factor V G1691A; Factor II G20210A; Methylene tetrahydrofolate reductase [MTHFR] C677T and Cystathionine beta synthase [CBS] 844ins68 mutations were studied by DNA techniques.

Results: It was observed that 4 patients had isolated reduction in PC levels (26.6%), whereas 5 patients (33.3%) showed significant reduction in both PC and PS levels on multiple occasions. 4 patients(26.6%) were heterozygous for CBS 844ins68 mutation whereas seven patients(46.6%) each were heterozygous and homozygous respectively for MTHFR C677T mutation [Four of them had both these mutations together]. No patient had Factor V G1691A or Factor II G20210A mutation.

Summary/Conclusion: Our data show that the two common mutations causing hyperhomocysteinemia play a significant role, in the genesis of stroke. PC and PS deficiency, possibly owing to ongoing consumption, appears to be an important additional cause in patients with SCD.

745 HEMATOLOGIC RESPONSE TO ANTI-APOPTOTIC CYTOKINE THERAPY: PROMISING RESULTS OF PENTOXIFYLLINE, CIPROFLOXACIN, AND DEXAMETHASONE IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background:

TNF-alpha mediated increased intramedullary apoptosis of hematopoietic cells has been thought to contribute to the ineffective hematopoiesis in myelodysplastic syndrome (MDS). The combination of pentoxifylline (P) and ciprofloxacin (C) has been shown to reduce serum level of TNF-alpha and an earlier trial of PC with dexamethasone (D) provided good palliation to patients with MDS, especially in RARS (Raza A, Blood 2000).

Aim:

The purpose of this study is to assess the hematologic response to PCD therapy in patients with MDS.

Methods:

Patients with MDS were treated with P 1200 mg/d, C 500 mg/d, and D 4 mg/d for 4 weeks. After this 4-week course, P 800 mg and C 500 mg were given 3 times a week with D 4 mg for 5 days every month for 2 months. Therapy has been continued for 1 year in responders. International Working Group hematologic improvement criteria were used to assess responses.

Results:

Between April 2002 and November 2003, 17 female and 8 male patients with MDS were enrolled in this study. Twenty-one of 25 patients, completed at least of 12 weeks of treatment, are evaluable for efficacy. At study entry, the median age was 60 yrs (18-75 yrs). WHO diagnoses included: RA (n=5), RCMD (n=10), RARS (n=1), RCMD/RS (n=1), RAEB (3), and CMML (n=1), divided into low (n=2), Intermediate-1 (n=16), Intermediate-2 (n=1), and high (n=2) IPSS risk groups. Eleven patients (52%) showed an improvement in cytopenia. Three patients had a triple lineage response, 1 had a dual lineage response, and 7 had a single lineage response. No differences were noted in response rate among the 3 lineages. Although there were no differences in response rates between FAB subtypes, all the patients with RS component responded. Median time to response was 2 weeks (2-12 weeks), and interestingly, 9 of 11 patients who had response remain without relapse with a median of 177 days (78-634 days)

Conclusions:

These preliminary results indicate that anti-cytokine therapy with PCD is an effective and well tolerated palliative treatment for patients with MDS. Further accrual is ongoing and several biologic correlates of this anti-apoptotic cytokine therapy are also under investigation.

746 ANALYSIS OF CHEMOKINE RECEPTOR GENES EXPRESSION IN LEUKEMIA/LYMPHOMA DERIVED CELL LINES

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Background. The region on 3rd chromosome, which was regularly lost following tumour growth in immunodeficient mice, includes the cluster of chemokine receptor (CCR) genes. Since CCRs play an important role in the maturation, proliferation and mobilisation of haematopoietic progenitors their loss could lead to the development of haematological malignancies.

Aims. This study aims to investigate a role of the chemokine receptors of chromosome 3 in development of leukaemia and lymphoma.

Methods. The CCR1, CCR2, CCR3, CCR5 and CCR6 genes expression has been analysed in 24 leukaemia/lymphoma derived cell lines of T- and B-origin, two HTLV-I transformed cell lines and six EBV immortalised lymphoblastoid cell lines (LCLs) using RT-PCR. As the controls peripheral blood lymphocytes (PBL), B- and T- lymphocytes isolated by negative CD19 and positive CD3 selection, respectively, as well as B-cells isolated by CD10 positive selection were used.

Results. The results revealed the lack of CCR3 expression in all examined cell lines. There are no or very low levels of CCR1 transcript in

9/18 and 4/6 leukaemia/lymphoma derived cell lines of B- and T-origin, respectively. Lack of CCR2 expression was found in 12/18 and 3/5 leukaemia/lymphoma derived B- and T-cell lines, respectively. CCR6 was not expressed in 8/18 leukaemia/lymphoma derived B-cell lines, whereas in all leukaemia/lymphoma T-cell lines high levels of CCR6 expression were observed. Expression of CCR5 was detected only in one leukaemia/lymphoma derived cell line and in two HTLV-I transformed cell lines. We also have found correlation between mRNA expression of CCR1 and CCR2 genes and B-cell plasmoblast-like phenotype of the group III Burkitts lymphoma (BL) and LCL cell lines.

Conclusions. Thus, the lack of the CCRs gene expression in majority of leukaemia/lymphoma derived cell lines indicates involvement of these genes in the development of haematological malignancies. However, the observed switch off of these chemokine receptors could be also associated with the differentiation stage of the cells. The predominant CCR1 and CCR2 expression in LCLs and group III BLs may be related to the plasmoblast-like stage of B-cell differentiation fixed through EBV immortalisation.

747 THE INFLUENCE OF THE INFLAMMATION ON THE IRON

DEFICIENCY MARKERS, IN CHRONIC HAEMODIALYSIS PATIENTS

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Background: Inflammation and iron deficiency are important causes of erythropoietin resistance in haemodialysis patients. Soluble transferrin receptors constitute a new indicator of iron deficiency as well as a parameter of erythropoietic activity. Soluble transferrin receptors concentration is not influenced by inflammatory states, malignancy or liver disease.

The aim of this study was to evaluate the influence of the inflammation on the iron status markers, in chronic haemodialysis patients.

Patients and methods: We studied 47 patients with a mean age 62.76 ± 14.6 years and a mean duration of haemodialysis 63.57 ± 37.2 months. Serum iron, total iron binding capacity (TIBC), transferrin saturation (SAT), ferritin, soluble transferrin receptors (sTfR), haemoglobin (Hb), erythrocyte indices (MCV, MCH, MCHC, RDW-CV), reticulocyte count (RET#), reticulocyte subpopulations (HFR, MFR, LFR), C-reactive protein (CRP) and weekly erythropoietin dose (EPO/kg) were measured. Patients were divided into two groups: group A (CRP negative), group B (CRP positive).

Results: CRP exhibited a significant positive correlation with: ferritin (r=0.3) and EPO/kg (r=0.39). In contrast, neither sTfR nor SAT were correlated with CRP. Soluble transferrin receptors were correlated negatively with SAT (r=-0.30), MCHC (r=-0.33), iron (r=-0.31) and positively with EPO/kg (r=0.44), RET# (r=0.47), HFR (r=0.31), RDW-CV (r=0.47).

Comparison between the results of group A (CRP-, n=31) and the results of group B (CRP+, n=16) (x±SD, statistically significant difference p≤0.05, ns=no sense):

Age years: Group A: 62.3513.82, Group B: 63.5616.44 (ns)

Hb g/dl: Group A: 11.881.02, Group B: 11.081.74 (ns)

RET# x106/μl: Group A: 0.0540.01, Group B: 0.0650.02 (ns)

RDW-CV%: Group A: 14.41.04, Group B: 16.211.63 (p=0.0001)

MCHC g/dl: Group A: 32.110.96, Group B: 31.551.49 (ns)

SAT%: Group A: 33.8720.05, Group B: 23.1812.39 (p=0.05)

Ferritin g/ml: Group A: 419.41221.70, Group B: 540.5306.18 (ns)

EPO/kg: Group A: 103.6745.04, Group B: 171.7793.98 (p=0.0039)

sTfR mg/l: Group A: 2.250.74, Group B: 2.520.82 (ns)

In Group A, the sTfR had a positive correlation with the indicators of erythropoietic activity: RET# (r=0.57), HFR (r=0.45), MFR (r=0.51) and a negative correlation with LFR (r=-0.50).

Conclusion: The positive correlation of CRP with ferritin and the absence of any correlation of CRP with sTfR make sTfR a more reliable indicator of iron deficiency in inflammatory states. However, during EPO therapy, where sTfR levels are elevated due to enhanced erythropoiesis, their diagnostic power in detecting iron deficiency is limited. Moreover, in absence of inflammation, the significant positive correlation of sTfR with RET#, HFR and EPO/kg suggests that sTfR are increased due to EPO treatment.

748 ABNORMALITIES OF CHROMOSOME 22 AMONG MYELODYSPLASTIC SYNDROME (MDS) PATIENTS PRESENTING A COMPLEX KARYOTYPES

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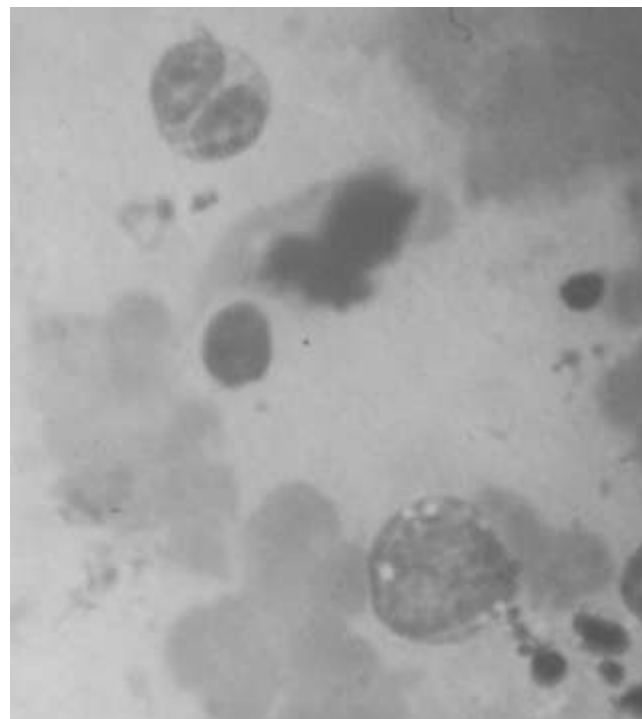
Multiple chromosome rearrangements (MCRs) are found in 15-30% newly diagnosed patients (pts) with myelodysplastic syndrome (MDS). In this group of pts, recurring single way translocations are rare and molecular mechanisms involved are largely unresolved. MCRs detected by G-banding technique exclusively remain unclear with various abnormalities of unknown origin. Spectral analysis (SKY) overcome this limitations and allow us precise delineation of the karyotype. We report eight MDS and MDS related to acute myeloid leukaemia patients who were referred to the Hematology Department UJ with MCRs. Chromosome analysis were performed at diagnosis on bone marrow cultures according to the standard protocol using G-banding. Nearly in each karyotype, the rearrangements of chromosome 22 were presented. For the reason of several markers presenting in following karyotypes: 48,XY,der(1)(q23-q33?),der(2)(?),der(3)(?),der(4)(q31?),i(6)(p10),+18[pt N1]; 42-47,XY,-3,del(5)(q31),+11,-17,-18,+21,+mar1,+mar2[pt N2]; 47,XX,+1,-4,-5,+8,-16,-17,-18,t(22;?)9p10;?),+mar1,+mar2,+mar3,+mar4[pt N3] the spectral karyotype analysis (SKY) were used for re-evaluation of G-banding results. The involvement of chromosome 22q rearrangements were presented in all three cases, namely t(2;22), t(3;22) in the first one, the remaining showed t(10;22) and t(21;22), respectively. Similarly to the previously published cases, we summarise that 22q abnormality it seems to be one from common in MCRs. In our pts disease was not curable with short overall survival time (1,5 months) for all the three regardless of WHO subtype. We speculate a special role of mentioned changes of chromosome 22 in MDS process.

749 ATYPICAL LYMPHOID CELLS IN MODIFIED CULTURAL SYSTEMS OF BLOOD, BONE MARROW AND CEREBROSPINAL FLUID OF PATIENTS WITH MALIGNANT LYMPHOMA AND ITS VALUE FOR THE DIFFERENTIAL DIAGNOSIS AND PROGNOSIS

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In spite of the variety of the methods inculcated in clinical practice for the investigation of patients with enlarged lymph nodes and/or splenomegaly or leukemoid reactions with lymphocytosis sometimes the differential diagnosis is being a problem. To work out the additional criteria for the diagnosis of malignant lymphomas we investigated the blood, bone marrow and cerebrospinal fluid cells in short- and long-term culture systems modified by us. In blood leukocyte cultures of patients with Hodgkins lymphoma Hodgkins cells and significant decrease of macrophage-lymphocyte rosette formation pointing to the low immune reactivity of organism are observed. Besides macrophages lack the proliferate activity. In cultures of patients with non-Hodgkins lymphoma proliferation of lymphoid cells is observed. Unlike to acute lymphocytic leukemia cases where majority of cells in vitro are lymphoblasts, in the cases of non-Hodgkins lymphoma most of cells are morphologically normal lymphocytes. The presence of atypical lymphoid cells with deformed, misshaped or fragmented nucleus or with two nuclei and figures of asymmetrical pathological mitosis shows the malignant nature of disease. The presence of the definite amount of lymphoblasts, prolymphocytes and plasma cells in vitro depends on the lymphoma cell variant. In the cases of non-Hodgkins lymphomas proliferation of macrophages in vitro is inhibited and they show low phagocytic activity as well. Amount of macrophage-lymphocyte rosettes may be normal, low or high. Normal indices of macrophage-lymphocyte rosettes and low proliferate activity of atypical lymphocytes in vitro was correlated with good outcome, while low or high amount of macrophage-lymphocyte rosettes was a bad prognostic marker. High proliferate activity of atypical malignant lymphoid cells and frequent figures of pathological mitosis predicted leukemization of non-Hodgkins lymphoma or represented the terminal stage of disease. In cultures of patients with leukemoid reactions and lymphadenopathy maintenance of lymphocytes and proliferation of macrophages with high ability of phagocytosis is observed. Atypical lymphocytes and figures of pathological mitosis are absent in these cultures; amount of macrophage-lymphocyte rosettes depends on the character of main disease expressing the immune reactivity of organism. Results of our studies show that cultural investigations may be helpful and necessary for the differential diagnosis of lymphomas and leukemoid

reactions and for the prognosis of malignant lymphomas as well. Low proliferate activity of pathological cells in cultures of patients with Hodgkins and non-Hodgkins lymphoma that represents low proliferate activity of malignant cells in vivo can be considered as one of the reason of the greatly improved cure rates for these patients. See Image 1.



Caption 1: Fig.1 Lymphocyte with two nuclei (up) and lymphocyte

750 ABSOLUTE NEUTROPHIL COUNTS AND ORTHOPAEDIC MANIFESTATIONS OF SICKLE CELL DISEASE

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Neutrophils defend the body against infection but they can be destructive to tissue. In sickle cell disease, the interaction of granulocytes, sickle cells, endothelial cells lead to the release of injurious cytokines, which contribute to the pathophysiology of the disease and high granulocyte counts have been shown to be a risk factor for death. Although patients with sickle cell disease (SCD) are susceptible to infections due to a variety of reasons, traditionally they are followed up with routine blood and reticulocyte counts and not much attention has been paid to the absolute neutrophil count (ANC) as a means of monitoring disease morbidity routinely.

Our aim was to correlate the absolute neutrophil count with various orthopaedic manifestations of SCD.

We reviewed all patients with SCD who presented at the National Orthopaedic Hospital Lagos between January 1997 and December 2002 with orthopaedic complications who had complete blood counts performed on them.

There were a total of 80 patients, 44 males, 36 females, and Mean Age 18.22 years, Age Range 1.25- 51.

There was an astronomical increase in the neutrophil count level in patients with bone pain crisis, multifocal chronic osteomyelitis, chronic leg ulcers and avascular necrosis of the femoral head. The highest count of 34.927 x 10⁹/L. was recorded in a patient with pyomyositis, multifocal osteomyelitis, underlying anaemia and septicaemia. Bilaterality of any of the conditions seemed to increase the ANC as patients with bilateral AVN and leg ulcers had ANC > 4.0 x 10⁹/L while none of the patients had less than 2.0 x 10⁹/L.

Infection and high leukocyte counts are independent causes of disease-related morbidity and mortality in SCD2. Our patients with multifocal

COM, Bilaterality of orthopaedic complications and chronic leg ulcers had very high ANC indicating severe complications.

We conclude that in SCD patients with orthopaedic manifestations, the absolute neutrophil count is useful in monitoring disease-related morbidity and response to therapy.

Reference:

1. Platt OS, Brambilla DJ, Rouse WF et al. Mortality in sickle cell disease: Life expectancy and risk factors for early death. *N Engl J Med* 330:1639, 1994.
2. Attah Ed B, Ekere MC. Death patterns in sickle cell anaemia. *JAMA* 1975; 233:889-890

751 CORRELATION OF REACTIVE PLASMACYTOSIS WITH UNDERLYING DISEASES

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Background: The number of plasma cells in BM is normally less than 5%. The most common causes of reactive plasmacytosis are infections, especially HIV, autoimmune diseases and neoplasms.

Aim: During the study of BM aspiration, we found a frequent occurrence of non-specific increases in the number of plasma cells. The goal of the study was to determine the correlation of reactive plasmacytosis with the respective underlying diseases.

Method: We studied 66 patients, 32 males, 34 females mean age of 72.3 years, who underwent the bone marrow procedure due to a mild anemia and an elevated ESR. On each patient we performed a quantity determination of immunoglobulins. On the basis of the number of plasma cells, we divided the patients into three groups:

A 5-10%; B 10-20%; C 20-30%. We recorded the diagnosis and the quantity of immunoglobulins.

Results: Group A: Patients 52

26 infections: RT (5), UT(9), GT (2), sepsis (5), brucellosis (3), fibrile of unknown reason (1), and erysipelas (1)

2 breast cancer

8 connective tissue disease: SLE (2), polyarthritis syndrome (2), RA (1), dermatomyositis (1), temporal arteritis (1), Behcet disease (1).

16 others: cirrhosis (4), anemia due to chronic renal failure (2), diabetes mellitus (2), hypertension (1), iron deficiency anemia (5), deep vein thrombosis (1), epilepsi (1).

For 37 patients, (71%), the immunoglobulins levels were normal, for 15 (29%), we observed a slight increase of IgA (mean value of 425 mg/dL, normal range 80-400 mg/dL), while in 6 (11.5%) of them a simultaneous increase in IgG and IgA

Group B: Patients 12

7 infections of which: 3 viral (HIV (1), pericarditis (1), gastroenteritis (1)), and 4 bacterial (sepsis (2), urinary track infection (1), TBC spondylitis (1))

3 carcinoma

1 connective tissue disease: polyarthritis syndrome

1 cirrhosis

Among these 12 patients, four patients (33%), had normal immunoglobulin levels. While six patients, (50%), had elevated only the IgA (mean value 671 mg/dL), 1 patients IgA and IgM, while another 1 IgG, IgA and IgM.

Group C: Patients 2

1 Febrile of unknown reason with elevated levels of IgG

1 sepsis caused by klebsiella and enterobacter cloacae with elevated IgG.

The distribution and morphology of plasma cells in Group A were normal. In Groups B and C the plasma cells formed large aggregates, with occasionally binucleated and trinucleated plasma cells, as well as flame cells.

Summary: 1 The large majority of patients, 78.8%, showed increase in plasma cell numbers of up to 10%.

2. The most common causes of reactive plasmacytosis were: infections (53%), connective tissue disease (13.6%), neoplasms (7.5%), and cirrhosis (7.5%).

3. The quantity determination of immunoglobulins showed significant increases of IgA levels in all three patient groups, with a simultaneous slight increase of IgG.

4. Where the proportion of plasma cells exceeded 10%, we have mainly observed disorder in the distribution and, to a lesser extent, in the morphology of the plasma cells.

752 TEMPLE STUDY: TRANSFUSION EFFECTS IN MYELODYSPLASTIC PATIENTS, LIMITING EXPOSURE

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Background. Myelodysplastic syndromes (MDS) are clonal disorders characterised by dysplasia in at least 2 myeloid cell lines. MDS patients are predominantly treated with blood transfusions to improve health-related quality of life (HRQoL). Concerns about the transfusion-related complications, such as infections, tumour behaviour and immunomodulatory effects, and the costs, necessitated a re-evaluation of the transfusion practice.

Aims. The goal of this study is to evaluate if a restrictive transfusion policy (Hb transfusion trigger <4.5 mmol/l) reduces the amount of red cell transfusion compared to a liberal transfusion trigger (Hb <6.0 mmol/l) without a decrease in HRQoL. Because of concerns about the feasibility of this study early results were analysed and are presented in this abstract.

Material and Methods. After a run in period of 3 months (Hb transfusion trigger of Hb <6.0 mmol/l) patients are randomised for the restrictive or the liberal transfusion policy. Patients are followed then 12 months. HRQoL is measured after inclusion, after randomisation, 6 weeks, 3, 6, 9, and 12 months after randomisation. Also anaemia related complications and red cell antibodies are scored. Hb values were blinded for the patients during the study period.

Results. From 10-7-2002 till 11-11-2003 15 patients were included (4 RA, 5 RARS, 4 RCMD, 1 RAEB, 1 CMMoL) in 2 general hospitals and 1 university hospital. Two patients died in the run in period. Eight patients were randomised for the restrictive transfusion policy and 5 patients for the liberal transfusion policy. The mean follow up period in the liberal group was 6.2 months (inclusive run in period) and 7.4 months for the restrictive group. Two patients in the liberal group died after randomisation. One patient received growth factors. In the restrictive group 2 patients finished the study, 1 received growth factors and 1 patient withdrew informed consent. The mean Hb level was lower in the restrictive group and after randomisation about 55 % reduction in amount of transfused red cells was found (1.8 units per pt per month in the liberal group vs 0.8 in the restrictive group). No anaemia related complications were found, e.g. cardiac failure and cerebro vascular ischemia nor a decrease in activity performance.

Conclusion. There were some concerns after introduction of the restrictive transfusion policy. This preliminary results show, however, that a restrictive transfusion policy leads to a diminished use of red cell transfusion without an increase of cardiac complications or a decrease in activity performance. This study will be continued to compare HRQoL scores in both groups.

753 DETECTION AND CHARACTERIZATION OF THREE HEMOLYSINS IN PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA

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Background: Some patients with autoimmune hemolytic anemia (AIHA) have in their sera autohemolysins. These autoantibodies, by definition, are able of lysing red blood cells (RBC) in vitro by activation of C1 - 9. Detection of in vitro hemolysin activity is not used as a routine laboratory test in the diagnostic and follow-up of AIHA.

Aims: In the following, we describe 3 autohemolysins in patients with AIHA and we study clinical correlations.

Methods: 32 patients with AIHA followed in the Department of Hematology AOH / Tunis city, were included in our study. Samples of Blood performed in the progressive course of the disease during the period between July 2001 - September 2002, were examined in the Immuno-Hematological Laboratory of NCBT / Tunis city. For each patient, we achieved systematically Direct Antiglobulin Test (DAT) by gel-filtration, elution and serologic investigations (antibodies screening, in vitro hemolysin activity, thermal amplitude, titre and specificity).

Results : 3 autohemolysins were detected in the sera of 3 patients : 2 warm and 1 mixte. All of these autohemolysins were likely IgM autoantibodies and reacted in vitro only with enzyme-treated RBC. The 2 warm autohemolysins were detected in patients with warm-type AIHA and showing a DAT positive for IgG, IgM and C3d in patient 1 and for IgG, IgM, IgA and C3d in patient 2. The first warm autoantibody active only at 37C was neutral, lysing RBC at pH = 7,3. It had a low titre (pur). The second warm hemolysin active optimally at 37C, with a wide thermal amplitude reaching 22C and a titre of 16, was acid (pH = 6,5). The specificities of all these warm autoantibodies were not serologically classified. In patient 3 with cold hemagglutinin disease and a C3d positive DAT, the serum showed an in vitro hemolysin activity at 4 C and 22 C at acid pH. The thermal optimum was at 4 C and the titre 64. This in vitro hemolysin activity was also detected at 37C with the same titre (64), but only at neutral pH. Hemolysis at low temperature and neutral pH was not tested. The specificity revealed to be anti-I. So, is it the same autoantibody having a mixte hemolysin activity or is there 2 distinct hemolysins, a cold acid reacting optimally at 4C and a warm neutral one ?

Summary / Conclusion : In our study, the prevalence of hemolysins in AIHA was approximately 9 %, which is a not inconsiderable percentage. In contrast to the literature, although these autoantibodies were incomplete (hemolysing in vitro only enzyme-treated RBC), they were associated for all of the 3 patients with the occurrence of severe hemolytic episodes requiring sometimes, intensive therapy such as blood transfusion support. So, isn't it likely important to detect in vitro hemolysin activity in patients with AIHA, and to consider this serologic evaluation as a potential prognostic feature ?

754 RETICULOCYTE MATURITY INDEX, RETICULOCYTE SUBPOPULATIONS AND RETICULOCYTE COUNT IN HAEMOGLOBINOPATHIES

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Background: Reticulocyte measurement with flow cytometry is a sensitive and precise method. With this automated analysis, reticulocyte can be classified into three groups (high, medium, low) by the fluorescence intensity of auramin-O staining according to the amount of intracellular RNA. The fluorescence intensity reflects maturity of reticulocyte. Reticulocyte maturity index is an indicator for estimating qualitative abnormality of erythropoiesis. The aim of this study was to investigate the reticulocyte maturity index (RMI), the reticulocyte subpopulations [high (HFR), medium (MFR) and low fluorescence ratio (LFR)] and the absolute reticulocyte count (RET#) in haemoglobinopathies.

Patients and methods: We studied 120 β -thalassaemia carriers (Group A), 15 α -thalassaemia carriers (Group B), 23 sickle cell carriers (α 2 β 2 β 6Glu $\rightarrow$ Val) (Group C), 29 O-Arab carriers (α 2 β 2 β 121Glu $\rightarrow$ Lys) (Group D) and 78 healthy individuals (Group E). Blood samples were analysed on Sysmex SE-9500+RAM1. RMI is determined as follows: [(HFR+MFR)x100]/LFR.

Results: (xSD)

Group A n=120: Hb g/dl=10.871.35, RMI%=17.638.47, HFR%=1.401.28, MFR%=13.224.95, LFR%=85.365.83, RET#x106/ μ l=0.0770.03

Group B n=15: Hb g/dl=11.361.21, RMI%=8.474.31, HFR%=0.450.69, MFR%=7.263.51, LFR%=92.283.65, RET#x106/ μ l=0.0400.01

Group C n=23: Hb g/dl=12.316, RMI%=21.710.0, HFR%=2.592.48, MFR%=14.694.5, LFR%=82.76.6, RET#x106/ μ l=0.0560.01

Group D n=29: Hb g/dl=12.511.54, RMI%=17.6711.8, HFR%=2.152.44, MFR%=12.136.03, LFR%=86.08.31, RET#x106/ μ l=0.0510.02

Group E n=78: Hb g/dl=13.71.1, RMI%=13.536.51, HFR%=1.491.32, MFR%=10.193.82, LFR%=88.314.81, RET#x106/ μ l=0.0480.01

Comparison between the mean values of the results of groups A, B, C, D and the mean value of the results of group E (Student's test, statistically significant difference p $\leq$ 0.05, ns=no sense):

Group A: RMI $\uparrow$ (p=0.001), HFR ns, MFR $\uparrow$ (p=0.001), LFR $\uparrow$ (p=0.001), RET# (p=0.001)

Group B: RMI $\uparrow$ (p=0.01), HFR $\uparrow$ (p=0.005), MFR $\uparrow$ (p=0.005), LFR (p=0.005), RET# ns

Group C: RMI $\uparrow$ (p=0.001), HFR $\uparrow$ (p=0.005), MFR $\uparrow$ (p=0.001), LFR $\uparrow$ (p=0.001), RET# ns

Group D: RMI (p=0.025), HFR $\uparrow$ (p=0.05), MFR $\uparrow$ (p=0.05), LFR $\uparrow$ (p=0.05), RET# ns

Conclusion: Erythropoietic activity indicated by RMI is increased in -thalassaemia trait and in haemoglobinopathies with haemoglobin structural variants (sickle cell trait, O-Arab trait). The high proportion of immature reticulocyte (high value of HRF fraction) that is observed in

sickle cell trait and O-Arab trait is probably due to increased haemolysis. The haemoglobinopathies with haemoglobin structural variants we studied, behave similarly according to RMI, reticulocyte fractions and absolute reticulocyte count. On the opposite, there is no similarity found between the two thalassaemic syndromes.

755 THE DEVELOPMENT OF TUMOR CELL DRUG RESISTANCE TO VINCISTINE ON CONDITION OF P-GP FUNCTION SELECTIVE INHIBITION

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Background: P-glycoprotein 170 (P-gp) is responsible for the antitumor drug transport to the cell and therefore belongs to the specific intracellular systems determining the cancer cell drug resistance. The clinical significance of P-gp expression and functional activity has been shown. As a result the ways of P-gp activity inhibition are widely investigated. Hence, it is necessary to study other drug resistance mechanisms which can appear in the conditions of P-gp activity suppression.

Aim: To study the probability of drug resistance maintenance in the conditions of P-gp functional activity suppression.

Methods: Human B lymphoblastoid cell line IM-9 was used as an experimental model system. This line was cultured in complete RPMI-1640 medium at standard conditions. Parental cell line IM-9 was cultured for 3 months in the complete medium with gradually increased vincristine concentrations for the induction of resistance to vincristine (subline IM-9/VR). In parallel parental line IM-9 was cultured with increased concentration of both vincristine and cyclosporine A (CsA) for the concurrent suppression of P-gp functional activity (subline IM-9/VR+CsA). The test with Rhodamine-123 (Rho) was used for the estimation of P-gp functional activity by means of FACS analysis. The assessment of cell line metabolic activity was realized by means of cytotoxicity test (MTT-test).

Results: MTT-test results showed, that sublines IM-9/VR and IM-9/VR+CsA acquired increased vincristine resistance. The drug concentration which leads to 50% cell metabolic activity inhibition (IC50), was 7,51,9 nM for IM-9, 23,12,4 nM for IM9-VR and 20,42,1 nM for IM-9/VR+CsA subline. Thus, the drug resistance to vincristine was independent of CsA presence in the cell culture during the resistance development. FACS analysis showed that sublines IM-9/VR and IM-9/VR+CsA are characterized by significantly higher levels of Rho efflux in comparison with the parental cell line IM-9 and they are differed from each other. The Rho efflux for IM-9/VR and IM-9/VR+CsA sublines was 2,8%0,3 and 32,9%3,6 respectively in comparison with IM-9 line. So, the high level of Rho release determines one of resistance mechanisms to vincristine. However, it maybe not necessarily concerned only P-gp activity. It was also noted the decrease of Rho cell efflux after the addition of CsA to the established sublines and to the parental cell line. It would be expected that CsA increases accumulation of vincristine in cell and promotes the decrease of resistance to this drug. However, IM-9/VR+CsA cells still maintained high level of drug resistance under the combined action of vincristine and CsA (IC50 was 20,42,1 nM and 16,22,5 nM respectively before and after action).

Conclusions: It was established the possibility of vincristine resistance development in lymphoblastoid cells of IM-9 line in the conditions of P-gp functional activity suppression. Obviously, the maintenance of tumor cell resistance to vincristine, in case of P-gp functional activity loss, is connected with substitution of this drug resistance mechanism for others which determine resistance of tumor cell to the damage action.

756 LEVELS OF SOLUBLE TRANSFERRIN RECEPTORS AS AN INDICATOR OF IRON DEFICIENCY IN HAEMODIALYSIS PATIENTS ON ERYTHROPOIETIN TREATMENT

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Background: The diagnosis of iron deficiency in chronic haemodialysis patients on erythropoietin treatment is usually difficult using the common laboratory tests. Soluble transferrin receptors (sTfR) constitute an indicator of erythropoietic activity. The levels of sTfR are increased in iron deficiency as well as in encephalopathy.

The aim of this study was to evaluate the levels of soluble transferrin receptors as an indicator of iron deficiency in chronic haemodialysis patients on erythropoietin treatment (EPO).

Patients and methods: We studied 47 patients with a mean age 62.7 ± 14.6 years and a mean duration of haemodialysis 63.5 ± 37.2 months. All patients received EPO treatment for >6 months and have had stable haemoglobin (Hb) levels for 3 months. Hb, erythrocytes indices (MCV, MCH, MCHC, RDW-CV), reticulocyte count (RET#), reticulocyte maturity index (RMI), reticulocyte subpopulations (HFR, MFR, LFR), ferritin, sTfR, folate, vitamin B12, iron, total iron binding capacity (TIBC) and transferrin saturation (SAT) were measured. Patients were divided into two groups: group A (MCHC>31.4 g/dl) n=11, group B (MCHC≤31.4 g/dl) n=16. Results: Detection of iron-deficient patients according to the cut-off values of the individual tests and their combinations (cut-off values for iron deficiency detection: SAT≤20%, MCHC≤31.4 g/dl, ferritin<200 ng/ml, sTfR>1.76 mg/l): 1/ SAT: n=17 2/ MCHC n=14, 3/ Ferritin: n=6, 4/ sTfR: n=34, 5/ SAT+MCHC: n=12, 6/ MCHC+sTfR: n=13, 7/ SAT+sTfR+MCHC: n=9. None of the patients have had neither vitamin B12 - folate deficiency nor haemoglobinopathy.

Soluble transferrin receptors showed a significant negative correlation with: SAT ($r=-0.30$) and MCHC ($r=-0.33$). Soluble transferrin receptors exhibited a significant positive correlation with: RET# ($r=0.49$), HFR ($r=0.31$) and EPO/kg ($r=0.44$). There is no correlation found between sTfR and ferritin.

Comparison between the results of group A (MCHC>31.4 g/dl) (n=31) and the results of group B (MCHC≤31.4 g/dl) (n=16), (xSD, statistically significant difference $p \leq 0.05$, ns=no sense).

Age years: Group A: 60.6115.18, Group B: 66.9312.83 (ns)

Hb g/dl: Group A: 11.841.26, Group B: 11.161.42 (ns)

SAT%: Group A: 35.2220.40, Group B: 20.567.36 ($p=0.009$)

RET# $\times 10^6/\mu\text{l}$: Group A: 0.05720.0214, Group B: 0.06060.0248 (ns)

RDW-CV%: Group A: 14.651.41, Group B: 15.571.50 ($p=0.005$)

sTfR mg/l: Group A: 2.120.62, Group B: 2.770.86 ($p=0.008$)

Ferritin ng/ml: Group A: 420.8181.9, Group B: 537.81355.74 (ns)

EPO/kg: Group A: 105.0445.04, Group B: 173.6997.07 ($p=0.04$)

Conclusion: The correlation of soluble transferrin receptors with other markers of iron status shows that their estimation contributes to the diagnosis of iron deficiency. However, the positive correlation with RET#, HFR and EPO/kg demonstrates that the high levels of sTfR are due mainly to increased erythropoietic activity.

In haemodialysis patients undergoing EPO therapy, the evaluation of sTfR overestimates the number of iron-deficient patients; therefore, soluble transferrin receptors levels do not constitute a reliable indicator of iron status.

757 FAMILIAL B CHRONIC LYMPHOCYTIC LEUKAEMIA IN TWO FAMILIES

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Background. Familial chronic lymphocytic leukemia (B-CLL) is particularly interesting from the genetist's point of view because of the phenomenon of anticipation which is expressed in younger generations: The onset of the disease happens in earlier age and is combined with more severe clinical features and course. No particular genetic damage or important immuno-phenotypic, karyotypic or molecular differences between familial and not familial B-CLL has been detected in 80 families known to have been studied until now.

Aims. The study of phenomenon of anticipation and clinic, immuno-phenotypic, karyotypic and molecular types in two families with B-CLL.

Material-methods. We studied in four members of two families with familial B-CLL, [family A: father, son, (vertical appearance in two generations), family B: two brothers (horizontal appearance in the same generation)] : a) the percentage of CD38 + B lymphocytes using flow cytometry b) the b2 microglobulin and LDH using classic methodology c) cytogenetics (C) on unstimulated bone marrow cultures using G-banded chromosome preparations d) histologic type of the infiltration of the disease (HTI) in the bone marrow and e) immunoglobulin heavy chain variable region genes (IGHV) somatic mutation status in clonal rearranged IGHV genes after RT-PCR amplification, direct sequencing, sequence alignment to the BLAST/IMG databases and analysis of the distribution of mutations.

Results. Family A. The senior member (father) presented the disease at the age of 67 years and remains stable without treatment 10 years later.

His son (second generation) presented the disease recently in the age of 47 years, deteriorated rapidly, presented resistance to any treatment and died in one year from the diagnosis. The prognostic indicators of disease in the case of the son were bad (Table), while the prognostic indicators in the case of the father good.

Family B. Both brothers (same generation, horizontal appearance) presented bad prognostic indicators, except the low percentage of CD38 + B-lymphocytes the one (Table) and was given therapy from diagnosis.

The three out of four patients presented unmutated (U) IgHV genes status, (VH homology to germline > 98%) and the one mutated (M).

Conclusions. In family A the typical expression of anticipation phenomenon consisted in rapid development of the disease in the member of the second generation, resistance in treatment, appearance in considerably younger age, fulminant development of the disease, bad prognostic indicators as well as unmutated IgHV genes.

Family	Age	RAI	b2M	LDH	HIT	CD 38+	C	IgH
A Father	67	I	N	N	ND	5%	N	M
A Son	47	IV	↑	↑	D	74%	P	U
B Brother 1	76	III	↑	↑	D	57%	-	U
B Brother 2	55	II	↑	↑	D	2%	P	U

ND: Nodal, D: Diffuse, N: Normal, P: Pathologic, M: Mutated, U: Unmutated

Caption 1: Table

758 CLADRIBINE COMBINED WITH ETOPOSIDE IN THE TREATMENT OF REFRACTORY HEAVILY PRETREATED LOW-GRADE LYMPHOMAS

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Background : The purine nucleoside analogues fludarabine (FA), cladribine (2-CdA) and 2'-deoxycytosine represent a novel group of cytotoxic agents with high activity in low grade lymphoid malignancies. Combined use of purine analogs with other cytotoxic agents may be more effective than monotherapy. Pre-clinical experiments indicate, that cytotoxic agents including etoposide (VP16) act additively or synergistically with 2-CdA and FA .

Aims: The aim of the study was to evaluate efficacy and toxicity of combination of cladribine and etoposide in relapsed and refractory low-grade non-Hodgkin lymphoma.

Methods: Adult patients with relapsed or refractory low grade non-Hodgkin's lymphoma (LG-NHL) or B-cell chronic lymphocytic leukemia (CLL) entered the study. The treatment consisted of 2-CdA given at a dose of 0.12 mg/kg/d for 5 consecutive days. and VP16 at a dose 100 mg i.v. for 5 days.

Results: Twenty one patients were treated according to the regimen. Twenty patients were eligible for evaluation of response. Median age of the patients was 59 years (range 41-80). Female/male ratio was 5/ 16. Six patients were diagnosed with LG-NHL, 14 with CLL and 1 with hypereosinophilic syndrome. Fifteen of the patients were refractory to prior chemotherapy and 6 were recurrent. All patients received 3 or more cycles of chemotherapy previously (median 8, range 3-19). Thirteen of the patients received 2-CdA before, 4 of them together with rituximab. Overall response rate was 6/20 (30 %, 95% CI 10%-50%) including complete response in 1 patient (5%) with CLL and partial response in 5 patients (25%, 95% CI 6%-44%) (4 'CLL, 1-LG-NHL). Stabilisation of the disease occurred in 3 patients (15%, 95% CI 0%-31%) and 11 patients (55%, 95% CI 33%-77%) did not respond. Neither of patients with mantle cell lymphoma responded. Three of the patients (2-CLL, 1- LG-NHL) receiving 2-CdA previously, achieved a PR. Neither of the patients treated earlier with combination of 2-CdA and rituximab responded.

Myelosuppression was the major toxicity of the regimen. Thrombocytopenia occurred in 9 patients and neutropenia in 8. Infections occurred in 8 patients, including pneumonia in 6 patients, sepsis in 1 and herpes zoster in 1.

Conclusions: Combined use of 2-CdA and VP-16 seems to be effective treatment in heavily pretreated patients with CLL or LG-NHL, even those refractory to 2-CdA in monotherapy. The regimen seems however to be not active in case of refractoriness to 2-CdA and rituximab combination.

759 11Q ABERRATIONS IN PATIENT WITH HEMATOLOGICAL MALIGNANCIES

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Background: Translocation, deletions and other structural rearrangements involving chromosome band 11q23 have been observed in various hematological malignancies, associated with bad prognosis and poor clinical outcome. The great majority of chromosomal changes involve the MLL gene.

Aims. The aim of this study was: 1. investigation of 11q rearrangements, including translocations, deletions and insertions, in bone marrow samples of patients with different hematological malignancies. 2. mapping and identification of deletions and breakpoints using molecular cytogenetic techniques. 3. correlation of clinical characteristics, evaluation of outcome and survival of patients according with the type of 11q rearrangement

Methods: Using classical cytogenetic techniques we examined bone marrow cells of 32 patients (14 male, 18 female, median age 41.7) with structural rearrangements of 11q or complex rearrangements of chromosome 11 with different hematological malignancies (22 myeloid, 10 lymphoid). Fluorescence in situ hybridization (FISH) with locus specific MLL dual color probe (VYSISIM) for 11q23 region, locus specific ATM probe (VYSISIM) for region 11q22 and locus specific Cyclin D1 probe (VYSISIM) for region 11q13 were used for determination of the breakpoints or detection of deleted segments. In addition 56 patients with CLL were examined using FISH with MLL and ATM probes. Multicolor FISH (mFISH) was performed to confirm the complex translocations.

Results: According to the results of cytogenetic, FISH and mFISH analyses three groups of patients with 11q rearrangements were identified: 1st group patients with the breakpoint in MLL gene (12 patients, median age 27.4, 5 with AML, 6 with ALL and 1 with MDS), 2nd group - patients without MLL rearrangement but with structural rearrangement of 11q (3 male, 6 female, median age 37.8) and 3rd group patients with deletion of MLL and ATM gene (6 male, 5 female, median age 60.5, 8 patients with MDS, 2 with AML and 1 with ALL). In a group of patients with CLL deletion of MLL gene was proved in 7 of them, in 6 of them patients deletion of ATM gene was found together with deletion of MLL.

Summary: Patients with structural rearrangements of 11q represent genetically heterogeneous group. MLL rearrangements was proved in myeloid as well as in lymphoid malignancies. According to our results it seems that also other breakpoints than 11q23 may play important role in the leukemia process. Correlation of clinical characteristics and cytogenetic findings was analysed and will be presented in the poster.

This work was supported by grant GA CR 301/04/0407.

760 VASCULAR ENDOTHELIAL GROWTH FACTOR IN PATIENTS WITH MYELOPROLIFERATIVE DISEASES

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Background. The vascular endothelial growth factor (VEGF) promotes angiogenesis and is associated with the growth and dissemination of cancer. The prognostic value of serum concentrations of VEGF in patients with myeloproliferative diseases (MPD) has not been thoroughly studied.

Aim : The purpose of this work was the study of the serum concentrations of VEGF in patients with chronic myelogenous leukemia (CML), essential thrombocytemia (ET), polycythemia rubra vera (PRV) and their correlation with the levels of bcr-abl transcripts, the number of leucocytes, thrombocytes and the levels of haemoglobin in the blood.

Material Methods: We studied 46 samples of patients with MPD in different phases of their diseases 16 with CML, 16 with PRV, 14 with ET, and 110 samples of healthy persons (controls).

We measured: a) the serum concentrations of VEGF using Elisa, b) the levels of bcr-abl transcripts using real time quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) methodology, c) the number of leucocytes, thrombocytes and haemoglobin using automate hematology analyser.

Results: a) the mean value of the serum concentrations of VEGF was X=349.86pg/ml in healthy people and X=1353.9pg/ml in patients with MPD overall p<0.001, b) the mean value of the concentrations of VEGF was X=1573.6 pg/ml in CML, X= 1348.8 pg/ml in ET and X=1189.9 pg/ml in patients with PRV, c) patients with CML found to have strong correlation between the concentrations of VEGF and bcr-abl transcripts P<0.01 and between the concentrations of VEGF and the leucocytes of blood P<0.01, d) we have not found any correlation between the concentrations of VEGF and the haemoglobin in patients with PRV. We have neither found any correlation between the concentrations of VEGF and the number of thrombocytes in patients with CML, ET and PRV nor between the concentrations of VEGF and the number of leucocytes in patients with ET and PRV.

Conclusions: Our study confirms that patients with MPD, have high concentrations of VEGF in their serum unrelated to the number of thrombocytes. The high concentrations of VEGF are correlated with the levels of bcr-abl transcripts and the number of leucocytes in patients with CML. The high concentrations of VEGF in myeloproliferative disorders might have a special biological role in the pathogenesis of these diseases.

761 DELETIONS AND STRUCTURAL REARRANGEMENTS OF CHROMOSOME 7 IN HEMATOLOGICAL MALIGNANCIES

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Background. Deletions of the long arms of chromosome 7 are the consistent aberrations in all types of myeloid disorders, associated with poor prognosis. The finding of acquired chromosome deletions indicates the presence of a tumor suppressor genes, which, when deleted, contributes to tumor initiation or progression. Different segments, 7q22, 7q31-q33 and 7q35 have been implicated as critical regions of the gene loss, associated with these disorders.

Aims. The aim of our study was the identification of cryptic 7q deletions in patients with complex rearrangement of chromosome 7 and mapping of the deleted segments 7q using molecular cytogenetic techniques. Correlation of clinical characteristics, outcome and survival of patients according to cytogenetic findings and diagnoses were evaluated.

Methods. Using classical cytogenetic techniques we examined 13 patients with different hematological malignancies (11 myeloid, 2 lymphoid), whose bone marrow cells contained 7q deletion or complex rearrangements of chromosome 7. Fluorescence in situ hybridization (FISH) with locus specific probe for 7/7q31 region (VYSIS), and 7q22/q35 specific probe (QBiogene) were used to confirm the deletion and to prove the breakpoints. Multicolor FISH (mFISH) was performed for identification of chromosomes involved in complex translocations.

Results. Using FISH with locus specific probes 7q deletion was confirmed in 4 patients (1 male, 3 female), cryptic 7q deletions with complex rearrangements of 7q were proved in 6 patients (2 male, 4 female). Deletion was found only in patients with myeloid malignancies, in 1 patient as a sole chromosomal aberration, in 2 patient together with other aberrant chromosomal clones, in 7 patients with complex rearrangements of the karyotype. The size of deletion varied among patients, del(7)(q22q35) was the most frequent one (4 patients). No statistical difference in survival time was proved due to low number of patients. It is necessary to examine large series of patients to obtain and explain all clinic characteristics associated with this aberration.

Summary. Karyotypic abnormalities of chromosome 7 are often associated with a poor prognosis, increased risk of infection, rapidly progressive disease and poor response to treatment. Identification of the deleted genes that are localized at different chromosomal regions would serve in the future for therapeutic interventions. For this purpose it is important to observe the large cohort of patients with 7q deletion and to use molecular cytogenetic techniques to reveal cryptic deletions.

This work was supported by grant IGA MZCR NR 7995-3 and GA CR 301/04/0407.

762 THE ROLE OF FLOW CYTOMETRY IN STAGING OF B-CELL-NON-HODGKIN'S LYMPHOMA

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Background: In staging non-Hodgkin's lymphoma (NHL) histology of a bone marrow biopsy is the golden standard. Cytology of an aspirate has some disadvantages: The sensitivity (morphology of the lymphocytes is not distinctive for all types of NHL) and negative selection on aspiration. With multiparameter flow cytometry monoclonal B-cells can be detected with a sensitivity of at least 0.1%.

Aims: Comparison of the results of cytology, histology and flow cytometry of bone marrow specimens in staging of newly diagnosed patients with B-cell B-NHL.

Methods: Of all patients between 2000 - 2003 the results of bone marrow cytology, histology and flow cytometry of 113 patients were compared. Of these patients the WHO classification was as follows: Diffuse Large B-cell lymphoma 57, follicular lymphoma 20, Burkitt lymphoma/leukemia 8, (extra)nodal marginal zone lymphoma 11, mantle cell lymphoma 4, lymphoplasmacytic lymphoma 5, others 3 (precursor B-lymphoblastic leukaemia/lymphoma 1, mediastinal (thymic) large B-cell lymphoma 1, Post transplantation lymphoproliferative disease 1). Flow cytometry was performed with double staining with monoclonal antibodies against the heavy and light chains of immunoglobulins or against other discriminative markers, after gating of the B-cells with CD19 en CD45.

Results: The results of histology compared to FC are shown in table 1. Cytology: 95 patients had negative cytology (71 biopsy and FC negative; 7 biopsy negative, FC positive; 9 biopsy positive, FC negative; 8 biopsy and FC positive; 1 biopsy negative, FC positive), 26 had positive cytology (25 biopsy and FC positive, 1 biopsy negative, FC positive).

Conclusion: In determining the presence of lymphoma in bone marrow of patients with the newly diagnosed B-cell NHL the standard (histology) is not superior to flow cytometry. In 13% a discrepancy between both types of investigation was found, in 6.2% bone marrow infiltration was missed by histology. Cytology does not contribute to the discovery of bone marrow localisation.

Patients	N =	biopsy+, FC +	biopsy+, FC -	biopsy -, FC +	biopsy-, FC -
All	113	34	8	7	64
DLBCL	57	7	5	4	41
Follicular	20	6	3	2	9
(extra)nodal MZ	11	2	0	0	9
Burkitt	8	5	0	0	3
Splenic	5	5	0	0	0
Lymphoplas- macytic	5	4	0	1	0
Mantle cell	4	4	0	0	0
Others	3	1	0	0	2

Caption 1: table 1

763 RITUXIMAB PLUS ICE REGIMEN FOR RELAPSED AND REFRACTORY AGGRESSIVE NON-HODGKIN LYMPHOMAS

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Background: High-dose chemotherapy (HDT) or radiotherapy followed by autologous or allogeneic stem cell transplantation (SCT) is a potentially curative treatment approach for patient with relapsed and refractory aggressive non-Hodgkin lymphomas (NHL). However, its applicability is restricted to patient responding to second line treatment (SLT). To date, there has been no SLT regimen that has proven to be superior with regard to response rate and to stem cell toxicity.

Aims: To evaluate effects of Rituximab added to SLT regimen (ICE) in relapsed and refractory aggressive Non-Hodgkin lymphomas.

Methods: We report our initial results of a SLT regimen consisting of Rituximab and ICE(R-ICE) in the first 8 evaluable patients, previously treated with a single standard anthracycline-containing regimen for aggressive lymphomas at the Clinic of Hematology, Medical Faculty-Skopje. Patients diagnosed as CD20+ aggressive B-NHL, with the median age 47.4 (range 29-62) were either in first relapse (5 patients) or refractory to treatment (3 patients). Patients received Rituximab 375/m

on day (d) 1, Etoposide 100 mg/m² d 3-5; Ifosfamide 5g/m² d4 mixed with the equal dose of MESNA and Carboplatin at AUC 5 d4, repeated every 14 days for a total of 3 cycles. Leukapheresis was performed after the third cycle.

Results: The median duration of treatment was 41day (range 35-52). The regimen was very well tolerated and no serious side-effects were observed. The overall response rate was 75% (6 patients) with 50% (4 patients) in complete remission. A median of 4.4 millions CD34+cells/kg were collected in a median of 2 apheresis (range 1-4) and all patients were eligible for HDT/SCT.

Conclusion: Our initial results suggest that R-ICE regimen is a very effective for cytoreduction and mobilization in patients with relapsed and refractory aggressive NHL and support the thesis that the addition of Rituximab to chemotherapy significantly enhances the response rate in NHL patients presumably as a result of the synergistic effects of the Rituximab with standard SLT regimens.

764 ACTIVITY OF SERUM THYMIDINE KYNASE IN ADULT ACUTE MYELOID LEUKEMIA.

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To determinate the prognostic value of serum thymidine kynase (TK) in adult acute myeloid leukemia (AML), TK was measured in 48 patients with AML (age range 17-63 years) in the time of pretreatment, remission and relapse.

Serum TK levels were determined by radioimmunoassay with 5125I - iododeoxyridine.

All patients were treated with standard induction therapy (daunorubicin 60 mg/m²/day for three days and cytarabine 100 mg/m²/day for seven days). The level of TK in the group of healthy persons was 0-6 units/l.

TK levels in 21 patients with AML who obtained complete remission after the first course of chemotherapy, was 9.8+/-5.0 units/l on the time of pretreatment and 4.4+/-0.8 units/l in the 21st day after completing the course. Patients, who achieved remission within 2-3 courses of the standard induction therapy (14 persons), had TK level 15.3+/-4.5 units/l and 7.7+/-2.7 units/l accordingly and those, who were resistant to standard chemotherapy (9 person), had TK level 30.35+/-17.95 units/l in the time of pretreatment and 97.9+/-78.6 units/l on the 21st day after completing the course. The level of TK in 29 patients in the state of stable remission was 1.4+/-6.6 units/l. Five persons who relapsed, had serum TK levels from 20.9 to 206.9 units/l. The level of TK didn't correlate with the number of leukocytes in blood and with the percentage of marrow blasts in the time of diagnosis. The levels of TK also correlated well with stage of the disease (remission, relapse).

765 DETECTION OF AML1/ETO, CBFb/MYH11 AND PML/RARA FUSION TRANSCRIPTS IN ACUTE MYELOID LEUKEMIA: COMPARISON OF CYTOGENETICS AND MOLECULAR GENETICS

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Background: Multiple recurrent chromosome and gene rearrangements have been identified in acute myeloid leukemia (AML) including t(8;21) inv(16), and t(15;17). These abnormalities result in the formation of AML1/ETO, CBFb/MYH11 and PML/RARa fusion genes respectively and account for approximately one-third of the chromosome aberrations found at diagnosis in patients with AML. It has been reported that patients with these translocations have a favorable prognosis. Therefore, the accurate identification of all patients with these rearrangements before initiation of therapy is of utmost importance.

Cytogenetic analysis can identify these abnormalities and provides prognostic information available, predicting outcome of both remission induction and post remission therapy. The fusion genes formed by the above chromosome translocations can be detected by reverse-transcriptase polymerase chain reaction (RT-PCR), which will indicate the presence of these genetic alterations in some patients in whom standard cytogenetics was technically inadequate.

Aim: To investigate the prevalence of AML1/ETO, PML/RARa and CBFb/MYH11 fusions in Iranian de novo AML patients by cytogenetic and molecular genetic techniques.

Subjects and Methods: We analyzed 150 (71 female, 79 male) consecutive newly diagnosed, untreated de novo AML patients with an age range of 3-90 years (median 34 years) for one of the AML1/ETO, PML/RARa and CBF β /MYH11 fusions. We used two steps RT-PCR and compared the results with those obtained by conventional cytogenetics.

Results: Using RT-PCR, AML1/ETO, PML/RARa, and CBF β /MYH11 Chromosomal aberration were found in 47/150 (31.3%) patients. AML1/ETO fusion transcripts were detected in 14/150 (9.3%) patients. In 19/150 (12.6%) patients PML/RARa fusion transcripts were found by PCR. Eight patients showed bcr3 (short form) and 11 patients showed bcr1 (long form) transcripts. In 13/150 patients (8.6%) the PCR analysis showed CBF β /MYH11 fusion transcripts. Seven patients had type A, 4 patients type E and 2 patients showed type D transcripts. In one patient (0.6%) PML/RARa and AML1/ETO was detected simultaneously. All patients with cytogenetic evidence of t(8;21), t(15;17) and inv(16) were positive by RT-PCR for the corresponding fusion transcripts. However, RT-PCR was also positive in one cases of AML1/ETO, two cases of CBF β /MYH11 and two cases of PML/RARa without the corresponding cytogenetic abnormalities representing an increase in the overall detection rate of t(8;21), inv(16) and t(15;17) from 8.6% to 9.3%, 7.3% to 8.6% and 11.3 to 12.6 respectively.

Conclusion: Both RT-PCR and cytogenetics identify most patients with CBF and AML1/ETO but, RT-PCR is a rapid and sensitive method for the detection of fusion genes in leukemia and the only technique that defines the PML and MYH11 breakpoint types and that allows the definition of a correct strategy for subsequent minimal residual disease (MRD) monitoring. We suggest that all the primary AML patients should routinely be tested for the presence of these fusion genes by molecular screening in order to improve genomic stratification of patients with AML into risk related treatment group. ymort@yahoo.com

766 TELOMERE LENGTH REFLECTS A PORTION OF AUTOLOGOUS HEMATOPOIESIS IN PATIENTS WITH ACUTE LEUKEMIA AND MDS TREATED BY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALHSCT)

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Background: Telomeres have an important role in chromosome function. Rapid erosion of telomeres in hematopoietic cells significantly contributes to arising of extensive genome instability and therefore might be one of the critical factors in leukemic transformation. Eroded telomeres are present in a majority of patients with untreated acute leukemia and are connected with the progression of MDS towards more advanced forms and overt leukemia.

In patients treated by allogeneic hematopoietic stem cell transplantation (alHSCT) molecular monitoring of cell chimerism provides distinguishing between original hematopoietic cells of the patient and his/her donor by DNA profiling. The presence of mixed chimerism in PB/BM cells after alHSCT indicates restoration of autologous hematopoiesis (AH).

Aims: To investigate variability of telomere length in hematopoietic cells of patients with mixed chimerism treated by allogeneic HSCT for MDS and acute leukemia with objective to comprehend, whether dynamics of telomere length reflects a portion of autologous hematopoiesis.

Methods: The study was performed on 15 samples of 5 patients treated for MDS, AML and JMML by allogeneic HSCT. High molecular weight DNA was obtained from BM/PB mononuclear cells. Chimeric status after alHSCT was detected by amplification and genotyping of informative high variable DNA polymorphisms chosen from the panel including 7 VNTR, 8 STR and 3 sex specific loci (sensitivity of investigation: 0.1 - 0.0001%). In cases of mixed chimerism (presence of both autologous and allogeneic DNA profile) the ratio of allogeneic and autologous hematopoiesis was estimated from the density of bands representing specific alleles of donor and recipient using software UVP GelBase. Length of telomeric and adjacent sub-telomeric regions was determined as terminal restriction fragment (TRF) performing Southern blot analysis of DNA digested by frequent cutters (TeloTAGGG Telomere Length Assay). We postulated cases with TRF shorter than 7.5 kbp (mean TRF from 45 age matched healthy individuals, Me = 38.5 years) as cases with reduced telomeres.

Results: The highest difference in TRF was shown in bone marrow samples of patient with RAEB when compared the diagnosis (TRF=8.9kbp) and relapse after alHSCT (TRF=3.8kbp; 90% AH). Significant decreased in TRF was observed also in MDS-RAEBt patient

in relapse (TRF=6.4kbp; 82% AH) compared to sample at the diagnosis (TRFdg=8.2kbp). In the case of JMML after alHSCT prolongation of TRF was detected (TRF=7.3kbp; 0% AH complete chimerism) in contrast to relapse where we found even shorter telomeres (TRF=4.8kbp; 84% AH) than at the diagnosis (TRFdg=5.8kbp). Patients with AML-M1 and M2 after transplantation showed gradual telomere shortening in agreement with increasing portion of autologous hematopoiesis.

Summary/conclusions: The status of mixed chimerism after alHSCT in PB/BM cells of patients with acute leukemia and MDS is connected also with dynamics of telomere length. Besides other markers, it might indicate a higher risk of disease relapse and serve as an unspecific prognostic factor and further insight into patients response to treatment that might be helpful for assessment of an optimization of treatment strategy.

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The study was supported by the grants of NC-7606-3 and CR23736001

767 TWO OF THE MORE COMMON FUSION GENES AML1/ETO AND PML/RARa IN A PATIENT WITH APL MORPHOLOGY

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Background: The chromosomal translocations t(8;21)(q22;q22) and t(15;17)(q22;q21) are most frequent karyotypic abnormalities in acute myeloid leukemia (AML) resulting in AML1/ETO and PML/PARa chimeric fusion genes. However, co-expression of both abnormal fusion genes has also been reported in a single patient.

Aim: To report an AML patient with both translocations t(8;21) and t(15;17). This 42-year-old male with anemia, thrombocytopenia, malaise, gingival bleeding, epistaxis and easy bruising was referred to our hematology section in August 2002.

Methods: Chromosome specimen was prepared by short-term culture of bone marrow cells, karyotype analyses by R-banding and fusion transcripts by reverse transcriptase-polymerase chain reaction (RT-PCR).

Results: Hematological studies showed a leukocyte count of 27x10⁹/L with 84% blasts in the peripheral blood. Hemoglobin was 10g/dl and platelet 112x10¹²/L. Bone marrow aspiration was hypercellular with 84% blast cells. Many Auer rods were observed in the blasts. Bone marrow karyotype was carried out on thirteen metaphase spreads and showed 48, XY, +8, t(8;21)(q22;q22), t(15;17)(q22;q21), +21 in 3 metaphases and 47,XY,t(8;21)(q22;q22), t(15;17)(q22;q21),+21 in 2 metaphases. Molecular analysis using RT-PCR was positive for AML1/ETO and PML/RARa (short isoform) chimeric genes. A diagnosis of AML-M3 was made based on the cell morphology.

The patient received As2O3 and achieved complete remission. After consolidation therapy with courses of As2O3, two steps RT-PCR was used on BM sample to detect the AML1/ETO and PML/RARa fusion transcripts. The PCR was negative for both transcripts fourteen months after the start of the therapy. The residual disease was below the detection limit of 1 in 104 cells for PML/RARa and 1 in 105 cells for AML1/ETO.

Conclusions: Our results indicate that long term remission can be achieved by As2O3 even in such complex cases. We suggest that AML with both t(8;21) and t(15;17) might be considered as a novel subtype of acute leukemias. drostamy@yahoo.com

768 HYPEREOSINOPHILIC SYNDROME (HES). ONE CENTER EXPERIENCE

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Introduction: The hypereosinophilic syndrome (HES) is an heterogeneous group of disorders characterized by the presence of unexplained eosinophilia, and defined by 3 clinical criteria: eosinophilia > 1500/ L for at least 6 months, absence of known causes of eosinophilia and presence of signs and symptoms of end-organ dysfunction due to eosinophilic infiltration and mediator release.

Patients: The current study population included 6 patients with HES that were evaluated at the 'Cl nica Universitaria de Navarra' between 1991 and 2003, in whom there was absence of an underlying cause of hypereosinophilia despite extensive diagnostic evaluation.

Results: There were 4 males (66%) and 2 females (33%), with a median age of 53 years (range 31-82). At diagnosis leucocytes (109/L) were 23,4 (range 7-35) and eosinophils 4,54 (range 1,3-16). Clinical features at diagnosis were arthromyalgia (50%), skin lesions (33%) and dyspnea (33%). Bone marrow aspiration disclosed eosinophilia > 15% in 5 patients. Cariotype was performed in 5 patients, being normal in 4 of them, whereas in 1 patient the t(9;22) p190 was found. TC was normal in 3 patients (50%), adenopathy, lung and liver infiltration was found in 2 patients (33%) and 1 patient (16%) presented splenic infarcts. Echocardiography was normal in 4 patients (66%), whereas in 2 patients (33%) mitral valve disease was demonstrated. One patient achieved a spontaneous remission without treatment. In the other 5 patients initial treatment was corticosteroids, achieving analytical and clinical improvement in all them, although the response was maintained in 3 of 5 patients (40%). One of the patients without maintained response suffered a disease progression and died from respiratory infection, and the other 2 patients initiated treatment with Hidroxiurea, without response, so they were subsequently treated with α Interferon, with initial good response in both cases. One of them discontinued the treatment because of adverse events, taking place a rapid clinical and analytical relapse. The other one relapsed after 3 months of combined therapy (Corticosteroids, Hidroxiurea and α Interferon), so the patient was then started with Imatinib (Glivec®) with transitory response, so an homologous stem cell transplantation was performed. Up to now, 6 months after the bone marrow transplantation, she is in complete remission.

Discussion: The HES is characterized by its great clinical and prognosis variability, ranging from paucisymptomatic patients who require no therapy and associate prolonged survival, to a rapidly fatal disease course in a short period of time. Response to initial treatment with corticosteroids uses to be good, although not always maintained, so an escalated therapy must be considered, including chemotherapeutic agents and, in selected cases, the performance of a stem cell transplantation.

769 THERAPY RELATED SECONDARY ACUTE MYELOID LEUKAEMIA AFTER TREATMENT FOR BREAST CANCER: AN INCREASING PROBLEM?

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Between January 2000 and December 2002, 4 cases of therapy related secondary acute myeloid leukaemia (sAML) following adjuvant chemotherapy for breast cancer presented to our department. The median age was 59 years. Two cases were FAB type M4 and two were FAB type M5. Chromosomal translocations involving 11q23 (MLL), associated with prior treatment with topoisomerase inhibitors (anthracyclines and etoposide), were found in 3/4 cases with normal cytogenetic seen in the remaining case (negative for 11q23 rearrangements by FISH). All 3 cases with 11q23 rearrangements had prior topoisomerase inhibitors (epirubicin in 2 cases and doxorubicin in 1 case). The remaining case had had treatment with both alkylating agents and topoisomerase inhibitors (epirubicin); however the likely causative agent was felt to be the alkylating agent regimen in view of the cytogenetics and a latency period of 3-4 years. As expected the prognosis of these patients was poor. Although complete remission was achieved in 3/4 patients (with 2 patients resistant to initial induction therapy), the median disease free survival was 6 months and median overall survival was 9 months; with only 1 patient alive and in remission at 16 months. Overall during this period we had 28 cases of newly diagnosed AML giving an annual incidence of 2.8/100,000 comparable to other published rates. sAML is said to account for approximately 5% of all AML; however during this period sAML accounted for 14% of cases of AML presenting to us. Based on the accepted latency periods of sAML (20-34 months following topoisomerase inhibitors and 36-48 months following alkylating agents) we looked back at the number of women between 1996 and 2000 who received adjuvant chemotherapy for breast cancer in our catchment area. Approximately 500 women received adjuvant chemotherapy, with the majority also receiving radiotherapy (which is also associated with an increased risk of AML and may be synergistic with chemotherapy). This gives an incidence of AML in this population of 0.8%, which is comparable to a number of recently published studies. Based on our annual incidence this is approximately 100 times expected. The oncologists in our area use(d) different anthracyclines, although generally 1/3 of patients received anthracycline based regimens. Epirubicin is thought to be more leukaemogenic in vitro and as stated 2/3 of the anthracycline related cases had had epirubicin. With the increasing use of adjuvant chemotherapy in the treatment of breast cancer we feel that sAML, together with its poor prognosis, is likely to become an increasing

problem, which may be exacerbated by the increased use of epirubicin. Since the beginning of 2002, within one oncology practice in our area >95% of chemotherapy regimens have been epirubicin based.

770 PRIMARY BONE LYMPHOMA: REPORT OF 13 CASES

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INTRODUCTION

Primary bone lymphoma (PBL) is a rare entity observed in only 4-5% of extranodal non-Hodgkin lymphomas. Predominant manifestations at presentation are pathological fracture, pain or the appearance of a soft tissue mass. Approximately, 60% of patients presented with stage I and 40% percent with stage IV. Histopathologically, PBL represents diffuse large B cell lymphoma.

PATIENTS AND RESULTS

We have reviewed the clinical manifestations, outcome and treatment of thirteen patients diagnosed with PBL between 1979 and 2004 in our center. Nine patients were male and five were female aged between 35 and 74 (median: 48). The inaugural symptom was pain (10 patients), pathological fracture (2 patients) and the onset of a soft tissue mass (1 patient). Disease localisation at diagnoses were femur (42%), vertebra (33%), pelvis (17%) and mandible (8%). None of them presented B symptoms. Fifty four percent of the patients presented with stage I and 46% with stage IV. Initially, two patients were just treated with local radiotherapy. The rest were treated with chemotherapy: CHOP (7 patients), BACOD-M-YCAV (2 patients), CVP (1 patient), and megaCHOP (1 patient), all of them followed by radiotherapy to the primary site. Consolidation therapy with autologous blood stem cell transplantation was performed in two patients. Ten patients out of 13 cases (77%) achieved a complete response (CR) and relapse was observed in three patients (30%). It is important to note that the two patients who were treated with radiotherapy alone relapsed, but achieved CR following the administration of chemotherapy.

Five patients died due to progression of disease or infectious complications.

Up to now, eight patients are alive with no evidence of disease (61%).

DISCUSSION

Nowadays, the optimal regimens for the treatment of PBL have not been firmly established. Anyway our observations and literature data indicate that the response rate and the disease-free survival is higher with the combination of radiotherapy and chemotherapy.

771 FLUDARABINE ADMINISTERED IN ORAL FORM IN COMBINATION WITH CHEMOTHERAPY/CHEMOIMMUNOTHERAPY - WELL TOLERATED OUTPATIENT REGIME IN TREATMENT OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND FOLLICULAR LYMPHOMA

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Background: Fludarabine in treatment of patients with B-cell chronic lymphocytic leukemia (B-CLL) and follicular lymphoma (FL) extends the interval of disease-free survival and may extend the overall survival.

Aim: We report our own experience with fludarabine administered in oral form in combination with chemotherapy or immunotherapy, which we administered to outpatients with B-CLL and FL.

Patients and methods: We started to use fludarabine in oral form in our department in 2002. The study group consisted of 21 patients with B-CLL and 5 patients with FL. We treated 22 patients with fludarabine in combination with cyclophosphamid (FluCFA) and 4 patients in combination with mitoxantron, 3 patients received the combined chemoimmunotherapy (FluCFA + rituximab). The median age of patients was 57 years (39-72), F/M ratio 7/19. 5 B-CLL patients were in clinical stage A, 10 in stage B and 6 patients in stage C according to the Binet staging system. Among the FL patients 2 patients were in stage III and 3 in stage IV according to the Ann Arbor system. 12 patients were not pre-treated, 14 patients were treated for relapsed or a refractory disease. Cytogenetic examination and FISH examination were performed in all patients with B-CLL. Fludarabine was administered orally in a dose of 40mg/M2/D for 3 days, CFA 150-300 mg/D for 3 days or mitoxantron 20 mg for 1 day, rituximab was administered in a dose of 375 mg/M2 4 doses. Treatment was repeated every 4 weeks, median number of administered cycles was 5 (interval, 1-6).

Results: Treatment response was achieved in 16 patients with B-CLL, overall response rate 84%, 2 patients had stable disease, disease progressed in 1 patient (all 3 of these patients had adverse cytogenetic changes), in 2 patients the effect of treatment has still not been evaluated. CR was achieved in all 5 patients with FL. Treatment of 3 patients with B-CLL had to be prematurely terminated, 1 due to progression of the disease, 2 for autoimmune disease as a complication of the treatment. 2 patients refused to continue with treatment, the treatment of 2 patients had to be terminated due to infectious complications (all 4 achieved treatment response). The main adverse effect of the treatment was myelosuppression, in 54% of patients prophylactic administration of G-CSF was necessary.

Conclusion: We see the advantage of the above-described regime as the possibility of out patient care, reduction of dependency on hospitalization, improved quality of life of patients, higher number of treatment response, good level of tolerance and small toxicity. Eventual infectious complications were not serious.

772 COMPARISON OF IN VITRO EFFECTS OF CYCLOSPORIN A (CYA) AND FK506 ON CORD BLOOD HEMATOPOIETIC CELLS

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Background: Umbilical cord blood (UCB) is a rich source of hematopoietic stem/progenitor cells (HSPC) and can be used in clinical settings for hematopoietic cell transplantation. One of the serious and challenging complications following hematopoietic cell transplantation is GVHD. Cyclosporin A and FK506 (Tacrolimus) are potent immunosuppressive agents widely used as prophylaxis against GVHD.

Aim: The aim of this study was to assess the in vitro effects of CyA and FK506 on cord blood hematopoietic cells.

Methods: Effects of immunosuppressants, CyA and FK506 on cord blood mononuclear cells were assessed following 7-day liquid culture in the presence of early acting growth factors such as SCF, FL, TPO and IL6 by flow cytometry, clonogenic assay and determination of Myeloid/Erythroid (M/E) ratios. Growth of hematopoietic progenitors was assessed using semisolid cultures in two stages (before and after 7-day liquid culture).

Results: Under the mentioned culture conditions, the expansion of CD34+ cells were stimulated by both agents in a dose-dependent manner, but there was no changes in the M/E ratios. Growth of the colonies stimulated only in the second stage of the clonogenic assay.

Conclusions: These findings demonstrate that CyA and FK506 both have stimulating effects on expansion and clonogenicity of cord blood mononuclear cells as well as being immunosuppressive agents. We suggest replacing of CyA with FK506 for GVHD prophylaxis due to the similar effects of these agents on clonogenicity and expansion of CD34+ cord blood mononuclear cells and low toxicity of FK506.

773 UNEXPLAINED PROLONGED PANCYTOPENIA IN PATIENTS IN REMISSION OF LYMPHOID MALIGNANCY POSSIBLY ASSOCIATED WITH HLA-DR15+

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Three patients with an unusual clinical course attracted our attention.

1. A 44 y old man with CLL underwent HSCT from HL-A-identical brother, and was treated for disease progression and decreasing chimerism with donor lymphocyte infusions (DLI) and anti CD52 mAb. Very severe transfusion-dependent pancytopenia and BM aplasia lasting >1 year ensued, during which chimerism and BM function gradually recovered, while CLL disappeared. As of now the patient has mild, non-transfusion dependent pancytopenia, full chimerism and no signs of CLL.

2. This 21 year old female was transplanted from a haploidentical donor in second chronic phase of CML post lymphatic blast crisis. She experienced graft failure, was treated with DLI and a second stem cell dose and has now prolonged unexplained pancytopenia in spite of increasing donor chimerism.

3. A 47 y old man diagnosed with mantle cell lymphoma and pancytopenia with BM aplasia was treated with CHOP and anti CD20 mAb. He remained severely pancytopenic in spite of disappearance of lymphoma. All three patients are HL-A DR 15+.

Despite some differences, these three patients with lymphoid malignancy have unexplained long lasting pancytopenia, not related to persistent disease. They are HLA DR15+. Interestingly, we found a higher than expected frequency of DR15+ not only in 45 AA patients, as expected

(44% versus 27% in controls (p=0.0025), but also in 18 CLL patients (44% versus 27% (p=0.05)).

We propose that the association between unexplained pancytopenia, hematopoietic tumor regression and HL-A DR15 is not random. Further study of this AA-like syndrome is warranted.

774 ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN ADULTS: LONG-TERM RESULTS OF TREATMENT BY THE MODIFIED GMALL-04/89

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Aim: analyzing the modified GMALL-04/89 regimen effectiveness in treating ALL adults.

Methods: the group under study was formed of 48 ALL patients (25 M., 23 F., median age-35yrs, range 15-68) treated between 01.92 and 12.99. Immunophenotype was available in 39 patients (81.25%): prepreB4, common20, preB5, T-10. Cytogenetics revealed: Ph+ in 5 patients of 17 with common-ALL (29.4%), t(4;11) in all prepreB-ALL cases. Standard risk (SR) patients 13 (27.1%), high risk (HR) 35 patients (72.9%). HR factors: L>30x109/l, age>35 yrs, t(9;22) or t(4;11), complete remission (CR)>4 weeks.

The therapy was administered according to the modified GMALL-04/89: Etoposide (120 mg/m2) was used instead of Teniposide in consolidation; Prednisolone instead of Dexamethasone in reinduction course; central nervous system prophylaxis (CNSP) was changed. From 01.92 to 05.96, CNSP included triple intrathecal therapy (TIT). From 06.96 to 12.99, CNSP was differentiated according to the risk of ALL relapse development. SR patients received the first prophylaxis variant (TIT). In HR patients cranial irradiation (CI) (24 Gy) was given after the II phase of induction (TIT+high-dose (HD) therapy+CI).

Consolidation in t(4;11) and t(9;22) patients: HD Cytarabine (1 g/m2 every 12 h, days 1-4) with Mitoxantrone (10 mg/m2 days 2-5), for other HR patients: HD Methotrexate and L-asparaginase (1,5 g/m2 and 10,000 units/m2 respectively, once a week N3).

Results: CR: 72.92% (35 patients); in the '<50 yrs' group 84.63% (33/39), in the '>=50' 22.22% (2/9). Among the SR patients, CR was achieved in 92.3% of cases (12/13), among the HR ones 65.7% (23/35).

4 patients (8.33%) did not respond to the therapy: 2 (5.12%) of them <50 yrs, 2 (22.22%) >=50. SR resistant patients-7.7%, HR ones-8.6%. Early death rate was 18.75% (9/48): '<50' 10.25% (4/39), '>=50' 55.6% (5/9). 2 patients in remission (5.7%) died during chemotherapy, both cases were Ph+. Relapse occurred in 22 patients (62.8%) within 270 months from remission. In HR group 93.2% of relapses (15/16) occurred within 3 years, in SR 42.9% (3/7); 57.1% (4/7) had relapse in 36 years of CR. No relapse occurred after 6 years of CR. CNS relapse was diagnosed in 4 patients (including 1 with initial CNS involvement) among the group receiving the I CNSP variant; CNS recurrence frequency - 11.4%. After the II CNSP regimen (with CI), no CNS relapse developed.

5-years disease-free survival and 5-years overall survival: the whole group 40% and 29.2%; '<50' 42.2% and 35.9%; '>=50' 0% for both; SR group 66.7% and 61.5%, HR '<50' 28.6% and 19.2%; prepreB-ALL 50% and 40%, T-ALL 57.1% and 40%. Remission (59140 months) continues in 10 patients. In HR common+preB groups all patients died in 3 years. In patients with L>30x109/l, only 3/22 had continuous CR>5 years (T-ALL-1, t(4;11)-2; the T-ALL patient relapsed after 5 years).

Conclusions: Long-term survival depends on risk factors. In SR patients relapses often occur after 3 years, in HR within 3 years. The modified GMALL-04/89 regimen is effective in treating ALL patients <50 years. In older patients it proved to be highly toxic.

775 FERROXIDASE ACTIVITY OF CERULOPLASMIN-LACTOFERRIN COMPLEX

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Ceruloplasmin (Cp; EC 1.16.3.1) - a 132-kDa multifunctional copper-containing protein of the blood plasma with ferroxidase activity. Cp has antioxidant properties as it oxidizes Fe2+ to Fe3+ that is accompanied by 4-electron transfer to oxygen. Aceruloplasminemia is an inherited disorder of iron metabolism caused by synthesis of truncated and inactive Cp, which results from mutations in the Cp gene. We first reported about interaction of Cp with lactoferrin (LF), major antibacterial 78-kDa protein of neutrophilic granules, milk and other body fluids. LF belongs to the transferrin (Tf) family and is able to bind metals ions (Fe3+, Cu2+), forming strong complexes of these ions and HCO3-. Cp and LF interact to form a complex (Cp:Lf=1:2) with a Kd=1.8 x 10-6 M (Zakharova et al.,

2000). Lf is able to scavenge the so-called 'pro-oxidant' copper of Cp released from the protein upon its proteolytic degradation (Pulina et al., 2004).

Aim. Study of ferroxidase activity of Cp and its complex with different LF forms.

Methods. Ferroxidase activity of Cp was evaluated by measuring the velocity of Fe²⁺ oxidation. The method is based on spectrophotometric measurement of colored complex of Fe²⁺ with ferrozine [Brown et al., 2002]. Velocity of the ferroxidase reaction for various amounts of the enzyme used in experiments was calculated from the decrease with time of Fe²⁺ measured spectrophotometrically ($\epsilon_{564}=27900 \text{ M}^{-1}\text{cm}^{-1}$). A mixture containing spontaneously oxidized iron and CP initially inhibited by sodium azide (0.1 μM) served as a control. Lf from human milk was purified according to Zakharova et al. (2000), stable Cp from human plasma according to Sokolov et al. (2004).

Results. Different forms of Lf increased ferroxidase activity of Cp in different way. A 1.5-fold and 3-fold increase was observed with iron- or copper-containing Lf (1:1-Cp:Lf and 1:2-Cp:Lf, respectively). A 1.1-fold and 2.2 increase with apo-Lf (1:1-Cp:Lf and 1:2-Cp:Lf, respectively). Further increase of molar stoichiometry (1:2) did not increase Cp activity. Different forms of Tf, the Fe³⁺-binding plasma protein, did not increase ferroxidase activity of Cp.

Conclusions. Different forms of Lf increase ferroxidase activity of Cp, but other Tfs are unable either to interact with Cp or to increase its activity. Cuprous and ferrous ions are growth-limiting for pathogenic microorganisms and, hence, should be tightly bound by plasma proteins in inflammation foci. Unlike Tf, Lf is able to bind these ions under acidic pH that is typical for inflammation sites. Besides, incorporation of Fe³⁺ into Lf is realized when the latter stimulates Fe²⁺ oxidation by Cp.

776 NON-STABILITY OF CERULOPLASMIN IN PATIENTS WITH HEMOPHILIA A

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Ceruloplasmin (Cp) - a 132-kDa multifunctional copper-containing protein of the blood plasma with ferroxidase activity. It affects iron metabolism and behaves as antioxidant through the oxidation of Fe²⁺ to Fe³⁺. Aceruloplasminemia is an inherited disorder of iron metabolism caused by synthesis of truncated and inactive Cp, which results from mutations in the Cp gene. It is manifested by iron accumulation that provokes free-radical reactions in brain and other organs. Coagulation factors Va and VIIIa contain A domain that is highly homologous to Cp. Therefore, the latter can compete with F Va and F VIIIa for binding to protein C, which reduces their inactivation.

Aim. To study Cp in case of hemophilia A (HA) i.e. deficiency of factor VIII.

Materials and methods. Cp in sera of 14 patients with diagnosis of HA was analyzed with respect to its content and degradation. 11 patients were young and suffered from frequent hemorrhages and 3 were elderly patients with degenerative processes and accompanying chronic diseases. Activity of F VIII in all patients was less than 1 %. HA was diagnosed on the basis of clinical symptoms and laboratory tests. The control group was composed of 25 donors.

The Cp from donors' and patients' sera was isolated by affinity chromatography on Protamine-Sepharose [Sokolov et al., 2004]. Western blotting [Anderson et al., 1982] confirmed proteolytic degradation of Cp previously revealed by SDS-PAGE [Laemmli, 1970]. Cp content was determined in reaction of p-phenylenediamine oxidation [Ravin 1961]. Total proteolytic activity (TPA) of sera from donors and patients was determined as the function of tyrosine yield from cleaved casein (mM/hour).

Results. Concentration of Cp in sera of patients without hemorrhage was $30 \pm 1 \text{ mg\%}$ while those with hemorrhage had $25 \pm 1 \text{ mg\%}$. In sera of donors mean Cp concentration is $45 \text{ mg\%}$. All patients had two-fold increase of TPA as compared with donors (0.18 ± 0.02 and $0.09 \pm 0.01 \text{ mM tyrosine/hour}$, respectively). In case of HA Western blotting confirmed the peculiar degradation of Cp. In addition to 3 fragments with Mr 132, 110 and 19 kDa found in donors Cp, protein of the patients had fragments with Mr 67, 52, 28 and 25 kDa. Increased proteolytic fragmentation of Cp may result from higher activity of endogenous proteases in case of HA. One cannot exclude that storage at -700C is more damaging for Cp from patients than for donors Cp. Anyhow, Cp of the patients with HA is less stable, than donors Cp.

Conclusions. 1. In patients with HA a two-fold increase of TPA is observed.

2. Concentration of Cp in patients with severe HA on the average is 2 times lower than in healthy donors.

3. Western blotting evidenced that Cp of patients with HA is more proteolyzed than Cp of the donors.

777 QUALITY OF LIFE IS A KEY POINT OF DECISION MAKING PARADIGM IN HEMATOONCOLOGY

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During the last decades health related quality of life (QoL) of patients is considered as an important outcome of cancer treatment. However, in everyday practice physicians rarely consider QoL in decision making.

The present work aimed to develop the new paradigm of decision making with QoL as a key point in treatment of hematologic malignancies.

Decision making paradigm in hematooncology consists of three major milestones: choice of the strategy of treatment, identification of treatment methods and determination of treatment outcomes.

The paradigm is based on the principle of balance of treatment strategies. Treatment strategies are determined by the treatment goals.

I. These goals are as follows:

1.To cure and maintain QoL after curation

2.To improve survival and maintain QoL

3.To improve quality of life

4.To test new methods of treatment or medications.

II. Treatment approach is chosen depending on treatment goal (s). The goal and the approach are specified by the type and stage of hematologic malignancy.

-At the initial stages the first two goals dominate and the approach to be chosen is curative treatment.

-At the advanced stages combination of treatment goals and approaches takes place and in doing so the importance of QoL issues increases.

- For terminally ill patients the major goal is to support QoL and in this case palliative treatment is the dominating approach.

It is worth mentioning that palliative care of cancer patients is to be started from the early stages with its proportion increasing with the disease progression. It is important to emphasize that psychological, spiritual and social support of family members from the initial stages of the disease and after the patient's death is an inalienable paradigm component.

Taking into account cancer treatment goals it is seen that at any stage of the disease QoL of a patient is among the key issues to be analyzed and in doing so QoL is to be measured.

In conclusion, the paradigm gives grounds for the unified approach to treatment of hematologic malignancies. It is of value in identifying end-points and outcomes in clinical trials and might be used by physicians in their routine practice.

778 SEVERE BLEEDING TENDENCY CAUSED BY AN ACQUIRED INHIBITOR OF FACTOR XIII

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Factor XIII inhibitors are exceedingly rare with only 20 previously reported cases. They prevent factor XIII mediated fibrin cross-linking leading to increased clot fragility (1). This functional defect may cause a clinically severe bleeding diathesis with life threatening haemorrhage.

We report a case of unusual bleeding in a 69 year old male caused by an acquired inhibitor to factor XIII. He had no prior history of bleeding diatheses.

He presented with marked haematuria. Physical examination revealed painless extensive bruising on his lower back and left thigh. A fall of 4 g/dl in Haemoglobin occurred in the first day of his admission.

Extensive investigation of the intrinsic pathway revealed normal studies. Factor XIII was measured by clot solubility test in 5% monochloroacetic acid to demonstrate fibrin cross-linking. A photometric determination using Dade Behring Berichrom on the Sigma Amax 400 revealed levels of $<0.13 \text{ IU/ml}$

Clinically his bleeding episode resolved spontaneously. However he again presented a month later to another hospital with a retroperitoneal haemorrhage. Again his routine coagulation tests were normal, however

mixing studies revealed an inhibitor at 1:1 dilution. This has been consistent throughout medical management.

Treatment with oral cyclophosphamide, plasma and steroids has been clinically successful and no further bleeding has occurred.

1. Factor XIII: Inherited and Acquired deficiency. P.G. Board, M.S. Losowsky. K.J.A. Miloszewski. Blood Reviews (1993) 7, 229-242

779 LONG-TERM SURVIVALS IN ACUTE PROMYELOCYTIC LEUKEMIA (APL); THE EXPERIENCE OF THE CLINIC OF HAEMATOLOGY FUNDENI ON THE PERIOD 1986-2003.

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The aim of the paper is to reveal and analyze the clinical, cytological and therapeutical characteristics of APL cases with long survival. Out of 187 APL patients, in 31 cases (17%) a long-term survival (40 – 207 months) has been recorded. The patients age varied between 16-60 years (y) with a median of 30y; 21 were females and 10 males. At the admission when the diagnosis was established, the evaluation of peripheral blood showed the following median values: 2.500 leukocytes/ μ L (range 500-30.000), platelets 20.000/ μ L (range 10.000-107.000), blast cells 1.000/ μ L (range 0-12.300). The cytological examination of peripheral blood and of bone-marrow detected a macrogranular (M3) form in 29 patients and a microgranular variant (M3v) in 2 cases. The median value of the fibrinogen was 220 mg/dL (range 60-445 mg/dL). Nine of 31 patients (29%) belonged to the low risk group ($L \leq 10.000/\mu$ L, $Plt > 40.000/\mu$ L), 19 (61%) fell into the intermediate-risk group ($L \leq 10.000/\mu$ L, $Plt \leq 40.000/\mu$ L) and only 3 (10%) into the high-risk group ($L > 10.000/\mu$ L). In 90% (28/31 cases) the induction consisted of ATRA plus chemotherapy (CT)(DNR) very early introduced; in 10% (3/31) only CT was administered as induction (DNRARA-C). The consolidation was performed with DNR-Ara-C and maintenance therapy with 6MP plus MTX with intermittent administration of ATRA. The median survival was 75 months (mo) (40-206 mo). ATRA plus chemotherapy induce the greatest percentage (> 90%) of complete remissions (CR) in APL, as it was proved in our own experience as well as in the literature. Chemotherapy added very early to ATRA at the induction and the maintenance program with 'low-dose' continuous chemotherapy and intermittent ATRA may reduce the incidence of relapses and consequently may prolonge the survival.

780 THE EFFECT OF RALOXIFEN UPON ENDOTHELIAL, OXIDATION AND COAGULATION CIRCULATING MARKERS IN HEALTHY POSTMENOPAUSAL WOMEN.

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Introduction: Raloxifen, a selective estrogen receptor modulator, has been proposed as a viable alternative to hormone replacement therapy for the prevention of atherosclerosis although its effect on vascular wall has not been yet fully explored.

In this study we examined the effect of raloxifen upon a wide spectrum of lipid, endothelial and coagulation parameters in a population of healthy postmenopausal women

Methods: Fifty postmenopausal women, free of any chronic disease, were included in this study, which was designed as a randomized double blind clinical trial. All participants were randomly assigned to receive either raloxifen, 60mg/day per os (raloxifen group, 25 women) or bisphosphonates, 10mg/day (control group, 25 women).

Both groups were followed for 3 months. The following parameters were measured:

-Lipid parameters: Total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL), LDL-cholesterol (LDL), Apolipoprotein A-I (ApoA), Apolipoprotein B-100 (ApoB), Lipoprotein (a) (Lpa).

- Soluble endothelial markers: Tissue plasminogen activator (t-PA), Plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWF), Thrombomodulin (TM), intercellular adhesion molecule-1 (ICAM), E-Selectine (SEL).

- Lipid peroxidation markers: Oxidized LDL-cholesterol and autoantibodies against Cu++- oxidized LDL-cholesterol

- Coagulation markers : Factor VII and Factor VIII.

In addition, flow-mediated (FMD) vasodilation of the right brachial artery was measured at baseline and at the end of follow up.

Results: No changes were found in the control group during the follow up. However, raloxifen administration was associated with an improvement of the lipid profile (TC: 251 $\pm$ 56.4mg/dl before vs 233.4 $\pm$ 52.1 mg/dl after raloxifen, $p=0.006$, LDL: 172.4 $\pm$ 48 mg/dl before vs 152.6 $\pm$ 42.4 mg/dl after, $p=0.004$, ApoA: 159.7 $\pm$ 26 mg/dl before vs 178.1 $\pm$ 25.9 mg/dl after, $p=0.012$, ApoB: 111.3 $\pm$ 29.1mg/dl before vs 106.8 $\pm$ 25.9 after, $p=0.047$ and Lpa: 12.3 $\pm$ 13.2mg/dl before vs 6.4 $\pm$ 5.3 after, $p=0.044$) and a statistically significant decrease of selectine levels (48.7 $\pm$ 38.9 mg/dl before vs 35.5 $\pm$ 25.5 mg/dl after treatment, $p=0.033$). In addition, an increase in factor VIII levels was also noted in the raloxifen group (105.7 $\pm$ 49.9 % before vs. 126.2 $\pm$ 36.4 % after treatment, $p=0.043$). Furthermore, FMD and other endothelial and oxidative markers such as vWF, TM and oxidized LDL remained unchanged.

Conclusions: Raloxifen, despite its favorable effect on the lipid profile and on some endothelial markers, does not improve flow-mediated vasodilation. Moreover, it may increase thrombogenicity and thus we should remain sceptical about its potential use for the prevention of atherosclerosis.

781 TRISOMY 8 IN MYELOYDYSPLASTIC SYNDROMES: IMPACT ON CLINICAL PRESENTATION AND COURSE OF THE DISEASE (A SINGLE CENTRE EXPERIENCE)

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Background: Trisomy for chromosome 8 is one of the most common chromosome abnormalities detected in myeloid disorders. However, and despite its great incidence, its biologic significance remains poorly understood.

Aims: To study the role of trisomy 8 in pathogenesis, clinical presentation and progression of myelodysplastic syndromes.

Results: At presentation, 109 of 271 successfully karyotyped patients (40.2%) had abnormal karyotypes, with the aberrations of chromosomes 5, 8 and 7 as the most frequent. Ten patients had trisomy 8 as the sole aberration, whilst additional three cases had trisomy 8 included in complex karyotypes (≥ 3 chromosomes). Cases with constitutional trisomy 8 mosaicism (CT8M) were excluded using chromosome analyses of PHA-stimulated blood cultures. According to FAB classification, four pts had RA, three pts had RAEB, and one patient each had RARS, RAEB-t and CMML. All pts with trisomy 8 as the sole aberration have been scored into 'intermediate' IPSS risk group. During extended cytogenetic follow-up, trisomy 8 was not recorded in evolving karyotypes. In contrast, in two cases trisomy 8 disappeared during sequential cytogenetic studies, 23 and 72 months from diagnosis, in one patient accompanied with complete hematological remission. No differences regarding age, sex, cytopenia, blood and marrow blast count or response to treatment was found between patients with trisomy 8 as the sole aberration compared to those with normal cytogenetics. Median actuarial survival of patients with trisomy 8 as the sole aberration was 27 months, as compared to 32 months in patients with normal cytogenetics ($p=0.468$).

Summary: Disappearance of metaphase cells with trisomy 8, particularly when associated with hematological improvement, indicate that trisomy 8 in two our MDS patients was possibly not the primary event in the genesis of the disease, and that there may have been competition between a normal karyotype clone and a trisomy 8-positive clone. Other our results suggest that the presence of a clone with trisomy 8 is not always a sign of disease progression or of poor prognosis in MDS patients.

782 FACTORS AFFECTING THERAPEUTIC RESPONSE AND SURVIVAL IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Acute lymphoblastic leukemia (ALL) represents 20% of cases of acute leukemia in patients aged over 15 years. The first aim of ALL treatment is to eradicate the malignant cell population as effectively as possible without inducing major therapy-related toxicity, to ensure a long disease free survival.

Aims. Assessment for evolution and therapeutic results and identifying the prognostic factors which can influence therapeutic response, time to relapse and survival in patients with ALL diagnosed and treated in Department of Hematology Iasi.

Patients and methods. The study (a retrospective one) comprised initially 86 patients with ALL diagnosed in our department between 1997-2002. The group comprised 46 males and 40 females, with median age 49 years (range 16-84 years). We analysed the clinical, hematological and biochemical parameters of all patients. Sequential chemotherapy with cyclophosphamide, adriablastin, vincristine and cortisone doses was administered as induction therapy. After that, treatment was continued according to the response. Twenty-six patients died at the onset or during the induction therapy and they were removed from the final group. This one comprised 28 males and 32 females with median age 38 years (16-78).

Results. Complete remission (CR) was achieved in 25 patients (42%), partial remission (PR) in 18 patients (30%), 17 patients (28%) were resistant. Splenomegaly ($p=0.020$), leucocytosis over 10.000/mm³ ($p=0.077$), over 50% blast cells in peripheral blood ($p=0.022$), and thrombocytopenia under 50.000/mm³ influenced negatively the response. Reinduction treatment was administered in 31 patients, achieving 12 CR and 8 PR. Twenty-eight of 37 patients who achieved CR after induction and reinduction therapy have relapsed in bone marrow, CNS or both. For these patients a new treatment was initiated with achievement of 7 CR and 11 PR. Median overall survival for entire cohort was of 11 months (range 2 and 72 months), 18 months for those who achieved CR and 8 months only for those without CR. Overall survival was 57.5% at 12 months and 35% at 24 months. Age over 40 years ($p=0.0019$), leucocytosis over 10.000/mm³ ($p=0.0689$), and especially, the lack of CR ($p=0.0000$) have influenced negatively survival. Thirty-six patients (60%) died. The first causes of death were infectious and hemorrhages/blast involvement of CNS.

Conclusions. ALL remains a very serious disease and health problem with very increased mortality, especially, the early mortality. Avoiding early morbidity and mortality and achieving a persistent CR after induction and consolidation therapy remain essential elements for long disease free survival and overall survival.

783 5Q- IN MYELODYSPLASIA - THE ISRAELI EXPERIENCE

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Myelodysplastic syndrome (MDS), a heterogenous group of hematopoietic neoplasm associated with mono-, bi-, or pancytopenia, is a clonal stem cell disorder. Del(5q) is a frequent karyotypic abnormality within MDS and occurs in 10-15% of patients, as an isolated abnormality or in a complex pattern. MDS accompanied by an isolated del(5q), as first described in 1974 and 1975 by Van den Berghe and Sokal, is a separate entity: '5q- syndrome', as we find it in the new WHO classification of MDS. It is characterized by hypoblobulated megakaryocytic hyperplasia, micromegakaryocytes, erythroid hypocellularity, macrocytic anemia, normal to elevated platelet count, normal to moderately lowered leukocyte count. According to the literature, this entity is associated with a better prognosis, although this issue has not always been agreed upon.

Data of 39 patients, from 8 medical Israeli centers, with MDS and del(5q), as a single (20 patients) or in a complex with additional chromosomal abnormalities (19 patients), were analyzed. We tried to answer the next questions (using the group of isolated 5q- vs the group of MDS with additional chromosomal abnormalities): median age at diagnosis, female:male ratio, ethnic origin of the patients, CBC at diagnosis, cellularity and the amount of dysplasia in bone marrow different cell lines, blood transfusion requirements and prognosis. We found, except for the consonant features with those described in other studies, in our series of isolated 5q- MDS a female preponderance (female:male 4:1). The great majority was Ashkenazi patients and a minority was Sefaradi; no Arab or Druze patients were observed. The median survival was significantly shorter, 51 months, than the median survival described in certain studies, but similar with those found in other series of patients. The great majority of patients were blood-transfusion dependent, not at diagnosis, but after a median period of 16 months. The percentage of disease-related death was higher in our patients, 50%.

In the second group, those with 5q- among other chromosomal abnormalities, the female:male ratio was 1:1, the Ashkenazi patients still predominates, but Arab patients were also reported. The percentage of blood-transfusion dependent patients was greater in this group, as the disease-related death.

784 CLINICAL SIGNIFICANCE OF IMMUNOPHENOTYPING OF MYELOID CELLS IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background: Knowledge of the blast phenotype in myelodysplastic syndromes (MDS) would be valuable, as in other malignancies, but remains sparse.

Aims: To explore the immunophenotype of myeloid cells in adult primary myelodysplastic syndromes (MDS) and its clinical implications, particularly implications on prognosis.

Methods: The bone marrow mononuclear cells (MNC) from 39 adult MDS patients were isolated at presentation, and labeled using a panel of monoclonal antibodies. Immunophenotyping and flow cytometry methods were used to establish expression levels of membrane Ags on bone marrow cell populations (expressed as % of labeled cells in myeloid compartment gate). Survival of patients and risk for leukemic transformation was plotted according to the Kaplan-Meier method.

Results: A distinct finding in MDS pts was significantly higher expression levels of CD13, CD33, CD38, CD14, and CD15 Ags on myeloid compartment bone marrow cells in correlation to findings in healthy control group. CD34 Ag expression was in a positive linear correlation to HLA-DR Ag, and in negative correlation to CD11b and CD15 Ags. Myeloid ratio (HLA-DR/CD11b) was significantly higher in patients with MDS in comparison with control group, particularly in pts with advanced disease. However, the type of CFU-GM colony growth and the presence of chromosomal aberrations were unrelated to the CD34 antigen overexpression and elevated myeloid ratio. Median survival of patients with CD34 expression was 7 months, in comparison with 31 months for patients without CD34 overexpression ($p=0.0375$). Pts with CD34 overexpression also had significantly higher leukemic transformation rate ($p=0.046$). Median survival of patients with elevated myeloid ratio was 7 months, in comparison with 33 months for patients with myeloid ratio within normal range ($p=0.015$). Pts with elevated myeloid ratio also had significantly higher leukemic transformation rate ($p=0.041$).

Summary: Detection of immunophenotype of myeloid cells in MDS cells clearly contribute to the diagnosis and predicting prognosis. Particular importance had the presence of CD34 antigen overexpression and elevated myeloid ratio.

785 CLINICAL RESISTANCE TO IMATINIB (STI571) AND DETECTION OF BCR-ABL GENE MUTATIONS IN KOREAN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Imatinib mesylate (Gleevec, STI-571), targeting the tyrosine kinase activity of BCR-ABL oncogenic fusion gene which is responsible for the induction of chronic myeloid leukemia (CML), inhibits the growth of cells expressing BCR-ABL in vitro and induces remarkable hematologic and cytogenetic remission in clinical trial. But, imatinib-resistance has been observed in a significant proportion of CML patients. Among the proposed several mechanisms in vitro, the point mutations of BCR-ABL catalytic domain has been clinically identified and verified by many additional reports. We evaluated the clinical resistance of imatinib in Korean CML patients and searched the point mutation of BCR-ABL gene as possible mechanism in clinically resistant patients.

Method: For the presence of imatinib resistance we evaluated the clinical data and cytogenetic studies in 32 CML patients treated imatinib during at least 3 months, who were requested for the bone marrow and chromosome analyses from Jan 2002 to Aug 2003. Mutational analyses for the point mutation of BCR-ABL kinase domain in hematologic and cytogenetic resistant patients were tested using RT-PCR and direct sequencing method.

Results: All chronic phase CML patients were induced complete hematologic remission, but 2 patients relapsed to blastic crisis during continuing treatment. Major cytogenetic response was observed in 67% of chronic phase patients. In 2 patients among the patients with complete cytogenetic response, Philadelphia chromosomes reemerged in follow-up chromosome study. Four of 6 accelerated phase or blastic

crisis patients were induced complete hematologic remission lasting 4 weeks, but, 2 among these patients relapsed. All accelerated and blastic phase patients requested for cytogenetic analyses had no cytogenetic response. Mutational analyses for 11 patients with hematologic and cytogenetic resistance showed the point mutations in 2 patients. One hematologic relapsed patient had the substitution of 351st amino acid of BCR-ABL kinase domain, methionine to threonine (M351T), and the other cytogenetic relapsed patient had the substitution of methionine into leucine (M351L).

Conclusion: The frequency of imatinib resistance in Korea is similar to that of well-controlled western studies. Point mutations of BCR-ABL kinase domain were detected in two patients. M351T substitution was reported in many reports as the cause of imatinib resistance, and our result shows the same conclusion. In contrast with M351T, a novel mutation (M351L) is thought to have controversial clinical impact on hematologic relapse in the consideration of the several comparative functional studies, but, additional functional study and clinical observation are needed. Based on these result, more sensitive and extensive study for the imatinib-resistant patients will be helpful to reveal the another mechanism of imatinib resistance and to establish more effective treatment plan.

786 STUDY OF SOLUBLE VASCULAR CELL ADHESION MOLECULE ONE (SVCAM-1) AND VON WILLEBRAND FACTOR (VWF) IN CHILDREN WITH BETA THALASSEMIA MAJOR.

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Background: There is a strong evidence of endothelial cell activation in beta thalassemia and vascular complications in these patients are more frequent. Objective: To study the levels of circulating soluble vascular cell adhesion molecule (sVCAM-1) and Von Willebrand factor (vWF) as parameters of endothelial cell activation in a group of children with B-thalassaemia major, and their relation to duration of the disease. Subjects: the study was conducted on 35 patients with B-thalassaemia major, recruited from the hematology outpatient clinic of Alexandria University Children's Hospital, who were divided into two groups. Group I included 15 young children with a disease duration ≤ 5 years and group II included 20 older children and adolescents with disease duration > 5 years. Ten apparently healthy children of matching age and sex served as control group. Methods: Full clinical examination with special emphasis on signs of vascular affection. Estimation of serum levels of: sVCAM-1 by enzyme-linked immunosorbent assay (ELISA), vWF by radial immunodiffusion. Results: Four of the studied patients had pulmonary hypertension and one patient suffered from left lower limb deep vein thrombosis. Soluble vascular cell adhesion molecule-1 results demonstrated significantly higher levels in both thalassaemic groups in comparison with controls and non-significant difference between the two groups themselves. A positive correlation was found between sVCAM-1 levels and serum ferritin in both groups, and with vWF in group II only ($P=0.007$, 0.042 and 0.031 respectively). There was non-significant difference between the levels of vWF in both thalassaemic groups and controls ($P=0.093$). There was no statistical significant difference as regards sVCAM-1 and vWF levels in non-splenectomized and splenectomized children in both groups ($P=0.545$, $P=0.219$). Conclusion: Measurement of sVCAM-1 might be a useful marker for the follow up of vascular disease in transfusion dependant thalassaemic patients.

787 COTRANSPLANTATION OF HEMATOPOIETIC STEM CELLS AND CULTURE-EXPANDED MESENCHYMAL STEM CELLS IN SCID MICE

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Background : Hemopoiesis is sustained by two main cellular components, the hematopoietic stem cells (HSC) and the mesenchymal stem cells (MSC). MSC are multipotent and are the precursors for marrow stroma. Aims : Animal studies have indicated that MSC can engraft in bone marrow. In this study, we hypothesized that cotransplantation of HSC and MSC would facilitate the engraftment of HSC.

Methods : Mononuclear cells from the various sources were seeded in long-term culture for ex vivo MSC isolation and expansion. The MSC purity was assessed by flow cytometry. A total of 1×10^7 MSC plus HSC/uL were injected to tail vein of SCID mice. After 3-4 weeks later, homing and engraftment of human cells were determined by flow cytometry and fluorescence in situ hybridization studies.

Results : The engraftment of CD45+/CD34+ cells significantly increased in cotransplanted mice. The number of human cells counted by XX or XY fluorescence signals were also higher in case of cotransplantation.

Summary : The results presented here suggest that MSC are capable of enhancing the hematopoietic engraftment. Now we are planning to transfer genes associated with stem cell homing, e.g., CXCR4, TPO, GM-CSF into ex vivo expanded MSC and then do cotransplantation with HSC.

788 LYMPHOPROLIFERATIVE DISORDERS AND OTHER TUMORS COMPLICATING IMMUNODEFICIENCIES

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Background. Lymphoproliferative disorders and selected carcinomas occur as severe, lifethreatening complications of primary and secondary immunodeficiencies (ID) with an incidence of 1-25 %.

Aims. The aim of this retrospective study was to establish the frequency and cyto-histopathologic features of malignancies complicating primary as well as acquired ID during childhood.

Methods. The analysis was performed on 629 subjects, divided in two cohorts: group A consisting of 57 patients with primary ID and group B counting 572 patients with secondary ID (AIDS). As inclusion criteria we considered the age of the patients below 18 years and the diagnosis of ID prior to the diagnosis of malignancy in order to exclude ID secondary to cancer or its therapy.

Results. In group A the frequency of malignancy was 10.52 % (6/57), occurring at a mean age of 5.7 years. It consisted of: non-Hodgkins lymphoma (4 cases - 7.01 %); acute nonlymphoblastic leukemia (1 case - 1.75 %) and erythrophagocytic hystiocytosis (1 case - 1.75 %). Patients who developed malignancy belonged 1 case to each of following ID: ataxia teleangiectasia, Nijmegen syndrome, common variable immunodeficiency (CVID), IgA deficiency, Bruton agammaglobulinemia and Kostmann agranulocytosis. The mortality rate due to malignancy was 66.66 % in group A. In group B the frequency of malignancy was 4.37 % (25/572), occurring at a mean age of 9.7 years. It was dominated by malignant lymphoproliferation (16 patients with NHL and 1 patient with ALL) and Kaposi sarcoma (6 cases); beside them, 1 case of soft tissue sarcoma and 1 case of malignant hystiocytosis were also identified. The mortality rate in this group was 84%.

Conclusions. Historically, the succes of treatment of lymphoproliferative disorders and other types of cancer in immunodeficiencies with conventional multiagent chemotherapies and/or radiation therapy has been limited by unfavorable tumor response rate and high morbidity and mortality related to opportunistic infection. Unfortunately this is reflected also by our experience. Maybe with improvements in supportive care and the use of recombinant biologic response modifiers survival of immunodeficient hosts following tumor diagnosis will improve.

789 FLOW CYTOMETRIC EVALUATION OF MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA PATIENTS USING CD19, CD56, CD38, AND CD138

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We have examined the co-expression of CD19, CD38, CD56, and CD138 molecules in plasma cells of bone marrow aspirates and their relation with bone marrow infiltration, M-protein, LDH and treatment in patients with multiple myeloma by flow cytometry. Twenty-nine bone marrow aspirate (BM) samples were assessed from 29 patients, mean age 56.83 $\pm$ 9.1, male : female ratio 2.6, at diagnosis and on follow-up. They were recieved at least 4 courses of VAD-like regimens and 11 of them were also treated with high dose melphalan and PBCT (peripheral autologous stem cell transplantation). The median follow-up period was 19.1 $\pm$ 22.7 months. According to light microscopy the bone marrow smears from patients on follow up were classified into one of three categories: complete remission(CR) ($< 5\%$), partial remission(PR) ($> 5\%$ and $< 30\%$), and wide infiltration(WI) ($> 30\%$). The flow cytometric analysis revealed that only the mean value of CD38 expression was significant. We correlated infiltration ratio with each parametries and only M-protein relation was found statistically significant ($p=0.01$). We also correlated independent variables with each other and found a relation between CD138 and M-protein ($p=0.01$), CD138 and LDH ($p=0.00$), M-protein and LDH ($p=0.00$), CD38 and CD19 ($p=0.005$). We also defined the groups as

treated with peripheral autologous transplantation or not and compared the independent variables between them in which CD138 was statistically significant ($p=0.02$) respectively. We suggest bone marrow plasma cells expressed mainly by CD138, and M-protein value with CD138 expression may be considered in follow up for minimal residual disease in myeloma patients.

790 ABNORMAL CHROMATIN CLUMPING SYNDROME IN LEUCOCYTES INDUCED BY MYCOPHENOLATE MOFETIL

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Background

Abnormal chromatin clumping (ACC) is a sign of severe dysplasia described in the abnormal chromatin clumping syndrome by Gustke (1). This entity can be observed in myelodysplastic syndromes, myeloproliferative syndromes, acute myeloid leukemias and reactive dyshemopoiesis. It is characterized by nuclear abnormalities in mature neutrophil granulocytes as a loss of segmentation and an abnormal clumping of nuclear chromatin. But this syndrome can be also acquired during treatment with mycophenolate mofetil (mmf) and disappears after its reduction or discontinuation. Eleven cases have been published, ten in adults and one pediatric which is the only one to have occurred after bone marrow transplantation. To our knowledge, we report the first adult case of ACC under mmf therapy in a bone marrow transplant patient.

Methods / Results

We report a case of splenic marginal zone lymphoma (World Health Organization classification) with mini allo-graft. This patient was treated with immunosuppressive drugs against Graft Versus Host Disease (GVHD). Ciclosporine was administrated and three months later replaced by mmf (Cellcept®) because of gastric GVHD. Three months and three weeks later the first administration of mmf (2g/day), blood smear analysis and bone marrow examination revealed an ACC in granulocytes and a few neutrophils showed nuclear loss of segmentation (pseudo-Pelger abnormality) and Dhle bodies. The bone marrow karyotype study was normal. With a follow-up of six months, the patient remains asymptomatic.

Conclusion

ACC in granulocytes of patients under mmf therapy is a rare adverse event. The mechanism of appearance of ACC remains to be defined : it could be a direct hematological toxicity or an inhibition of guanosine nucleoside synthesis. Actually, it seems that ACC is a secondary reversible effect of mmf without any clinical symptoms. So when an ACC is noted in a similar context it appears to be necessary to follow-up the patient to confirm the disappearance of this anomaly.

(1) GUSTKE, S.S., BECKER, G.A., GARANCIS, J.C., GEIMER, N.F., PISCIOTTA, A.V. (1970) Chromatin clumping in mature leucocytes : a hitherto unrecognized abnormality. Blood, 35, 637-658.

791 EFFECTS OF RITUXIMAB ASSOCIATED TO FLUDARABINE AND CYCLOPHOSPHAMIDE ON HEMATOPOIETIC STEM CELL (HSC) MOBILIZATION IN PATIENTS WITH FOLLICULAR NON HODGKIN'S LYMPHOMA (NHL)

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Introduction: The combination of rituximab with fludarabine and cyclophosphamide is effective for the treatment of follicular NHL. Adverse events related to the antibody or fludarabine could be delayed neutropenia and absence of HSC mobilization.

Patients and methods: We studied 21 patients aged 33-59 years. Nine of them received six courses of FND (fludarabine, 25 mg/m² x 3 days, mitoxantrone 10 mg/m² x 1 day, dexamethasone 20 mg/day x 5 days) and rituximab (375 mg/m²). After induction therapy, lfo-Vp16 (ifosfamide 3.3 g/m² x 3 days and etoposide 150 mg/m²/12 h x 3 days) plus G-CSF 5 mcg/kg/day were used for mobilization. Twelve patients received six courses of FluCy (fludarabine 25 mg/m² x 3 days and cyclophosphamide 1000 mg/m²) plus rituximab as induction therapy; G-CSF 10 mcg/kg/d x 4 days was used for mobilization.

Results: Higher levels of CD34⁺ cells were obtained in patients receiving lfo-VP16 with respect to patients mobilized with G-CSF alone. Differences were also significant for the number of leukocytes previous to the apheresis ($p < 0.005$). In the group mobilized with G-CSF alone, only three patients achieved enough cells for autologous transplantation, whereas

five obtained less than 0,7 x10⁶/kg CD34⁺ cells (in 1-2 apheresis). These six patients had prolonged neutropenia, lymphocytopenia and hypogammaglobulinemia. Mobilization was not possible in four additional patients four months after the end of therapy; they also had prolonged neutropenia, lymphocytopenia and hypogammaglobulinemia.

Conclusion: Although the combination of FluCy and rituximab is effective in patients with follicular NHL, HSC mobilization is very difficult and will be associate to prolonged neutropenia. The reason for that could be an immune reaction against neutrophils and/or committed hematopoietic progenitors.

792 PRIMARY PANCREATIC LYMPHOMA. REPORT OF FIVE CASES

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Primary pancreatic lymphoma (PPL) is a very rare disease and fewer than 150 cases have been reported in the english-language literature. We retrospectively analysed the records of 630 patients with non-Hodgkin's lymphoma (NHL) diagnosed and treated at our Institution from 1987 to 1997. A series of five patients with PPL was detected (0,7% of all NHL). None of these patients had evidence of extrapancreatic disease and they were categorized as PPL involving the pancreas only (stage IE, 3 patients) or the pancreas and peripancreatic lymph nodes (stage IIE, 2 patients). There were four men and one woman, mean age 65 years. The most common symptoms at diagnosis were abdominal pain and weight loss. Abdominal ultrasound (US) and computed tomography (CT) scan showed a mass in the pancreatic head in all cases. Bilirubin was elevated in three patients and two of them showed intra- and extra-hepatic bile ducts dilatation secondary to the pancreatic mass. The histological diagnosis was made by US-guided fine-needle aspiration biopsy (FNAB) and tissue core fine-needle biopsy (FNB) in three patients and by surgery in the remaining two patients. All cases were B-cell NHL, three diffuse-large cell lymphoma and two lymphoplasmacytic lymphoma/immunocytoma. Three patients received chemotherapy as front-line therapy and two of them received also local radiotherapy; one of these patients is still alive in complete remission at 69 months of follow-up, one died of an unrelated disease at 67 months and one died of lymphoma relapse at 88 months. Two patients underwent pancreaticoduodenectomy plus adjuvant chemotherapy; one of them died of recurrent cholangitis 8 months after surgery while the other one is still alive in complete remission after 160 months. These study shows that: 1- PPL is a very rare disease with a non-specific clinical presentation; 2- imaging techniques are unable to distinguish PPL from the most common pancreatic adenocarcinoma; 3-histological diagnosis can be obtained by percutaneous US-guided biopsy; 4- surgery can be avoided both for diagnosis and treatment.

793 IMATINIB UNEXPECTEDLY ELIMINATES A WHOLE CHROMOSOMAL COMPLEX TRANSLOCATION INCLUDING PHILADELPHIA CHROMOSOME IN A PATIENT WITH ESSENTIAL THROMBOCYTHEMIA

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Imatinib Mesilate (Glivec) is a signal transduction inhibitor, specifically targeting Bcr-Abl, the molecular abnormality in Chronic Myeloid Leukemia (CML). It functions as a competitive antagonist by occupying the ATP-binding site of the Bcr-Abl tyrosine kinase domain. It inhibits the fusion product of Ph chromosome by impairing Bcr-Abl-mediated transfer of phosphate to its substrate. Inhibition of Bcr-Abl reverses the effect of Abl kinase activity, which is the expansion of pluripotent stem cell with a preference for myeloid lineage differentiation.

Essential Thrombocythemia with Philadelphia chromosome (ET/Ph pos) is a rare but well documented entity, even if disputed by some hematologists.

We present such a case, in a 54-year-old woman, with a composite translocation: 46,XX,t(1;9;16;22)(p13;q34;q24;q11), including Bcr-Abl rearrangement.

Imatinib treatment resulted in progressive reduction in the number of metaphases Ph positive, till complete cytogenetic response, and later complete molecular response.

Nine months after Imatinib was started, the patient had a normal karyotype, for up to 16 months, that is until today.

We train to explain the mechanism responsible for the elimination of whole complex translocation and not only of the Ph chromosome/Bcr-Abl.

794 ERYTHROPOETIN IN THE TREATMENT OF MULTIPLE MYELOMA-RELATED ANEMIA

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Anemia is a frequent problem related to patients with multiple myeloma and sometimes requires regular blood transfusions. Aim of the study: We analyzed the effect of erythropoietin-beta on hemoglobin level in patients with myeloma-related anemia. Material and methods: Five patients (3M/2F) with myeloma-related anemia, median age 63 years (range 44-78) were evaluated. Clinical staging according to Durie-Salmon classification was: II-B one patient, III-A one patient and III-B 3 patients. Three patients were in the onset of the disease, one was with advanced myeloma and one was in remission. They were all treated with conventional chemotherapy (4 pts according to VMCP and one patient according to MP). The median time from diagnosis to initiation of erythropoietin was 20 months (range 3 months to 6 years). The mean Hb level was 7.7g/dl (range 6.9-8.6). Epoetin beta (Recormon-Roche) was administrated at a dose of 10,000 IU 3 times/weekly. Iron supplementation was not given to any patient because there was no evidence of ferro deficit. Results: Three out of five patients (60%) responded. Major and minor responses were defined as no need for transfusions and an increase of Hb level by >2gr/dl and >1gr/dl respectively. There were 2 major and one minor response. One patient achieved an increase of Hb level >2gr/dl within 4 weeks and the other within 6 weeks. The third responder became transfusion independent within the first 4 weeks of treatment but achieved an increase in Hb level >1gr/dl even within 6 months and >2gr/dl within 10 months. No side effects were detected in these patients. Conclusion: Epoetin beta is a potent and safe agent that determines a rapid erythroid response in a high percentage of patients with myeloma-related anemia and enables these patients to increase their quality of life.

795 MALIGNANT THYMOMA COMPLICATED BY AMEGAKARYOCYTIC THROMBOCYTOPENIC PURPURA, PURE RED CELL APLASIA AND MYASTHENIA GRAVIS

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We report a case of 41-year-old man who suffers from sequences of paraneoplastic syndromes of malignant thymoma including amegakaryocytic thrombocytopenia.

A 41-year-old man presented with severe anemia (Hb 4.78g/dL). He had been diagnosed as having myasthenia gravis 10 years earlier. His symptoms controlled with pyridostigmine bromide 120 mg/day. There were neither reticulocytes on peripheral blood smear, nor other evidence of hemolysis or clinically frank bleeding. A bone marrow examination revealed absence of erythroid precursors. Pure red cell aplasia was diagnosed and the patient was treated with periodic transfusions and oral steroids. The screening tests for etiologies of pure red cell aplasia including parvovirus were negative. Despite normal chest roentgenogram, the simultaneous combination of myasthenia gravis and pure red cell aplasia motivated us to proceed to a computed tomography scan of the thorax. Computed tomography scan showed an anterior mediastinal mass, which later was diagnosed to be thymoma. Pure red cell aplasia was resistant to partial tumor resection and chemotherapy. Three months after chemotherapy courses were stopped widespread muco-cutaneous bleeding appeared. Severe thrombocytopenia (PLT 4000/ml) was diagnosed. Bone marrow aspirate showed absence of megakaryocytes with normal maturation and differentiation of white blood cell precursors. The bleeding was controlled by periodic random donor thrombocyte infusions. There is a well-recognized association between thymoma and myasthenia gravis and between thymoma and pure red cell aplasia. Amegakaryocytic thrombocytopenia is not known as a paraneoplastic syndrome complicating thymoma.

796 FLUDARABINE, CYCLOPHOSFAMIDE AND MITOXANTRONE IN THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Fludarabine is the most effective of purine analogues, used in chronic lymphocytic leukemia (CLL). Combining fludarabine with other cytotoxic agents may improve the quality and duration of responses, and prolong survival.

The aim of the study was to assess the efficiency and toxicity of fludarabine combined with mitoxantrone and cyclophosphamide.

The study included 62 CLL patients (M/F: 36/26). Median age was 59 years (age range: 34 - 75 yrs). According to the Binet classification 40 patients were in stage B, 22 pts were in stage C.

30/62 CLL pts were untreated. 32/62 pts were pretreated with chlorambucil or CHOP. All pts were treated with FCM and they received this regimen with fludarabine 25 mg/m² intravenously (IV), d 1-3, cyclophosphamide 200 mg/m² IV, d 1-3, mitoxantrone 6 mg/m² IV, d 1. FCM regimen was given every 4 weeks, but no more than 6 courses. The median number of courses given was 4 (range: 2-6). 30/30 (100%) previously untreated CLL pts responded to FCM: complete remission (CR) was achieved in 60% (18/30) pts, partial remission (PR) was achieved in 40% (12/30) pts. The overall response rate in pts with previously treated CLL was 78% (25/32) with 32% (10/32) CR and 46% (15/32) PR, 3 pts (10%) were relapsed. The main hematological toxicity was neutropenia, the main non-hematological toxicity was infections, documented in 32% pts.

Therefore, chemotherapy, including fludarabine, cyclophosphamide and mitoxantrone (FCM) is very effective in patients with CLL. The most effective response on therapy, resulting in CR, was in previously untreated patients, compared with pretreated ones. Predominant toxic effects were myelosuppression and infections. Thus, FCM regimen had a moderate toxicity, especially when it was used in patients with previously untreated CLL.

797 EVALUATION OF FERRITIN AND TRANSFERRIN AS ACUTE PHASE PROTEINS IN CHILDREN AND ADULTS

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Introduction: Ferritin and transferrin as proteins for the storage and transfer of iron respectively, exhibit a variant behavior in the case of infections as markers for the diagnosis and monitoring of the latter.

Aim: The determination of the ferritin and transferrin levels, considered as positive and negative markers respectively, in the existence of infections and their possible variations by age.

Method - Material: Our material consisted of 91 serums of children (average age: 4.4 years) who were hospitalized in the Karamandaneio Children's Hospital of Patras exhibiting infection symptoms (CRP > 1mg/dl) and 45 serums of adults who were hospitalized in the General Hospital of Kyparissia with similar symptoms. The measurement of ferritin was performed by the microenzymic immunoassay (MEIA) and of transferrin by immunoturbidimetry.

Results: 29 out of the 91 pediatric samples (31.8%) showed high ferritin (>88ng/ml) and low transferrin (<190mg/dl) values. 42 samples (46.2%) showed ferritin and transferrin within the normal range and 20 samples (22%) showed high ferritin values and normal transferrin values. 28 out of the 45 adults' samples (62.2%) showed high ferritin values (>175ng/ml) and low transferrin values (<190mg/dl). 17 samples (37.8%) showed both markers within the normal range. Ferritin in all samples (136) was >20ng/ml. The evaluation of the results was accomplished on the basis of the patient's age and sex.

What arises from the above is that in a significant percentage of children (31.8%) ferritin constitutes a positive infection marker and transferrin a negative one. Comparatively, this percentage is much higher in adults (62.2%).

This finding can be read as a result of pre-existing iron deficiency, which is typical in the age group of children, which were included in our study so that the final profile of ferritin and transferrin respectively appear to be normal.

In addition, it was observed that ferritin responds as a positive infection marker in the pediatric samples at a higher percentage (53.8%) comparing to transferrin (31.8%).

Conclusion: Ferritin and transferrin are markers of acute infection (ferritin as positive, transferrin as negative one). However, they should be always evaluated also in relation to other parameters such as the patient's age or a possible iron deficiency.

Iron DeficiencyInfectionFinal Result

Ferritin' Normal

Transferrin ↑'Normal

798 LAMIVUDINE PROPHYLAXIS IN A PATIENT WITH ACUTE MYELOGENOUS LEUKEMIA AND CHRONIC HEPATITIS B

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Chemotherapy treatment in hepatitis B virus (HBV) carriers could cause in a significant percentage of patients viral reactivation and hepatitis B which can even progress to fatal liver failure. Over the last few years the prophylactic administration of lamivudine as a potent antiviral drug has been tested in this type of patients. The cases that have been reported in the literature are relatively few. They refer almost exclusively to lymphomas and other non-hematological malignancies which were treated with chemotherapy of moderate intensity. To the best of our knowledge, this is the second reported case of a patient with acute myelogenous leukemia and chronic hepatitis B to whom lamivudine was prophylactically administered.

A 47-year-old female was admitted to our clinic and diagnosed with acute myelogenous leukemia. Her medical history showed HBsAg positivity for the past seven years. Biochemical blood tests revealed normal function of liver but serological studies detected positive HBsAg, anti-HBc and anti-HBe with negative anti-HBc-IgM, HBeAg, anti-HCV. PCR method revealed an HBV-DNA titer higher than the highest detectable limit of our laboratory, namely $> 4 \times 10^7$ copies/ml (normal $< 1 \times 10^3$). Lamivudine therapy was initiated at a dose of 100 mg p.o. daily, two days before chemotherapy treatment. Remission induction chemotherapy consisted of cytarabine 100 mg/m² i.v. (continuous infusion) days 1-7, idarubicin 10 mg/m² days 1-3 and cytarabine 2g/m² days 8-9. After complete remission was achieved, the patient was initially treated with cytarabine 200 mg/m² i.v. (continuous infusion) days 1-5 and idarubicin 12 mg/m² i.v. days 1-2. The second cycle of postremission chemotherapy included cytarabine 2 gr/m² /12 hours i.v. days 1-3 and etoposide 100 mg/m² i.v. days 4-5. Throughout the three cycles of chemotherapy and the long periods of severe aplasia (25-30 days) there were no clinical and/or laboratory findings of liver dysfunction. A new viral DNA study 2.5 months after the first evaluation showed an impressive decrease of viral load (4.7×10^3 copies/ml).

Conclusively, lamivudine prophylactic administration was proven to be highly efficacious at preventing hepatitis B flare-up during intensive chemotherapy in a HBsAg positive patient with acute myelogenous leukemia.

799 COGNITIVE DECLINE IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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BACKGROUND: Anemia is a common complication in patients with hematologic malignancies and has great influence on QoL.

AIMS: To determine cognitive functions in patients with hematologic malignancies and evaluate the use of Complex Reactimeter Drenovac (CRD) versus Folstein Mini-Mental Status Examination (FMMS) to measure cognitive functioning in cancer trials.

PATIENTS AND METHODS: A total of 150 pts with hematologic malignancies in various stage of disease were seen for baseline evaluation. For each patient we collected clinical data, laboratory tests and performed tests measuring cognitive functions (CRD and FMMS), ability to perform activities of daily living (ADLs) and quality of life (QOL), repeated them monthly during six months. In the same time control group (50 healthy pts) of the same age, sex, and education were compared.

RESULTS: We found cognitive decline in all hematologic pts. Performance on the tests were strongly related to anemia, cancer treatment, and stage of disease. The worst cognitive functions had pts with acute leukemia and multiple myeloma, especially when anemic. Successful treatment of anemia had great influence on cognitive improvement and depended upon achieved hemoglobin level.

CONCLUSION: We applied and compared two tests to determine cognitive changes related to hemoglobin level. Results emphasized necessity of early correction of anemia in hematologic patients.

800 CLINICAL SIGNIFICANCE OF P53 EXPRESSION IN MULTIPLE MYELOMA

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Background: Mutations of p53 are one of the most often acquired genetic changes in malignant tumors. According previous reports, the mutations of p53 are rare events in patients with newly diagnosed MM. However, there are a few literature data about clinical significance of this protein in MM.

The aim of this study was to evaluate clinical significance of p53 expression in MM.

Patients and methods: 59 patients with newly diagnosed MM (27 females and 32 males, median age 62 years) were enrolled in the study. The diagnosis of MM was made according to the criteria of Chronic Leukemia-Myeloma Task Force. Clinical staging was done according to the Durie and Salmon classification (4 patients had disease stage I, 15 patients stage II and 39 patients stage III). The histological grade and histological stage (i.e. volume of myeloma infiltration) were determined according to the models proposed by Sailer and Bartl, respectively. We used standard immunohistochemical analysis with p53 antibody on B5-fixed and paraffin-embedded bone marrow specimens to evaluate expression of p53 in myeloma cells. The specimens were considered positive when equal or more than 5% plasma cells showed nuclear positivity.

Results: p53 expression was detected in 9 (15.25%) patients. p53 expression did not correlate significantly with clinical stage (I+II vs. III), 2-microglobulin level (≤ 6 mg/L vs. >6 mg/L), histological grade (I vs. II+III), histological stage ($< 20\%$ vs. $21-50\%$ vs. $> 50\%$) and the extent of osteolytic lesions (≤ 3 vs. > 3 lesions). Median survival of patients with p53 immunoreactivity in $\geq 5\%$ plasma cells was 10 months, whilst median survival of patients with p53 immunoreactivity in $< 5\%$ plasma cells was 35.8 months. However, this difference was not significant ($p = 0.2$).

Conclusion: Frequency of p53 expression in our group of newly diagnosed MM was relatively low. p53 expression was not associated with clinical and histological features of more aggressive disease, nor with the short survival.

801 GLUTATHIONE PEROXIDASE (GPX) ACTIVITY AND GLUTATHIONE (GSH) LEVEL IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA (MM) TREATED WITH ARSENIC TRIOXIDE (ATO)

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Background: Multiple myeloma (MM) remains an incurable B cell malignancy with median survival rates of 4-6 years. Arsenic trioxide (ATO) is a novel therapeutic option in the treatment of MM. This agent has multifaceted mode of anticancer action. One of them is induction of apoptosis by inhibition of the GSH cellular redox system. Arsenic suppresses glutathione peroxidase (GPx) which is the major route for detoxification of reactive oxygen species. Elevated glutathione (GSH) level has been associated with the MM chemoresistant phenotype. Reduced GSH level enhances the apoptotic effects of ATO.

Aim: The aim of the study was to assess clinical efficacy of ATO and to evaluate activity of GPx and level of GSH in patients with relapsed MM treated with ATO and ascorbic acid (AA).

Methods: 7 patients (4 women and 3 men, mean age 59 years) with relapsed MM were treated with 6 cycles of ATO (Vipham, Poland) 0.25mg/kg iv five days in the week in a 2 weeks on/ 2 weeks off schedule. In addition ascorbic acid 1000mg p.o. was given. All patient were in advanced stage of disease III A acc. Durie and Salmon.

GPx activity and GSH levels were measured by spectrophotometry (spectrophotometer SP-8-100 Pye Unicam). Normal range for GPx 27.5 - 73.6 U/g Hb and for GSH 25.5 - 50.0mg%. Statistical analysis was made using Wald-Wolfowitz test ($p < 0.05$).

Results: 1 patient achieved partial response and 1 patient had stable disease according SWOG criteria. The common drug related adverse events were mild and resolved spontaneously: cytopenia (3 patients), QT prolongation (1 patient), abdominal pain and diarrhoea (1 patient), peripheral neuropathy (1 patient). In all patients reduced GPx activity was observed. Only in 2 out of 7 patients (one with partial response and one with stable disease) reduced GSH levels were observed. Glutathione (GSH) level and glutathione peroxidase (GPX) activity are presented in the table.

Conclusion: Obtained data although based on relatively small group of MM patients suggest that ATO in combination with AA has clinical activity in multiple myeloma and significantly decreases activity of GPx.

	GSH mg%	GPx U/g Hb
Before ATO + AA	36,34	49,91
After 6 cycles of ATO + AA	31,68	24,07
p	NS	0,003

Caption 1: Glutathione (GSH) level and glutathione peroxidase

802 EXPRESSION OF KILLER INHIBITORY RECEPTORS: CD94 AND CD158B IN NATURAL KILLER CELLS IN MULTIPLE MYELOMA AND NON-HODGKIN'S LYMPHOMA

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Background: Natural killer (NK) cells express MHC class I-specific inhibitory receptors, termed 'killer inhibitory receptors' (KIRs). Upon binding to MHC class I molecules on target cells, these receptors deliver a negative signal that prevents the NK-mediated lysis of target cells. Different MHC class I-specific killer inhibitory receptors (KIRs) are expressed in vivo by NK cells. There are some reports that expression of CD94 is increased in multiple myeloma (MM) patients. The role of KIR in non-Hodgkin's lymphoma (NHL) is not clearly established.

Aim: The aim of this study was to investigate the expression of KIRs (CD94-NKG2-A receptor complex and CD158b) in NK cells of B cell malignancies: MM and NHL patients.

Methods: 32 MM and 19 NHL patients were studied. Clinical data are showed in the table 1. Patients were studied before any chemotherapy. The control group consisted of 10 age-matched normal donors. The presence of CD94 and CD158b was evaluated in NK cells isolated from MM, NHL patients and normal donors.

Peripheral blood mononuclear cells (PBMCs) were obtained by density-gradient centrifugation (Ficoll-Hypaque) of heparinized venous blood. These were then counted microscopically. Three colour immunofluorescence staining was performed. 7 ml of monoclonal antibodies conjugated with FITC and PE in the case of double colour staining or additionally conjugated with Cy-Chrome (three-colour staining) was added to each tube. We used the following monoclonal antibodies specific for: CD14, CD45 (DAKO, Denmark), CD3, CD56, CD94, CD158b and NKG2A (Becton Dickinson, USA). Tubes were then agitated and incubated for 20 min. at 4°C in the dark, after which 5 ml of PBS-Ca2+Mg2+ containing 0,1% NaN3 (Sigma Chemical Co., St Louis, Mo, USA) was added to each tube and pelleted by centrifugation (1200 rpm for 10 min). The supernatant was removed and the pellet resuspended in 0.2 ml PBS-Ca2+Mg2+ with 0,1% NaN3 paraformaldehyde. Twenty thousand labelling events were routinely accumulated and analysed for fluorescence on PAS flow cytometer (Partec, Munster, Germany) using FloMax software. The results were statistically analysed using test ANOVA rang Kruskal-Wallis. Results: Results are showed in the table 2

Conclusion: In our study we have demonstrated that the mean number of NK cells expressing CD94 and CD158b not differ statistically in MM and NHL patients compared with normal donors.

MM		NHL		Control
Mean age/ Sex	Stage acc Durie and Salmon	Mean age/ Sex	Stage Ann Arbor	Mean age/ Sex
65 years/ 21 Female 11 Male	I - 5 II - 8 III - 19	61 years/ 8 Female 11 Male	III - 1 IV - 18	59 years/ 5 Female 5 Male
	NK cells [3/4]	NK cells CD94+ [3/4]	NK cells CD94+HK32A + [3/4]	NK cells CD158b+ [3/4]
NHL	0,359453	0,180631	0,117747	0,106208
MM	0,322615	0,186313	0,092499	0,124814
Control	0,172565	0,091928	0,081941	0,071086
p	NS	NS	NS	NS

Caption 1: Clinical data and results

803 CYTOTOXICITY OF MONONUCLEAR CELLS (MNC) AND LYMPHOKINE ACTIVATED KILLER (LAK) CELLS AGAINST AUTOLOGOUS AND ALLOGENEIC TUMOR CELLS BY ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) IN CHILDREN

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Background: acute lymphoblastic leukemia may adversely affect the variety of immune functions. The possibility of recover cell immunity and augment cell cytotoxicity can provide therapeutic tool for tumor treatment.

Aim: to assess the cellular cytotoxic activity of blood/bone marrow MNC and LAK cells derived from children with ALL against allogeneic and autologous tumor cells.

Patients/methods: 38 patients (age 0-14 years, median 6,7) with ALL were enrolled in the study. MNC were separated from blood and bone marrow and were tested on both allogeneic blasts of K562 cell line and autologous lymphoblasts. Cytotoxic activity of MNC was assessed in acute period of disease before any treatment as well as after induction chemotherapy in remission. LAK cells were obtained in remission by in vitro incubation of MNC with interleukin-2 and were tested on the same target cells. Additionally, cytotoxicity of LAK cells from healthy volunteers was measured on K562 cell line and ALL patients' tumor cells. MTT was used to assess cytotoxic activity. Results: cytotoxicity of blood MNC against K562 line cells increased from 28,7% initially to 36,6% in remission (from 20,2% to 31,9% for bone marrow MNC, respectively). Blood MNC cytotoxicity against autologous blasts increased from 22,8% in acute period to 25,4% in remission (from 14,9% to 22,9% for bone marrow MNC, respectively). LAK cell cytotoxicity was 26,9% by testing against allogeneic tumor cells and 49,1% - against autologous blasts. Cytotoxic activity against K562 cell line as well as against autologous tumor cells had negative correlation ($p < 0,05$) with age of patients and initial white blood cell count. Cytotoxicity of LAK cells obtained from MNC of healthy volunteers was 72,3% in test against tumor cells from patients with ALL. Conclusions: cytotoxicity of blood and bone marrow mononuclear cells is decreased in acute period of ALL and becomes significantly higher in remission. MNC cytotoxicity against allogeneic tumor cells is higher then that against autologous ones. It could be due to low susceptibility of autologous blasts to effector cells and due to immunosuppressive effects of tumor cells. MNC cytotoxic activity can be augmented by generation of LAK in presence of interleukin-2.

In comparison to ALL patients LAK cells from healthy volunteers are significantly more cytotoxic against allogeneic blasts.

804 IMMUNOPHENOTYPICAL FEATURES OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. In the past years the immunophenotyping of leucocytes has won a central place in the diagnosis of chronic lymphocytic leukemia (CLL). But immunophenotype in CLL is usually heterogeneous and few markers have an important prognostic significance.

The aim of the study was to investigate immunophenotypical features of B-CLL and evaluate the prognostic power of some molecules.

Material and methods. The study included 180 B-CLL pts (M/F: 96/84), average age was 58 yrs (age range 38–75 yrs). Immunophenotypical studies were performed in peripheral blood smears using monoclonal antibodies 'DAKO' (Denmark).

Results. We divided our pts into 2 groups. Pts of group 1 (62/180 pts) were characterized by typical CLL antigens: CD5+, CD19+, CD20+, CD22+, HLA-DR+, CD23+, CD10-, sIgslow. Pts of group 2 (118/180 pts) among typical for CLL antigens had activation antigens: CD11c, CD11b, CD25, CD38. The number of CD38-positive cells more than 30% was correlated with progressive course of the disease. The percentage of CD95-positive cells was significantly lower in CLL pts when compared with healthy individuals ($p=0.002$). Presence of activation antigens was significantly associated with low percentage of CD95+ cells. The percentage of CD95+ cells was correlated with stage of CLL and type of bone marrow infiltration by CLL cells. All pts received CT with fludarabine. We compared response to CT and immunophenotypical features of CLL. In group of pts with atypical immunophenotypical features overall response rate was significantly lower compared with ones without atypical immunophenotypical features (58% and 88%, respectively).

Conclusion. Our results show that immunophenotypical features may be used as prognostic factors of course of the disease and efficiency of therapy.

805 DEPRESSION/ANXIETY SYMPTOMS AND NEUROTICISM IN PATIENTS TREATED DUE TO HAEMATOLOGIC CANCERS

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BACKGROUND: Haematological treatment is associated with excessive distress due to severe life consequences of the illness and due to side effects of cytostatics. It is a cause of anxiety or depressive states in vulnerable persons.

AIM: The purpose of the study was to determine relation between neuroticism, distress and severity of depressive/anxiety symptoms in patients treated due to leukaemia and lymphoma.

METHODS: 45 patients with leukaemia or lymphoma, 46 treated due to other internal diseases and 45 healthy persons were assessed. The following research instruments were used: the Eysenck Personality Questionnaire (EPQ-R), the General Health Questionnaire (GHQ-30), criteria scales (ICD-10) for depression and anxiety.

RESULTS: the two groups of patients differed significantly from control group in scores of depression, anxiety and distress (GHQ). Differences in neuroticism level were small and not relevant statistically. The group with haematological illness had higher score of anxiety than the group other internal illnesses. The difference depended on psychic symptoms of anxiety, the score of somatic symptoms of anxiety was similar in all the groups. The level of neuroticism was similar in all the groups. It correlated with depression and anxiety scores in the groups of haematological patients (Pearson product-moment correlation $r = 0.59$ and 0.58 respectively), and in the groups of other internal patients ($r = 0.54$ and 0.54) but not in the control group ($r = 0.1$ and 0.15).

CONCLUSION: The groups of haematological patients have higher, depressive and anxiety scores than controls and slightly higher than other patients with internal diseases. This is result of psychological stress coming from awareness of being sick and biological stress coming from and illness itself and from medication. Neuroticism score is the measure of stable personality trait and is not close related to symptoms nor stress. So neuroticism score could be a predictor of vulnerability to depressive and anxiety states in the course of internal illnesses.

806 THE COMPARISON OF SPONTANEOUS LDH RELEASE ACTIVITY FROM CULTURES PBMC WITH SERA LDH ACTIVITY IN NON-HODGKIN'S LYMPHOMA PATIENTS

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Based on consideration that intracellular characteristics of lactate dehydrogenase (LDH) enzyme are very sensitive indicators of the cellular metabolic state, aerobic or anaerobic direction of glycolysis, activation status, malignant transformation, as well as membrane damage following cell death process, aim of this study was to correlate values of the spontaneous LDH release from circulated PBMC with their sera LDH activity in 43 different subtypes of lymphoma. The diagnosis of NHL patients was based on histological proven of the lymph node and bone marrow biopsy for appropriate patients, while clinical staging was performed according to the Ann Arbor system based on combination of clinical routine examination, blood and ultrasound analyses, chest ray and computerized tomography (CT) scan. The spontaneous LDH release after cell separation and 2h cultures of PBMC (at concentration 8, 4 and 2 x 10⁶/ml of culture medium) was determined by spectroscopic methods using substrate mixture on the micro-titer plate reader (Behringer EL-311, Germany) expressed as changes in absorbance at 492-630 nm. The results display that patients in advanced clinical stage (III and IV) have a significantly increase of sera LDH activity ($p<0.05$, Mann-Whitney U-test) in comparison to those in early stage (I and II). However, mean values for sera LDH activity, in low, intermediate and high grade malignancy in early stage (I and II) display mean values of LDH which is no significantly differ ($p>0.05$, Mann-Whitney U-test) in comparison to reference values (320 U/ml) although, some individual patients have elevated sera LDH activity. The investigation of spontaneous LDH release activity from cultures PBMC, expressed as changes in absorbance/min is a significantly differ among all observed groups based on histology and clinical stage ($p<0.01$, Kruskal-Wallis ANOVA). In addition, there are a significant positive correlation between spontaneous LDH release determined from cultures PBMC and serum LDH level in individual NHL patients ($n=43$, $r=0.493$, $p=0.001$). Based on obtained data, we conclude that the evaluation of the spontaneous LDH release from cultures cells appears to be an additional parameter that correlates with particular subtypes and forms of lymphomas and, together with other findings may aid in their diagnosis, especially in patients with early stage of disease.

807 AUTOIMMUNE HEMOLYTIC ANEMIA IS NOT MORE FREQUENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH FLUDARABINE

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Background: Autoimmune hemolytic anemia (AHAI) is a frequent event (10-20% of cases) in Chronic Lymphocytic Leukemia (B-CLL) and can appear at any time during the course of the disease. The mechanisms causing production of antibodies in B-CLL are not fully understood, some studies asserting the higher frequency of this one in patients treated with fludarabine.

Aim: Our study has a retrospective character wishing to evaluate the relation between LLC and AHAI considering the therapy with fludarabine.

Methods: Between 1987 and 1999, AHAI was observed at our institution in 25/165 (15,15%) patients with B-CLL (15 men, 10 women); median age 59 years, range (21-74).

Results: AHAI was observed at times ranging from diagnosis to 68 months from diagnosis; in 24% patients before treatment initiation and in 76% after. In the later cases, 47,36% patients had received chemotherapy with chlorambucil and prednisone, 31,57% followed CVP (cyclophosphamide, vincristine, and prednisone) and 21,05% had received fludarabine. There are not significant differences in the incidence of the AHAI occurrence at the three groups of patients. The low hemoglobin value observed at onset of AHAI was 5,2 g/dL (range 4,2-8,6 g/dL) but there are not important disputes between the lot of patients treated with FAMP (mean 6,3 g/dL) and the ones treated with alkylating drugs (mean 4,9 g/dL). The direct antiglobulin test was positive with anti IgG + anti C3d antiserum in 72% patients; in one (4%) patients with anti IgG + anti IgA + C3d antiserum; in 16% patients with anti C3d antiserum. All the patients undergoing treatment with fludarabine belong to the first lot but again the significant limit hasn't been attained. Only 24% cases required

packed red blood cell transfusions, two belonging to the group treated with fludarabine. All the patients were treated with corticosteroids (PDN 1mg/kg/day for up to three weeks), as for the lot treated with fludarabine this one was interrupted and switched to another antineoplastic therapy. Complete hematological response to AHAI was observed in 80% cases, 16% patients had partial response and 1 (4%) patient died of hemolytic crisis (fludarabine group). 20% of complete responders had AHAI relapse. Splenectomy is performed in 12% patients in two cases for multiple relapses after PDN and chemotherapy and one for incomplete response, none belonging to the group treated with fludarabine. Other three patients had received PDN in low doses for three to six months (between chemotherapy) for incomplete response of AHAI. Overall survival of the patients was 46 months from diagnosis of malignancy. Conclusions: Our data suggest that AHAI is more frequent in previously treated patients, with progressive B-CLL, than in untreated patients. AHAI had a low incidence in patients treated with fludarabine without presenting important differences comparing with the rest of the patients with LLC and AHAI.

808 THE EFFICIENCY OF SPLENECTOMY IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA (IPT) RESISTING TO CORTICOTHERAPY

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Background: Splenectomy is the second therapeutical line in patients with IPT after failure of steroids or after 2-3 relapses in 6 to 12 months. The postsplenectomy rate response is between 65-75% in most of the literature studies.

Aim: The aim of this work is to evaluate the efficiency of splenectomy at the level of our lot of patients with IPT resisting to corticotherapy.

Methods: Our lot consists of a number of 56 patients with IPT diagnosed beginning with the year 1983. The median age was that one of 28 years old. (12) 21,42% of the patients belong to the male sex. The platelet count at the presentation at the time of splenectomy, the sex, the age, the months of evolution before surgery, the number of previous relapses, response and number of relapses after surgery were evaluated. CR was defined as normal platelet count for ≥ 1 month on no treatment and without relapse and PR as platelet count $\geq 50 \times 10^9/L$ at least 1 month after splenectomy and no criteria for CR.

Results: 16 (28,57%) of patients with IPT were splenectomized. The platelet count at the time of the surgery indication varied between 12.000 and 38.000/mm³ platelets with an average of 21400 mm³ of platelets. 4 (25%) of the splenectomized patients were male and 12 (75%) female. Median age at diagnosis: 26 years (14-54). Months from diagnosis until splenectomy was 28,7 (7-210). Indication of surgery was: 7(43,75%) patients were refractory to previous treatment and 9 (56,25) patients presented different number of relapses (3-4). There was no death after the surgery and morbidity is materialized in complications like: one case with left side pleurisy, a subphrenic abscess and two cases of the thrombophlebitis of the leg. In our series, total response rate, including CR or PR was achieved in 81,25% of the patients: 8 (50%) CR and 5 (21,75%) PR, with a median following time of 88 months (12-250). No-responding patients underwent the immunosuppressive therapy with good results in two of the three cases.

Conclusions: The indication of splenectomy in our patients with IPT has a percentage of 28,57%. Total response rate (CR+PR=76,18%) to splenectomy seems to be similar to that reported in the literature, although a lower CR rate is observed.

809 FOUR CYCLE OF COPP/ABVD FOLLOWED BY LOW DOSE INVOLVED FIELD RADIATION THERAPY (LDIF RT) ARE INAPPROPRIATE IN UNFAVOURABLE SUPRADIAPHRAGMATIC CLINICAL STAGES I-II HODGKINS DISEASE

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Background. The patients with Hodgkin disease(BH), stage I and II have a high rate of complete remission and a long time survival. The identification of some risk factors in this group of patients allowed different therapeutical approach.

Aim. Our study has retrospective character and wants to emphasize that four cycles of COPP/ABVD plus LDIF RT represent an inappropriate therapeutical modality for unfavourable supradiaphragmatic clinical stages I and II Hodgkin's disease.

Methods. The study group includes 63 patients with HD stage I (9 patients) and stage II (54 patients), except bulky and II B. Nodular sclerosis is present in 65.07% of cases. The mean age is 37 years and males are slightly predominant (34/29). The therapy consists of four cycles of COPP (before 1994) or ABVD combination chemotherapy followed by LDIF RT (24-32 Gy) in patients with complete response (CR). Patients with stable or progressive disease after four cycles of chemotherapy were switched to extended field radiotherapy (36-40 Gy). Minimum follow-up was 2 years, with a median of 51 months.

Results. From the 63 patients with BH in stage I and II with supradiaphragmatic disease, 31,74% present at least one unfavorable prognostic factor: the age ≥ 50 years, stage II with more than four involved areas, stage A+VSH ≥ 50 mm/h or stage B+VSH ≥ 30 mm/h. CR after polichimiotherapy has been attained in 40 cases (93,02%) for BH in stage I and II with supradiaphragmatic disease and favorable prognostic and only 75% of the patients with Hodgkin disease stage I and II with unfavorable prognostic (Fisher exact $p=0,01$). It means that the proposed protocol for BH in stage I and II, non bulky was completed for only 75% of the patients with BH stage I and II with unfavourable prognostic. The association of the extended radiotherapy increases the CR rate to 97,67% for the first lot and to 85% for the lot with risk factors (Mantel Haenszel $p=0,05$). The survival rate free of the disease after 4 years was of 95,23% for the lot of patients with favourable prognostic and of 76,47% for those ones with risk factors (Mantel Haenszel $p=0,03$). None of the patients with BH stage I or II and risk factors undergoing extended radiotherapy had a relapse (Fisher exact $p=0,04$).

Conclusions: The differences of therapeutical response to the applied protocol are significant between the two lots: the patients with BH with unfavorable prognostic present a lower rate of complete remissions after the 4 cures of COPP/ABVD, the relapse rate at 4 years is higher and all these patients belong to the lot undergoing LDIF RT. It means that four cycles of COPP/ABVD followed by LDIF RT is not enough for unfavorable supradiaphragmatic clinical stages I-II Hodgkins disease.

810 DISSEMINATED INTRAVASCULAR COAGULATION AND SERUM VEGF LEVELS IN PATIENTS WITH ACUTE LEUKEMIA

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Background: Laboratory findings of disseminated intravascular coagulation (DIC) are common in patients with acute leukemia. Tissue factor is a major activator of pathologic coagulation process in DIC. Tissue factor antigen is elevated in patients with acute leukemia and DIC. Recent studies have shown that tissue factor may be involved in tumor angiogenesis and metastasis and is implicated in regulation of vascular endothelial growth factor (VEGF) - a most important angiogenic factor.

Aims: To determine laboratory parameters of DIC in acute leukemia patients and to correlate them with serum VEGF levels and patient survival time.

Methods: 35 adult patients with acute leukemia were studied (3 with acute lymphoblastic leukemia, 32 with acute myeloblastic leukemia). The following coagulation tests were performed on diagnosis, before induction therapy, using routine laboratory techniques: Prothrombin time (PT), activated partial thromboplastin time (PTT), fibrinogen, platelet count, thrombin time (TT), D-dimer, fibrinogen split products (FSP). Serum VEGF level were assayed concomitantly using a commercial quantitative sandwich enzyme immunoassay technique.

Results: Abnormal PT was found in 16(46%) patients, prolonged PTT in 1(2.8%) patient, decreased fibrinogen level in 2(5.7%) patients, D-dimer was elevated in 16 patients (46%) and FSP in 13 (37%). The mean serum VEGF level was 45 pg/ml (range 4.45-139). A borderline correlation was found between PTT and log serum VEGF level ($p=0.086$) and fibrinogen and log serum VEGF level ($p=0.097$). No correlation was found between serum FEGF level and PT, TT, D-dimer, and FSP levels. Furthermore, no correlation was found between VEGF levels and patients survival time.

Conclusions: Coagulation abnormalities consistent with DIC are prevalent at diagnosis in acute leukemia patients (37-46%). No correlation was found between them and serum VEGF levels, suggesting that released tissue factor, reflected by the onset of DIC in patients with acute leukemia, does not trigger angiogenesis in these patients.

811 THE HAEMOPHAGOCYTIC SYNDROME (HPS) A CLINICOPATHOLOGIC STUDY OF 12 PATIENTS IN AN ASIAN POPULATION

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Background:

HPS is a clinico-pathologic entity characterised by systemic proliferation of non-neoplastic histiocytes showing phagocytosis of haemopoietic cells, resulting in blood cytopenia. It has been associated with a variety of infections, collagen vascular diseases and malignancies, especially lymphomas.

Aims:

The clinical spectrum and outcome of 12 cases of HPS in Singapore between 1998-2003 are reviewed.

Methods:

The investigation is a retrospective study of the clinical characteristics and outcomes of 12 patients with HPS diagnosed on bone marrow aspirate over a 5-year period.

Results:

HPS affected both sexes of a wide age range. 8 cases were associated with malignant lymphoma, 2 were associated with infections including 1 case of disseminated tuberculosis, 1 patient had acute leukaemia and 1 had no obvious underlying disease found. All patients presented as cases of pyrexia of unknown origin with high spiking fever, cytopenia, abrupt drop in blood counts, and deranged liver function and coagulation profiles. 10 including all 8 lymphoma cases died of acute diseases and only the 2 patients with infections are still alive. All lymphoma cases had a systemic presentation, poor outcomes and lymphoid infiltration of the bone marrow at presentation, although significant lymphadenopathy were disappointingly rare. HPS associated with viral infection is uncommon as compared with western populations.

Summary:

A review of the literature revealed that most cases of HPS have fulminant courses. The 8 cases of lymphoma had a median survival of only 47.3 days from onset of symptoms. As tumour masses indicative of lymphoma were not striking in most of the lymphoma cases, bone marrow studies appear to be of great value for the diagnosis of HPS-associated lymphoma.

Conclusion:

HPS is an uncommon disorder. A vigorous search for an underlying malignancy or infection is warranted once diagnosis is made. It may obscure the diagnosis of an underlying and treatable infectious disease. An early diagnosis and prompt initiation of chemotherapy may improve the prognosis of HPS-associated lymphoma.

812 NON HAEMATOLOGICAL MALIGNANCY MASQUERADING AS ACUTE LEUKAEMIA: THE LIMITATIONS OF IMMUNOPHENOTYPING

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Background:

In the UK, guidelines for the diagnostic immunophenotyping of haematological malignancies (Bain et al 2002) have been produced by the British Committee of Standards in Haematology (BCSH). A stepwise approach is undertaken, with morphological assessment and the primary antibody panel used to establish lineage specificity. This will in turn determine the choice of secondary panel antibodies. If plasma cell differentiation is suspected, the secondary panel includes detection of cytoplasmic (cyt) immunoglobulin (Ig) heavy and light chains, cyt CD79a and surface CD138.

Aims:

To highlight the pitfalls of immunophenotyping, despite adherence to the BCSH guidelines, when dealing with a non-haematological malignancy.

Case report:

A 56 year-old man presented with a 1 month history of malaise, lethargy, easy bruising, epistaxis and 10kg weight loss. His past medical history was unremarkable. He was taking no medications. He was an ex-smoker (stopped 20 years ago) of 10 pack years. Although he worked as a building site manager, there was no known exposure to toxic chemicals, including asbestos. The significant findings on examination were: cachexia, bruising on the trunk and limbs, oral candidiasis, a craggy mass in the right upper quadrant, proven on imaging to be hepatomegaly.

Haemoglobin measured 8.0g/dL, WCC 8.7 x 10⁹/L and platelets 50 x 10⁹/L. A blood film showed blasts and myeloblasts accounting for 1% of white cells. A bone marrow aspirate showed normal marrow elements effaced by sheets and clusters of what appeared to be small blasts. The patient was referred to our care with a presumptive diagnosis of acute lymphoblastic leukaemia (ALL).

Immunophenotyping results:

The primary panel revealed a CD13+, CD117+, CD45-, HLA-DR- population, which was negative for B- and T-lineage antigens, as well as cytMPO and nTdT.

The secondary panel showed the cells to be SmlgM+, cytlgM+, intracellular-k restricted, CD138+ and CD56+.

Interpretation of data

The presentation and cell morphology were consistent with an acute leukaemia. However, the primary panel excluded the possibility of ALL, while CD45 and HLA-DR negativity suggested a plasma cell dyscrasia or a non-haematological malignancy, rather than AML. Overall, the immunophenotype was strongly suggestive of a plasma cell dyscrasia - CD45-, CD56+, CD117+, CD138+, SmlgM+, cytlgM+, HLA-DR-. Indeed, the strong cyt-k restriction would be regarded by the BCSH guidelines as being plasma cell lineage-specific.

Final diagnosis:

The trephine biopsy was hypercellular, with diffuse involvement by non-haematological cells staining for NCAM, but negative for LCA and MN116. A CT scan of the chest revealed a right hilar mass. A diagnosis of bronchial small cell carcinoma was made.

Conclusion:

This case highlights:

- 1) Non-haematological malignancy may present clinically and morphologically as acute leukaemia.
- 2) The limitations of immunophenotyping, which suggested a plasma cell dyscrasia.
- 3) Cytoplasmic light chain restriction is not always lineage specific for plasma cells.
- 4) Unusual clinical findings (hard, irregular hepatomegaly) and CD45 and HLA-DR negativity were indications for the need to exclude a non haematological malignancy.

813 MYELOBLASTOMA AS INITIAL MANIFESTATION OF ACUTE MYELOID LEUKEMIA

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Background: Myeloblastoma (chloroma, granulocytic sarcoma) with no demonstrable abnormalities in the peripheral blood (PB) and bone marrow (BM) is a rare initial manifestation of acute myeloid leukemia (AML). The majority of patients (pts) present with AML very soon after myeloblastoma diagnosis.

Aims: We are presenting the analysis of clinical and laboratory data for 5 pts with extramedullary AML (myeloblastoma).

Methods: The diagnosis of myeloblastoma was established by immunohistochemical investigations (IH) (CD34+, CD117+, MPO+). BM analysis included morphology, immunophenotype and cytogenetic studies.

Results: Five pts (male 2, female 3; age : child 7 yrs, adults 25-66 yrs) presented with myeloblastoma of the female genital tract (uterus- 1 pt, ovary with abdominal lymph nodes and peritoneal tissue- 1pt), cervical lymph nodes (1 pt), subcutaneous tumor in the parietal region of the head (1 pt) and orbital tumor (1 pt). At the moment of myeloblastoma diagnosis all pts had normal PB counts, with normal BM investigations (mentioned above) done in 2/5 pts. All pts were treated with radical surgery for tumor. But, 2-12 months (Me 7,5), after operation there were signs of AML in PB and BM in 4/5 pts (2 pt M2 and 2 pt M4 FAB type, with pathologic karyotype in only one M4 pt- inv16) without extramedullary tumors of any site. AML was diagnosed even in pt treated with one cycle of AML induction chemotherapy after operation , but 12 months after initial diagnosis. Pt with uterus myeloblastoma (66 yrs) is still alive (9 months) after radical operation, without chemotherapy and without AML signs. AML treatment was successful in 2/4 pts, with complete remission (duration 2 and 8 months) and overall survival of 5 and 18 months. Two pts died due to resistant AML (7 and 17 months after myeloblastoma diagnosis).

Conclusions: Our experience confirms the significance of IH investigations for the myeloblastoma diagnosis, with complete BM studies, followed

by intensive AML chemotherapy in young pts, due to bad prognostic influence of this extramedullary AML

814 QUALITY OF LIFE DURING THERAPY FOR AGGRESSIVE NON-HODKINS LYMPHOMA IN PATIENTS WITH DIFFERENT AGE

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Background: Although patients age is one of the recognized prognostic factors in aggressive Non-Hodgkins Lymphoma (NHL) and the choice of treatment is often modified due to it, it is not stated yet if the patients need a Quality of Life (QOL) evaluation with age-specific instrument.

Aims: The aims of this study are to evaluate the impact of the lymphoma characteristics on the QOL of patients and to see if it is dependant on their age.

Methods: A group of 109 patients (median age 59 years, range 18-83) with aggressive NHL is presented, as well as its two subgroups of different age, separately: 58 patients younger than 60 years and 51 patients older than 60 years. The disease characteristics and the patients QOL have been assessed using the International prognostic index (IPI), age-adjusted IPI (aaIPI) (Shipp, 1995) and the QLQ-C30 (Aaronson, 1993).

Results: The majority of the patients (33,0%) and the majority of the elders (33,3%) had high IPI, while the younger ones had the high-intermediate IPI more frequently (29,3%). In the group analyzed as a whole, patients with the high IPI, experienced all their functional abilities as significantly lower, compared to those of better prognosis. The exception was their cognitive functioning (CF), that did not depend on the index ($p=0,12$). The patients personal assessment of the most of their physical disabilities (fatigue, nausea, pain, dyspnoea, appetite loss) also depended on the IPI ($p<0,01$). When evaluating the subgroup of patients younger than 60 years, the only functional abilities found independent on the aaIPI were the CF ($p=0,16$) and the social functioning (SF) ($p=0,09$). The half of the investigated physical disabilities (fatigue, dyspnoea, appetite loss, insomnia) were experienced as significantly dependant on the aaIPI by younger patients ($p<0,01$). On the contrary, the only QOL components experienced as dependant on the aaIPI by the elders, were the SF ($p=0,04$), the global assessment of QOL ($p=0,008$) and the fatigue ($p=0,01$).

Summary: The changes in the physical health lead to the persons experience of lowering own functioning abilities and global QOL but the data suggest that the perception, the experience and/ or the communication of QOL seem to be age-dependent. An age specific instrument for the QOL assessment in the NHL patients seems to be needed.

815 CLINICAL EVALUATION OF ONCE WEEKLY DOSING

OF EPOETIN ALPHA IN MULTIPLE MYELOMA PATIENTS: IMPROVEMENT IN HEMOGLOBIN ARE SIMILAR TO THREE-TIMES-WEEKLY DOSING

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Introduction. The prevalence of anemia in patients with multiple myeloma is greater than 80%. Erythropoietin (EPO) leads to achievement and maintenance of high haemoglobin levels, which are of considerable prognostic significance in patients with multiple myeloma. The efficacy of single, weekly doses of EPO has been recently confirmed.

Purpose of this study was to evaluate the clinical effects of single, weekly doses of EPO in anemic patients affected by multiple myeloma (MM).

Patients and Method. We studied 43 anemic MM patients, 23 men and 20 women, who were diagnosed and treated in our clinic during the past five years. Their mean Hb level before EPO treatment was 10.5 gr/dl (range: 6.3-12 gr/dl). Patients were randomized in two groups and EPO was administered subcutaneously, at a dose of either 10.000 IU three times a week or 40.000 IU once weekly. Hb level response was evaluated 4 weeks and 3 months after treatment initiation. A major response was regarded an increase of untransfused Hb levels equal or greater than 2 gr/dl, while a minor response was regarded an increase in Hb levels of at least 1 gr/dl. No response was defined as a response less than a minor response.

Results. Twenty-six patients received EPO at a dose of 10.000 IU three times a week. According to defined response criteria, at 4 weeks after treatment initiation, 19 patients (73%) responded to EPO treatment. Eight patients (30.8%) attained a major response, while 11 patients (42.2%)

attained a minor response. Seven patients (27%) did not respond to EPO treatment. Three months after treatment initiation, Hb levels remained stable or slightly raised in 7 major responders, while 1 patient presented with decreased Hb levels, due to disease progress and 9 minor responders attained major response. The remainders required an increase of EPO dose to 150 U/kg 4 times a week. Seventeen patients received EPO at a dose of 40.000 IU once weekly. At 4 weeks after treatment initiation, 12 patients (70.6%) responded to EPO treatment. Six patients (35.3%) attained a major response, while 6 patients (35.3%) attained a minor response. Five patients (29.4%) did not respond to EPO treatment. Three months after treatment initiation, Hb levels remained stable or slightly raised in 5 major responders, while 4 minor responders attained major response. The remainders required an increase of EPO dose to 150 U/kg 6 times a week. Between the two groups, compliance was equal but convenience was superior in the group receiving the single weekly dose. **Conclusions.** Our study shows significantly increased and sustained response of anemic MM patients to recombinant human erythropoietin treatment and suggests that 40.000 IU of EPO given once a week is at least as effective as the more frequent three times a week administrations of the drug usually employed in MM patients and optimizes patients convenience.

816 QUALITY OF LIFE IN PATIENTS WITH AGGRESSIVE NON-HODKINS LYMPHOMA AND THE THERAPY OUTCOME

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Background: The gain in the Quality of Life (QOL) is an attainable aim when aggressive Non-Hodgkins Lymphoma (NHL) patients are treated. It is important to know which QOL domains depend on the therapy outcome.

Aims: The aims of this study are to see which are the QOL domains that differ significantly between responders and non- responders and if those differences last.

Methods: A group of 109 adult patients with aggressive NHL is presented. The patients QOL has been assessed using the QLQ-C30 (Aaronson, 1993) and The Experience of Quality of Life Questionnaire (EQLQ) (Berger, 1989) at the time of diagnosis, the therapy evaluation and six months later. EQLQ measures the patients perception of his health, his life, his family and social relationship and orientation towards the future.

Results: Patients that achieved complete remission (CR) assessed almost all the components of their QOL as better when compared to patients with no remission (NR) and they experienced less physical disabilities. The experience of family relationship (EFR) was the only QOL domain that did not differ in the two subgroups ($p=0,54$). In the sixth month of the follow-up neither the experience of social relationship ($p=0,11$) nor EFR ($p=0,63$) differed between them significantly. At that time of evaluation, patients in remission assessed their functional abilities as better comparing to non-responders, but the differences between them were of minor statistical significance comparing to the estimations made six months before. As for the physical symptoms, there were no differences in the patients experiences regarding their hematological status any more.

Summary: The results suggest that the family coherence did not depend on the therapy outcome. This is important because of the possibilities that doctor-family cooperation means for the treatment. Patients in CR have more difficult social tasks; some domains of functioning (like role and cognitive) may get better in spite of lymphoma resistance; non-responders may have more intensive medical care and attention and the late toxicity effects have their impact on all the patients. These are the possible explanations for the fact that QOL of responders and non-responders seem to become more similar during the follow-up.

817 CHEMOTHERAPY IMPACT ON GASTROINTESTINAL SYSTEM'S MUCOSA ABSORPTION IN PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA (AML)

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Background. The role of leukemic cells and the chemotherapy impact on the gastrointestinal system's mucosa is not entirely clear. It is still unknown whether patients with AML have small intestinal malabsorption, which also can be changed by antileukemic therapy or whether that

malabsorption might have impact on patients' malnutrition. Neoplastic cachexia in leukemic patients has been observed very often especially in those cases with high expression of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α).

The aim of the study was to assess an ability to absorption in small intestine by D-xylosis test in AML patients before and after induction chemotherapy and compare with body mass index (BMI) and serum concentration of IL-6 and TNF α .

Patients and methods. Twenty-three patients with AML we enrolled in the study: 11 females and 12 males aged 25-77, mean 48 years. According to FAB classification: 1 patient represented AML-M0, 4 patients- M1, 8 patients- M2, 9 patients 'M4 and 1 patients-M6. All patients received standard chemotherapy including farmorubicine and Ara-C. D-xylosis tests were performed before and 14-18 days after first course of chemotherapy. Concentration of IL-6 and TNF- was measured by ELISA method in the same time.

Results. There was no statistical difference between D-xylosis elimination, BMI and concentration of IL-6 and TNF- α before and after chemotherapy. Concentration of IL-6 was statistically higher before than after treatment (22.344.1pg/ml vs. 6.89.3pg/ml; $p=0.01$). There was no difference between TNF- α concentration in patients before and after treatment.

Conclusion. The data indicate that further examination on the chemotherapy role on malabsorption should be performed in patients with AML.

818 CLINICAL COURSE OF CML IN PATIENTS WITH COMPLEX PH CHROMOSOME TRANSLOCATION TREATED WITH IMATINIB THE ROLE OF CONVENTIONAL CYTOGENETICS AND RESIDUAL DISEASE MONITORING USING REAL-TIME QPCR

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Background: Chronic myelogenous leukemia (CML) takes a natural course with evolution from chronic (CP) towards acceleration phase (AP) and blast crisis (BC) characterised by fatal outcome. Introduction of imatinib, and the development of reliable methods for quantitative PCR (qPCR) have significantly influenced the course of CML. Treatment with imatinib induces major or complete cytogenetic remissions in more than 40% of patients resistant or intolerant to IFN α . qPCR is frequently used for monitoring patients in addition to or instead of conventional cytogenetics. Additional chromosomal abnormalities detected at diagnosis or arising during the CML treatment may have a prognostic value, and could be undetected when using qPCR alone in the assessment of residual disease during therapy.

Aims: to compare the course of CML in patients with complex and 'classical' Ph translocation treated with imatinib, and evaluate the role of qPCR and conventional cytogenetics in monitoring of residual disease in these patients.

Methods: Forty three patients treated with imatinib for CML in different stage were prospectively followed in three months intervals with RT-PCR, qPCR and karyotyping for the follow-up of 34 (mean 13) months. Comparisons were made between qPCR and karyotyping results from the bone marrow. Sokal index, Euro score, treatment response and progression-free survival was assessed.

Results: We detected complex Ph chromosome in 14% (6 out of 43) of patients at start of imatinib therapy. After 3 months of treatment 5 out of 6 patients (83%) (all from CP group and one from the BC group) achieved complete hematological response (CHR). One patient with CP achieved CCyR after 6 months of treatment with more than 2 log reduction of BCR-ABL transcripts in qPCR, being positive in RT-PCR. In one patient there was a 2 log reduction of BCR-ABL transcript in qPCR despite persisting of the complex Ph chromosome in the conventional cytogenetics. Two patients died because of CML progression; one from the CP and one from the BC group. In two patients (one with CP, one with BC) we were able to detect simultaneously p210 and p190 BCR-ABL transcripts by RT-PCR. The analysis of the Sokal and Euro score, the response rate to imatinib, clinical course and PFS, didnt differ significantly form patients with 'classical' Ph chromosome.

Summary/Conclusion: The course of CML in our patients with complex Ph seems to be similar to patients with 'classical' Ph, however the clear conclusion regarding the risk profile cannot be made because of their small number. Our observation of 2-log BCR-ABL transcript reduction in qPCR in patient with residual complex Ph is in accord with other studies reporting the occurrence of clonal cytogenetic abnormalities in the Ph-negative cells, which appeared after suppression of the Ph-positive clone by imatinib, and supports number of data suggesting that cytogenetics

should not be completely replaced by qPCR. False reassurances of successful imatinib treatment by qPCR can result in disease progression and delay of patients referral for alternate therapies.

819 -THALASSEMIA INTERMEDIA AND MALIGNANT LYMPHOMA: A REPORT OF TWO CASES

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Introduction. Malignant lymphomas are rare among β -thalassemia patients. We describe two patients with β -thalassemia intermedia who presented with non-Hodgkin lymphoma and Hodgkins disease, respectively.

Case reports. We refer to a male and a female patient, 37 and 52 years of age, respectively. They both suffered from β -thalassemia intermedia and were splenectomized at the age of 14 and 21, respectively. Since then, they were both on a regular transfusion schedule of 2 units of red packed cells every 3 weeks, in order to maintain a pre-transfusion hemoglobin concentration of 9-10gr/dl, and were treated with desferoxamine for iron overload. The male patient had chronic hepatitis C infection and was on antiviral therapy with ribavirin and interferon- α . The patients were followed-up monthly, in the Thalassemia Unit of our hospital, with detailed history and clinical examination, complete blood count and laboratory tests for renal and liver function.

During a scheduled follow-up, in June 2003, the male patient presented with cervical, axillary and inguinal lymphadenopathy, persisting for more than 3 weeks, without co-existence of constitutional symptoms. Cervical lymph node biopsy revealed a marginal zone B-cell lymphoma. The clinical stage was IIIA.

The female patient was referred to the Thalassemia Unit in November 2003, with a left supraclavicular lymph node. Physical examination revealed the presence of bilateral axillary and inguinal nodes. Constitutional symptoms were absent. Supraclavicular lymph node biopsy showed lesions compatible with the non-classical Hodgkins disease of the nodular lymphocyte predominant subtype. The clinical stage was IIIA.

Conclusion. On reviewing the literature, only five cases of thalassemia and lymphoma are reported worldwide, thus making our cases the sixth and seventh report of this rare combination of diseases. The increase of mean survival in β -thalassemia patients due to the current medical facilities, the chronic blood transfusions, the state of altered immunological reactivity and the chronic infections may have an impact on the incidence of malignant lymphomas among these patients. Further studies are needed in order to understand the relationship between the two entities.

820 AUTOLOGOUS STEM CELL TRANSPLANTATION FOLLOWING EFFECTIVE TREATMENT WITH THALIDOMIDE OF PATIENTS WITH MULTIPLE MYELOMA RESISTANT TO CONVENTIONAL CHEMOTHERAPY

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Background: Thalidomide has proven to be effective in treatment of myeloma. It inhibits angiogenesis and has an immunomodulatory effect. The aim of this study was to assess the effectiveness of mobilization and collection of progenitor stem cells of haematopoiesis as well as haematological recovery following autologous transplantation of progenitor cells in patients with resistant multiple myeloma, effectively treated with thalidomide.

Material and Methods: Autologous transplantation (PBSCT) was conducted in 10 patients treated in our Department for multiple myeloma resistant to standard chemotherapy. 7 males and 3 females aged at average 55 yrs (range 42-64) were treated. The patients had the following types of MM: 4 IgG kappa, 2 IgG lambda, 2 IgA kappa, 1 IgA lambda and 1 IgD lambda. All the patients had reached the IIIA clinical stage according to Durie Salmon staging system. Positive response to treatment with Thalidomide (at least PR according to SWOG) was achieved in all patients. The initial dose of 200mg/d of thalidomide was increased to 400mg/d after 4 weeks of treatment. Administration of thalidomide was combined with dexamethazone at a dose of 40mg/d for 4 days every 28 days. After a period of between 8-24 weeks of treatment with thalidomide (approximately 17 weeks), when best results were achieved, patients were qualified for stem cells mobilization. Cyclophosphamide at a dose of 4g/m² and G-CSF (Neupogen) at a dose of 5 mgm/kg/day were employed in the course of mobilization.

Results: Mobilization was successful in all cases, 2-3 cytaphereses were conducted at the average. The following results were obtained:

2.74x108/kg MNC (1.67-3.21), 4.45x106/kg CD34+ cells/kg (1.86-15.3), 30.85x104/kg CFU-GM cells/kg. Regeneration of granulopoiesis and platelets was achieved in all patients; median time to ANC>0.5G/l was 18 days, PLT>50G/l was 17 days, respectively. The mean time of observation following autoPBSCT is 10 months (4-18). Complete remission was achieved in 3 patients and partial remission in 7 patients. Relapse occurred in 3 cases, respectively 8 and 13 months after transplantation. Conclusions: Mobilization of progenitor stem cells, following effective with thalidomide and Dexamethazone is possible in resistant types of multiple myeloma. The effects of mobilization and haematological recovery in patients treated with thalidomide and those treated with conventional chemotherapy are comparable.

821 A STUDY OF HAEMOGLOBIN DISORDERS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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BACKGROUND- To study various Haemoglobin disorders using High performance liquid chromatography and to analyse the severity of illness in the backdrop of the quantitative defects obtained using the variant analysis. To study the epidemiological profile of various hemoglobin disorders among patients reporting to our hospital.

MATERIAL & METHODS: -50 patients with various haemoglobin disorder upto age of 12 years were detected using HPLC in Hamidia hospita BHOPAL M.P INDIA. Patients with other causes of anemia were excluded out.

OBSERVATIONS: -Out of 50 cases 58% were of thalassemia (22% of thalassemia minor), 38% were of sickling disorder with maximum were of sickle beta thalassemia. 2 patients had HbE beta thalassemia. There was a male preponderance of 2.6:1 with Hindus comprising 86% and 64% of cases were native of Bhopal (M.P.). There was a high prevalence of thalassemia 27.6% in Sindi community and sickling disorder in Tribal & Harijan community (84.2%). Progressive pallor & Abdominal and joint pains were the commonest presenting complaints in thalassemia, sickle cell anemia and sickle beta. Thalassemia respectively. In thalassemia major 88.9% received transfusion below 1 year of age. For nutritional status large number of patients were in the normal range. Caloric intake in patients with sickle disorder was approximately 83% of the expected calories contributing to reduced anthropometer parameters. 89.6% cases of thalassemia showed mild hepatomegaly (<4cm). 88.9% and 66.6% cases of thalassemia intermedia & major had spleen size between 4-8 cm. 44% cases of thalassemia major had hemoglobin value less than 5 gm/dl and means Hb in sickle was 7.5+1.72 gm% TRBC count in thalassemia was 2.28+0.39 m/Cu mm and in sickle cell was 2.92+0.46 m/Cu mm. 20.7% and 15.7 case of thalassemia and sickle cell disorder respectively had malaria. HbF values in thalassemia minor, major and intermedia were 6.5+3.5 (n=11), 90.1+2.99 and 62+15.4%, 3.01+1.27% 3.83+0.92% in thalassemia minor, intermedia, major and in sickle beta thalassemia.

CONCLUSION:

Percentage of foetal Hb as determined by HPLC was found to be higher and more in concordance with clinical status and severity of illness of the patients in comparison to the values of obtained by Alkali denaturation test.

822 INTRAVENOUS IRON SACCCHARATE FOR THE TREATMENT OF IRON DEFICIENCY ANEMIA IN CHILDREN

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BACKGROUND: Iron deficiency is the most common cause of anemia worldwide, particularly in children. Although often associated with bowel and kidney disorders, iron deficiency is frequently caused by insufficient dietary intake, especially in infants and young children. Oral supplementation can efficiently replace stores. However, in many cases parenteral iron is needed, due to the severity of iron deficiency or to childrens low compliance, for a rapid and efficient iron store replacement. Unfortunately, some adverse reactions reported with parenteral iron therapy have led to its limited use in children.

AIM: The aim of this study was to evaluate the safety and efficacy of intravenous iron saccharate administration in iron-deficient anemic children, who are unable to tolerate (gastrointestinal dysfunction or iron

malabsorption), or failed to respond (mental retardation, bad compliance) to oral iron supplementation.

METHODS: Five children aged 2.5-11 years old (median: 6.4 years), 3 boys and 2 girls, received intravenous infusion of iron saccharate. Total iron to be infused was determined by the formula: Total iron[mg] = Weight[kg] x (expected Hb* patient Hb) [g/l] x 0.24 + iron stores* [mg], (*expected Hb = 130 g/l, *iron stores = 15 mg/kg). Three children suffered from other diseases (cerebral palsy, Aicardi syndrome, ulcerative colitis) and 2 had no underlying problem (bad compliance). Intravenous iron saccharate was given at the hospital. Hemoglobin values, reticulocytes count, serum iron and serum ferritin were determined before iron administration (day 1), as well as 15 and 45 days after iron administration.

RESULTS: Tolerance of intravenous iron saccharate was good in all children, and no adverse reactions were noticed. Intravenous iron led to a significant increase in hemoglobin concentration (median-day 1: 6.34 g/dl, median-day 45: 9.34 g/dl). One child, suffering from ulcerative colitis, did not respond to parenteral iron therapy, even though she received the same dose three times in a period of 6 months.

CONCLUSION: Intravenous iron supplementation with iron saccharate leads to a rapid and efficient replacement of iron body stores, and significantly increases the hemoglobin concentration. Unfortunately, the experience in children is poor and the possible occurrence of allergic reactions emphasizes the need for close medical supervision.

823 EBV DRIVEN B CELL NHL ON A BACKGROUND OF PERIPHERAL T CELL NHL

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Peripheral T cell lymphomas are amongst the most aggressive forms of Non-Hodgkin lymphomas (NHL). Epstein Barr virus (EBV) driven lymphoproliferative disorder has been reported in angioimmunoblastic NHL but is rare in Peripheral T cell NHL (PTCL). We present two cases of PTCL unspecified with EBV-infected B cell blasts.

Both cases presented with widespread disease. Immunohistochemical staining of node biopsies identified two distinct lymphoid cell populations. The majority of the biopsies comprised small CD3 positive T cells. In addition, both patients had a population of large pleiomorphic cells which stained positive for B cell markers CD20 and CD79a. These cells also expressed CD30 and were positive for EBV. Standard clonality testing revealed both immunoglobulin heavy chain (IgH) and T cell Receptor (TCR) clonal rearrangements in patient 1 and a TCR monoclonal population in patient 2. Cytogenetic analysis of cultured lymphocytes from the nodes revealed two apparently unrelated abnormal clones in both patients. Combined immunofluorescence and fluorescent in situ hybridisation (FISH) on formalin fixed, paraffin-embedded tissue sections was undertaken. This enabled us to relate the phenotype of the abnormal cells to specific clonal populations of cells which revealed these phenomena were due to two distinct abnormal populations - one B cell, one T cell - rather than reactive B cell hyperplasia or biphenotypic evolution from a common ancestral lymphoma.

In both cases a true B cell malignancy appears to have emerged within a pre-existing but unrelated T cell lymphoma. Although immune dysregulation is known in association with angioimmunoblastic lymphoma it has not been commonly reported with peripheral T cell NHL. We hypothesize that both patients had a primary T cell NHL and the consequent impairment of T cell function permitted emergence of a B cell lymphoma from hyperplastic EBV-driven B cells.

824 NON-STEROIDAL ANTIESTROGENS AS POTENTIAL ANTILEUKEMIC AGENTS: MODE OF ACTION

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BACKGROUND: Our previous studies have shown that non-steroidal antiestrogens such as tamoxifen have multifactorial activities that are mediated by estrogen-receptor independent pathways. These activities include apoptosis, an antiproliferative effect and synergism with other drugs as well as reversal of drug resistance. **AIM:** In the present study, the mode of action of two non-steroidal antiestrogens, clomiphene and nafoxidine, on leukemic cells was investigated. **METHODS:** T lymphoblastic leukemia cells, CCRF-CEM, which lack the estrogen receptor, were treated with clomiphene and nafoxidine at different concentrations for 24 and 48 h. Cell viability was determined by XTT reduction, and apoptosis was determined by acridine orange and ethidium bromide staining. Translocation of PKC-epsilon was examined in subcellular fractions as measured by western blot analysis. Reactive

oxygen species (ROS) generation was measured by the fluorescent dye 2', 7'-dichlorofluorescein diacetate (DCFH-DA) using FACS analysis. RESULTS: Both clomiphene and nafoxidine induced apoptosis in CCRF-CEM cells. These drugs caused early translocation of PKC-epsilon from cytosol to the membrane which occurred after 30 min, while no change in PKC-alpha redistribution was observed. The L428 cell line which does not express PKC-epsilon was found to be less sensitive to these drugs. In addition, early changes in mitochondrial membrane potential were observed, which suggests the involvement of mitochondria in the cytotoxic effect of these drugs. The drugs also induced a slow increase in ROS production which was detected at longer periods of incubations. The antileukemic effect of these drugs was abrogated by addition of the antioxidants alpha-tocopherol and N-acetyl L-cysteine. These results indicate that ROS play a role in the apoptotic effect. Additionally, inhibitors of NAD(P)H oxidase had no effect on antiestrogen activity which points to another source of ROS production that is responsible for the cytotoxic effect of antiestrogens on leukemic cells. CONCLUSION: The antiestrogens, clomiphene and nafoxidine, induce apoptosis via an estrogen receptor-independent mechanism which involves PKC-epsilon translocation, mitochondrial activation and ROS production.

825 NECROSIS A MODE OF CONTROLLED CELL DEATH REGULATED BY PROTEOLYTIC ENZYME

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BACKGROUND: Necrosis and apoptosis are morphologically different manifestation of cell death. However, recent evidence suggests that necrosis may share common features with apoptosis and perhaps common mechanism. In order to regulate these forms of cell death it is of the utmost importance to understand the mechanisms underlying these processes. There are accumulating evidence-indicating involvement of serine proteases in apoptosis. However, much less is known about their role in necrosis. AIM: In the present study we investigated the involvement of chymotrypsin-like serine proteases (CLPs) in both necrotic and apoptotic cell death. METHODS: Necrotic and apoptotic cell death in U-937 cells were stimulated by different inducers. Cell death mode was determined by double staining with acridine orange and ethidium bromide. Proteolytic CLP-activity was assayed with synthetic substrate selective for chymotrypsin-like proteases. Another approach to examine proteolytic CLP-activity in necrotic cell death was by running cell extracts on gelatin substrate gels. Reactivation of proteolytic activity on gels was done in the presence or absence of specific protease inhibitors. RESULTS: It was found that TPCK (N-Tosyl-L-phenylalanyl-chloromethyl ketone) an inhibitor of CLP protects U-937 monocytic cell line from undergoing apoptosis and necrosis. TPCK inhibited apoptosis induced by anti-Fas or Staurosporine (STS). Moreover, TPCK inhibited necrosis induced by same inducers in the presence of oligomycin in glucose-free medium. However, TLCK (N-tosyl-L-lysine chloromethyl ketone) an inhibitor of trypsin-like proteases with a chloromethyl ketone group was not inhibitory in both apoptotic and necrotic system. 3, 4-Dichloroisocoumarin (DCI) another inhibitor of CLP that does not possess a chloromethyl ketone group had also inhibitory effect on potassium cyanide (KCN)-induced cell death. Induction of apoptotic and necrotic cell death was accompanied by increased CLP activity, as measured using specific chymotrypsin substrate. Our results indicate that cellular localization of the induced chymotrypsin-like enzyme/s is present in particulate and cytosolic fractions, but not in mitochondrial fraction. The purified enzyme was found to be of lysosomal origin.

CONCLUSIONS: These results indicate that CLP(s) play a role at an early stage of both apoptotic and necrotic death processes as demonstrated by inhibition of cell death using different CLP inhibitors as well as by an early induction of CLP activity induced by different stimulators. The results also imply the possible function of CLP in a common pathway leading to necrotic and apoptotic cell death.

826 ANALYSIS AND COMPARISON OF THE PRODUCTION OF CFU-GM AND BFU-E IN PATIENTS WITH DIFFERENT SUBTYPES OF MDS ACCORDING THE NEW WHO CLASSIFICATION

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Introduction: The new classification of MDS was proposed by WHO in 1999. It considers RAEB-t to be AML and also eliminates CMML, which is

included among myelodysplastic-myeloproliferative disorders (MDS/MPD). Aim: The aim of the study was to comparison the production of CFU-GM and BFU-E in patients with different subtypes of MDS according the new WHO classification and MDS/MPD (CMML1 and 2).

Methods: The study group contained of 54 patients with MDS and 5 with MDS/MPD. There were: 7 patients with RA, 17 RCMD, 2 RARS, 5 RCMD-RS, 3 5q-, 1-MDS-U, 10-RAEBI, 9-RAEBII, 1-CMML with WBC<13 G/L and 4-CMML with WBC>13 G/L. The diagnosis of MDS and MDS/MPD was established on the basis of generally agreed criteria: bone marrow cytology, histology, karyotype and peripheral blood count. Bone marrow mononuclear cells (BMMC) were isolated using density gradient centrifugation (Lymphoprep 1.077 g/ml). The interface was collected and washed twice using IMDM medium. The cells were resuspended to 2x10⁶ /ml in IMDM and seeded in semisolid methylcellulose (Methocult 4230 or 4434) for 0,1 ml to 3 ml of medium. The cells were placed in two dishes containing Methocult 4230 with G-CSF (100 ng/ml) or GM-CSF (100 ng/ml), or Methocult 4434 - SCF (50 ng/ml) plus IL-3 (10 ng/ml) plus EPO 3 U/ml and GM-CSF (10 ng/ml). Control dishes contain only medium and cells for detection of endogenous CFU-GM. Cultures were incubated for 14 days at 37C in humidified 5% CO₂ air atmosphere and then examined for CFU-E at day 7, and CFU-GM and BFU-E at day 14. CFU-E with 6 and more cells and CFU-GM with 50 or more cells were counted.

Results: Results of production of cell cultures in each group (subtype of MDS according WHO classification) were contained in tabl. 1.

Summary: The autonomous growth of CFU-GM was observed only in patients with CMML with WBC>13 G/L compared with patients with MDS (all subtypes) and CMML with WBC<13 G/L.

Subtype (number of patients)	Autonomous Mean* [min (PTS)-max]	+ G-CSF Mean* [min (PTS)-max]	+ GM-CSF Mean* [min (PTS)-max]	4434+SCF+IL-3 CFU-GM Mean* [min (PTS)-max]	BFU-E Mean* [min (PTS)-max]	CFU-E Mean* [min (PTS)-max]
RA (n=7)	3 [0(4)-17]	5 [0(3)-17]	33 [0(2)-165]	31 [0(1)-179]	76 [3-165]	n
RCMD (n=17)	5 [0(1)-20]	31 [0(4)-239]	71 [1-399]	119 [0(2)-701]	21 [0(2)-62]	57 [2-252]
RARS (n=2)	0(2)	32 [0(1)-16]	32 [16-48]	n	n	2 [0(1)-5]
RCMD-RS (n=5)	19 [0(2)-136]	27 [0(1)-31]	72 [2-421]	153 [5-399]	44 [5-612]	n
5q- (n=3)	0(3)	3 [2-6]	5 [5-146]	128 [17-257]	4 [0(1)-103]	n
MDS-U (n=1)	4	25	58	160	199	n
RAEBI (n=10)	15 [0(5)-60]	34 [0(3)-112]	55 [2-232]	197 [5-659]	44 [0(1)-147]	n
RAEBII (n=9)	0 [0(0)-29]	0 [0(4)-50]	27 [0(1)-125]	n	n	55 [0(2)-205]
CMML1 (n=1)	0	0	n	0	0	0
CMML2 (n=5)	146 [1-402]	31 [5-212]	186 [5-425]	36 [125-614]	8 [0(2)-26]	n

PTS - number of patients without growth (number of cells cultures = 0)
n - it means that cells cultures weren't done.
* - mean of the number of cells cultures

Caption 1: Results of growth of CFU-GM and BFU-E in each grp.

827 BONE MARROW ANGIOGENESIS- COMPARISON OF THREE METHOD OF IMMUNOHISTOCHEMICAL STAINING ENDOTHELIAL CELLS

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Background:

Bone marrow is the one of the first place of activity diseases in hematological malignancies. Angiogenesis means branching and growing of existing vasculature, which are built of vascular endothelial cells. The extent of angiogenesis in bone marrow can evidence intensity of disease process. The identification of pathological microvessels can be consist in immunohistochemical analysis of bone marrow biopsy and application monoclonal antibodies for endothelial cells glycoprotein.

Aims:

The estimation of bone marrow angiogenesis and comparison sensitivity and speciality of used methods.

Material and method:

The application of three monoclonal antibodies IgG1 kappa:

-anty von Willebrand factor (vWF),

-anty CD31 antygen,

-anty CD34 antygen.

Seventy paraffin-embedded bone marrow core specimens from adult patients were studied in three groups (according to histopathological diagnosis):

1.normal bone marrow- 20 specimens
2.bone marrow with infiltration of limphatic cell or increase number of plasma cell- 40 specimens
3.bone marrow with plasmocytoma- 10 specimens
For each trepanobiopates sections were performed immunostaining for vWF, CD31, CD34 and negative control by a labeled streptavidin-biotin peroxidase method. Detection system-LSAB +Kit (Dako) was used for antigen visualization.

A optic microscopy was used in the identification by two independent viewers, who each evaluated slides in a blinded fashion. Slides were scanned at 100x and 200x magnification , and three areas of maximal microvesells ('hot spots') were counted at 400x magnification. In this way was defined mean number of vessels per area in each sample and noted as microvessel density (MVD).

Results:

(Tab. 1)

1.The negative control was corect in each groups.
2.In each groups we finded the non-staining vessels, who were identified on the basis of their morfology.

Summary

1.All immunostaing method of microvessels confirm increase angiogenesis in pathological bone marrow biopsy.
2.We obtained the best immunohistochemical resaults with anti-CD34 monoclonal antibody. This method has the best sensitivity for each groups.
3. The immunostainig for CD31 detects the smallest number of microvessels in each groups. This method has the worst sensitivity in our study.
4. The unequal distribution of angiogenesis in the single group can be resaulting to diffrent grading of clinical progresion of desease.

group	number of microvessels		
	vWF	CD31	CD34
1	0-5	0-1	0-1
2	0-5	0-3	0-21
3	0-15	0-5	0-25

Caption 1: Tab 1: Results

828 CLINICAL SIGNIFICANCE OF P53 EXPRESSION IN MULTIPLE MYELOMA

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Background: Although mutations of p53 are one of the most often acquired genetic changes in malignant tumors, these mutations are rare events in patients with newly diagnosed multiple myeloma (MM). Moreover, there are a few literature data about clinical significance of overexpression of this protein in multiple myeloma.

Aim: The aim of this study was to evaluate clinical significance of p53 expression in MM.

Patients and methods: 59 patients with newly diagnosed MM (27 females and 32 males, median age 62 years) were enrolled in the study. The diagnosis of MM was made according to the criteria of Chronic Leukemia-Myeloma Task Force. Clinical staging was done according to the Durie and Salmon classification (4 patients had disease stage I, 15 patients stage II and 39 patients stage III). The histological grade and histological stage (i.e. volume of myeloma infiltration) were determined according to the models proposed by Sailer and Bartl, respectively. We used standard immunohistochemical analysis with p53 antibody on B5-fixed and paraffin-embedded bone marrow specimens to evaluate expression of p53 in myeloma cells. The specimens were considered positive when equal or more than 5% plasma cells showed nuclear positivity.

Results: p53 expression was detected in 9 out of 59 patients (15.25%). p53 expression did not correlate significantly with clinical stage (I+II vs. III), 2-microglobulin level (≤ 6 mg/L vs. >6 mg/L), histological grade (I vs. II+III), histological stage ($< 20\%$ vs. $21-50\%$ vs. $> 50\%$) and the extent of osteolytic lesions (≤ 3 vs. > 3 lesions). Median survival of patients with p53 immunoreactivity in $\geq 5\%$ plasma cells was 10 months, whilst median survival of patients with p53 immunoreactivity in $< 5\%$ plasma cells was 36 months. However, this difference was not significant ($p = 0.2$).

Conclusion: Frequency of p53 expression in our group of newly diagnosed MM was relatively low. p53 expression was not associated with clinical and histological features of more aggressive disease, nor with the short survival.

829 SECOND CANCERS IN PATIENT WITH MULTIPLE MYELOMA AND WALDENSTROM MACROGLOBULINAEMIA

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Background: It was previously reported that patients with multiple myeloma (MM) and Waldenstrom macroglobulinaemia (WM) have an increased incidence of solid cancers.

Aim: The aim of this study was to analyze the incidence and localization of solid cancers in patients with MM and WM.

Patients and methods: We retrospectively analyzed the occurrence of solid cancers in 48 patients with MM and 28 patients with WM between 1994 and 2002.

Results: Solid cancers were observed in 5 out of the total of 48 MM patients (10.41%). At the time of diagnosis of MM, four pts were in clinical stage III, and only one in clinical stage I according to Durie-Salmon classification system. All patients were treated with melphalan-prednisol chemotherapy for two years. The median age of MM cases at the time of diagnosis of solid cancers was 76 years. Associated tumors included: breast carcinoma (2), endometrial carcinoma (1), bladder carcinoma (1), prostate carcinoma (1) and skin carcinoma (1). The coexistence of two solid cancers was diagnosed in one patient (breast and endometrial carcinoma). In one case solid cancer was diagnosed simultaneously with MM and in four cases subsequently after median of four years from diagnosis of MM. All patients died, four because of the progression of the solid cancer (after 14, 19, 40 and 12 months) and one because of progression of myeloma (24 months after establishing diagnosis of skin carcinoma). Solid cancers were observed in 6 out of the total of 27 WM patients (22.2%). Five patients were treated with chlorambucil-prednisol chemotherapy and one patient was treated with melphalan-prednisol chemotherapy. The median age of WM cases at the time of diagnosis of solid cancers was 68 years. Associated solid malignancies included: carcinoma of the colon (2), prostate cancer (2) breast cancer (1) and bladder cancer (1). In all cases second cancers was diagnosed subsequently after median of four years from diagnosis MW. At the time of the analysis, two cases died because progression of the solid cancer (21 and 70 months after establishing diagnosis of second cancer).

Conclusion: The occurrence of solid cancers in our relatively small group of patients with MM and MW is not rare. The solid cancers can develop simultaneously or, more often, subsequently after median of four years from establishing an diagnosis of MM or MW. Although aged patients are in higher risk for development of solid cancers in general population, it seems that the treatment with alkylating agents and the imunoproliferative disease itself contribute to the development of solid cancers.

830 TUNNELED CENTRAL LINES COMPLICATED BY THROMBOSIS IN CHILDREN WITH CANCER

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Children with cancer require a long term vascular access to administer chemotherapy, fluids, blood products, drugs and parenteral nutrition. The use of indwelling catheters (IC) provides an important means of venous access however implantation of IC can cause thrombotic complications, even in patients with thrombocytopenia.

Aim of the study: to report on our data of thrombotic complications associated with IC implantation in children with cancer.

Patients: 119 children with cancer (40 girls, 79 boys aged 1- 18 years), who underwent IC implantation.

Method: a retrospective analysis of data collected from medical records of children with cancer treated in our institution between January 2000 and December 2003.

Results: Mean platelet count prior to canulation was 132 G/l (3-469 G/l). In children with pre - implantation platelet count below 50 G/l the procedure was proceeded or accompanied by platelet transfusion. The total rate of thrombotic events was 46.2% (55/119). Subclavian vein thrombosis (SVT) occurred in 2.5% (3/119), thrombosis of the catheters lumen (diagnosed upon the inability to aspirate blood with or without inability to flush occluded catheter) occurred in 43.7% (52/119).

The patients with SVT were treated with tPA and/or LMWH. tPA alone in continuous infusion was used for the treatment of thrombosis of the catheter. In 21.8% (12/55) cases the catheter had to be removed due to rupture of occluded lumen or ineffective thrombolytic treatment. Conclusions: The thrombosis of central veins and/or the lumen of the catheter is the frequent complication of central vein cannulation. However it results in the necessity of catheter removal in 21.8% of patients with thrombosis.

831 PULMONARY INVOLVEMENT IN PLASMA CELL DYSCRASIAS: A SET OF FOUR CASES

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Background: Pulmonary involvement in plasma cell dyscrasias is quite rare and often mirrors high biological aggressivity of the malignant clone. It can be divided into three main subtypes: 1) solitary pulmonary plasmacytoma which can be completely cured by radiotherapy, 2) pulmonary or pleural mass in typical myeloma which does not necessarily worsen the prognosis, 3) myelomatous pleural effusion that is often encountered in the terminal phase of the disease.

Aims: To demonstrate the diagnostic and therapeutic approach to such patients.

Material and Methods: Authors describe four cases of patients treated at Charles University Teaching Hospital in Hradec Kralove. Patient No.1 presented with exertional dyspnea, cough and hemoptysis. He underwent bronchoscopy which revealed an intrabronchial tumour. Surprisingly, biopsy showed infiltration by plasma cells and solitary intrabronchial plasmacytoma was diagnosed. The patient was treated by bronchoscopic laser evaporation and subsequently by radiotherapy. Six years after the diagnosis he is fit and well. Patient No.2 developed a pulmonary infiltrate during a typical course of multiple myeloma. Myelomatous infiltration was verified biotically. The patient received four regimens of standard VAD-like chemotherapy and after that high dose chemotherapy (Mel 200) followed by autologous transplantation. Two years after he is in complete remission and receives maintenance treatment consisting of CED regimen once every four months. Patients No. 3 and 4 presented with myelomatous pleural effusion in their terminal phase. Despite repeated intrapleural bleomycin and hydrocortisone instillation and thalidomide treatment, their survival did not exceed four months from the diagnosis of the effusion. Conclusion: Pulmonary or pleural involvement in multiple myeloma is rare. Nevertheless, it is important to think of this possibility when differential diagnosis of pleural or pulmonary mass at a chest X-ray or CT scans is being made. Solitary pulmonary plasmacytoma is curable with radiotherapy alone. Pleural or pulmonary mass in typical multiple myeloma often resolves when treated by standard chemotherapy. Myelomatous pleural effusion usually represents the terminal phase of the disease and the prognosis is poor.

832 EVOLUTION OF HEMOSTASIS IN 90 CASES OF ACUTE PROMYELOCYTIC LEUKEMIA (APL) TREATED WITH ALL-TRANS RETINOIC ACID(ATRA)

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Background: APL is characterized by a complex alteration of hemostasis combining thrombocytopenia and severe coagulopathy triggered by several mechanisms: disseminated intravascular coagulation (DIC), fibrinolysis, proteolysis.

Aims: follow-up of the evolution of hemostatic changes during induction therapy with ATRA +/- chemotherapy.

Methods: we analyzed 90 cases with APL de novo. Evaluation of hemostasis: clinical, platelet count, PTTa, PT, AP, fibrinogen, FDP, D-dimers, soluble fibrin monomers, ATIII, euglobulin clot lysis time, morphologic assessment of the presence of schistocytes, DIC score of the ISHT.

Results: Hemorrhage was present in 82 patients (91%) and severe coagulopathy was found in 30 cases (33.3%) at onset. The severity of abnormalities of hemostasis was correlated with high numbers of leukocytes and peripheral blasts. The main mechanism of coagulopathy was DIC present in 84 cases (83.3%), including 12 patients with overt DIC. Remission rate was 82.2%. Treatment with ATRA resulted in rapid improvement of hemostatic disturbances. Despite this evolution 52.2% of cases presented bleeding during the induction period, 73.3% had

alteration of hemostasis mainly correlated with hyperleukocytosis and the introduction of chemotherapy. The major cause of early deaths was cerebral hemorrhage. Anticoagulant therapy had no significant impact on the evolution of the coagulopathy.

833 ABI PRISM7000 AND LIGHTCYCLER TECHNOLOGIES FOR THE QUANTIFICATION OF MINIMAL RESIDUAL DISEASE IN CHRONIC MYELOGENOUS LEUKEMIA (CML)

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Background: Quantitative data for minimal residual disease (MRD) in CML following therapy with Imatinib mesylate or bone marrow transplantation can be obtained by Real-Time quantitative PCR (RQ-PCR) analysis of BCR-ABL fusion gene transcripts using the commercially available apparatuses for Real Time PCR.

Methods: We compared the flexibility and sensitivity of two widely used Real Time PCR instruments: the ABI PRISM7000 (Applied Biosystems, USA) and the LightCycler (Roche, Switzerland). The RNA was extracted by the guanidinium isothiocyanate method from 3x10⁶/ml K562 cells or from PB and BM cells of CML patients on Imatinib mesylate therapy. In vitro reverse transcription of 1 µg of total cellular RNA to cDNA was performed using random hexamers as primers and Moloney Murine Leukemia Virus reverse transcriptase. In both detection systems we performed the same MRD assay using the primers and hydrolysis probes for the control gene (ABL) and the fusion gene (M-BCR-ABL) from the EAC (Europe Against Cancer) standardized RQ-PCR protocol. (FAM (6-carboxy fluorescein) was the reporter dye at the 5'-end of the probes and TAMRA (6-carboxytetramethyl rhodamine) was the quencher dye at 3'-end.) The cDNA used was the same for both assays. A standard curve for control (ABL) and fusion gene (M-BCR-ABL) was prepared as a test for the sensitivity of the assay and as a reference for the quantification. For the preparation of the standard curve we used serial dilutions of a plasmid suspension containing inserted cDNAs corresponding to the above mentioned genes (Ipsogen, France). The PCR conditions were modified in order to be adapted in the two different detection formats. Within this context BSA was added to the LightCycler reaction mixture and ROX (passive dye) to the ABI PRISM7000 reaction mixture. All experiments were performed in triplicate.

Results: Plots of the standard curves (Ct values against log copy number) showed good linearity for control gene and fusion gene in both different detection formats. Differences of Ct values were not higher than 1.5 Ct on the lowest dilution point and less than 0.5 Ct on the high concentration points with both instruments. The K562 cell samples were tested at neat, 10⁻¹, 10⁻² and 10⁻³ dilutions. Analysis of the results was done on the basis of the ratio of copy numbers of BCR-ABL/ABL expressed as a percentage. The analysis showed a good agreement between the two different technologies and the estimated ratios of BCR-ABL/ABL were reproducible. Of the 8 samples obtained from CML patients 6 gave reproducible BCR-ABL/ABL ratios, 2 showed discrepancies of the calculated BCR-ABL/ABL ratios of the order of 5%-8% between the two instruments.

Conclusion: Both real-time PCR systems provided similar, reproducible, specific and sensitive results and prove suitable for the MRD quantification in the range required for clinical decision-making within ongoing therapeutic protocols. The quantification assays can be easily transferred from one detection system to the other by appropriately adapting the PCR conditions for each technology.

834 IN VITRO TESTING FOR IMATINIB RESPONSIVENESS IN CML

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Background: Imatinib mesylate (STI571; Glivec; Gleevec) is a selective inhibitor of the protein tyrosine kinases ABL, PDGFR α , KIT, ARG and PDGFR β and has proven to be much more effective for the treatment of chronic myelogenous leukemia (CML) than conventional therapy. However, although approximately 90% of patients in the chronic phase of CML have initially a satisfactory response to imatinib therapy, the number of patients who later cease to respond is steadily increasing. During the accelerated phase or blast crisis of CML most patients appear to have developed resistance to Imatinib. Moreover, in a small percentage of chronic phase patients the resistance to imatinib is primary. The most common mechanisms of resistance are: (i) point mutations of the BCR-ABL tyrosine kinase domain, (ii) genomic amplification or overexpression of the BCR-ABL hybrid and (iii) clonal evolution with additional karyotypic aberrations.

Methods: We have developed two in vitro methods to assess the Imatinib mesylate sensitivity in CML patients. Mononuclear cells (MNCs) were obtained from peripheral blood (PB) or bone marrow (BM) of CML patients. The MNCs were placed in liquid cultures and in methylcellulose medium with growth factors and treated with 0, 1.0 and 5.0 μ M Imatinib. The response to Imatinib was evaluated on the basis of the number of colonies and the number of viable cells which were counted over 14 to 21 days (for the liquid cultures) and on days 7 and 14 (for the methylcellulose cultures) in comparison to cultures from the same patients containing no Imatinib and cultures of MNCs from normal individuals grown in presence of Imatinib. Cell viability was determined by the trypan blue assay. **Results:** Cultures from CML patients in chronic phase (7 patients) grown in presence of Imatinib showed a reduction of both the number of colonies and that of viable cells in comparison with the control cultures from the same patients and the cultures from normal individuals treated with Imatinib. In contrast, the MNCs cultures of 2 out of 3 CML patients in blastic crisis failed to show any changes in response to Imatinib despite dose escalation; one additional patient with CML in blastic crisis displayed the chronic phase pattern, while a last one in chronic phase but with additional chromosomal aberrations on chromosome 16 failed to show any response. The study progressively recruits more patients. **Conclusions :** In vitro cultures of MNCs from the peripheral blood and bone marrow of patients with CML may prove useful for the identification of patients who could have or may develop resistance to Imatinib therapy. Furthermore, they may provide indications for the potential response to combinations of imatinib with other therapeutic agents.

835 GENETIC RISK FACTORS OF ACUTE LEUKAEMIAS REALIZATION IN CHILDREN SUFFERED FROM CHERNOBYL ACCIDENT

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Background: The prevalence of acute lymphoid (ALL) and myeloid (AML) leukaemias among oncological diseases in children causes a necessity of studying of different prognostic factors of pathology development. Fusion genes and HLA- genes are genetic markers of leukemia process.

Aim: to study the diagnostic and prognostic significance of fusion genes and HLA system genes in development of acute lymphoid and myeloid leukaemias in children.

Methods: 85 children, 1-18 years age permanently living in territories contaminated with radionuclides (contamination of soils from 10 to 492 kBq/m²), children of clean-up workers (parents doses 16-75 cSv) and Kiev citizens. There were 56 cases of acute lymphoid and 29 cases of acute myeloid leukemia (M1, M2, M4 and M5 variants) under investigation in different treatment phases for 2 years period. Fusion genes (BCR/ABL, MLL/AF9, MLL/AF4, AML1/ETO, CBFB/MYH11, E2A/PBX1, TEL/AML1) expression were detected by RT-PCR method. The molecular HLA- genotyping on loci HLA-A *, HLA-B *, HLA-Cw *, HLA-DRB1 *, HLA-DQA1 *, HLA-DQB1* was carried out.

Results : Fusion genes expression has been revealed at 18 of 85 patients: at 14 from 29 AML cases and 4 from 56 ALL cases. No one case of tumor clones elimination had been revealed for supervision period. The significance of blood immunogenetic factors as prognostic markers of acute leukemia development in children is established.

Summary: Setting the simultaneous investigations of fusion genes and HLA system genes will allow to isolate patients groups with common genetic features and improve the leukemias treatment effectiveness.

836 COMPARISON OF THREE DIFFERENT TBI DOSE IN UNRELATED MATCHED MARROW TRANSPLANTATION FOR ADULT PATIENTS WITH SEVERE APLASTIC ANEMIA

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Background and Aims: To determine the optimal total body irradiation(TBI) dose as conditioning regimen in marrow transplantation from matched unrelated donor(MUD) for adult patients with severe aplastic anemia(SAA), we conducted a prospective study to evaluate minimal dose of TBI sufficient to achieve sustained engraftment when it is used in combination with cyclophosphamide(CY).

Methods: Between May 1998 and December 2003 twenty-eight patients were enrolled with a median age of 23(20-44). Conditioning regimen consisted of CY(120mg/kg) plus TBI and they were divided into 3 group according to fractionated TBI dose: group 1(1200cGy, n=5), group

2(1000cGy, n=9), and group 3(800cGy, n=14). Donor/recipient pairs were matched for HLA-A, -B by serology and -DRB1 by low-resolution DNA typing(n=15) or HLA-A, -B, and -DRB1 by high-resolution typing(n=13). Patients received CsA+MTx(n=4) or FK506+MTx(n=24) as GVHD prophylaxis. All patients were multiply transfused and 18 had received 1 or 2 course of intensive immunosuppressive therapy.

Results: All patients except one(group 2) achieved sustained engraftment. The incidence of acute GVHD more than grade II was not significantly different(60% in group 1, 38% in group 2, and 43% in group 3), but chronic GVHD developed more frequently in group 1 and 2(80%, respectively) compared to group 3(8%). Patients who received 800cGy(group 3) had significantly better survival than those 1000 and 1200cGy(93% in group 3, 44% in group 2, and 40% in group 1; group 3 vs group 1 and 2, p<0.05).

Conclusion: Thus, a TBI dose of 800cGy in combination with CY(120mg/kg) was sufficient to allow for engraftment in adult patients with SAA who received MUD marrow transplantation. Outcome in patients who received 800cGy of TBI was superior to survival that among patients 1000 and 1200cGy.

837 QUALITY OF LIFE IMPAIRMENT AND PREVAILING SYMPTOMS IN PATIENTS WITH ADVANCED HEMATOLOGICAL MALIGNANCIES A.A. Novik¹, T.I. Ionova², X.C. Wang³, T. Mendoza³, S.A. Kalyadina², M.O. Ivanova², O.Y. Uspenskaya², A.V. Kishitovich², C.S. Cleeland³

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Introduction: Patients with advanced hematological malignancies are burdened by symptoms related to the disease itself or the toxicities of treatment. Assessment of quality of life (QoL) impairment and identification of symptoms which result in QoL worsening provide the basis for effective symptoms control.

The goal of the research was to assess QoL impairment in patients with advanced hematological malignancies as compared with population norms and to reveal predominant symptoms in the above patients group.

Patients and methods: 98 patients with advanced hematological malignancies (33% -lymphoma, 22% - chronic lymphocytic leukemia, 20% - chronic myelocytic leukemia, 17% - multiple myeloma; male/female - 61/37; mean age 61.3, stages IIB-IV). All the patients underwent conventional treatment in the city hospitals of St.Petersburg. To compare with normative data the sample of St.Petersburg population (n=283) adjusted to age and gender was used (male/female 187/96; mean age 60.8 (SD 4.7). The Russian versions of SF-36 and M.D. Anderson Symptom Inventory (MDASI) were used for QoL and symptom assessment respectively. To obtain the QoL index (the higher the index the better QoL is) and the coefficient of QoL profile deformation (DC, the higher the coefficient the more deformed QoL is) the integral profile method was used. Statistical significance of differences between groups was tested by Mann-Whitney U-test.

Results: The QoL index in the patients with hematological malignancies was much less than in population sample (0.26 vs. 0.37; p<0.01). In addition, DC was significantly higher in patient group (1.1 vs. 0.7). Three most severe symptoms in patients with hematological malignancies were fatigue (mean 4.9; prevalence 93.2%), sleep disturbance (3.1, prevalence 75%) and pain (2.5, prevalence 60 %) were found. Other marked symptoms were shortness of breath (2.3), remembering (2.3) and distress (2.2). Symptoms interference was 2.9.

Conclusion: Patients with advanced hematological malignancies exhibit significant impairment of QoL as compared with healthy population. In addition, deformation of QoL profile takes place in the patients. Fatigue, sleep disturbance and pain are the predominant symptoms that contribute to QoL impairment. This data are of great importance for decision making in oncohematology.

838 AN UNUSUAL PRESENTATION OF AN IDIOPATHIC MYELOFIBROSIS PATIENT

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Extramedullary haematopoiesis (EMH) is one of the most frequent finding of idiopathic myelofibrosis (IMF) and generally occurs in liver and spleen. Rarely EMH may also develop in lymph nodes and other organs in association with liver and spleen involvement. However EMH in other organs without spleen and liver involvement is a very rare

finding. Here we present a case of 42-year-old woman with IMF who developed EMH only in lymph nodes after receiving granulocyte colony stimulating factor (G-CSF) for severe neutropenia. After the diagnosis of myelofibrosis on bone marrow biopsy etiological tests for secondary myelofibrosis were all examined and found in normal levels. Her clinical and bone marrow biopsy findings were also not compatible with AML-M7. So that she was diagnosed as IMF. During the three month follow-up period her pancytopenia progressively increased with a neutrophil count of 100/mm³ and a platelet count of 15000/mm³. As she was a young IMF patient bone marrow transplantation was decided. During this time because of severe neutropenia she began to receive G-CSF. After G-CSF therapy her neutrophil count progressively increased but at the same time bilaterally cervical multiple lymphadenopathy (LAP) developed. Pathological examination of lymph node biopsy revealed diffuse EMH. After cessation of G-CSF therapy her LAP progressively regressed. In literature there were two metastatic breast cancer patients with EMH in lymph nodes after G-CSF therapy. In both of these patients there were no bone marrow fibrosis. In our case expansion of EMH in lymph nodes after G-CSF therapy may be because of the existence of microscopic EMH focuses in lymph nodes prior to therapy. Development of LAP after growth factor therapy without liver and spleen involvement in an IMF patient is also an interesting finding. According to this finding microscopic EMH focuses in lymph nodes may be more sensitive to G-CSF therapy than spleen and liver.

839 PRETREATMENT EXPRESSION OF APOPTOSIS-REGULATING PROTEINS IN LEUKEMIC CELLS: THE POTENTIAL PROGNOSTIC SIGNIFICANCE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: There is indisputable need for a marker of the effectiveness of leukemia treatment that can rapidly report the response of tumor cells to chemotherapy. Because during chemotherapy these cells die by mode of apoptosis, the status of apoptosis-regulating machinery in na@ve leukemic blasts seems to be crucial for the response to cytotoxic treatment.

Aims: The aim of this study was to investigate the potential prognostic significance of pretreatment expression of apoptosis-regulating proteins in leukemia cells as well as frequency of their spontaneous apoptosis in patients with acute myeloid leukemia (AML).

Methods: Twenty one newly diagnosed patients with AML were entered to the study. Apoptosis was detected by two independent flow cytometry assays: Annexin-V/propidium iodide staining and detection of caspase activation by fluorescent pan-caspases inhibitor FAM-VAD-FMK. Additionally, expression of several pro-apoptotic (p53, Bak, Bax, Bid, Bcl-XL) and anti-apoptotic (Bcl-2, Bcl-XS, FLIP, XIAP, Mcl-1) proteins were assessed using flow cytometry. The percentage of spontaneous apoptosis (apoptotic index, AI) and the level of protein expression were correlated with the response to induction chemotherapy and routinely used prognostic factors, such as age, cytogenetics, leukocyte count and the number of chemotherapy cycles to achieve complete remission (CR).

Results: In the study group, 16 patients (76.2%) achieved CR after induction chemotherapy and five (13.8%) did not respond to treatment. The median time of follow up in the whole group was 34 weeks (range 2-65 weeks). At the time of statistical analysis, 10 patients (47.6%) remained in lasting CR, with the median time free of disease 29 weeks (range 11-42 weeks). The positive correlation was found between spontaneous AI of leukemic cells and early effect of induction chemotherapy, defined by the rapid increase in absolute blast count ($p < 0.01$). Higher pretreatment AI correlated also with the achievement of CR, however, the differences were not statistically significant ($p > 0.05$). High probability of long term CR had patients with upregulation of apoptosis promoters Bak ($p = 0.043$) and Bid ($p = 0.025$) in na@ve leukemic blasts. Tumor cells from those patients had also lower expression of Bcl-XL and FLIP proteins than other patients (n.s.; $p > 0.05$). Significant Bcl-XL upregulation was found in patients with high absolute leukocyte number (more than 30.0 G/L) at the diagnosis ($p = 0.012$). There was no significant correlation between protein expression and age over 60 years, unfavorable cytogenetics at the diagnosis or the number of chemotherapy cycles needed to achieve CR. The follow up of patients is continuing in our Department.

Conclusion: Results of this preliminary study indicate that the pretreatment profile of 'high Bak/Bid' and 'low XIAP/Bcl-XL' expression in leukemic blasts can serve as an independent indicator of good response to induction chemotherapy in AML patients.

This work was supported by the grant No 502-11-829 (157) from the Medical University of dz, Poland

840 R-FMC IS AN EFFECTIVE SALVAGE REGIMEN FOR FOLLICULAR AND MANTLE B CELL LYMPHOMAS

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Follicular and mantle cell lymphomas are usually chemosensitive at diagnosis but become progressively refractory to further therapies. Recently the combination of Rituximab with Fludarabine, Cyclophosphamide and Mitoxantrone (R-FMC) has been reported as effective in relapsed or refractory follicular and mantle cell NHL. We evaluated the toxicity, feasibility and effectiveness of the R-FMC salvage regimen in eleven previously treated patients (pts) (5 females and 6 males, median age 61, ranging from 47 to 77 yo) with follicular (9 cases) and mantle cell (2 cases) NHL, treated between January 2003 and January 2004. At diagnosis two pts were in stage I-II and nine in stage III-IV, whereas by the time of R-FMC treatment one were in stage I-II and ten in stage III-IV; only two patients had B symptoms. When starting R-FMC, no pts were considered low risk according to IPI, seven patients were intermediate and four were high risk. Pts had received a median of two (one to four) prior regimens; ten were pretreated with anthracyclines, five received purine analogues and four Rituximab. The treatment regimen consisted of Rituximab 375 mg/m² on day1, Fludarabine 25mg/m² on days 1, 2 and 3, Mitoxantrone 8 mg/m² on day 1 and Cyclophosphamide 200mg/m² on days 1, 2 and 3, delivered every four weeks. Four cycles were planned in each pt and prophylaxis with Acyclovir and Cotrimoxazol was given as recommended. In a total of 36 cycles delivered to eleven pts (since one pt progressed after 2 cycles and four are still on treatment), the major toxicity observed was mielossuppression. Seven patients needed G-CSF during treatment. Despite growth-factor support, neutropenia (< 1500 neutrophils/l) occurred at least once in nine pts and was severe (< 500 neutrophils/l) in three. Lymphocytopenia was observed in all patients who completed therapy, with a median of 500 (range 100 to 700) lymphocytes/l 3 to 4 weeks after the end of chemotherapy. Although five infectious episodes requiring antibiotic treatment occurred, only one patient was hospitalised with a CMV infection that resolved with Gancyclovir. Anemia (< 10 g/dl) and thrombocytopenia (< 100 000/l) were observed in two and three pts, respectively but only one needed erythrocyte and platelet transfusions. No pt died during therapy. Of seven pts evaluable for response, five entered a complete and one a partial remission (86% response rate), while another progressed and lately died after failing two more chemotherapy regimens. After a median follow up of 6 months, four pts are still on treatment, four are in complete remission, one is dead of progressive disease and two progressed and are receiving other therapies. The R-FMC salvage regimen is highly effective and may allow prolonged remissions with an acceptable toxicity in heavily pretreated, intermediate and high-risk NHL pts.

841 ATORVASTATIN MODIFIES THE EXPRESSION OF BOTH ENDOTHELIUM- AND LIVER- DERIVED COMPONENTS OF THROMBOSIS/FIBRINOLYSIS SYSTEM DURING THE ACUTE PHASE OF UNSTABLE ANGINA

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Background: Evidence suggest that lipid-lowering treatment with statins reduces mortality and morbidity in patients with coronary artery disease. The effects of lipid-lowering treatment during the early phase of the acute coronary syndromes are unknown. In this study we investigated the effect of atorvastatin treatment on plasma levels of tissue plasminogen activator (tPA), von Willebrand factor (vWF) and factors V (fV) and VII (fVII), in patients with unstable angina.

Methods: Forty-six patients (34 males 12 females, aged 64±8 years old) with unstable angina, were randomly divided into 2 groups and received atorvastatin 10 mg/day (n=23, ATR group) or no statin treatment (n=23, ATR group) for 6 weeks during and after admission. Plasma levels of tPA, vWF, fV and fVII were measured at baseline, at 1 week and at 6 weeks after the admission. Levels of the above thrombotic markers were determined by ELISA. All values are expressed as means±SEM.

Results: At baseline, levels of tPA, vWF, fV and fVII were not significantly different between ATR (10.030.78 ng/ml, 1076.5%, 1257.8% and 924.4%) and the control group (10.81.0 ng/ml, 109.39.2%, 1268.1% and 90.76.7%, $p = NS$ for all). After 1 week, serum levels of tPA, vWF, fV and fVII were significantly increased in control group (13.31.3 ng/ml $p < 0.01$, 1278.5% $p < 0.05$, 1527.4% $p < 0.001$ and 1048.6% $p < 0.05$ respectively, compared to baseline). This increase was prevented in

ATR group (12.71.8ng/ml, 1259.7%, 1345.8% and 1097.7% respectively, p=NS compared to baseline). However, at 6 weeks, plasma levels of tPA, vWF, fV and fVII returned to their baseline values in both ATR group (11.20.9ng/ml, 91.68.9%, 125.37.8% and 94.86.7% respectively, p=NS for all compared to baseline) and control group (11.40.95ng/ml, 98.810.6%, 1186.9% and 11431%, p=NS for all compared to baseline). Conclusions: During the first week of treatment, atorvastatin decreases the levels of factors V, VII, von Willebrand factor and tissue plasminogen activator in patients with unstable angina. These findings suggest that early administration of atorvastatin in patients with unstable angina, may be beneficial by affecting the thrombotic/fibrinolytic process.

842 PROGNOSTIC RELEVANCE OF CD34/HLA DR EXPRESSION IN ACUTE MYELOID LEUKEMIA PATIENTS

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Immunophenotyping is an usefull method for diagnosis and classification of acute myeloid leukemia (AML). In AML patients a variety of clinical and biological parameters, including phenotype have been examined for potential value in predicting treatment response. We investigated the prognostic importance of CD34 expression in 66 de novo AML patients (Male 41, Female 25 patients with a median age of 40 ;range 16 - 84) . Complete remission (CR) rate of post-induction therapy with Ara-C and daunorubicin was 55 %. CR rates were significantly high in patients with < 60 years of age and WBC count < 30000/mm3 We could be able to make cytogenetic analysis to only 37 patients and 6 of them were in unfavorable group. CR rate for favorable and intermediate cytogenetics was 65%. Among 6 patients with unfavorable cytogenetics, only 3 of them achieved CR after induction therapy but during follow-up 5/6 patients died within 6 months. At diagnosis CD34 expression was positive in 42 % of patients. The CR rate was not significantly different for patients expressing CD34. When we excluded 10 patients with AML M3 from the study group, positive HLA DR expression did not cause any difference for CR rates in other AML subtypes. Coexpression of CD34/HLA DR also did not cause any significant difference for CR rates (p> 0.05). Several studies reported a poor response to induction chemotherapy in patients with CD34 positive AML. In a recent study it has been reported that when HLA DR was added to the model CD34 expression lost its significance. In this study HLA DR+ or CD34+/HLA DR+ immunophenotype has been shown as an independent adverse risk factor from the multifactorial analysis for achieving CR. As a single institution size of our patient population studied and methodological differences may contribute to the controversy from the literature.

843 INCIDENCE OF SECOND MALIGNANCIES IN A COHORT WITH HODGKIN'S DISEASE ALONG TWENTY YEARS.

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Background: Hodgkin s lymphoma (HL) is a rare malignancy with an incidence rate of about 2.4 per 100.000 per year. With standard therapy 85% of cases can be cured. Nowadays the importance of late effects of treatment have become more apparent (second malignancies and ischaemic heart disease). Purpose of study: to determine the incidence rate of second malignancies in HL patients and to compare with cancer incidence rate in general population during a period of 20 years.

Methods: since 1978 to 1998 a cohort of 295 patients diagnosed at having in the Hematology Department of Miguel Servet University Hospital was followed-up to 1998 (population at risk: 533,946 inh). A longitudinal, retrospective and descriptive study has been performed. Data source: clinical reports and population Cancer Registry of Zaragoza. Variables: demographic data (age, gender), date of HL diagnosis, histological subtype, stage, treatment schedule (chemotherapy, radiotherapy, combined), date of cancer diagnosis, subtype and location of cancer. Cohort was stratified according to age, gender and schedule of therapy. Other risk factors related to cancer were considered (smoke, toxic contacts, previous neoplasia). The variables age, gender, histological subtype, stage, treatment schedule (chemotherapy, radiotherapy, combined), toxic records and time since complete remission until second malignancie appearance were considered as predictive factors in a bivariate study and we perform a lineal regression model and its coefficient have been done.

Results: The incidence rate of HL in our population is 2.4 cases/105 inh/y (F:1.87; M:3.03), as previously reported (Blood,2000); estimated

cancer incidence in our area was 255.2 SD 32.3 cases/105 inh/y for the period 1978-1998. Mean age: 38.3 SD 26 years. Gender: 67.0% M/ 33.0%. F. HL subtype: nodular sclerosis (71%), mixed cellularity (22%), lymphocyte-rich (24); Ann Arbor staging system: IA (12.7%), IB (1.6%), IIA (27.9%), IIB (5%), IIIA (16.9%), IIIB (14.4%) IVA (9.3%) IVB (11.8%). Radiotherapy alone 16.1%, chemotherapy alone 39.8%, combined therapy 44%. Location of irradiation: mantle field 50%, inverted-Y field 11.8 %, both: 1.6% Type of radiotherapy: cobalt teletherapy (59.3%) linear accelerator (4.2%). The range total dose was 20-36 Gy. Chemotherapy regimen: ABVD (14.4%), MOPP (15.5%), CMOPP (23.7%), CMOPP/ABVD (18.6%), ABVD/MOPP (15.2%). Relapsed patients: 10.1%, mean: 49 months. During this period 15 HL developed a second neoplasia (12.7%). In 11 cases a non-hematological neoplasia was developed adenocarcinoma (colon, breast, lung, oropharynx, skin, cavum, parotida, thyroid) and a hematological neoplasia were observed in 4 cases (acute myeloid leukaemia and non Hodgkin's lymphoma). The second neoplasia appears along the follow-up (mean 102.3 m; range 9-285). The risk of cancer in patients with HL was 10.0 and 6.5 when only non hematological tumor were considered. No variable with predictive value was found in the model.

Conclusions: The incidence of second malignancies among long-time survivors of HL is higher than in normal population. In other essays the major risk has been observed in patients treated with radiotherapy either alone or combined with chemotherapy.

844 THE BCL-2 PROTEIN EXPRESSION IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background:

The mitochondrial bcl-2 protein is an important inhibitor of programmed cell death – apoptosis. Its high expression increases the malignant cells vitality and decreases their chemosensitivity.

Aim:

To determine the bcl-2 protein expression level in the mononuclear cells obtained from bone marrow from children with acute lymphoblastic leukemia (ALL).

Material and methods:

This study included 65 patients (28 girls/37 boys, age range 1-19 y.) with ALL. Only patients with first manifestation of disease were included in this group. Twenty-six healthy children were examined as a control group. Mononuclear cells were separated from bone marrow (or peripheral blood from healthy controls) by Ficoll gradient centrifugation. Bcl-2 expression was analyzed using the anti-bcl-2 monoclonal antibody (DAKO) by indirect immunofluorescence (flow cytometry). HLA A, B, C antigens were examined as a positive control.

Results:

The expression of bcl-2 protein was characterized by percentage of bcl-2 positive cells in the examined bone marrow sample. The results are presented in the table 1. We observed increased bcl-2 expression in the children qualified to standard risk group (BFM) than patients from high risk group 32,2±20,0 % vs. 21,1±18,9% p=0,04 (U Mann-Whitney Test). We also found the negative correlation between bcl-2 expression and white blood cells count (R= -0,33; p=0,03). The bcl-2 expression in leucemic cells was higher than in mononuclear cells separated from healthy controls (27,9±20,5% vs. 19,5±9,4 %; p=0,05).

Summary:

? We observed no correlation between the bcl-2 protein level and event free survival or total free survival although increased bcl-2 level may be an important reason of chemoresistance of leucemic cells. The bcl-2 level can not be a risk factor in children with ALL.

? No correlation between expression of bcl-2 and sex, age, FAB classification type was found. The expression in the malignant cells from B-cell progenitors was higher than from T-cell progenitors but without statistical significance (29,720,1 vs. 19,521,2; p=0,1).

? The increased bcl-2 level in leucemic blasts comparing the normal mononuclear cells may suggest the role of this protein in malignant transformation and the biology of neoplastic cell.

	average	median	range
ALL group	29,53 %	27,00 %	0,7-79,7 %
Control group	19,48 %	18,75 %	6,4-34,9 %

Caption 1: The expression of bcl-2 protein

845 MABTHERA FOR IMMUNE CYTOPENIA IN ADULTS: IDIOPATHIC THROMBOCYTOPENIC PURPURA AND AUTOIMMUNE HEMOLYTIC ANEMIA.

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To evaluate the efficacy of rituximab for the treatment of adult patients with immune cytopenia, including idiopathic thrombocytopenic purpura (ITP) and autoimmune hemolytic anemia (AIHA). **PATIENTS AND METHODS:** We treated 11 patients with rituximab 375 mg/m², once weekly for four weeks. Seven 7 patients had active and symptomatic autoimmune thrombocytopenia that had relapsed or was refractory to standard therapies and 4 patients had autoimmune hemolytic anemia (one with cold agglutinin disease, three with warm antibody autoimmune hemolytic anemia). Data regarding age, diagnosis, date of diagnosis, previous treatments, comorbid conditions, blood cell counts before and response to previous treatment were analyzed. **RESULTS:** Rituximab proved to be active in all patients, with 7 complete and 4 partial responses. Of 7 patients treated for ITP, all were receiving corticosteroid-based treatment either alone or combined with other immunosuppressive therapy at the time they received rituximab. Complete remission occurred in 5 (71%) of 7 patients with ITP and in 2 (50%) of 4 patients with AIHA. Complete response was often durable in ITP and AIHA (follow-up 18 months). Treatment was well tolerated and no acute or delayed toxic events were recorded. **CONCLUSIONS:** Rituximab appears to be a promising immunotherapeutic agent for the treatment of autoimmune cytopenias.

846 EXPRESSION OF CD123 IN ACUTE LEUKEMIAS

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Background: In normal hematopoiesis survival, proliferation and differentiation of hematopoietic cells are regulated by several hematopoietic growth factors like interleukin-3 (IL-3) and colony-stimulating factors. IL-3 shows its activity through a specific receptor called IL-3 receptor. In our study we evaluated the expression of IL-3 R α chain (CD123) in acute leukemias and their prognostic significance. **Methods:** Bone marrow samples from previously untreated 28 AML, 14 ALL patients and 10 healthy persons used as controls were analyzed for the expression of CD123 by flow cytometry. Positive was defined as brighter staining intensity than normal controls in greater than 20 % of blasts. Several clinical characteristics such as age, sex, white blood cell (WBC) count, platelet count, hemoglobin level, LDH level, FAB morphology and karyotype were analyzed. All patients received remission induction therapy. After completion of remission induction therapy patients were evaluated for response of therapy. **Results:** CD123 was negative in healthy control group. CD123 expression was present in 79 % of AML and 64 % of ALL patients. Expression of CD123 was detected in all morphological subtypes of AML. We did not found a clear correlation between CD123 expression and complete remission rate, WBC count, hemoglobin level, platelet count, LDH level but age was significantly correlated with CD123 expression. We found no differences of CD34 expression between patients with or without CD123 expression. **Conclusions:** High expression of CD123 in a significant proportion of patients with acute leukemia may suggest a possible role of IL-3 in leukemogenesis. Our data revealed that CD123 has no prognostic significance in acute leukemia.

847 ASSESSMENT OF CANNABINOID RECEPTORS EXPRESSION IN BONE MORROW TREPHINE BIOPSY OF PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

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Cannabinoid receptors CB1 and CB2 are part of endocannabinoid system discovered during the last two decades in humans and animals. Apart from their neurocrine effects, cannabinoid receptors play an important role in the process of proliferation and apoptosis of neoplastic cells. Chronic lymphocytic leukaemia (CLL) is one of the diseases in which these processes are altered.

The aim of our study was the assessment of cannabinoid receptor expression on the lymphocytes B in bone marrow trephine biopsy derived from patients with diagnosed chronic lymphocytic leukaemia. The influence of purine nucleoside analogues treatment (fludarabine or 2-chlorodeoxyadenosine) on the cannabinoid receptor expressions was evaluated.

We assessed 18 untreated patients and 13 after the treatment with nucleoside analogues containing regimens. Bone marrow trephine biopsy was taken routinely from the posterior iliac crest. Standard immunohistochemical staining procedure for paraffin embedded sections was applied. The lymphocytes forming neoplastic infiltration were recognised by bounding with CD20 monoclonal antibody. The cannabinoid receptors were detected using specific primary polyclonal antibody CB1 and CB2. The antibody binding was detected by the biotin-streptavidin-peroxidase method using LSAB+ HRPkit with DAB (3,3-diaminobenzidine) as a chromogen. The cellularity of bone marrow tissues and the number of neoplastic cells with the positive reaction were analysed using morphometric analyser set.

The results show that both types of cannabinoid receptors are expressed on the surface of neoplastic cells infiltrating bone marrow. The expression of CB1 receptor was statistically significantly lower than CB2 (19.13.4% and 91.52.0% respectively). The applied treatment caused a significant decrease in lymphoid infiltration (77.66.1% to 29.75.9%, $p < 0.001$) and CB1 receptor expression (19.13.4% to 8.22.6%, $p < 0.01$) but did not affect CB2 expression.

The study provides original evidence for the existence of cannabinoid receptors on lymphocytes B in the patients with CLL. CB2 receptor, which is expressed in great number on the neoplastic cells may be a new particular surface structure modifying a course of the disease. The presence of CB2 receptor on the lymphocytes of patients after chemotherapy may be considered as a new target in the treatment of residual disease. However, further studies are needed to explain the role of cannabinoid receptor in the CLL and their potential usefulness in the cure of the disease.

848 AGGRESSIVE NON HODGKIN'S LYMPHOMAS - CLINICO-BIOLOGICAL CHARACTERISTICS AND THERAPEUTICAL RESULTS

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Background: Aggressive Non Hodgkin's Lymphomas (NHL) are severe diseases with aggressive and biological findings and a natural evolution through dissemination and death. NHL can be very sensitive to chemotherapy having a large percent of complete remissions, but also can have relapses that are hard responsive to treatment. **Aims:** The purpose of this study is to evaluate the complete remission rate, death rate and overall survival rate in aggressive NHL patients that were followed up in our clinic. **Methods:** We studied 85 patients that were diagnosed and treated in the Hematology Clinic of Timisoara between 06.1998 - 06.2003. The inclusion criteria were the clinical, histological and immunological findings specific for aggressive NHL. Staging in this group was according to Ann Arbor classification system and the histological differentiation was made according to the REAL classification system on morphological and immunohistochemical basis. Statistical work was made electronically in an organised file in the Microsoft Excel data base and using EPIINFO 6 and INSTAT programs. Overall survival analysis was performed by Kaplan-Meier curves statistically compared with the student test ANOVA. **Results:** From the 85 patients with aggressive were females with a median age of 48,4 years (aged 16-77 years). In this study group 64,8% of patients had initially periferic adenopathies, 32,8% had mediastinal adenopathies (10 patients had primary mediastinal tumor), 25% had abdominal adenopathies and 20% had retroperitoneal adenopathies. There were also 8,3% patients that had at the income generalized adenopathies (periferic, mediastinal, abdominal, retroperitoneal). Extranodal determination were found out in 60 % of the cases which were divided in 38% of primitiv extranodal determinations and 22% diffuse determinations of extanodal disease. The median value of the serum LDH was higher in patients with B signs. The group was made of 10 patients in stage I + IE; 24 patients in stage II + IIE; 18 patients in stage III + IIIE and 32 patients in stage IV. The most frequent histological type was the large cell diffuse one (68%) having an approximately equal distribution on the 4 clinical stages. In stage IV the most frequent histological type of lymphoma were those very aggressive anaplastic (CD30+) and limphoblastic. In this study group there were 81,25% cases of B phenotype and 15,62% T phenotype. The treatment was generation 1, 2 and 3 chemotherapy (CHOP, M-BACOP, COP-BLAM). **Conclusions:** We obtained a remission rate of 62,5%, death rate of 50,96% and an overall survival of 14,97 11,09 months in the study group. The modesty of the therapeutical results is a cause of the high number of patients in advanced stages of the disease and the use of mostly generation 1 and 2 chemotherapy.

849 SURVIVAL OF LYMPHOMA PATIENTS WITH RELAPSE OR PROGRESSION AFTER HIGH-DOSE CHEMOTHERAPY WITH AUTO TRANSPLANTATION

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Introduction: High-dose chemotherapy with autologous stem cell transplantation (SCT) is effective in the management of patients (pts) with relapsed and resistant lymphoid malignancies. Unfortunately a significant number of pts relapsed despite performed autologous SCT. Within those pts the outcome is generally poor with median survival of 10,5-25 (Hodgkin disease-HD) and 3-7,5 months (non-Hodgkin lymphoma-NHL), respectively. **Aim:** To assess survival of pts with HD and NHL whose disease had progressed after autologous SCT. **Patients and methods:** Between January 97 and October 03, a total of 36 pts with lymphomas, 16 HD and 20 NHL (aggressive NHL 75%) underwent auto SCT in our institution. Among them, 20% of NHL and 25% of HD were transplanted with resistant disease. In all pts source of stem cells were mobilized peripheral blood. The conditioning regimen was BEAM in HD and CVB in NHL pts. **Results:** In both groups, 10 pts each relapsed or progressed, among HD 10/16 (62,5%) and NHL 10/20 (50%). The mean time to relapse was 14,5 and 12 months for HD and NHL. The median survival from relapse or disease progression was 18 months for HD and 7,5 months for NHL. In NHL pts longer postrelapse survival was associated with indolent histologies. The median post-transplant survival was 29 (range 2-82) and 18 (range 2-73) months for relapsed pts with HD and NHL. Among relapsed pts 4 (1 NHL and 3 HD) are still living more than 60 months. **Conclusion:** The short-term prognosis for pts with progression of disease after SCT were little better for HD compared with NHL but long-term survival is generally poor in both groups. Relapse or progressive disease after this treatment imply difficult decisions for further strategy: conventional dose chemotherapy with largely disappointing results or a second transplantation.

850 CML AND IMATINIB MESYLATE: QOL AND RESPONSE RELATED TO KARYOTYPE.

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Background: Imatinib mesylate (STI571), as a new specific inhibitor for tyrosine kinase product of oncoprotein Bcr/Abl has been reported. We present our experience on this treatment analyzing hematological and cytogenetic response related to karyotype and quality of life related to therapy (QOL). **Patients and methods:** We evaluated 25 patients diagnosed as having CML between 1990-2003 with Imatinib 400 mg/day (mean time on therapy: 0-27 months). Previous therapy: alpha-interferon 18, pegilated interferon 1, alpha-interferon plus citarabine 9 and on first line 5. When Imatinib was started, 3 patients showed signs of accelerated phase, 18 were on chronic phase and 4 in post-blast crisis chronic phase. Patients were stratified according to cytogenetic abnormalities in two groups: Group A: typical translocation (9;22) in 18 patients, Group B: variant or complex karyotypes: 7. **Hematological response (HR)** was evaluated (clinical and blood counts) monthly and cytogenetic response (CR) in bone marrow every 3 months in the first year evaluating karyotype, percentage of metaphases Ph, FISH and quantitative PCR study. Cytogenetic response (CR) was quantified by number of metaphases Ph in bone marrow: 0% complete (CCR), 1-35% partial (PCR), 36-95% minor (MCR) and > 95% failure (F). **Molecular response (MR):** normal karyotype (Bcr/Abl negative and quantitative PCR<5 copies). A generic quality of life (QOL) questionnaire SF-36 and specific FACT-BRM were filled by the patients evaluating physical functioning, role emotional, mental health and global perception of health previously to the beginning of therapy and 6 months later. **Results:** 17M/ 8F (5 blast crisis and died), mean age 55 (r30-74). In 20 patients Imatinib was applied as second line of therapy by loss / non response (12), intolerance to interferon (3), accelerated phase (2), chronic phase post blast crisis (3). **Group A:** 3 months HR 89%, 6 months CR 71.4% (1 MR), 12 months CR 70% (3 MR), 18 months CR 66%. **Group B:** 3 months 100%, 6 months CR 66%, 12 months CR 50%, 18 months CR 50% (1 MR). **Side effects** in 16 cases: hematological toxicity in 12 (grade I-II: 10, grade III:2), edemas in 8, urticaria in 4, diarrhoea in 2 and muscle-skeletal pain in 2. SF-36 scores were similar as general population except physical pain, social function and emotional role with lower values. Items of FACT-BRM were:

physical function 77, social/ family environment 65, emotional state 58, capacity of personal functioning 63 and others worries 67. **Conclusions:** At 3 months after starting Imatinib hematological response was better in B Group but CR was better in A group. The follow-up at 6 and 12 months shows persistently a higher CR in A group than in patients with complex or variant karyotypes. Further studies must be done in order to establish a statistical difference between both groups. QOL assessment showed a good acceptance and tolerance of this treatment with few interferences in their life style.

851 AGGRESSIVE NON HODGKIN'S LYMPHOMA WITH EXTRANODAL INCOME. CLINICO-BIOLOGICAL CHARACTERISTICS AND THERAPEUTICAL RESULTS

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Background: Extranodal sites income in the pathology of Non Hodgkin's Lymphomas (NHL) is 35-40% of the cases, being in a continuous rising because of the high incidence of immunodepressed patients by gained immunodepression (HIV infection) or secondary to organ transplants. On the other hand there are a lot of diagnostic methods that have been developed: histological and imagistical ones. **Aims:** The purpose of this study is to analyse the group of extranodal aggressive NHL patients according to their clinical, biological and therapeutical particularities and the study of the general survival and disease free survival rates. **Methods:** We analysed a number of 38 patients diagnosed and treated between 06.1998 - 06.2003 in the Hematology Clinic of Timisoara with extranodal aggressive NHL. The selection of the extranodal income patients was made according to the presence of lymphoid tumor in a extranodal site, the absence of peripheral, mediastinal and abdominal adenopathies, the absence of the hepatosplenomegaly and the normal aspect of peripheral blood and bone marrow. **Histopathologic classification** was made according to REAL classification on morphological and immunoistochemical basis and staging was made according Ann Arbor classification. We considered stage IE - single involvement of one extranodal organ; stage II - multiple involvement of one extranodal organ; stage IIE - association and involvement of one nearby nodal group; stage IIIE - extranodal involvement and upper and under diaphragmatic nodal groups involvement and stage IV - diffuse involvement of an extranodal organ. **Results:** Median age of the group was 49,5 years with male/female percent of 63,4%/36,6% (24M/14F). The most frequent extranodal site as income was the gastrointestinal tract - 13 cases (28,88%) followed by the Waldayer lymphatic ring, spleen, brain, sinus, thyroid glands, bone, other localizations in 2 to 6 cases. The most frequent histologic type was diffuse large cell lymphoma (DLCL) and the major phenotype was B cell lymphoma (91,1% B lymphomas/ 8,9% T cell lymphomas). The other histological types were in a smaller number of patients: immunoblastic lymphoma (DIBL), anaplastic lymphoma (DAPL), Burkitt lymphoma, lymphoblastic lymphoma (DLBL). There were more stage I, IE, II, IIE patients, 73,2% patients had B signs at income. There were 60% of the patients with Karnowsky scor under 70%, the medium value of LDH was 591,56UI. Surgical treatment was used for bioptic and histological purpose, but also for total or partial resection of the localized tumor (gastrointestinal tract, spleen, thyroid, bone). Chemotherapy was used after surgery such as CHOP, CHOP + Bleo, special protocols for several localizations (cerebral localization - De Angeli protocol) or High dosis chemotherapy. Radiotherapy was used in selected cases for the consolidation of surgical treatment. **Therapeutical results** were 62,22% of complete remissions, 15,21% partial remissions, 10,34% non responders and 12,23% progressive disease. Overall survival was 12,31% 10,32 months. **Conclusions:** Aggressive NHL with extranodal income had different localizations, but the most frequent were the gastrointestinal ones. Complete remission rate, overall survival and disease free survival were good as a result of precocious positive diagnosis and an adequate therapy.

852 DISTRIBUTION OF HLA I AND II CLASS SPECIFICITIES AND CYTOKINE PRODUCTION BY MONONUCLEAR CELLS IN PATIENTS WITH SMALL B-CELL LYMPHOCYTIC LYMPHOMA (B-SLL)
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Considering that small B-cell lymphocytic lymphoma (B-SLL) is rather uncommon event in some world regions, its family occurrence and nearly 2-fold predominance in male patients, lead us to suppose the important role of genetic predisposition in pathogenesis of B-SLL, particularly ' of HLA system. We have studied the distribution of HLA I class (A- and B-loci) and II class (DRB1) in 117 patients with B-SLL, citizens of St.-Petersburg, Russia, and compared it to corresponding population control group (n=3028). Also we have evaluated the ability of peripheral blood mononuclear cells to spontaneous and induced production of cytokines - TNF α , IL-1b, IL-6 in 56 B-SLL patients.

According to Clinical classification of lymphoma (2002) all examined patients were divided into two groups: 1 - with indolent (86,3%) and 2 - with aggressive (13,7%) lymphoma. Study of HLA distribution in these two groups revealed the decreased incidence of HLA-A1 (12,9% vs. 21,1%, $c^2=3,98$) and increased incidence of HLA-B14 (8,9% vs. 3,8%, $c^2=6,74$) and B62 (16,8% vs. 8,2%, $c^2=9,37$) in patients with indolent lymphoma, as compared to healthy control group. The same tendencies were revealed in B-SLL patients in whom disease manifested in age >60 years. While aggressive lymphoma group differed from indolent lymphoma revealing higher, than in 1st group, frequency of HLA-A24 (43,8% vs. 13,9%, $c^2=8,37$) and B18 (43,9% vs. 14,9%, $c^2=7,55$). Thus, the indolent character of disease is associated with HLA-specificities, which we have revealed as provoking genes, while aggressive lymphoma is associated with another HLA-specificities.

Comparison of cytokine production in two B-SLL groups showed, that in indolent lymphoma spontaneous IL-1b production was 1,2-fold increased, as compared to normal values, while in aggressive lymphoma - 1,8-fold. LPS-induced synthesis of IL-1b was less markedly decreased in 1st group than in 2nd - 3,7 and 8,6, respectively, as compared to normal level. IL-6 synthesis was increased 18,4 and 24,4, respectively in 1st and 2nd groups. Thus, in patients with aggressive B-SLL we have revealed more marked disturbances in IL-1b and IL-6 synthesis by peripheral blood mononuclear cells.

853 DIAGNOSTIC AND CLINICAL SIGNIFICANCE OF ARGYROPHILIC PROTEINS IN PATIENTS WITH ACUTE LEUKAEMIA
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Background: The distribution of nucleolar acidic proteins, selectively stained by silver (AgNORS) has become an important parameter for the cytological diagnosis of malignancy. However, little information is available regarding their utility in hematological disorders. Objective: to study the possible relation of AgNORS to immunophenotypic markers and clinical outcome. Subjects: The number (amount) of Argyrophilic proteins (AgNORS) were estimated in bone marrow smears of thirty-six patients with acute leukemia at initial diagnosis: 24 children (10 males 14 females) and 12 adults (6 males and 6 females) as well as 12 matched controls. Methods: Silver staining of AGNORS was applied to bone marrow smears, and immunophenotyping was done by FACS flow cytometry. Results: Statistically significant difference in mean AgNORS count/nucleus was observed in patients versus control ($p<0.05$), and a different staining pattern was observed in ALL (fine dots) versus AML patients (coarse dots). Positive correlation was found between AgNORS and bone marrow blast percent, while negative correlation was found with age, hemoglobin concentration and platelet count. Strong AgNORS staining was associated with immaturity markers and poor response to treatment. Eighty percent of AML and 70% of ALL patients with mean AgNORS<2.5 achieved complete remission. Conclusion: determination of AgNORS has a major predictive value and adds an additional tool to differentiate ALL from AML smears. Hence, it should be included in the diagnostic and prognostic workup of patients with acute leukemia.

854 THE RAT ADULT BETA-GLOBIN PROXIMAL PROMOTER: COMPARISON TO HUMAN AND MURINE
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Among mammals studied so far, rats have the largest number of active adult β -globin genes. We have presumed that the transcriptional regulation of rat adult β -globin genes is different, compared to those of the human and murine. Besides the locus control region (β LCR), promoter is the key regulatory region for β -globin gene transcription. Comparison of the proximal promoters of the rat, human and murine β -globin genes reveals certain similarities, but we have also observed differences that might be relevant to the regulation of transcription of these genes.

We have identified and characterized a DNA-binding activity with specificity for the β DRE from rat β bmny-globin promoter, an evolutionarily conserved transcriptional regulatory element in mammalian adult β -globin promoters. We have also confirmed that β DRE regulatory element contributed to the transcriptional inducibility of rat β bmny-globin gene in murine (MEL) and rat erythroleukemia cells (REL). By using gel mobility shift and South-Western blot competition studies we have shown that 60 kD erythroid-specific transcription factor and 35 kD and 20 kD ubiquitous factors from REL cells specifically bind to the rat β DRE. Mouse β maj-globin β DRE promoter element resembles the same regulatory element from the rat proximal promoter. It contributes significantly to erythroid-specific induction of transcription and β DRE binding activity is detected. On the contrary, the human β -globin minimal promoter provides erythroid-specific induction of transcription only if combined with the β LCR, and there was no evidence of DNA-binding activity with specificity for the β DRE.

Additionally, we have found that -120 GATA element contributes to the transcriptional inducibility of rat β bmny-globin gene in MEL and REL cells. We have also characterized the transcription factor that specifically bind to the GATA motif as GATA-1, by using gel mobility shift competition and supershift studies. The -120 region from the human β -globin promoter has a weak binding site for GATA-1. Mouse β maj-globin promoter does not contain GATA-1 binding site at the position -120.

Further analysis of the interactions of β LCR with the promoter elements that play different roles in different species will provide better insight into the regulation of transcription of β -globin genes. poznanic@eunet.yu

855 IDENTIFICATION OF MOLECULAR THROMBOPHILIC ALTERATIONS (FVLEIDEN AND P20210) IN PATIENTS WITH THROMBOTIC DISEASE AND FAMILIAL HISTORY. DEFINITION OF RISK PROFILE AND THROMBOTIC LOCALIZATION.
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BACKGROUND: Thrombotic disease is multifactorial, and results from multiple interactions between inherited and acquired risk factors. It is necessary to know risk factor to act in function of risk profile.

AIMS:

1) Evaluate the efficacy of thrombophilic studies in a population of thrombotic risk. 2) Know the incidence of inherited thrombophilic defects and realize the stratification in risk categories according to the presence of risk factors. 3) Identify the incidence of genetic defects how responsible of thromboses in unusual localizations.

MATERIAL AND METHODS:

In our institution were studied 321 patients from 2001 year. 195 patients had thrombophilic criteria (previous thrombosis at age < 50 years, repeated thrombotic events, unusual thrombotic manifestation) and 58 patients were studied because they presented familial history. We define the following categories of risk: 1) High: deficiency of natural anticoagulant proteins, homozygous Factor V Leiden, antiphospholipid antibodies, or the combination of more than one. Or recurrent thromboses. 2) Intermediate: Heterozygous Factor V Leiden or prothrombin gene mutation. Or Thrombosis in a life-endangering localization (visceral vein thrombosis and massive pulmonary embolism). 3) Low: with transitory risk factors. Genetic study included assay of Factor V Leiden mutation (FV-Q506) and Factor II gene mutation (G20210A), using PCR methodology (reagent of VIENNA LAB). Plasmatic analysis included Protein C and S, Antithrombin III, APCR and lupic anticoagulant determination.

RESULTS:

1) In the 33% of the thrombophilic patients was detected some genetic defect. In familial history group, the mutations were present in the 51% of the serie. There were double carriers in both groups: 8% and 5% respectively. 2) Factor V Leiden and FII mutation was detected in 16% and 32% of the group of thrombophilic patient, and 17% and 19% and familial history group, respectively. 25%, 47% of patients of our study were in high-risk, intermediate-risk category, respectively. The patients that belonged at low-risk category were excluded. 3) Factor V Leiden mutation was responsible of the 42% thrombotic events, with the following localizations: venous thromboembolic disease (VTD) 22%, cerebral thrombosis 10%, arterial thrombosis 2%, and atypical thrombosis (retinal, portal thrombosis) 8%. Factor II gene mutation was responsible of the 47% thrombotic events, with the following localizations: venous thromboembolic disease (VTD) 24%, cerebral thrombosis 17%, and atypical thrombosis (retinal, portal thrombosis) 6%. 4) We detected 12% of plasmatic thrombophilic alterations (Protein S and C deficiency, and lupic anticoagulant).

CONCLUSIONS:

1)Genetic study allow identify the cause of the thrombosis until 56% of studied population. 2)Genetic study help to define the thrombotic risk and modify the prophylactic and therapeutic recommendations in this group. 3)A high percentage of the cerebral thromboses in younger patients are explained by non-classical risk factors.

856 ENDOTHELIAL FACTORS AND ADHESION MOLECULES IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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BACKGROUND: Several studies have reported a serious dysfunction of the vascular endothelium in children with acute lymphoblastic leukaemia (ALL), that could be caused by the disease itself, therapy, or other conditions (sepsis, catheters).

AIM: The aim of this study was to determine prospectively the activation markers of the vascular endothelium in children with ALL, in order to define if any of them could be used as predictors of the disease course, or response to therapy.

METHODS: For this purpose, 50 children with ALL were studied (44 B- and 6 T-cell) at the disease onset (before initiation of treatment) and at the 1st relapse of the disease. Eighteen of them were girls and 32 boys, aged 1-13 years (mean age: 5.8 years). Twelve children were treated with UCALL-XI and 38 with BFM-95 protocol. Three children died during the acute phase of the disease. Nineteen children who had been treated for ALL were also studied (control group), 1 to 10 years after the end of therapy. In all children the levels of endothelial factors (NO, ET-1, PGDF, vWF, Thrombomodulin), adhesion molecules (sVCAM-1, LFA-1, LFA-2, LFA-3, sP-selectin), and acute phase reactants (ESR, CRP, IL-6), were assessed.

RESULTS: Abnormal findings were as follows: a)At the disease onset we found significantly increased levels of NO (45,3 µmol/L, p<0,005), vWF (168,5%, p<0,001), thrombomodulin (23,2 ng/ml, p<0,005), as well as, ESR, CRP, IL-6 and decreased levels of integrins LFA-1 and LFA-3, compared with those of the control group, b)in patients during 1st relapse we found significantly increased levels of only vWF (174,2%, p<0,001) and thrombomodulin (20,18 ng/ml, p<0,005), compared with those of the control group, and c)in children of control group all findings were normal.

CONCLUSION: Abnormal values of the parameters determined at the onset of the disease showing serious dysfunction of the vascular endothelium, evidently result from the disease itself. Normal values of most parameters during 1st relapse may indicate favorable prognosis and abnormal values of some parameters, such as vWF and thrombomodulin may be attributed to the treatment.

857 MEDICOLEGAL AND ETHICAL GUIDELINES WITHIN THE ONLINE COMMUNITY FOR TELEHEMATOLOGICAL CONSULTATIONS

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Medicolegal and ethical aspects of telehematology reflect an important cluster of issues already well described in telemedicine, however, integration and adaptation of these regulations in the developing variety of telehematological systems, as well as telehematological legislation, remains a complex task yet to be approached and addressed on a larger scale.

Issues targeted by the Online Community for Telehematological Consultations include differentiation of subjects involved in telemedical consultations, their deontology and patients rights. Based on a number of prerequisites from the technical and medical domain, several limitations of responsibility have been defined in relation with possible risks of telemedical management. Lack of international (and national) telemedical legislation, directly influences the value of telemedical advice, even in optimal technical and medical conditions achieved by telemedical subjects. Therefore, professional groups, compliant with own standards and regulations, tend to increase the value of telemedical management by developing guidelines and strategies aimed at a certain level of excellence. However, the experience within the Online Community for Telehematological Consultations showed that such approach also restricts the potential development of the Telehematology Service - by decreasing the number of potential collaborators enabled to follow such policy, or simply by decreasing our ability to assist remote GPs, diagnose and treat remote patients.

Therefore, we believe that doctor and patient education, and informed consent remain quite important, integrative points of all medicolegal and ethical aspects related to the use of telemedicine. (telehematology.net).

858 INTERMITTENT DOSAGE OF IMATINIB - A FEASIBLE STRATEGY FOR PATIENTS WITH THE HISTORY OF SIGNIFICANT HEMATOLOGIC TOXICITY

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There is growing evidence that hematologic toxicity in patients with CML treated with standard continuous dosing of imatinib (IM) is associated with inferior prognosis. In order to avoid prolonged interruptions in treatment and, possibly, to prevent induction of resistance in such cases we adopted a strategy of intermittent treatment (I-T): 300-600 mg of IM once to five times weekly. Here we report the experience with I-T in 9 patients (6 women and 3 men) aged 25 to 74 (m=48) years treated for 11,7 (2-21) months in average. Patients have 4-152 (m=38,9) months long history of CML at the initiation of IM therapy that was indicated for accelerated phase (in 4 patients), cytogenetic (3) or hematologic (1) resistance and toxicity after interferon (1). 6 of them were previously treated with interferon, 2 with hydroxyurea and one with busulfan only. Two patients have the history of autologous peripheral stem cell transplantation. Additional cytogenetic aberration (+8) was present before IM therapy in one patient and in other two cases interstitial deletions of 9q were identified. 4 patients had high and all other intermediate Sokal score, while the value of Euro score was high in each case. All patients received initially the standard treatment (300-600mg daily) with IM for 1-6,5 (m=2,6) months resulting in hematologic remission with various degree of myelosuppression in each of them. Hematologic toxicity was the reason for the discontinuing of therapy in 7 patients for 0,5-4 months. One patient was treated before introduction of I-T with daily dose of 100-150mg of IM for 11 months. In 7 cases complete hematologic remission was maintained by I-T and in one of them steady regeneration in blood counts allowed reintroduction of full dose standard therapy. Hematologic progression occurred in two patients. Both of them were switched to full daily dosing of IM. Two patients improved their cytogenetic response while on I-T. In two cases major cytogenetic remission achieved with standard dosing was maintained. A significant reduction of the clone with +8 was achieved in the patient with longest duration of I-T. Using

interphase FISH the level of cytogenetic response was below 10% in 6 patients at the latest examination. In one patient the development of +8 in BCR/ABL negative cells was observed. In some cases in vitro tests for evaluating the sensitivity to IM and screening for BCR/ABL tyrosine-kinase mutation were performed. We conclude that the I-T with IM might serve as a reasonable and cost-effective alternative in heavily pre-treated or in high-risk patients with CML who could not be offered allogeneic stem cell transplantation or experimental therapy. Surprisingly, with our strategy major cytogenetic remissions was obtained in two and maintained in other two patients. I-T allows safe tailoring the actual dose of IM in order to avoid excessive hematologic toxicity. Our observations need to be validated in larger cohort of patients during longer follow-up.

859 LATE EXPRESSED THALASSEMIA INTERMEDIA WITH HETEROZYGOUS IVS II NT 850 G>A MUTATION

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1. Heterozygous IVS-II-850 nt G>A mutation is not known to be responsible for clinic anaemia. Here is a case report of this intermedia b thalassemia with very late expression.

2. An 81-years-old Caucasian patient with vascular background (ischemic cardiopathy, arteritis) presents splenomegalia at examination. For 6 months he's needed one RBC transfusions each 2 months because of cardiovascular bad tolerance for anaemia. Before any transfusion, hemogram reveals anaemia: Hb 8.8g/l, MCHC 29.4g/l, MCH 19pg, MCV 65fl, reticulocyte count 166.109/l. Haemoglobin electrophoresis: HbA2 5.9%, no detected HbF. Bilirubin 11mg/l, LDH 240 IU/l, ferritin 440ng/l. Seric B12 vitamin and folate are in the normal. Urea is 0.5g/l, creatinine 17mg/l, and assessed clearance 37.11 ml/mn. Renal echography doesn't reveal any obstacle. Seric erythropoietin is 23.3 mIU/ml (N 3.5-11mIU/ml). No sign of myelodysplastic syndrome is detected on myelogram. The usual b thalassemia gene mutations (multiplex hybridization, b-glob Assay, VIENNA LAB) and a gene study (DGGE-PCR on the following regions: distal promoter, Exon 1 + TATA box, Exon 2, Exon 3, PolyA, proximal part of intron 2) have been carried out but revealed nothing. β gene sequencing shows IVS-II-850 nt G>A mutation, associated with an heterozygous triplicate a (aa/aaa) (detected by multiplex PCR and DHPLC). Patient has been treated with erythropoietin injections (10 000 IU/ injection 3 times a week then 20 000IU/ injection once a week), then he has been free of transfusion for more than 6 months and has more than 9.5 g Hb/dl.

3. The IVS-II-850 nt G>A mutation has been described only once in an north European original family, but haemoglobin levels reported were between 10.8g/dl and 13.9g/dl(1). In our case, the a (aa/aaa) triplicate is a constitutional aggravating factor for b thalassemia. The recent needs for RBC transfusions are linked to the renal failure. This is shown by the fact that the EPO levels, which are lower than those reported in the literature when measured on intermedia b thalassemic patients but greater than in patients with only renal failure. (1). Cr k MA Et al. Hemoglobin. 1995

860 GENETIC POLYMORPHISMS OF OXIDATIVE AND ANTIOXIDANT ENZYMES AND ARSENIC-RELATED HYPERTENSION

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We assessed the association of four genetic polymorphisms, NAD(P)H oxidase, manganese superoxide dismutase (MnSOD), catalase, and endothelial nitric oxide synthase (e-NOS) with arsenic-related hypertension risk among 79 hypertensive cases and 211 controls in an arseniasis-hyperendemic area of Taiwan. Overall, MnSOD polymorphism significantly increased the risk of hypertension regardless of arsenic exposure. NADPH oxidase and eNOS polymorphisms were significantly associated with hypertension risk in the arsenic exposure group. But catalase polymorphism was not associated with hypertension. We further stratified groups by triglyceride levels to evaluate the four polymorphisms together. The adjusted ORs of the extreme genotype combinations of variant genotypes (individuals with two to four variant phenotypes) versus the wild genotype (individuals with no variant phenotypes) were 1.1 (95% CI 0.3-4.7) for individuals with low triglyceride levels (< 110

mg/dL) and 3.3 (95% CI 1.0-11.1) for those with high levels (≥ 110 mg/dL), respectively. These results suggested that NAD(P)H oxidase, MnSOD, and e-NOS, but not catalase, polymorphisms might play a role in the development of arsenic-related hypertension especially in subjects with high triglyceride levels.

861 THE EFFECTS OF CHEMOTHERAPY AND CLODRONATE TREATMENT ON BONE METABOLISM IN MULTIPLE MYELOMA PATIENTS

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Background: Pathological fractures are detected in almost 75% multiple myeloma (MM) patients due to osteoporosis and/or osteolysis. 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: To assess the effects of clodronate treatment as a supportive care on bone metabolism in MM patients. Material and methods: The study group comprised 30 patients (17F/13M) in the age 35-83 years (median-59 yrs). Diagnosis was established on the base of common rules. 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: 7-IA, 7-IIA, 2-IIB, 16-IIIA. In 26 patients bone change were detected in X-ray examination. Standard VAD chemotherapy treatment and auto-PBSCT were used in 17 patients. Thirteen patients were treated with conventional chemotherapy. 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 μ g/l (SD 71.67 μ g/l), after treatment-207.32 μ g/L (SD 131.72 μ g/L). In control group median-133.6 μ g/L (SD 42.42 μ g/L). Median concentration of BAP before treatment-37.8 μ g/L (SD 19.82 μ g/L), after treatment-38.65 μ g/L (SD 18.4 μ g/L). In control group median-20.73 μ g/L (SD 15.05 μ g/L). Median concentration ICTP before the treatment-3.76 μ g/L (SD 10.06 μ g/L), after the treatment-6.85 μ g/L (SD 14.27 μ g/L). In control group median-4.72 μ g/L (SD 2.43 μ 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 ICTP were higher in comparison to control group ($p < 0.05$). Concentration of BAP both before and after treatment were higher than in control group ($p = 0.003$ and $p = 0.018$ respectively). 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 ($p = 0.06$; $r = 0.16$). 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 ($p = 0.025$; $r = -0.97$). 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.

862 PROGNOSTIC FACTORS INFLUENCING ACHIEVEMENT MAJOR CYTOGENETIC RESPONSE FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN ACCELERATED PHASE TREATED WITH IMATINIB MESYLATE

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Background: It is known, that decrease of percentage of Ph+ metaphase in bone marrow less than 35% associated with increase survival for patients with chronic myeloid leukemia.

Aims: to find out the demographic features, disease history and pretreatment characteristics influencing achievement major cytogenetic response for patients with chronic myeloid leukemia in accelerated phase treated with imatinib mesylate.

Methods: We treated 78 patients (pts) with accelerated phase CML (median age, 44 years; age range, 19-77; 46 were females and 32 males). The median treatment duration was 24 months. Characteristics analyzed included age, sex, time from diagnosis of CML, LDH, WBC, Hgb, Plts, % blasts in peripheral blood or marrow, % basophils in peripheral blood or marrow, marrow cellularity, % Philadelphia chromosome positivity by metaphase or interphase FISH analysis, presence of additional cytogenetic abnormalities and dose of imatinib mesylate. We used correlation analysis by Spearman method. Differences between patient groups were evaluated by χ^2 .

Results: major cytogenetic response (MCyR) in 6, 12, 18 and 24 months showed 27% (21 pts), 23% (18), 31% (24) и 34% (26), respectively. Overall, 34 pts (44%) achieved a MCyR, including 27 (35%) with a complete response. The median of achievement MCyR was 6 months. In correlation analysis, the factors associated with worse major cytogenetic response rates were: splenomegaly >5 cm below the costal margin ($p=0.001$), blasts >1% in the peripheral blood ($p=0.01$), LDH >600 u/l ($p=0.04$), time from diagnosis of CML more than 3 years ($p=0.004$) and dose of imatinib mesylate less than 600 mg/day ($p=0.0001$). The expected MCyR rates by the presence of zero, one-two, three or more of these factors were 74%, 49% and 15%, respectively ($p=0.001$). The clonal evolution as the sole criterion of disease acceleration is not adverse factor for achievement major cytogenetic response: all of 5 pts with clonal evolution showed MCyR. MCyR's stability depended on time of its achievement. Major cytogenetic response received during the first 6 months from a beginning of imatinib treatment was stable with 86 % of patients, and under more later - with 46 % of patients ($p=0.0001$).

Summary: The received results allow to determine tactics of treatment for the patients with accelerated phase chronic myeloid leukemia. At presence of adverse factors or at absence MCyR in 6 months of therapy it is necessary to escalation dose of imatinib mesylate by 800 mg/day or imatinib mesylate combination regimens.

863 MANAGEMENT OF PREGNANCY IN WOMEN WITH INCREASED RISK OF VENOUS THROMBOSIS

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Background : Venous thromboembolism (VTE) is a multicausal disease resulting from the combined effect of congenital and acquired risk factors. Pregnant women presenting thrombophilia markers are more predisposed to thrombotic complications. The risk of maternal VTE in thrombophilic patients depends mainly on the personal history of VTE and the underlying thrombophilia predisposing factor. The protocols for managing pregnancies with an estimated risk of VTE have not been clearly established.

Aim : We report here the results of a single centre, retrospective study, of 119 consecutive pregnancies with thrombotic risk, utilising a two-step score based thromboprophylactic strategy .

Methods : Patients were followed up monthly and managed utilising a two-step score based thromboprophylactic strategy taking into account personal history of VTE, genetic or acquired thrombophilia risk factors and pregnancy related additional risks. The venous thrombosis risk score (VTRS) determined which women required treatment and which women required clinical surveillance in antepartum period. The second score entitled pregnancy related venous thrombosis risk score (PRVTRS) is decisive for the duration of antepartum prophylaxis. PRVTRS was evaluated monthly in addition to clinical follow up and prophylaxis was introduced in women scoring >3.

Results : in some cases score based strategy may allow a limitation in the number of women under heparin therapy : Of the 119 pregnancies, an anti-thrombotic prophylaxis by prophylactic doses of low molecular weight heparin (LMWH) was prescribed in 64 cases (53.78%) with a positive score (>3). In 46 cases VTRS score was negative (<3) and patients did not have an antenatal prophylaxis.

During the study period, there were only two episodes of recurrent antepartum venous thromboembolism (incidence of 1.68%). A patient with AT deficiency at 61%, had a proximal DVT at 12 weeks gestation, despite an antithrombotic prophylaxis with unfractionated heparin. Another case of proximal DVT was noted in a patient with no recognized thrombophilia marker but a history of venous thrombosis after abdominal surgery at the age of 12 years. She was referred to our centre at the time of DVT. No complication related to long term heparin therapy was noted.

Conclusion : The score based approach evaluated in this study is efficient, safe and allows to calculate an individual risk of thrombosis. Thus, it could be helpful for the decision of antenatal prophylaxis in women with increased risk of VTE.

864 AGGRESSIVE NON-HODGKIN LYMPHOMA AND BCL-2 PROTEIN EXPRESSION - CLINICAL SIGNIFICANCE

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Overexpression of bcl-2 gene leads to cellular resistance to programmed cell death and it is overexpressed in approximately 50% of high-grade lymphoma.

Aggressive Non-Hodgkin lymphoma (NHL) is heterogeneous group with respect to clinical, histopathological and evolutive features. The International Prognostic Index (IPI) has provided a widely accepted prognostic set of criteria to design therapy. However, IPI dose not determine whether routine phenotypic features, beside IPI, may influence survival. We evaluate the prognostic significance of Bcl-2 protein expression in relation to clinical presentation and outcome.

In the period 1989-2002, two hundred eleven patients with newly diagnosed aggressive NHL were recorded in our Department. In this study we included patients with available biopsy sample. Protein expression was analyzed on paraffin embedded tumor tissue by immunohistochemistry in relation to clinical factor and outcome. The expression of more than 20% of neoplastic cell was considered positive for bcl-2. Comparison was made by χ^2 test. Survival curves were considered by Kaplan-Meier method and compared by the long-rank test. Sixty seven patient were recorded and their characteristic were: median age 53 years, stage III and IV, 67%; present B symptoms 44%; bone marrow infiltrating 29%, elevated LDH 58%, performance status more then 1- 26%; extranodal sites more than 2 34%. All 62 patients received antracyclin based combination chemotherapy, 72% CHOP regimen.

Overall survival was 52% with a follow up 6 -183 months. Bcl-2 expression was observed in 35, 5% (22pts). Overall survival was influenced by bcl-2. Bcl-2 positive cases were significantly associated with lower overall survival (20% vs. 58%; $p<0.01$).

Bcl-2 monoclonal antibody immunostaging appears to be a simple and reproducible method of determining biological potential of tumor cells and provides useful prognostic information in patients with aggressive NHL.

865 R-CHOP IS SUPERIOR OVER STANDARD CHOP AS INITIAL CHEMOTHERAPY REGIMENT FOR PATIENTS WITH UNTREATED AGGRESSIVE NON-HODGKIN LYMPHOMA (SINGLE CENTER EXPERIENCE)

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Treatment of aggressive Non-Hodgkin lymphoma with standard CHOP regimen has yielded overall response rates of 80-90%, with complete response rates 45-55%, and long term 5-years survival is seen in 30-40% of patients. Attempts to improve these results by using more dose-intensive regimens have not resulted in a significant increase in the complete response rate, nor improved disease-free survival or overall survival. So, it is essential to developed new therapeutic approach for patients with aggressive-histology NHL. Rituxan is a chimeric murine/human monoclonal antibody that reacts specifically with B-cell antigen CD20 and affects both complementmediated 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. In this study we evaluate results of treatment with R-CHOP in comparison with standard CHOP as initial treatment for patients with aggressive NHL.

This study comprises 115 patients with histopathology diagnosis of aggressive B-cell Non-Hodgkin's lymphoma treated at the Department of Hematology in the period 1989-2002, which gave us the observation period of 6 to 183 months. This study consisted of two treatment groups; one comprises twenty patients initially treated with R-CHOP regimen with Rituxan 375mg/m² and six cycles of CHOP given 21 days in 18 patient and 8 cycles in two patients. The other group comprises 95 patients initially treated with standard CHOP regimen. There were no significant differences in sex, age, prognostic groups of patients

according to the IPI. Most of the patients were in the advanced clinical stage at the disease on the initial presentation of the disease, 24% of the patients in the third stage and 43% in the fourth stage. B symptoms were noted in 44% of the patients. Bone marrow infiltration was found in 29%.

After initial chemotherapy complete remission was achieved in 60%, partial response in 4% and there was no response in 32% with early deaths in 4% in group treated with standard CHOP regimen. In comparison, group of patients treated with R-CHOP achieved 80% complete remission, 15% partial response and 15% did not responded. Although the follow-up in group of patient treated with R-CHOP is still, there is significant improvement in event-free survival and overall survival.

Our study confirmed that the addition of monoclonal antibody Rituximab to CHOP marked a major advance in the treatment of aggressive lymphoma and should be standard initial treatment for previously untreated diffuse large B-cell lymphoma.

866 VARICELLA VACCINE. A CLINICAL STUDY IN IMMUNOCOMPROMISED CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA.

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Background: The increased number and long term survival of immunocompromised children with ALL might lead to their affection with fatal varicella. **Objective:** The safety and immunogenicity of varicella vaccine was studied in a group of children with ALL and non immune siblings of leukemic children. **Subjects:** Forty five Children with ALL 26 healthy siblings of children with ALL (group III) all with ve history of chicken pox, were immunized with live attenuated varicella vaccine (Oka strain). Thirty five were still receiving maintenance chemotherapy (group I) & 10 have completed their maintenance therapy at the time of the study (group II). The control group comprised 12 children with ALL who acquired natural varicella virus infection (group IV). **Methods:** Serum IgG antibodies against varicella zoster virus (VZV) were measured by ELISA at baseline and at 3 and 6 months after vaccination for leukemic children & at baseline and 6 weeks after vaccination in healthy siblings, and once for the control group. **Results:** Patients in groups II & III tolerated well the vaccine with no side effects. However, varicelliform rash occurred in 5 (14%) children out of 35 cases of group I. The rash appeared 25-45 days (median 33.5 days) following 1st dose of the vaccine. It was vesicular in 4 cases and maculopapular in one case. The number of lesions was 15-30 in 2 cases & extensive in 3 cases. Three of them were treated with oral acyclovir. Zoster occurred in 3 cases (8.5%) of group I, it's onset was 2-3.5 months following the 1st dose, there was no pain & fever occurred in only one case. It's duration was 6-15 days. The mean serum level of (VZV) was significantly lower in ALL in children after 6 months of vaccination than the control group ($P=0.000$). However, during 3 years follow up breakthrough varicella occurred in only one case of group II after household exposure. It was mild with no fever & with only 7 skin lesions. **Conclusion:** The vaccine-induced immunity appeared effective in preventing or modifying chicken pox after exposure to natural disease.

867 TREATMENT WITH IMATINIB MESYLATE FOR THE PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA (CML) IN CHRONIC PHASE (CP)-THE IMPORTANCE OF EVALUATION ABOUT THE TREATMENT EFFECTS AFTER THREE MONTHS FROM THE INITIATION-

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In order to establish an efficient treatment modality of imatinib mesylate, we explored the usefulness of imatinib treatment in 72 patients with CML-CP and tried to evaluate the effects of the dose escalation of imatinib in respect of the timing and cytogenetic state. Seventy two patients, 21 of whom were newly diagnosed and 51 had been treated with INF-a and/or

HU, were enrolled in the study. Imatinib was administered at the starting dose of 400 mg. 86% of enrolled patients, who were newly diagnosed and could be kept treated at the dose of 400 mg, got to CCR after 3 months. Percent bcr-abl positive neutrophils in the other 14% of the patients were also less than 5% after 3 months. 72% of the patients, who had been previously treated with the other agents and could be kept administered at the dose of 400 mg, got to the hematological state of less than 20% bcr-abl positive neutrophils after 3 months and got to CCR thereafter by 6 months. Four of the other 7 patients, whose bcr-abl positive neutrophils was more than 20% after 3 months, were treated with escalated dose of 600-800 mg imatinib and three out of the 4 patients could get to CCR thereafter by 12 months. However, 3 of 7 patients, who were kept treated at the initial dose of 400 mg, failed to reach CCR. The imatinib treated patients, who were identified to have entered into nearly CCR by FISH, were demonstrated to have 3-log reduction in bcr-abl positive cells by Q-PCR. Besides, by the continuous administration of imatinib for these patients, 4-5-log reduction of bcr-abl positive cells was obtained in most of the patients with one patient showing a negative nested PCR of bcr-abl RNA. Six out of 12 patients, who could not be treated with the standard dose of imatinib by the severe side effects, entered into nearly CCR even by the treatment with diminished dose (100-200 mg) or alternate administration of imatinib. One of the patients, who got to CCR after 3 months of imatinib therapy, developed lymphoblastic crisis after 6 months at the dose of 400 mg. Although this masked Ph patient did not show any point mutations on the region of abl kinase domain (reported in imatinib resistant patients), the abnormality of ASS region was demonstrated. In conclusion, since it is suggested that entering into CCR in short duration of imatinib therapy brings a better prognosis, dose escalation of imatinib is strongly recommended for the patients with CML-CP, who could not get to the hematological state of 20% or less bcr-abl positive neutrophils after 3 months of 400 mg imatinib therapy and were free from moderate to severe side effects. Considerable cytogenetic response could be observed in some patients, who had been treated with low dose or alternate administration of imatinib, which implies that low dose administration of imatinib is also recommended to the patients who does not have any donor for stem cell transplantation.

868 A PILOT STUDY OF TARGETTED ITRACONAZOLE PROPHYLAXIS IN PATIENTS AT HIGH RISK OF INVASIVE MOULD INFECTIONS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

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BACKGROUND

Patients with severe graft versus host disease (GVHD) requiring intensive immunosuppression are at high risk of invasive mould infections (IMI). Prophylaxis with an active, oral anti-fungal agent with reliable absorption in this context is desirable.

AIMS

Evaluate efficacy of itraconazole solution in high risk patients post-allograft.

METHODS

Forty-four patients at high risk of post-engraftment IMI received itraconazole solution 2.5mg/kg bd as prophylaxis. Itraconazole levels were monitored.

RESULTS

Two of the first nine patients, in whom bioavailability was compromised due to significant vomiting and/or diarrhoea, died of probable or proven invasive aspergillus. None of the subsequent 35 patients, some of whom with severe gut GVHD received liposomal amphotericin B prophylaxis until itraconazole was reliably tolerated and absorbed, developed IMI. The overall incidence of IMI was substantially lower than in historical controls. Itraconazole was generally well tolerated with 5 patients (11%) ceasing the drug due to intolerance or disturbed liver function. Patients with severe GVHD were more likely to have sub-therapeutic itraconazole levels than those with moderate GVHD ($p=0.005$).

CONCLUSION

Targetted prophylaxis with oral or parenteral anti-fungal agents in high risk allograft recipients appears to be effective in reducing the incidence of IMI.

869 COMPARISON BETWEEN THE REDUCED INTENSITY TRANSPLANTATION AND THE CONVENTIONAL MYELOABLATIVE ONE USING ALLOGENEIC UMBILICAL CORD BLOOD FOR ADULT HAMATOLOGICAL MALIGNANCIES

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Background/Aims: There has been reported that the allogeneic non-myeloablative stem cell transplantation is promising for the hematological malignancies. To make clear the feasibility of the reduced intensity hematopoietic stem cell transplantation (RIST) on the allogeneic umbilical cord blood setting, we compared the result of the transplantation between with the reduced intensity conditioning and with the conventional myeloablative one (CT) to evaluate the efficacy (engraftment, GvHD, and survival) and the safety (regimen-related toxicity, and transplantation-related mortality).

Methods: 9 cases, 36 years old in average (22-59), 4 with AML, 3 with ALL, 2 with NHL, were transplanted with CT. The preparative conditioning was that 6 with TBI+Ara-C+CY, 2 with TBI+VP-16+CY, 1 with TBI+CY. The prophylaxis of GvHD was that 6 with Cy-A, 2 with Cy-A+short term MTX, 1 with FK506+MTX. The transplanted total cell number of the umbilical cord blood was $2.61 \times 10^7/\text{kg}$ in average (1.97-4.09), and the number of CD34-positive cells was $0.90 \times 10^5/\text{kg}$ (0.70-2.24). HLA disparity on DNA level between the recipient and the transplanted umbilical cord blood was that 1 with 1 antigen-, 6 with 2 antigen-, and 2 with 3 antigen-mismatched. 6 cases, 52 years old in average (36-61), 2 with NHL, 1 with CLL, 1 with HD, 1 with MDS overt leukemia, 1 with CMML, were transplanted with RIST. The preparative conditioning was that 4 with Flu+L-PAM, 2 with 4 Gy TBI+Flu+L-PAM. The prophylaxis of GvHD was that 4 with Cy-A, and 2 with FK506. The transplanted total cell number of the umbilical cord blood was $2.91 \times 10^7/\text{kg}$ in average (1.86-4.09), and the number of CD-34 positive cells was $1.39 \times 10^5/\text{kg}$ (0.90-2.65). HLA disparity on DNA level between the recipient and the transplanted umbilical cord blood was that 1 with 1 antigen-, 3 with 2 antigen-, and 2 with 3 antigen-mismatched.

Summary/Conclusions: In CT arm, the engraftment of neutrophils was 24 days in average (13-61), of reticulocytes was 35.5 days (21-88), and of platelets was 54 days (27-145). Acute GvHD was observed in 5 cases with grade I-II, and in 4 with III-IV. In RIST arm, the engraftment of neutrophils was 17 days in average (11-19), of reticulocytes was 29.5 (24-32), and of platelets was 35.5 (30-41). Acute GvHD was observed in 4 cases with grade I-II, and in 2 cases with III-IV. In RIST cases early engraftment was observed. On GvHD, no significance was observed. Further study including the long-term survival is necessary for the determination of the feasibility of RIST in the umbilical cord blood transplantation.

870 SAFETY AND EFFECTIVENESS OF HIGH DOSE DEFERIPRONE IN PATIENTS WITH THALASSEMIA MAJOR: A 2-YEAR STUDY

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BACKGROUND

Thalassemia Major (TM) patients with iron overload who do not comply with or tolerate deferoxamine may alternatively benefit from the use of deferiprone (L1) at the widely used dose of 75mg/kg/day, but this dosage is not sufficient in all patients.

AIMS

To study the safety and effectiveness of deferiprone given at a dose of 100mg/kg/d in transfusion-dependent TM patients over 2 years.

METHODS

Eleven TM patients who were attending the Chronic Care Center in Beirut and intolerant to the side effects of deferoxamine were studied. Each received regular transfusions of red cells every 3 to 4 weeks to maintain hemoglobin concentration above 9g/dl. Deferiprone was administered orally in a total daily dose of 100mg/kg/d. At each monthly visit detailed clinical examination was performed, patients were monitored for side effects, and laboratory tests were checked. We assessed efficacy

of deferiprone treatment by periodic measurements of serum ferritin concentration.

RESULTS

The initial mean serum ferritin level was $2881 \pm 821 \mu\text{g/l}$ which dropped to $1172556 \mu\text{g/l}$ at the end of the trial ($p < 0.05$). Deferiprone was stopped for two months in 6 patients when ferritin level dropped below $1000 \mu\text{g/l}$. Transient alanine transaminase increase (8 patients), gastrointestinal discomfort (3 patients), and arthralgia (2 patients) were most commonly reported events, but these were well tolerated and did not require discontinuation of deferiprone. One patient had a transient decrease in neutrophil count that resolved after decreasing the dose to 75mg/kg/d. Compliance with therapy was excellent in all patients.

CONCLUSION

Deferiprone at a dose of 100mg/kg/d was effective in reducing iron stores, as determined by serum ferritin levels, in all eleven patients with few mild and reversible side effects. A serum ferritin level $< 1000 \mu\text{g/l}$ was achieved in 55% of the patients. A larger prospective randomized study evaluating deferiprone at this dose over a longer period of time seems necessary to assure efficacy and safety.

871 IMATINIB INDUCES HIGHER AND EARLIER CYTOGENETIC RESPONSE FOR CHRONIC-PHASE OF CHRONIC MYELOGENOUS LEUKEMIA IN JAPAN

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A multi-institutional retrospective study was performed after 2 years experiences of imatinib for Philadelphia chromosome positive chronic myelogenous leukemia (CML) in chronic phase (Kanagawa Study). We administered imatinib as the initial dose of 400mg/day in 87% of the patients. The dose was declined because of severe adverse effects; skin eruption, fluid retention, GI tract symptom and cytopenia. Hematological response and cytogenetic response were evaluated at 6th month and 12th month.

Thirty-seven untreated (early) and 53 previously-treated (late) cases (by interferon in 42 cases) were treated by imatinib. Four patients dropped out by severe skin manifestations.

Complete hematological remission was observed in 100% (37/37) and 92% (49/53) within 6 months in early and late cases, respectively. In early cases, complete cytogenetic response rate (CCR) in 57% and major cytogenetic response rate (MCR) in 32% were observed at 6 months and CCR in 88% and MCR in 8% at 12 months. In late cases, CCR in 64% and MCR in 15% at 6 months, and CCR in 82% and MCR in 12% were observed at 12 months. In three cases which the patients have not achieved CCR or MCR at 12 months, the daily doses were reduced to 200mg because of severe complications. One case who had achieved CCR at 6 months developed accelerated phase at 12 months. Median time to achieve CCR was 5 months (2 - 14 months) in early cases and 4 months (1 - 13 months) in late cases. When patient outcome was evaluated between the groups with 400mg/day of imatinib and 300mg/day, CCR occurred 43 cases of 49 patients (88%) who treated at 400mg/day, and 9/21 patients (43%) treated by 300mg/day at 6 months. At 12 months, CCR occurred in 40 cases of 42 patients (95%) by 400mg/day, and 10/17 patients (59%) by 300mg/day. Furthermore the median time to achieve CCR was 3 months (1 - 11 months) in patients treated by 400mg/day although 7 months (2 - 13 months) in patients treated by 300mg of daily doses.

In conclusion, imatinib induced CCR and MCR in most patients with chronic-phase CML, and the duration to achieve CCR were shorter in Japanese. Four hundred mg/day of imatinib compared to dose in previous reports might be effective enough for Japanese CML patients.

872 DEEP VEIN THROMBOSIS IN A YOUNG PATIENT HOMOZYGOUS FOR THE RARE HEMOGLOBIN VARIANT, HB CRETE (BETA129(H7)ALA-PRO) AND HETEROZYGOUS FOR THE THROMBOPHILIC MUTATIONS MTHFR 677C-T AND PROTHROMBIN 20210G-A

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Background: Numerous cases of deep venous thrombosis, pulmonary embolism and cerebral thrombosis have been reported in patients with hemoglobinopathies, particularly in splenectomized patients with b-thalassemia intermedia, leading to the recognition of a chronic hypercoagulable state in such patients. Factors which play a role in the pathogenesis of a hypercoagulable state in the hemoglobinopathies involve altered properties of the abnormal erythroid cells, platelets and endothelium.

Methods and Results: The patient, a 20 year old soldier, was hospitalized with symptoms including red facial skin color, generalized itching following hot baths and deep vein thrombosis in his left leg. Physical examination revealed hepatosplenomegaly, while main laboratory tests showed: Hb 177g/L, Hct 0.58v/v, MCV 71fL, MCHC 304g/L, HbA2 4.0%, and FXII 38.0%, indicating a diagnosis of polycythemia and thrombophilia. Further investigation of the patient indicated the presence of a hemoglobinopathy, associated with increased oxygen affinity (P50 17.5mmHg), and increased bone marrow activity, reflected by a 2-fold increase of erythropoietin levels (26.2IU/L) and a 3-fold increase of soluble transferrin receptor levels (3.6mg/L). Molecular studies demonstrated homozygosity for the rare beta hemoglobin variant Hb Crete, and additionally heterozygosity for the thrombophilic mutations 5,10Methylenetetrahydrofolate reductase (MTHFR) 677C-T and prothrombin 20210G-A.

Conclusions: Hb Crete is a high affinity unstable Hb variant associated with erythrocytosis and heterozygous thalassemia like hematologic phenotype in most cases. Congenital thrombophilic mutations, such as factor V Leiden, MTHFR 677C-T and prothrombin 20210G-A, represent risk factors that contribute to development of severe thromboembolic events. Patients with high oxygen affinity Hb variants and consequent erythrocytosis tend to have a hyperviscous state, although they do not always succumb to thrombotic events. On the other hand, inheritance of thrombophilic mutations may increase the tendency towards thrombotic events, although additional factors are usually required to trigger them. The severe thrombophilic status of the patient of this study is explained by the combination of erythrocytosis, exacerbated by instability of the hemoglobin variant, along with the co-inheritance of two thrombophilic mutations.

873 EXPRESSION OF PLATELET RECEPTORS IN MAJOR DEPRESSION

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Introduction: There are a growing number of reports suggesting that depression may increase the risk for thromboembolic events. Little is known, however, about the pathophysiologic mechanisms underlying this association. Several reports assumed that there is a relation between platelet activation and depression. The activation of platelets was demonstrated by the increase of expression of GPIIb/IIIa and P-selectins.

The aim of the study is to investigate into the possible relation between major depression and the expression of certain receptors (mainly GPIIb/IIIa and P-selectins) on patients' platelet's membrane.

Material: We studied 39 subjects; 22 hospitalized patients (10 males and 12 females with a mean age of 57±9,7 years) suffering from major depression with a score at least 30 to the 'Montgomery Asberg Depression Rating scale (group P) and 17 healthy persons as controls (group C).

Method: The expression of platelet's membrane receptors was assessed by flow cytometry using monoclonal CD41, CD42a, CD42b, CD61 and CD62 antibodies. Simultaneously we examined the aggregation of platelets using as stimulators Ristocetin (RISTO) and ADP.

Results: Mean Values (MV) and Standard Deviations (SD) in the levels of the determined receptors are presented in table I. The comparison of MV showed a significant increase of GPIIb/IIIa and P-Selectin in the P group. However, platelet aggregation in the two groups was found to be the same, as presented in table II. **Comments:** The platelet reactivity, expressed by increased levels of platelet receptors (GPIIb/IIIa and P-Selectins) appears to be induced in major depression. These changes may present a risk factor for thromboembolic events in depressed patients.

	GPIIb/IIIa	GPIIb	GPIIb	GPIIb	P-Selectin
P					
MV	96.09	76.75	93.8	96.5	15.9
SD	3.44	10.8	5.3	3.11	9.61
C					
MV	99.08	35.24	67.9	97.61	6.48
SD	0.75	30.38	28.01	3.3	5.54

Caption 1: Tabel 1

	RISTO (%)	ADP (%)
P MV	80.2	70.9
SD	18.1	9.5
C MV	80.38	69.95
SD	15.8	11.2

Caption 2: Tabel 2

874 FARNESYLTRANSFERASE INHIBITOR (FTI:ZARNESTRA TM) INDUCES APOPTOSIS OF MULTIPLE MYELOMA CELLS

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Multiple myeloma (MM) is a malignancy in which Ras gene activation is observed in about 40% of cases. Besides Ras, there are many other farnesylated proteins in cancer pathways which are potential targets of farnesyltransferase. Farnesyl transferase inhibitor (FTI) demonstrated significant antitumor activities both in vitro and in vivo. We have investigated the effect in vitro of FTI Zarnestra (Z; provided kindly by Johnson Johnson Pharmaceutical Research and Development) on growth and survival of multiple myeloma (MM) cells.

Fresh myeloma cells were collected from the bone marrow (BM) of 11 MM patients (5 men and 6 women), aged 41-79 (median 57). The above group consisted of 9 IgG cases, 1 IgA case, and in one case light chain disease, staged III by Durie-Salmon with massive plasmacytic bone marrow infiltration (47-90%) in trepanobiopsy and typical of proteinological abnormalities. The mononuclear cells were obtained by Ficoll-Hypaque separation, and staining for myeloma cells was

performed (anti-CD 38-FITC, anti-CD 138-FITC) by flow cytometric analysis. The expression of Fas Ag on MM cells was also evaluated. Cells were cultured in FBS medium with or without Z at the concentration range from 5.0×10^{-7} M to 1.0×10^{-8} M. Dual staining with annexin V -FITC and propidium iodide was used to detect apoptosis (Annexin V Kit, Pharmingen, USA). 10 000 stained cells were analyzed in each sample by flow cytometry (FACSCalibur Becton Dickinson) using Cell Quest Software (Becton Dickinson). Early apoptosis is defined by annexin V FITC+ and PI- staining. Background apoptosis in myeloma cells before treatment was less than 6%. Parallel experiments were performed on normal mononuclear cells derived from MM of subjects (N=5).

Percentage of the plasma cell in the patients samples varied from 18-92% determined by co-expression of CD 38 and CD 138. The Fas protein was spontaneously expressed at high level on 27,2% (3/11) of myeloma cells. We have found that Z induced a significant (45.5%), dose and time dependent apoptosis in the bone marrow plasmocytes population of 11 MM patients. Z induced high apoptosis rates in MM cells 24,1%, 56,2,0%, 94,0% of Annexin V+ cells at 24, 48 and 72 h respectively. Maximal apoptosis was observed at 1.0×10^{-7} M Z (94% A+/PI-cells), following 72 h of culture. Normal bone marrow cells were significantly less sensitive (4 fold) to Z than plasmatic cells of MM pts ($p=0.006$). No correlation was found between the expression of Fas antigen and the effect of Z on apoptosis.

Farnesyl inhibitors effectively block myeloma cell growth and their anti-myeloma activity is mainly caused by inhibition of Ras expression. We conclude that Z induces apoptotic processes in pathological plasmocytes MM in a dose- and time dependent manner. These observations suggest that Z might be an interesting therapeutical agent in the management MM.

875 CYTOMEGALOVIRUS (CMV) INFECTION IN PATIENTS TREATED WITH ALEMTUZUMAB AND OTHER NON-TRANSPLANT THERAPIES - A MANAGEABLE COMPLICATION WITH EARLY DETECTION BY PRE-EMPTIVE SCREENING

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Background: Cytomegalovirus (CMV) reactivation is a life-threatening complication of therapies that are heavily immunosuppressive, such as bone marrow and organ transplantation. Reactivation of CMV following stem cell transplantation and alemtuzumab therapy is recognised but increased awareness of this complication is required following other therapies for lymphoproliferative disorders (LPDs).

Aims: Increasing awareness of CMV reactivation following non-transplant therapies will enable the effective management of this complication.

Methods: We analyzed the records for non-transplant patients found to be CMV positive.

Results: We report a series of 17 patients with LPD who developed CMV reactivation as a consequence of therapies not including stem cell transplantation. The median age of the patients was 63 years. Twelve (71%) patients had B-cell chronic lymphocytic leukaemia (CLL), 2 (12%) had systemic marginal zone lymphoma (MZL), 1 (6%) each of angioimmunoblastic lymphoproliferative disorder (AILD), diffuse large B cell lymphoma and mantle cell lymphoma. CMV reactivation occurred whilst receiving alemtuzumab in 14 patients, fludarabine/cyclophosphamide combination in 2 and CHOP in 1. Thirteen (76%) experienced fever, 5 (29%) were hypoxic and 8 (47%) developed a cough. Six (35%) had radiological abnormalities. The median level of CMV DNA detected by real-time quantitative PCR assay was 27,600 copies per millilitre (range 850 to 320,000). Sixteen (94%) received antiviral therapy in the form of intravenous ganciclovir. Five (29%) of those treated were resistant to first line therapy, and 3 went on to receive foscarnet. Of the 16 patients treated, there were 2 (13%) deaths whilst receiving first line ganciclovir. Both of these patients were symptomatic prior to the diagnosis of CMV reactivation being made for 7 days and 8 weeks each. The remainder (87%) had a complete response with resolution of all evidence of reactivation. Of the 14 patients who received alemtuzumab, 11 had CLL, 2 had systemic MZL and 1 with AILD. After the first few patients were treated with alemtuzumab it became clear that CMV reactivation might be an issue and patients receiving alemtuzumab were pre-emptively monitored for CMV DNA by quantitative PCR assay. The rate of CMV reactivation in our center is 11 out of 91 (12%) CLL

patients receiving alemtuzumab. Four (29%) of the 14 patients receiving alemtuzumab who developed CMV reactivation had asymptomatic disease. CMV reactivation occurred at a median of 5 weeks (range 2 to 8) after the start of therapy in these patients. The alemtuzumab was stopped in 9 (64%) while on CMV therapy, and restarted in 7 after clearing the virus. All patients who developed reactivation of CMV after pre-emptive screening for CMV DNA was instituted during alemtuzumab were effectively treated, and most patients were able to either continue alemtuzumab therapy during their asymptomatic CMV reactivation or to recommence it after eradication of CMV.

Conclusion: It is important to be aware that CMV reactivation can occur during alemtuzumab and after other non-transplant therapies for LPDs, such as CHOP or purine analogue-based therapies. Pre-emptive screening for CMV reactivation during alemtuzumab therapy permits the safe and effective management of this complication.

876 STUDY OF THE POSSIBLE PRO-ANGIOGENIC EFFECTS OF RECOMBINANT ERYTHROPOIETIN THERAPY IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCY

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BACKGROUND:

Angiogenesis may play an important role in the pathogenesis of malignant haematological conditions (Ratajczak et al, BJH 1998;103;969). Vascular endothelial growth factor (VEGF) appears to be the most relevant of the known angiogenic growth factors and increased levels are associated with a poor prognosis in many haematological malignancies, including myeloma and myelodysplastic syndromes. In clinical practice, the use of anti-angiogenic agents is showing some success in haematological malignancy, in particular in myeloma.

However, epo is also a mediator of angiogenesis (Lab Invest 2000;80;501) and stimulates endothelial (EC) proliferation and migration, probably by a combination of direct action on ECs and indirectly via increased release of VEGF. Epo treatment of human BM ECs causes increased proliferation and increased expression of endothelial NO synthase. Epo stimulates proliferation and migration of human umbilical vein ECs, probably due to direct action of epo on ECs and/or indirect action via increased release of VEGF, bFGF, etc. (ABS 3418, ASH 2001). Interaction between rEPO and VEGF is instrumental in the growth promoting action of rEPO on bovine aorta ECs and is due to and is due to VEGFR upregulation (both KDR/flk-1 and flt-1) by epo via tyrosine phosphorylation (J An Soc Nephrol 1998;98;9) In a recent study, serum VEGF levels in peritoneal dialysis patients were significantly increased in those receiving rEPO (del Peso et al, Adv Perit Dial 2000;16;85). Serum and plasma levels of other pro-angiogenic factors, hepatocyte growth factor (HGF) and endothelin-1, are also increased in kidney dialysis patients on rEPO compared to those not receiving rEPO.

OBJECTIVE:

To measure the effects of recombinant erythropoietin (rEPO) therapy on markers of angiogenesis and endothelial cell function in patients with haematological malignancy receiving EPO. Evidence of stimulation of angiogenesis or perturbation of endothelial cell function would suggest a more cautious approach to rEPO therapy since angiogenesis has been associated with a poor prognosis in many haematological malignancies.

PATIENTS AND METHODS:

To date 5 patients predominantly with myelofibrosis and MDS in stable phase were studied (12 patients will be recruited in total). By standard ELISA techniques soluble markers of angiogenesis (serum EPO, VEGF, HGF, bFGF) and markers of endothelial cell proliferation (plasma VCAM 1, vWillebrand factor) were measured prior to starting rEPO therapy. Samples were repeated 6 weeks into treatment and then again 1 month later following withdrawal of treatment. A dose of 10,00 units of rEPO was administered 3 times weekly and patients were assessed every 2 weeks with a full blood count and a reticulocyte count to document a response to therapy

RESULTS:

Results are enclosed in tabulated form.

CONCLUSIONS:

Results to date in this ongoing study do not show any clear association between angiogenesis and rEPO therapy in our group of patients. This suggests that rEPO can be used safely and effectively without adversely influencing long term prognosis.

	VEGF CONC. (pg/ml)	HGF CONC. (pg/ml)	b-FGF CONC. (pg/ml)	VCAM CONC. (ng/ml)	VEGF/PLT
PATIENT 3					
PRE EPO	210.4	2130.25	3.73	885.002	1.27
6/52 POST EPO	178.6	1520.25	3.61	902.64	0.88
4/52 OFF EPO	222.2	1365.25	1.39	962.6	1.02
PATIENT 4					
PRE EPO	52.7	3612.75	0.07	3733.529	0.78
6/52 POST EPO	61.3	3271.5	4.58	2648.971	0.82
4/52 OFF EPO	89	2592.75	1.902	2776.91	0.54

Caption 1:

877 UNCOMMON MORPHOLOGICAL FEATURES IN PHILADELPHIA CHROMOSOME POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background:

Acquired Pelger-Huet anomaly appears mainly in diseases affecting granulopoiesis such as myeloproliferative disorders, myelodysplastic syndromes, acute myeloid leukaemias and rarely but has been described in association with therapy with several drugs, infectious mononucleosis, chronic lymphocytic leukaemia among others.

Aims:

We present a case of Philadelphia chromosome positive acute lymphoblastic leukaemia (ALL) of L2 FAB subtype presenting with acquired Pelger-Huet leukocyte anomaly.

Clinical presentation and Laboratory findings:

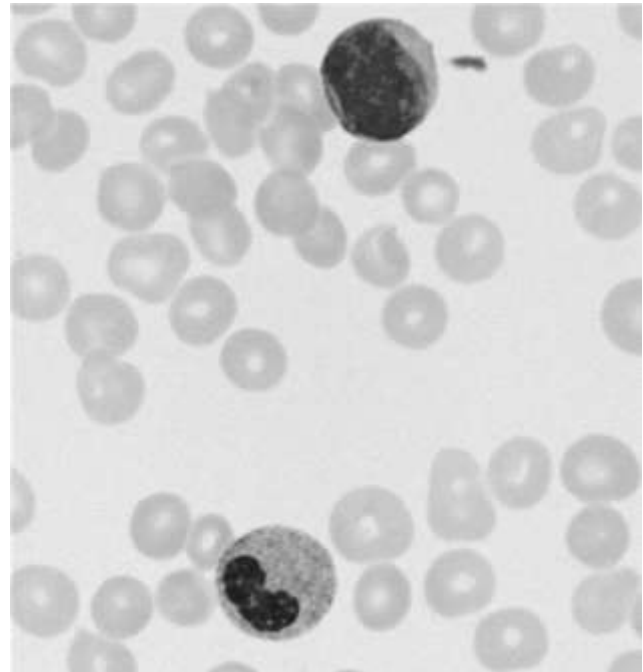
A 45 years old male patient was admitted to hospital for fatigue and blood cell count showing anaemia, thrombocytopenia and moderate leucocytosis with circulating blast cells. Physical examination was normal. Blood film examination showed a population of 43% blast cells medium in size, scanty cytoplasm with no azurophilic granulation and diffuse chromatin with 0 to 2 visible nucleolus. A high percentage of neutrophils and eosinophils showed decreased nuclear segmentation or non lobed nuclei and coarse chromatin with slightly increased cytoplasmic granulation and Dhle bodies. No signs of myelodysplasia at the platelet or red cell population was seen. There was failure to obtain a bone marrow aspirate (dry-tap). Blast cells were myeloperoxidase negative. Immunophenotyping of peripheral blood blast cells was consistent with the diagnosis of common B ALL. Cytogenetic analysis showed 4 of 17 metaphases with t(9;22) (q34;q11) and p190 BCR-ABL rearrangement was diagnosed by RT-PCR. The patient received chemotherapy following spanish multicenter trial for Ph⁺ ALL. At day 24 a diagnosis of neutropenic enterocolitis was made and despite systemic antibiotics, antifungal and surgical treatment the patient died within 72 hours at the intensive care unit.

Summary:

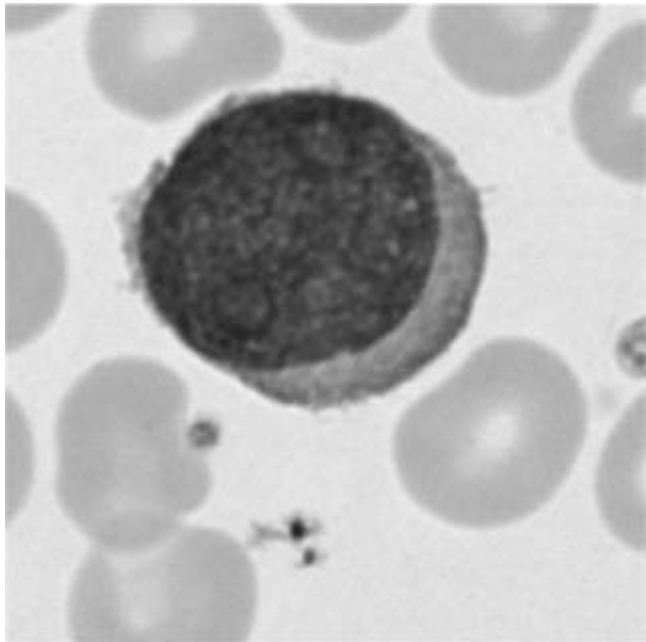
This case is significant because the association between acquired Pelger-Huet anomaly and lymphoid derived neoplasms is extremely uncommon. Regarding differential diagnosis: chronic myeloid leukaemia presenting as lymphoid blast crisis was not consistent with clinical data, physical examination and molecular findings. The patient was under treatment with thyroid hormones that have not been associated with this anomaly. There was no fever or other clinical symptoms of infectious diseases. Although a family study could not be performed before patient's death, neutrophils of congenital Pelger-Huet anomaly are usually bilobed with normal cytoplasmic granulation and without Dhle bodies.

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Caption 1: Lymphoblast and acquired Pelger-Huet neutrophil



Caption 2: Blast cell with two prominent nucleoli

878 FUNCTIONAL ANALYSIS OF T LYMPHOCYTES IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA (PNH) PATIENTS

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Paroxysmal Nocturnal Haemoglobinuria (PNH) is an acquired clonal disorder of haematopoiesis characterised by haemolytic anaemia, thrombophilia and cytopenia. Mutation in the PIG-A gene, responsible for glycosyl-phosphatidyl-inositol (GPI) anchor synthesis, is the hallmark of PNH cells. As a consequence, PNH cells are defective in

all GPI membrane-linked proteins. The presence of a residual normal haematopoiesis accounts for the mixed GPI- and GPI+ phenotype, usually found in peripheral blood cells of PNH patients. Some data suggest a close relationship between PNH and autoimmune diseases, therefore we have been investigating the functional behaviour of T lymphocytes in PNH patients in which are present both the GPI-defective and the normal T cell compartment. Our preliminary data indicate that the clonal PNH T lymphocytes compartment reveals decreased response to TCR-dependent T cell activation, as represented by reduced expression of activation molecules, proliferation and cytokine production. Interestingly, the normal T cell counterpart seems to show an increased frequency of autoreactive T cells and an altered distribution of the regulatory lymphoid populations (CD4+ CD25+ and CD3+TCR Va24+) as compared with a population of sex and age matched normal controls.

879 MORBID OBESITY AND THROMBOPHILIC FACTORS

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BACKGROUND: A predisposed factor of thrombosis is obesity. However, the exact role of known thrombophilic factors and their association with obesity are not yet determined. **AIM:** The aim of this study is to identify the possible relation between obesity and levels of thrombophilic factors. **METHODS:** 51 patients waiting to undergo bariatric surgery in our hospital (ages: minval=18 / average val=34,3 / maxval=58 years old), all suffering from morbid obesity (BMI>40 kg/m², minval=40 / average val=50,3 / maxval=69,5) were included in this survey, whereas 35 healthy (BMI<25 kg/m², minval=17,5 / average val=23,66 / maxval=25) volunteer individuals of similar age (ages: minval=20 / average val=33,8 / maxval=55 years old) were included as control. The PT, PTT, FIB, D-D, FVIII, vWF, AT III, PC, PS, APC-R, PLG, aPL, PAI-1 and tPA were measured for both groups, and the results were compared using the t-test method. We used clotting assays for PT, PTT, FIB, APC-R, FVIII and PS, ELISA for tPA, colorimetric assay for AT III, PC, aPL, PLG and PAI-1, and immuno-turbidimetric assay for D-D and vWF. **RESULTS:** The group of obese patients had higher average values for PT, PTT, FIB, D-D, PS, APC-R, PLG, aPL, PAI-1, tPA and vWF, while the healthy group had higher AT III, FVIII and PC, as shown on the results table. **SUMMARY/CONCLUSION:** Statistically significant differences (p<0,01) were found only in PTT, FIB, PAI-1, tPA, D-D, Leptin and AT III. In conclusion, we assume that the significant increase of FIB and the reduction of AT III might be responsible for the thrombophilic tendency of obese individuals. Moreover, the increase in both tPA and PAI-1, aPL in combination with the relative increase of D-D in obese individuals may be associated with an increased activity of the fibrinolytic system, as a reaction to the increased production of fibrinogen (which in turn is transformed to fibrin). En plus, our test results confirm the statistically significant difference in Leptin between healthy and obese individuals.

	PT	INR	PTT	FIB	D-D	FVIII	vWF	ATIII
BMI >40	12,9	1,02	33,6	385	0,4	118	145	86
BMI <25	12,6	1,02	31,4	297	0,3	120	111	103
p	0,05	0,9	0,004	3 x10 ⁻⁷	0,006	0,8	0,01	2 x10 ⁻⁵

	PC	PS	APC-R	PLG	aPL	PAI-1	tPA	Leptin
BMI >40	102	92	154	93	101	27,5	10	59,2
BMI <25	104	81	148	89	98	10,4	5	10
p	0,56	0,03	0,2	0,12	0,28	8 x10 ⁻¹⁴	3 x10 ⁻⁵	5 x10 ⁻¹⁸

Caption 1: results

880 COMPARISON OF TWO POINT OF CARE COAGULATION

MONITORS IN A HOSPITAL ANTICOAGULATION CLINIC

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Background: Point of care (POCT) anticoagulation monitoring systems are being introduced in European countries in order to accommodate the ever increasing size and workload of anticoagulation clinics. Their advantages are that the results are available virtually immediately and

with appropriate training they require minimal technical expertise. It is important clinically that the results are accurate, reproducible and dependable.

Aims: To find out how accurately two point of care monitors - CoagUChekS (Roche Diagnostics, Basel) and RapidpointCoag (Bayer) - display international normalised ratios (INRs).

Methods: Capillary whole blood samples were tested on the POCT monitor: either CoagUChekS (rabbit brain thromboplastin) or RapidpointCoag (human placental thromboplastin) and compared to a venous citrated plasma sample obtained at the same time and analysed on a CA6000 (Sysmex) using Innovin (human recombinant thromboplastin from Dade-Behring). The comparison took place over two months from a single hospital anticoagulant clinic. 181 patients were tested using the CoagUChekS and 149 patients using the RapidpointCoag.

Results: For the comparison of INRs between the CoagUChekS and the CA6000 there was a high correlation coefficient (r = 0.92, p<0.001). The mean INR with the CA6000 was 2.65 v 2.77 for the CoagUChekS (difference 4.5%, p<0.001). For the RapidpointCoag the correlation was much lower (r = 0.65, p<0.001). The mean INR with the CA6000 was 2.71 v 3.28 for the RapidpointCoag (difference 21.4%, p<0.001). For the CoagUChekS, 4/181 (2.2%) results were more than 2 INRs different compared to the CA6000. The equivalent number for the RapidpointCoag was 9/149 (6%). The comparison between the CoagUChekS and CA6000 revealed good agreement across an INR range from 1.0 to 7.5. Although there was a slight inverse trend (r = -0.2, p = 0.008), this would not be clinically significant. In contrast, the RapidpointCoag showed increasing deviation from the CA6000 across the INR range 1.0 - 10 (r = 0.32, p<0.001), particularly as the INR increased above 3.0.

Conclusion: The CoagUChekS gave results which, although statistically different from the CA6000, would not have significantly altered the clinical management of the patients. In contrast the results of the RapidpointCoag showed generally poor agreement with the CA6000.

881 SUBCUTANEOUS ALEMTUZUMAB WITHOUT DOSE

ESCALATION IN PATIENTS WITH PREVIOUSLY TREATED B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background

Alemtuzumab (MabCampath®) is an effective single agent for the treatment of chronic lymphocytic leukaemia (CLL). Subcutaneous (SC) administration of alemtuzumab as first-line therapy in CLL, starting at 3 mg and increasing to 30 mg per dose, usually within 710 days, produced transient but sometimes quite marked injection-site reactions. Systemic adverse events (AEs) were reduced, compared with intravenously treated patients.

Aims

This pilot study (ILEX Pharmaceuticals, L.P.-sponsored study) evaluated the safety and tolerability of SC alemtuzumab without dose escalation in relapsed, previously treated CLL patients. Preliminary data are reported.

Methods

Evaluations included safety and tolerability; assessment of injection-site reactions following an initial SC dose of alemtuzumab 30 mg (supplied as 30 mg/1.0-ml vial); and comparison of injection-site reactions after alemtuzumab 3 mg vs 30 mg SC. Ten patients with relapsed CLL requiring treatment were enrolled (mean age 63 [range 52-71] years; Rai stages 0, I, II, III, IV [n = 1, 6, 1, 1, 1, respectively]). Premedication was betamethasone (8 mg orally) and paracetamol (1 g orally). All patients received prophylactic trimethoprim/sulfamethoxazole and valacyclovir, while on therapy and for a minimum of 2 months after the last dose of alemtuzumab. On Day 1, 30 mg alemtuzumab was injected SC into one thigh, and 3 mg SC into the other thigh. The 30 mg alemtuzumab SC dose was repeated on Days, 3, 5, 8, 10 and 12.

Results

All patients were evaluated for injection-site reactions. The initial 30 mg injection produced only grade 1 reactions (as assessed by common cytotoxicity criteria [CTC]) at the injection site, consisting of edema or erythema, which was typically transient (2 days), in 7/10 patients. No injection site reactions or adverse events occurred in three patients. Injection-site reactions were generally limited to the first three injections.

Reactions to the 3 mg injection typically occurred 1 day later and resolved within 24 hours. Intensity of skin reactions was similar at the 3 mg and the 30 mg injection sites. Within the first 14 days following 30 mg SC alemtuzumab, four patients exhibited grade 1 edema on Days 12 post-dose, persisting 12 days, and one patient experienced intermittent grade 1/2 pruritus. One instance of grade 3 pain occurred after the initial injection. One patient experienced a grade 1 reaction lasting 7 days. Treatment was generally well tolerated, and three patients experienced no AEs during the study (Days 114). AEs were generally grade 1, with the exception of one case of grade 2 cramps and one case of grade 2 urticaria. Three cases of cytomegalovirus reactivation without clinical disease were observed, two on Day 22 and one on Day 25. All cases resolved with ganciclovir treatment. A Salmonella infection was reported in a patient at Day 56, which resolved after alemtuzumab was discontinued and treatment was provided.

Summary/conclusions

Treatment with SC alemtuzumab without dose escalation was well tolerated, and injection-site reactions were generally CTC grade 1 and transient. Considering the beneficial side-effect profile and cost-saving aspects, further development of SC alemtuzumab therapy is warranted.

882 IRON INFLUX TO STORAGE ORGANS INCREASES HEPICIDIN GENE EXPRESSION IN CASE OF BLOCKED HEMOPOIESIS

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Background

Hepcidin, a peptide produced in the liver, is a key regulator of iron kinetics. It reacts to body iron status and inflammation, and serves as a signal molecule mediating decrease in iron intestinal absorption as well as iron sequestration in macrophages. However, the exact mechanism and factors controlling its expression remain largely unknown.

Aims

We studied hepatic gene expression of hepcidin (Hamp and Hamp2) and other genes involved in iron kinetics and regulation (amount of particular mRNAs) in the situation of markedly increased influx of iron to the store organs, mainly the liver.

Methods

Mice, males C57BL/6 KaThy aged 8 weeks (n=5 in each group), were sublethally irradiated with 4 Gy (60Co) and 24 hours later phenylhydrazine (PHZ) was administered (50 mg/kg). Mice were sacrificed after 16 and 48 hours. Amount of mRNA for studied genes was determined by reverse-transcription real-time PCR and expressed as relative to the amount of mRNA for housekeeping gene beta-actin. Hematological parameters, plasmatic iron as well as iron content in the liver and in the spleen were determined.

Results

Irradiation followed by PHZ administration caused anaemia, an increase in iron in the liver and spleen and an increase in plasmatic iron concentration.

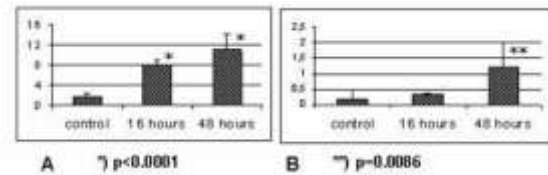
RBC count decreased after 16 hours from $8.18 \pm 0.50 \times 10^{12}/L$ to 6.58 ± 1.03 ($p=0.007$), and a pronounced anaemia was present after 48 hours (4.34 ± 1.31 vs. 8.18 ± 0.50 , $p=0.0001$). Iron content in the liver increased from 43.8 ± 9.5 µg/g wet tissue to 106.7 ± 13.9 µg/g 16 hours ($p<0.0001$) and to 147.3 ± 14.6 µg/g 48 hours after phenylhydrazine administration ($p<0.0001$). Plasmatic iron was 41.6 ± 10.4 µmol/L in controls, 72.5 ± 5.6 in the 16 hour interval ($p=0.0018$) and 58.8 ± 3.2 in the 48 hour interval ($p=0.0056$).

In the liver, hepcidin 1 mRNA content (relative to beta-actin) was similarly increased in both intervals (Fig. A) ' from 1.75 ± 1.03 in control group to 8.00 ± 0.79 after 16 hours, and to 11.05 ± 1.15 after 48 hours ($p<0.0001$). Hepcidin 2 mRNA (Fig. B) increased significantly only after 48 hours ' 1.22 ± 0.78 vs. 0.310 ± 0.31 in control group ($p=0.0086$). Hemoxygenase-1 was increased in both intervals. The amounts of mRNAs for other studied molecules ' DMT1, Ireg1, ferritin, frataxin, transferrin receptors 1 and 2, HFE and hemojuvelin were not significantly affected by the treatments. Amount of mRNA for TNFalpha was slightly increased only after 16 hours while that of IL-6 was unchanged in both intervals.

Conclusions

Hepcidin gene sensitively responded with increased expression to hemolysis in mice with inhibited erythropoiesis when iron was redistributed toward the liver. Surprisingly, other genes involved in iron kinetics were not significantly affected by this treatment.

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Caption 1: Hamp (A) and Hamp2 (B) expression

883 THROMBOPENIA IN PATIENTS WITH MALIGNANT LYMPHOMAS-KINETICAL AND IMMUNOLOGICAL EXAMINATION

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Trombocytopenia occurs frequently in patients with malignant lymphomas. Determining the causes of thrombocytopenia in these patients is often difficult.

Aim: The aim of this study was to assess the clinical impact of the platelet kinetic and immunologic studies for determination the mechanisms of the low thrombocyte counts in the management and treatment the thrombopenia in patients with malignant lymphoma.

Methods: Twenty six patients with malignant lymphoma associated with thrombopenia (12 with Hodgkin's disease and 14 with non-hodgkin's lymphoma) were investigated. The platelets were labeled with ^{111}In -oxine. Platelet survival was defined in daily blood sampling. Platelet distribution in vivo was determined by spleen/heart and liver/heart ratio. Platelets antibodies were measured by CELIA and MAIPA.

Results: According to our data the patients are divided into 3 groups. The results in group A established the platelet survival time and the sequestration indices near to the normal rate. Many chemotherapeutic agents used to treat malignant lymphoma have a myelosuppressive effect and depress megakaryocyte production. The data in patients group B showed a severe decreased platelet survival time mean 1,70 days (normal 8,10 days) and increase sequestration indices. In all examined patients the mean rate of platelet-associated antibodies was 6,07fg IgG/platelet (normal 1,56). Secondary immune thrombocytopenia can occur in association with lymphoma. In patients group C platelet survival time was negligibly decreased, mean 6,4 days, the spleen sequestration index was increased. The rate of platelet associated antibodies was normal. Patients with lymphoma often have splenomegaly and hypersplenism.

Conclusion: Thrombopenia is a common feature in patients with malignant lymphoma. Our results show, that it can be caused by platelet underproduction, increased platelet destruction, or disturbed platelet distribution (hypersplenisms). The determining of the mechanisms of the thrombopenia in patients with malignant lymphoma determine the choice of the most rational therapy (medication or splenectomy).

884 INCIDENCE OF THROMBOEMBOLIC EVENTS (TES) AND MALIGNANCIES. AN EPIDEMIOLOGICAL RETROSPECTIVE STUDY.

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Introduction: Thrombosis, both venous and arterial, is a common pathologic condition and a factor of important morbidity and mortality among the general population.

Aim: The aims of this retrospective epidemiological study are: 1) to reveal the frequency of TES among the hospitalized patients (hosppts) in AHEPA University hospital of Thessaloniki, during the last forty years and to compare this frequency with the one of malignancies of any body part or organ, during the same period. 2) to find out how many people died of TES in the west (industrialized) part of the city, during the last four decades and in the east (civil) part of the city, during the last two decades and 3) to compare this mortality with the one caused by malignancies in the same parts of the city and during the same period.

Material-Methods: The material consisted of 108.432 file records of the same number of pts hospitalized in AHEPA Hospital (mean age=46,8823,85). We studied the appearance of TES and of malignancies of any body part or organ in 1963, 1973, 1983, 1993 and 2002. Among the pts men were 63,75% (mean age=47,1623) and women 36,25%

(mean age=46,3923,85). Diagnoses were confirmed by the corresponding laboratory methods that were used each period of our study. We also studied 44.117 death certificates; the 8.656 were from the west-zone of Thessaloniki and referred to the years 1960-1999, while the rest 35.461 were from the east-zone and referred to the years 1980-1999.

Results:

(see Table 1)

(see Table 2, Ca= all kind of malignancies)

Conclusions: 1)a. The total number of hosp. pts has raised about 8,34 times during the period 1963-2002. b. In the same period the total number of pts with malignancies has increased by 7,66 times, while the number of pts with TEs has raised about 59,20 times. 2) In the west zone almost 1 person out of 3 die of TEs and 1 out of 4 of cancer. For the east zone the corresponding frequencies are 1 out of 4 for TEs and less than 1 out of 5 for cancer, indicating a potential correlation of death causes with the environmental factors.

Year	number of hosp pts	TEs	%	Malignancies	%
1963	4509	64	1,42	337	7,5
1973	16886	515	3,05	390	2,3
1983	18284	678	3,52	431	2,4
1993	31148	2897	9,30	2000	6,4
2002	37605	3789	10,07	2583	6,9

Caption 1: Table I. The number of TEs and malignancies and th

Years	west zone		east zone	
	Ca %	TEs%	Ca%	TEs %
1960-1969	18,4	17,4		
1970-1979	18,9	24,6		
1980-1989	19,5	31,2	18,9	15,9
1990-1999	24,3	34,4	18,4	23,4

Caption 2: Table II. The percentage rates of deaths caused by

885 DETECTION OF METHYLATION PATTERN OF P15INK4B TUMOR SUPPRESSOR GENE IN ACUTE LEUKEMIAS AND NON-HODGKIN LYMPHOMAS

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Background p15INK4B tumor suppressor gene inhibits the activities of cyclinD/CDK4 complex and negatively regulate cell cycle at the G1/S transition. Aberrant methylation of the 5'-CpG islands located within promoter regions has been proposed as a mechanism for the inactivation of the p15INK4B gene. Thus, loss of p15INK4B function in acute leukemias (AL) and Non-Hodgkin lymphomas (NHL) might lead to an escape from normal growth control by TGF-beta in bone marrow.

Aims We have studied frequency of abnormal methylation of 5'-CpG islands in promoter region of p15INK4B gene in a group of 39 adult patients (pts) at presentation, with diagnosis of AL (29 pts) and leukemic phase of NHL (10 pts).

Methods High molecular weight DNA was extracted from bone marrow MNC (24 pts), peripheral blood MNC (11 pts) or lymph node biopsy (4 pts), using standard protocols. DNA methylation patterns were determined by chemical bisulfite modification of unmethylated, but not the methylated cytosine to uracil and subsequent MS-PCR, using primer sets specific for either methylated or unmethylated modified DNA.

Results In AL group aberrant methylation pattern of p15INK4B gene was detected with higher frequency according to NHL group (17/29 pts, 59% vs. 5/10pts, 50%). Aberrant methylation pattern of p15INK4B gene in AL was detected with a lower frequency in ALL according to AML group (2/6 pts, 33% vs. 15/21 pts, 71%). In two pts with BAL aberrant methylation pattern was not detected. Normal unmethylated pattern of p15INK4B gene was present in every studied pt. All results were done at least in duplicates and were correlated with adequate positive and negative control samples.

Conclusion Abnormal p15INK4B gene methylation pattern was disclosed in all studied patient groups, but with the highest frequency in AML and NHL groups. High frequency of aberrant p15INK4B gene methylation may have potential implications as molecular marker for clinical monitoring of patients.

886 THE PRESENCE OF ANTI-ASPARAGINASE ANTIBODIES MAY INFLUENCE L-ASPARAGINASE ACTIVITY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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L-asparaginase is a basic agent of antileukemic treatment. Because of its high immunogenicity, allergic reactions are the most often side effect of the therapy with L-asparaginase. Some data suggest the correlation between the presence of anti-asparaginase antibodies and the clinical signs of allergy. It is still not clear; if there is a correlation between the development of antibodies and L-asparaginase activity during the treatment.

The aim of our study was to estimate the presence of anti-asparaginase antibodies and to compare these results with L-asparaginase activity in leukemic children. We examined 13 children; aged 3-15 years, with newly diagnosed ALL, treated according to the ALL-BFM 95 protocol. They received eight doses (5 000 IU/m²) of L-asparaginase (E.coli preparation) in the induction phase and four doses (10 000 IU/m²) of the same preparation in the reinduction phase of the treatment. L-asparaginase activity was measured photometrically; anti-asparaginase antibodies concentration was measured by ELISA assay.

The mean L-asparaginase activity in the induction phase was 273 IU/l, in the end of this phase no anti-asparaginase antibodies were detected. The mean L-asparaginase activity in the reinduction phase was 425 IU/l and anti-asparaginase antibodies in 5/13 children were found. Among these 5 children, 4 patients presented low L-asparaginase activity during the reinduction.

Our observation may indicate that the presence of anti-asparaginase antibodies could influence L-asparaginase activity. More data are needed to estimate the significance of the development of anti-asparaginase antibodies in childhood ALL.

887 IGE MRNA EXPRESSION IN IGE-PRODUCING CELLS IS INFLUENCED BY OVEREXPRESSION OF LATENT MEMBRANE PROTEIN 1 (LMP1)

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Background: Terminal differentiation process for IgE production in B cells involves germline Cepsilon transcription and isotype switching. CD40 signaling pathways, which are involved in isotype switching, are activated by the interaction of CD40 with CD40 ligand, and also mimicked by expression of Epstein-Barr virus latent membrane protein 1 (LMP1), one of the proteins encoded by the virus in latent infection. LMP1 is therefore assumed to influence IgE production in B cells. Aims: To elucidate whether IgE production in IgE-producing cells is influenced by overexpression of LMP1, IgE mRNA levels were examined in the rat IgE-producing hybridoma FE-3 cells and spleen cells derived from rats with or without immunization with allergen, following transient transfection with LMP1 expression vector. Methods: Immunization of Brown-Norway (BN) rats with 2,4-dinitrophenylated Ascaris suum antigen (DNP-As) was performed by subcutaneous injection of a mixture of DNP-As and killed Bordetella pertussis (Bp), followed by boosted injection of DNP-As alone. The IgE-producing hybridoma FE-3 cells and spleen cells derived from the rats with or without immunization with DNP-As were transfected with

LMP1 expression vector, pSG5-LMP1, using lipofection method. LMP1-transfected cells were cultured for 24 h and then collected to determine IgE mRNA expression using RT-PCR or Northern blot analysis. Results: Spleen cells were collected from the immunized rats at Day 7, since IgE levels were rapidly increased 6 days after first immunization (Day 6) and reached maximum at Day 15. In the non-immunized rats, IgE mRNA expressions were not altered by IL-4 treatment in the spleen cells transfected with pSG5 control vector as well as in those transfected with pSG5-LMP1. IgE mRNA levels were considerably higher in the spleen cells transfected with pSG5-LMP1 than in those transfected with control vector. In the immunized rats, IgE mRNA levels were significantly lower in the spleen cells transfected with pSG5-LMP1 than in those transfected with control vector. In the FE-3 cells, IgE mRNA levels were not affected by transfection with pSG5-LMP1. Conclusion: These results suggest that IgE mRNA expression in IgE-producing cells is influenced by LMP1 gene expression, depending on their differentiation stages following exposure to allergen.

888 PSEUDO-TUMORS AS COMPLICATION OF HAEMOSTATIC DISTURBANCES

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Pseudo-tumors are usually observed in the diaphysis of long bones, near the extremities. The appearance of pseudo-tumors in the anonymous bones of the pelvis due to psoas muscle hematoma, is rare. These pseudo-tumors are treated surgically with much difficulty. Here are presented 3 cases of pseudo-tumors (in different stage of evolution), which occurred in haemophiliacs and one case as complication of anticoagulant treatment in a thrombophilic. The first pseudotumor is in the very early phase and the periosteum just begins to detach. The second case is in the mid-phase and is still asymptomatic, without manifestation of any complications. Finally, the third one is presented radiologically with a major lytic defect in the left anonymous bone and is complicated with symptoms due to the pressure on the major vessels and nerves of the left leg. This pseudo-tumor was surgically treated in order to reduce the pressure on these organs. Four year and a half later the patient's clinical condition is quite good and he permanently takes substitution therapy with factor VIII concentrates, 40 U/Kg, twice weekly in order to avoid the hemorrhagic complications. The other two patients are followed-up for the possible evolution of their pseudo-tumors. As long as the 4th case is concerned, it is about a young man 29 years old with thrombophilia Leiden treated with Sintrom, who has a fracture in the middle phalanx of the finger. 20 days later the problem was complicated with sub-periosteal reaction and lytic lesions. According to our experience it is recommended for every patient, who has presented psoas hematoma in the past, to have x-ray of pelvis every two or three years. This is the only way to detect possible evolution to pseudo-tumor. We believe that it is wiser to wait for the development of the problem. Concerning the fourth case, of pseudo-tumor occurring in a thrombophilic, we believe that the description of all cases enhance our ability mainly to diagnose, but also to treat these problems.

889 CHARACTERIZATION OF SJOGREN SYNDROME ASSOCIATED WITH T-LARGE GRANULAR LYMPHOCYTIC LEUKEMIA AS A UNIQUE ENTITY

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T-large granular lymphocytic leukemia (T-LGLL) is a rare disease that has been found to be associated with several immune disorders including mainly cytopenias, rheumatoid arthritis, thyroiditis. The occurrence of Sjogren syndrome in T-LGLL has been occasionally described. During the 20 last years we diagnosed T-LGLL in 24 patients, only 4 of them were males. We were interested by the fact that many female patients who were referred for investigation of T-LGLL suffered from sicca symptoms, have positive Schirmer test and after performing minor salivary gland biopsy were definitely diagnosed as Sjogren syndrome. Thirteen patients were recognized. All were female, aged 43-73. The white cell count was increased in 3, normal in 6 and decreased in 4. The clonal dominant lymphocytic expression was CD8 in 9 patients, CD4 in 4 and CD56/57 in 6. Associated diseases were encountered in these patients including

pure red cell aplasia, ITP, vitiligo, Hashimoto and cytopenias. All the patients had sicca symptoms, 8 had positive Rose-Bangale test, 12 reduced salivary flow and all a positive minor salivary gland biopsy. By using PCR methods and primers for the corresponding genes gamma and delta, rearrangement of T cell receptor was found in all patients. The evolution of the disease during the period of 1 to 20 years was relatively benign. None developed a malignant expansion. Four patients received low-dose prednisone and 2 developed severe pure red cell aplasia requiring blood transfusion. In conclusion, our study emphasized a close association of T cell large granular lymphocytic leukemia with Sjogren syndrome in women affected by this disease.

890 GAMMA-DELTA T-CELL RECEPTOR (TCRGD) EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTE POPULATION OF LYMPHOMA MALIGNUM (NHL) PATIENTS

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Background: Gamma-delta T lymphocytes appear to possess intrinsic cytolytic antitumor activity in different carcinomas, sarcomas and leukemia. Aim: determine a mean percentage (%) of gd T cells in peripheral blood of untreated NHL patients and verify the impact of gd T cells presence in peripheral blood on the NHL clinical outcomes. Material and Methods: 22 newly patients (pts) with diagnosis of NHL (NHL-T 5 pts, NHL-B 17 pts), admitted to Department of Hematology, Blood Cancers and Bone Marrow Transplantation of Wroclaw Medical University between 2002-2004 and 14 healthy controls were included. The S. Murphy staging system was used for prognostic stratification. Samples of blood were taken at the moment of NHL diagnosis. TCR gd cells were estimated by flow-cytometry (FACS), using monoclonal antibodies (MoAbs: Ab-anti TCRgamma1-FITC (Becton-Dickinson), Ab-anti CD14-PE/CD45-FITC, CD2-PE, CD3-PE, CD4-PE, CD8-PE, CD16-PE, CD56-PE) and CD25-PE, CD69-PE for identify activated Tgd cells. Results: Mean % of gd T lymphocytes for all NHL was 2,54% and was lower than in control group (5,63%) (p<0,05). NHL with extensive tumor at diagnosis (stage IV) had a lower mean % of gd T cell (2,37%), than NHL in other stages (I-III) (3,2%) (p>0,05). In primary site in peripheral lymph nodes mean % of gd T cells was higher (2,59%) than in others manifestations (2,49%) (p>0,05), but lower than in control group (5,63%) (p<0,05). Mean % of gd T cells in NHL pts with LDH>500 U/l was 2,56%, LDH< 500 2,61% (p> 0,05), in NHL with CRP> 7mg% was 1,75%, CRP< 7mg% was 2,95% (p<0,05). Mean % of gd T cells in NHL pts with hepatomegaly was 2,60%, without enlarged liver is 2,81% (p>0,05). Mean % of gd T cells in NHL pts with splenomegaly was lower (2,17%), than in NHL pts without enlarged spleen (2,80%) (p>0,05). Pts with bone marrow infiltration had mean % of gd T cells lower (2,42%) than in pts with normal lymphocyte number in bone marrow (2,86%) (p>0,05) and than in control group (5,63%) (p<0,05). Mean % of gd T cells in pts with abnormal % all lymphocytes in blood smear was also lower (2,37%), than in pts with normal blood lymphocyte number (2,62%) (p>0,05) and than healthy controls (5,63%) (p<0,05). Conclusions: In less aggressive NHL the mean % of gd T cells is higher than, in advanced NHL at diagnosis, but lower than in healthy population (statistical significance). NHL patients with higher mean % of gd T cells in peripheral blood at diagnosis have better clinical outcomes and are classified into lower advanced stages of disease. This fact may have a crucial role in better prognostic factors estimation for lymphomas and may influence on overall survival, but it needs a further examination.

891 ENDOTHELIUM AND PLATELETS FUNCTION IN DIABETIC PATIENTS

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Introduction: Type I diabetes mellitus is a life long metabolic disorder that initiates in young ages and can lead to serious complications, affecting the blood vessels of the whole body, where it induces atherosclerosis.

Aim: The aim of this study is to investigate both into the function of platelets and the functional state of blood vessels' endothelium in diabetic patients, by determining the levels of specific haemostatic parameters.

Material-Methods: We studied a total of 108 subjects: a) 58 patients with type I diabetes (group D), with a mean duration of the disease 10.5 ± 4.4 years, b) 30 normal subjects (control group1, C1) in order to compare the function of their platelets with the one of the diabetics and c) 20 other normal subjects (control group 2, C2) in order to compare their levels of thrombomodulin (TM) and von Willebrand factor (vWFAg) with the ones of the diabetics. We evaluated the aggregation of platelets with 4 different stimulators: Ristocetin (RISTO), ADP, Collagen (COLLA) and Adrenaline (ADR), using the PICA® aggregometer of Chronolog co, and following directly the instructions for use given by its manufacturers. TM and vWFAg were determined using an immunoenzymatic method (ELISA).

Results: The results are recorded in Table I

Conclusions: 1) The comparison of MV between normal subjects and diabetics did not reveal any statistical significant difference. 2) However, in 8 out of 58 diabetics there were extremely high levels of TM. This fact together with the clinical manifestation of complications (diabetic nephropathy, neuropathy or retinopathy) in these patients imply a functional modification of their endothelium.

	RISTO	ADP	COLLA	ADR	vWFAg	TM
	%	%	%	%	%	mg/dl
D MV	71.04	65.2	63.7	45.5	106.2	5.2
SD	13.3	19.4	27.8	29.7	25.2	5.7
C1 MV	69.7	75.8	83.3	79.8		
SD	11.8	6.01	9.6	6.4		
C2 MV					119.6	3.7
SD					20.3	0.9

Caption 1: Table I: Mean Values (MV) and Standard Deviations

892 CONVENTIONAL CYTOGENETICS IS STILL THE FOUNDER PILLAR IN THE DIAGNOSIS OF LEUKEMIA

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Background:

Karyotyping is an important factor in the diagnosis of different type of leukemias and an independent prognostic indicator, with an impact on the choice of treatment. The discovery of recurring chromosomal abnormalities in the leukemic blasts of patients that depends on the types of hematopoietic cell lineages has identified a large number of genes involved in leukemogenesis. The patterns of the karyotypic alterations by conventional cytogenetics [CC] may provide insights into the genetic pathways of disease progression as certain specific genetic changes are related to prognosis.

Aim:

Present study is aimed to use CC in various hematological malignancies to rule out secondary structural rearrangements in addition to primary alterations.

Methods:

CC was carried out in 456 patients either from peripheral blood or bone marrow cells for various type of leukemias. 21 cases out of 235 cases were further selected for FISH study by using different Vysis FISH probes along with in-house FISH probes such as BACs, PACs, PCPs, and WCPs.

Results:

Out of 456 cases, 235 cases showed primary chromosomal rearrangements and 33 individual showed secondary rearrangements in addition to primary rearrangements. Remaining cases did not show any chromosomal changes suggesting either remission or apparently

no detectable chromosomal changes involved in the neoplastic process. FISH study confirmed the primary observation revealed by CC techniques in all the cases except two cases. One case of AML-M3 did not confirm the cytogenetic finding of t(15;17) by PML/RARalpha fusion signals as multiple cell lines were involved in the patient. Whereas in another case of CML in blast crisis, tetrasomy of chromosome 8 was confirmed by using CEP 8 which was missed by CC [68,XY,-17,iso(17)(q11.2),Ph',Ph'].

Conclusions:

This shows that diagnostic Karyotyping is an independent prognostic parameter in hematological malignancies, as observed in the present study. Many secondary rearrangements (14% in present study) could have been missed if molecular techniques such as FISH or PCR alone have been used for the diagnosis. This suggests that cytogenetic study has its own importance and cannot be replaced either by FISH or PCR but later can be used as supportive and confirmative diagnostic tool.

893 THALIDOMIDE, DOXIL AND DEXAMETHASONE (THADD) FOLLOWED BY INTERFERON OR INTERFERON PLUS THALIDOMIDE FOR ADVANCED AGE OR RELAPSED MULTIPLE MYELOMA (MM): AN ONGOING MULTICENTRE PHASE II-III STUDY

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A number of ongoing studies evaluates thalidomide in combination with various chemotherapy regimens in relapsed/refractory MM, demonstrating an overall response rate of 53 to 72%. However, myelosuppression and infection related to chemotherapy represent the most frequent adverse events.

We designed a protocol for patients with relapsed non age-limit MM and newly diagnosed patients aged > 65 years, all scheduled to receive induction therapy consisting of Doxil 40 mg/mq iv on day 1, dexametasone 40 mg/day iv on days 1-4 and 9-12 and thalidomide 100 mg/day. This regimen, namely ThaDD, was repeated every 28 days for 4-6 cycles. In eligible patients ThaDD was followed by autologous or allogeneic stem cell transplantation and/or by maintenance therapy including a-interferon 3 MU sc 3 times a week, dexametasone 16 mg/day im for 4 days in a month with or without pulsed thalidomide 100mg/day for 14 days in a month, randomly. So far, 36 patients have been enrolled and 30 are assessable for response and toxicity.

Median age was 71 years (range 57-78); 16 patients had IgG MM, 8 IgA, 3 Bence-Jones and 3 non-secretory MM. Thirteen patients had newly diagnosed MM whereas 17 had relapsed after a median of 3 prior regimens (range 1-6); 9 patients had received double autologous stem cell transplant. b-2 microglobulin level resulted > 3.5 mg/l in 11 patients (37%) as well as albumin level was < 3.5 g/dl and haemoglobin level < 11 g/dl. Median creatinine level was 1.0 mg/dl (range 0.6-8.7). All patients received antithrombotic prophylaxis with warfarin 1.25 mg/day per os. Response and toxicity were assessed according to Samson et al and to WHO criteria, respectively.

Out of 30 patients, 5 (17%) obtained a > 90% paraprotein decrease (2 CR and 2 VGPR), 16 (53%) had a PR and 4 (13%) MR. Overall response rate was 70% (77% in newly diagnosed and 65% in relapsed MM). Moreover, 2 patients (7%) progressed and 3 (10%) died after the first course of therapy because of tumor lysis syndrome in one and unknown reason in 2 patients.

Overall we administered 105 cycles of ThaDD therapy. Thalidomide toxicity was as expected at dose of 100 mg/day (quite all grade 2 common side effects) although one patient (3.3%) developed DVT and pulmonary embolism after the first course and refused to continue the therapy.

Among the commonest side effects of Doxil we observed a moderate-mild haematological toxicity (2 patients developed neutropenia and one thrombocytopenia > grade 2). However, immunosuppression caused 9 febrile episodes (grade 3 in 4 patients), including 6 FUO, 1 colitis, 1 septic shock and 1 pneumonia associated with VZV reactivation. None of these infections were fatal although they all caused therapy delaying.

Combination Thalidomide, Doxil and Dexamethasone is very active for both newly diagnosed elderly and advanced MM. Although myelosuppression was less than that observed in other protocols including thalidomide and chemotherapy, infectious complications keep on being the most dangerous adverse events requiring prophylaxis. So far, follow-up of maintenance therapy is too short for any analysis.

894 AUTOLOGOUS TRANSPLANTATION OF AC133+ CELLS IN PATIENTS WITH ACUTE AND RECENT MYOCARDIAL INFARCTION

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Background. Experimental and clinical studies suggest that transplantation of bone marrow derived stem cells beneficially affect left ventricular (LV) remodeling processes after acute myocardial infarction (AMI) and contribute to the regeneration of infarcted myocardium and neovascularization of ischemic myocardium.

Aims. The objective of the study was to assess the safety and feasibility of autologous transplantation by intracoronary infusion of bone marrow stem cells into the infarct-related artery in patients with acute and recent myocardial infarction.

Methods. We enrolled 6 patients with acute or recent (1 to 28 days), large myocardial infarction and successful reperfusion (PCI with stent implantation in AMI-related coronary artery) and LV ejection fraction < 40%. Approximately 12 hours before the cell injection procedure, bone marrow (600 - 650 ml) was aspirated from the posterior iliac crest under general anesthesia. Because of the high volume of bone marrow aspirate, all patients received an iso-group transfusion (500 ml). The cells were isolated by centrifugation, and the buffy coat was used. Buffy coat cells were incubated with 5% human intravenous immunoglobulin and AC133 beads (MACS) for 30 minutes, at 20°C. Then, the cells were washed with Clini MACS buffer 0.5% human serum albumin, centrifuged, and finally resuspended in the same buffer (final volume = 90 mL). This volume was introduced in the Clini MACS Plus magnetic cell sorter (Milteny Biotec, Germany) for 90 minutes. The positive fraction oscillated from 4.5x10⁶ to 8x10⁶ AC133+ stem cells (purity 92-95%). Viability testing was assessed with trypan blue exclusion (97-98%). The quantitative analysis of AC133+ stem cells was performed by immunophenotyping, using a flow cytometer FACS Calibur with 2 laser (Beckton-Dickinson), Cell Quest acquisition software, and Paint A Gate software for data analysis.

The cells were delivered by intracoronary injection into the infarct-related artery. Baseline evaluations included clinical and laboratory evaluation, ECG, 48 hour Holter monitoring, transthoracic echocardiography, stress echocardiography, SPECT with Technetium 99 Sestamibi, LV angiography.

Results. No major periprocedural complications occurred and no malignant arrhythmias on 48 hour Holter monitoring were observed early after intracoronary infusion of bone marrow stem cells. All patients were free of angina and had no signs of clinical worsening. At 1 month follow-up no adverse cardiac events occurred and no malignant ventricular arrhythmias on 48 hour Holter monitoring were found. At 4 months, SPECT analysis revealed a significant mobility of the infarcted area, and a significant increase of the LV ejection fraction (50+/-4%).

Conclusions. These results suggest that autologous bone marrow cells transplantation by intracoronary infusion proves to be a safe and feasible method. For the moment, the major limitations of this study are the small number of patients enrolled and the short term follow-up (1-4 months), but the method seems to improve the overall myocardial function.

895 THE INCIDENCE OF FACTOR V LEIDEN A1691G, METHYLENE TETRAHYDROFOLATE REDUCTASE C677T, AND PROTHROMBIN G20210A MUTATION IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic, systemic and inflammatory disease. Thromboembolic phenomena are more frequent in patients with RA than healthy individuals and several hemostatic factors increase in these patients with RA. The knowledge about FV Leiden A1691G, methylenetetrahydrofolate reductase (MTHFR) C677T, and prothrombin G20210A mutation in RA is very limited.

Aim: We aimed to evaluate the incidence of factor V Leiden A1691G, MTHFR C677T, and prothrombin G20210A mutation in patients with RA. **Methods:** Forty-seven patients with RA and 53 healthy individuals as control group are included to the study. The DNA of patients and control group were obtained from blood in tubes with EDTA by using pure polymerase chain kit. Then factor V Leiden A1691G, prothrombin G20210A, and MTHFR C677T mutation were investigated in DNA by using LightCycler-Factor V A1691G mutation, Prothrombin G20210A, and MTHFR C677T estimate kits. Yates chi-square and students t test for comparison of groups were used. **Results:** The mean age of patients and control groups were 48.812.2 and 48.87.9 years, respectively. There is no history or physical finding of venous thrombosis in patients and control group. The heterozygote FV Leiden A1691G point mutation was estimated in 9 patients with RA (6.3%) and 2 patients (3.7%) in control group (p<0.001). The homozygote FV Leiden and A1691G mutation were not determined among patients and control group. Heterozygote prothrombin G20210A mutation was estimated in 9 patients with RA (15%). There was no heterozygote prothrombin G20210A mutation in control group (p<0.001). Homozygote prothrombin G20210A mutation was not estimated among the patients and the control group. Heterozygote MTHFR C677T mutation was found in 40 patients (85%) with RA while in 13 patients (24%) of the control group (p<0.001). There was no homozygote MTHFR C677T mutation in patients with RA. While, homozygote MTHFR C677T mutation was determined in 2 patients of the control group (p>0.05).

Conclusion: The incidences of heterozygote prothrombin (G20210A) and MTHFR C677T are higher in patients with RA than healthy individuals. We do not know the importance of heterozygote prothrombin (G20210A) and MTHFR C677T in patients with RA. It may be useful to follow up closely in respect of further thromboembolic events in RA patients with heterozygote prothrombin (G20210A) and/or MTHFR C677T mutations.

896 RELAPSED/REFRACTORY AGGRESSIVE NON-HODGKIN'S LYMPHOMA PATIENTS: IPAD (IDARUBICIN, CISPLATIN, HD-ARA-C AND DEXAMETHASONE) REGIMEN AS SALVAGE AND MOBILIZING THERAPY

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Background. The IPAD chemotherapy regimen - a four-drug (idarubicin 12 mg/m² 1° day, cisplatin 50 mg/m² continuous infusion x 2 days, Ara-C 2g/m² every 12 hours 3° day, dexamethasone 40 mg x 4 days) combination scheme - was developed at our Centre as salvage therapy for patients (pts) with relapsed/refractory aggressive non-Hodgkin's lymphomas (NHL) needing disease debulking and, in bone marrow negative pts, PBSC mobilization.

Aims. To evaluate safety profile and efficacy in terms of debulking and mobilizing potential of the IPAD regimen.

Methods. Between March 1997 and January 2004, 39 pts (M 20, 19; median age 54 yrs, range 18-74), with relapsed/refractory aggressive NHL (29 DLCL-B including 4 primary mediastinal thymic; 5 T-large cell; 5 mantle cell NHLs) received the IPAD regimen as salvage therapy; in bone marrow negative pts, feasibility of PBSC harvest following IPAD was also assessed. A total of 80 courses were administered (median 2/pt). Response was evaluated after 2 courses of therapy using standard parameters.

Results. No toxic deaths were observed. Four pts are not evaluable: 3 of 4 are too early to be evaluated, 1 died because of disease progression while receiving chemotherapy. Among the remaining 35 pts, overall response was 51.5 % (18 pts, 7 CR, 11 PR); in 11 pts disease progression (P) was observed, while 6 had stable disease (SD) and were considered non-responders. Hematological grade IV toxicity was observed in 21 of 39 pts (54%); 4 pts (10%) developed either FUO or bacterial infections during neutropenia.

PBSC harvest was performed in 12 bone marrow negative pts and it was successful in all; among these pts median PBSC harvest was 6.5 CD34+ x 10⁶ /kg (range 2.5 - 90 x 10⁶ /kg). Eight pts underwent autologous bone marrow transplant after IPAD (2 CR, 4 PR, 1 P, 1 SD); 6 of 8 are currently in continuous complete remission (CCR) with a median follow-up of 13 months (range 1-27).

Of the 5 CR pts who did not undergo transplant, 1 progressed 2 mos from chemotherapy, 2 were in CR when they were lost to follow-up after 11 and 15 mos respectively and 2 are still in CCR after 4 and 76 mos of follow-up respectively.

Conclusions. These preliminary data show that the IPAD regimen is fairly well tolerated in pts with aggressive, relapsed-refractory NHL. In our series, response was observed in approximately half of this poor subset of pts. Moreover, our results show that IPAD combination is a predictable and highly effective mobilization regimen in view of high-dose consolidation procedures.

897 DERMATOLOGIC CHANGES IN CML PATIENTS TREATED WITH IMATINIB MESYLATE IN 200 PATIENTS: EXPERIENCE FROM INDIA

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We have prospectively evaluated a total of 200 patients with CML who were treated with Imatinib. There was lightening of the skin color (hypopigmentation) in 137 of the 200 patients (68.3%). This effect was also seen in de novo patients (i.e. with no previous exposure to hydroxyurea or any other chemotherapy). Imatinib inhibits the platelet derived growth factor receptor and the c-kit tyrosine kinases, the latter having an important role to play in the development of melanocytes and melanin expression. Inhibition of c-kit by imatinib is likely to have resulted in generalized hypopigmentation. Nine patients also complained of photosensitivity and 3 patients had phototoxicity. Dermatitis was seen in 55 patients (27.5%). Of these, 8 patients had Grade I, 39 had grade II and another 8 had grade III toxicity. In all patients with Grade I and 21 with Grade II dermatitis, topical steroids resulted in resolution of the skin lesions without need to discontinue imatinib. Imatinib had to be discontinued in 18 patients with grade II toxicity, following which there was complete response to continued topical steroids. Systemic steroids were used for all 8 patients with grade III toxicity with response in all of them. Imatinib could be restarted in all of them without reappearance of dermatitis. There was no correlation between hypopigmentation / dermatitis and cytogenetic response.

898 CD34+ SELECTED PERIPHERAL BLOOD AS SOURCE OF HEMATOPOIETIC PROGENITORS FOR ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH APLASTIC ANEMIA. RESULTS FROM A SINGLE INSTITUTION

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Allogeneic stem cell transplantation from HLA-identical sibling donors is the best therapeutic option for young patients with aplastic anemia (AA). Bone marrow and, more recently, peripheral blood stem cells are used for transplantation. The experience using CD34+ selected peripheral blood for transplantation in this setting is, however, limited. Eleven patients with AA (6F/5M; median age 17 years; range, 5-49) underwent CD34+ selected allogeneic peripheral blood stem cell transplantation from HLA-identical sibling donor at our institution between June 1996 and January 2004. Stem cells donors received G-CSF as mobilization regimen and median number of stem cell collections was 1 (range, 1-2). CD34+ cell selection was done by means of Ceprate (3 cases) or Miltenyi (8 cases) devices. To avoid a high risk of severe GVHD, the dose of CD34+ cells in the final product was adjusted to a value lower than $5 \times 10^5/\text{kg}$. Seven patients had an idiopathic AA (five severe and 2 moderate), two presented AA secondary to post viral hepatitis B and two had Fanconi's anemia. Median time between diagnosis and transplant was 1 month (range, 1-24), and median number of red blood cells and platelet transfusions administered before transplant was 19 units (range, 6-24). Overall, 8 of the 11 patients received CD34+ selected peripheral blood stem cell transplantation as first-line treatment. Two patients received combination therapy with anti-thymocyte globulin (ATG), cyclosporine A (CsA), prednisone and G-CSF and the remaining patient was treated with oxymetholone before transplant without response. Conditioning regimen consisted of cyclophosphamide 50 mg/kg/day, days -7 to -4 and ATG 30 mg/kg/day, days -4 to -2 in 9 patients. In the remaining 2 cases total lymphoid irradiation was added to the preparative regimen. Graft-versus-host-disease (GVHD) prophylaxis consisted of CsA and prednisone in every case. Median (range) number of CD34+ and CD3+ cells infused was $3.91 \times 10^6/\text{kg}$ (1.7-11.39) and $3 \times 10^5/\text{kg}$ (1-4), respectively. One patient died on day +2 and was not assessable for engraftment. Overall, median time to achieve a neutrophil count $>0.5 \times 10^9/\text{L}$ for the 10 remaining patients was 12 days (range, 9-13), and median time to recovery a platelet count $>20 \times 10^9/\text{L}$ was 13 days (range, 8-30). No patient developed late graft

failure. Two patients developed acute GVHD grade I responsive to treatment. There were 2 treatment-related deaths (18%). In both cases, patients were under mechanical ventilatory support during conditioning regimen due to the development of severe respiratory infections. One of them died on day +2 due to a bacterial pneumonia and the remaining patient developed pulmonary and cerebral hemorrhage and died on day +21. The remaining nine patients are alive with a median follow-up of 42 months (range, 1-94) and with a Karnofsky status of 90-100%. Although the number of patients is low, our results suggest that CD34+ selected peripheral blood from HLA-identical sibling donors is a valid source for stem cell transplantation in patients with AA. The procedure is associated with a rapid hematopoietic recovery posttransplant, a low transplant-related mortality and low incidence of GVHD.

899 IMMUNOCHEMOTHERAPY OF MALT LYMPHOMA- PRELIMINARY EXPERIENCE

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Mucosa-associated lymphoid tissue (MALT) lymphomas according to proposition R.E.A.L./WHO classification accounts to extranodal low-grade lymphoma with B-cell follicular marginal zone origin. Mature data taking into account the concept of MALT lymphoma as a distinct entity are still lacking in order to define the optimal approach to the management of this type lymphoma. The surgery has been widely used as front line therapy. GI localisation. Chemotherapy and radiation has been intensively investigated in extranodal gastric, but also extragastric origin. The monoclonal antibody rituximab targeting the CD20 antigen on B-cells may be active in this disease.

During two years period, at the Institute of hematology, Belgrade, 16 pts with MALT lymphoma were treated with combination of rituximab and chemotherapy. One patient with conjunctiva involvement were treated only with rituximab. Characteristics of patients were: male/female 9/7, median age 58.2 (range 31/77). Ten patients had primary gastric MALT lymphoma, and six patients had non-gastric lymphoma of MALT type. Five patient had H.pilory-negative primary gastric MALT lymphoma. Seven (43.7%) patients had bulky form of disease.

Rituximab (MabThera®), was given in standard dose: 375 mg/m² on the first

day of each cycle. Seven patients received CHOP regimen (6 cycles), tree patient received EPOCH regimen, and 5 patient was treated with Chlorambucil. Complete remission was achieved in 12/16 75%. Two patient died and stable disease was achieved in 2 patient. There were no serious adverse effects during therapy.

Conclusion: Immunochemotherapy is effective in primary gastric and extragastric MALT lymphoma.

900 FREQUENCY OF BREAST CANCER RESISTANCE PROTEIN (BCRP) POLYMORPHISMS IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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Breast cancer resistance protein (BCRP, 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. BCRP is presumed to play a protective role against a broad spectrum of toxic substrates in stem cells. 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 BCRP function may result in hematopoietic stem cell disorders. Several frequent single nucleotide polymorphisms (SNP) are described in the coding region of BCRP (V12M, Q141K, D620N), but the available information on the functional significance of the known SNPs is limited. The presence of Q141K was reported to decrease BCRP protein expression with unchanged mRNA levels. The aim of this study was to identify the frequency of V12M and Q141K polymorphisms in patients with chronic myelogenous leukemia (CML) comparing to healthy controls. V12M and Q141K were investigated in 149, unrelated consecutive blood donors, and in 138 patients with CML by PCR-RFLP and LightCycler allelic discrimination methods. The V12M and Q141K allele frequencies were 4.12.3% and 9.73.4% in the Hungarian control population and 5.42.7% and 9.43.6% respectively in patients with CML. Our data indicate that BCRP V12M and

Q141K polymorphisms are unlikely to play a role in the pathogenesis of CML. Further studies are performed to investigate the role of the above mentioned polymorphisms in acute myelogenous leukemia.

901 ENDOSCOPIC FINDINGS OF UPPER GASTROINTESTINAL SYSTEM IN MYELOPROLIFERATIVE DISORDERS

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Background: Gastrointestinal system (GIS) bleeding commonly occurs in myeloproliferative disorders (MPD), resulting from peptic ulcer, platelet dysfunctions and esophageal varices due to portal hypertension.

Aims: To investigate endoscopic findings of upper GIS and the incidence of *Helicobacter pylori* (Hp) in MPD.

Methods: We enrolled to this study 30 patients with MPD (14 essential thrombocytosis, 7 chronic myeloid leukemia, 5 polycythemia vera, 4 primer myelofibrosis, 19 women and 11 men, mean age: 5716 yrs) and 93 controls with irritable bowel syndrome (59 women and 34 men, mean age: 4413 years). The endoscopic examination of upper GIS was performed by Pentax EG-2940 video endoscope. CLO test (rapid urease/ campylobacter-like organisms) was used to identify Hp. One-way ANOVA, Mann-Whitney U and Yates chi-square tests were used for comparison of groups.

Results: Gastritis was detected in 27 (90%) patients with MPD and 27 (29%) controls ($p<0.001$). Bile reflux gastritis was found in 7 (23.3%) patients and one (1.1%) control ($p<0.001$). Peptic ulcer was noted in one (3.3%) patient and 4 (4.3%) controls ($p>0.05$). One patient (1.1%) had esophageal varices. Other endoscopic findings such as erosive findings, other gastritis were not different in-between patients and controls, as well as MPD subgroups ($p>0.05$). The incidence of Hp was 46.7% and 19.4% in patients and controls ($p<0.01$). All 5 patients with pangastritis and none of the patients with bile reflux gastritis were Hp positive ($p<0.05$). There was no influence of anagralide, hydroxyurea, acetylsalicylic acid, C-ara and steroid treatments on endoscopic findings and Hp positivity ($p>0.05$). New/former upper GIS bleeding was detected in 8 (26.7%) patients and no controls ($p<0.001$).

Conclusion: The results of this study suggested that the incidences of gastritis, upper GIS bleeding and Hp-positivity were higher in MPD patients compared to controls. MPD patients should be closely followed-up for potential upper GIS complications.

902 CAMPATH-1H PRODUCES A TRANSIENT REMISSION IN AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) COEXISTING WITH ANTIPHOSPHOLIPID ANTIBODIES (APA)

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Background: The antiphospholipid syndrome (APS) was reported in the early 1980s as the association of thrombosis, recurrent pregnancy loss in the presence of anticardiolipin antibodies (aCL) and/or lupus anticoagulant (LA). The antigenic targets for aPL are negatively charged phospholipids and serum phospholipid-binding proteins. Despite the strong clinical association between aPL and thrombosis, the pathogenic role of aPL in the development of thrombosis has not been fully elucidated. Proposed mechanisms include antibody-mediated interference with coagulation homeostasis, activation of platelets and endothelial cells and a T-cell immune response to serum phospholipid-binding proteins. The mainstay of therapy is anticoagulation, whereas immunosuppressive drugs seems to be ineffective.

Aims and methods: Herein, we present a 43 years old woman with AIHA unsuccessfully treated with steroids, splenectomy, imuran, cyclosporine A and danazol. Additionally, during the 5 years follow-up the antiphospholipid antibodies (APA) were found. Screening test performed showed the prolongation of APTT time (>300 sec), abnormal value of LA test and LA1/LA2 ratio (Dade Behring). Detailed examination revealed the presence of anti-beta2-glycoprotein I and anti-prothrombin antibodies. Mixing test studies and measurement of factor coagulant activity in the plasma suggested the presence of acquired inhibitor of factor VIII. However, the examination of amidolytic activity of factor VIII (Coamatic factor VIII, Chromogenix) revealed normal value (1,43 IU/ml). Because of intensive symptoms of hemolysis the treatment with Campath-1H has been introduced. The drug was given initially at dose of 3mg, and thereafter 10 mg daily intravenously three times a week (six doses). After ten weeks of treatment there was a dramatic improvement in general patient status and

hematological parameters (complete remission of AIHA). The significant improvement was also observed in coagulation test results (APPT 80 sec, normalization of LA test and LA1/LA2 ratio). Unfortunately, the remission of AIHA lasted ten weeks only. Also the screening tests results for APA became abnormal again (APTT 180-200 sec., LA1/LA2 ratio $>3,0$). Conclusion: the Campath-1H treatment may produce a significant response in patients with AIHA and APA. However, the schedule of drug administration should be further investigated.

903 CLINICAL MANIFESTATION OF THROMBOSIS IN PATIENTS WITH INHERITED DEFICIENCIES OF COAGULATION INHIBITORS AND RESISTANCE TO ACTIVATED PROTEIN C

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Background: It is known that thrombophilia is due to a deficiency in coagulation inhibitors and also to APC-R. AT III deficiency is associated with DVT, PE and, lesser frequent with cerebral vein thrombosis. The deficiency in PC and PS is correlated with superficial vein thrombosis, DVT and, more rarely, with cerebrovascular strokes(CVS), although APC resistance is correlated with venous and arterial thrombosis and to a lesser extent with CVS.

Aim: The aim of our investigation was to evaluate the sites of thrombosis in regard to deficiencies in particular coagulation inhibitors.

Method: Our study groups consisted of 150 patients suffering from thrombosis, 79 males and 71 females, with a mean age of 45 years. Patients with infections were excluded from the study. On each patient we measured the activity of AT III, proteins C and S. We evaluated the APC resistance. In 58 of these patients, (38.7%), 24 males 34 females mean age of 46.6 years, we found abnormally low levels of one or more of the evaluated parameters.

Results: The patients were divided into 5 categories:

A: 2 patients (3.45%) with ATIII deficiency, mean value 65%, (normal range 75-125%) with CVS. B: 3 patients (5.17%) with PC deficiency, mean value 52.7%, (normal range 70-140%), one CVS, one DVT and one PE. C: 37 patients (63.7%) with PS deficiency, mean value 52.5%, (normal range 70-120%), 19 CVC, 3 DVT, 3 PE, 5 CAD, 3 repeated loss of pregnancy and the rest Crohn disease, SLE, ophthalmic vessel thromboses. D: 2 patients (3.45%) with APC resistance, mean value 101.5 sec (normal >120 sec), 1 CVS 1 DVT E: 14 patients (24.14%) with multiple deficiencies, the most common combination was APC resistance with PS deficiency, (42.8%), followed by a combined deficiency in PC and PS, (28.8%), 5 CVS 4 DVT 2 PE and the rest repeated loss of pregnancy, and mesenteric conclusion.

Conclusions: 1. Of the patients we studied, we found that 38.6% had a deficiency of one or more coagulation inhibitors.

2. The most common clinical manifestations were CVS (48.3%), followed by DVT(15.5%), PE(10.3%) and coronary artery disease(8.6%).

3. The most common disorder was a deficiency in PS, followed by combined deficiencies in one or more of the following: PS, PC, ATIII and or APC-R.

4. The incidence of C.V.S was as follows: 100% among patients with a deficiency in ATIII; 51% among patients with a deficiency in PS; 50% among patients with APC-R resistance; 35.7% among patients with combined deficiencies; and 33% among patients with a PC deficiency.

5. Among our patient groups, females (58.6%) were more susceptible to thrombophilic disorders than men (41.4%).

904 CYTOGENETIC AND MOLECULAR CHARACTERIZATION OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH TRISOMY OF CHROMOSOME 12: REPORT OF 20 CASES

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Chronic lymphocytic leukemia (CLL) is characterized by clinical, morphologic, immunophenotypic and molecular heterogeneity. Trisomy 12 is among the more common recurring chromosomal defects in CLL (15-30% of cases) and has been correlated with atypical morphology and shortened survival; nevertheless, the negative prognostic impact may be attributed to case selection. In our Department, in a cohort of

ninety successfully karyotyped (with conventional cytogenetic analysis of mitogen-stimulated peripheral blood mononuclear cells) CLL patients (CD5+CD19+CD20+CD23+), twenty (22%) were identified with trisomy 12. This group included 12 males and 8 females with a median age of 65.5 years (range, 46-79); most patients were at early clinical stages (Rai stages 0-II: 15 pts; stages III-IV: 5 pts). After a median follow up time of 16.5 months (2-92), there was one treatment-related death, nine patients have progressed and required treatment, while the remainder have stable disease. Eleven cases were classified as morphologically atypical (>10% prolymphocytes and/or >15% cells with a cleaved nucleus and/or lymphoplasmacytic features). Thirteen out of twenty cases carried isolated trisomy 12; 3/20 showed co-existence of trisomy 12 and deletion 13q; 4/20 showed co-existence of trisomy 12 with various structural and/or numerical aberrations. There were nine IgM/Dkappa cases, one IgMkappa, three IgGkappa and seven IgM/Dlambda cases; nine patients had more than 30% CD38-positive cells. Ten out of nineteen analyzed cases carried mutated immunoglobulin heavy chain (IgH) genes (less than 98% homology to germline), while 9/19 carried unmutated IgH genes (4/9 using the VH1-69 gene and 2/9 the VH4-39 gene); concerning Ig light chain genes, 9/20 cases carried mutated genes while 11/20 carried unmutated genes (there was only one case with an unmutated A27-utilizing Vk rearrangement that had a mutated VH4-28-utilizing IgH rearrangement). No association was identified between Ig mutation status and morphology. Of the nine patients who progressed, 7 carried unmutated Ig genes; in contrast, 8/10 patients with stable disease carried mutated Ig genes. Statistical associations were explored between disease progression and: (i) karyotype (isolated trisomy 12 vs. trisomy 12 co-existing with other aberrations); (ii) CD38 expression; (iii) Ig mutation status; (iv) morphology (typical vs. atypical). Logistic regression analysis (forward stepwise) demonstrated that Ig mutation status and additional cytogenetic aberrations were significantly associated with disease progression. In conclusion, the present analysis provides evidence for the molecular and phenotypic diversity of CLL cases with trisomy 12; importantly, cases with trisomy 12 were evenly distributed among the mutated and unmutated subgroups. Although the follow-up time is short, thus precluding firm conclusions, Ig mutation status and additional cytogenetic aberrations were the only variables significantly associated with disease progression. Nevertheless, in the same context, it is noteworthy that almost half the cases with trisomy 12 in the present series exhibited disease progression within a relatively short period, (similar to the CLL1 trial of the German CLL Study Group).

905 GLYCOPROTEIN-P EXPRESSION ON MYELOBLASTS OF ADULT ACUTE MYELOID LEUKEMIA PATIENTS - HAS NO IMPACT ON PATIENTS OUTCOME

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Background: Expression of glycoprotein-P (Pgp) on malignant cells was considered by several authors as an important factor in development of multi drug resistance (MRD) in different cancers. In AML patients the correlation between Pgp expression and response rate to chemotherapy and DFS and OS was found. In our study we looked for correlation between Pgp expression and different laboratory and clinical parameters in AML patients treated in our department.

Methods: Pgp expression was evaluated on the bone marrow myeloblasts of 131 AML adult patients. Blast immunophenotype and presence of MDR product were determined using flow cytometry while taking advantage of double and triple staining with monoclonal antibodies for the particular CD-determinant and an extracellular epitope of Pgp (UIC-2, Immunotech). Out of the evaluated group - 20% pts (26/131) died during first induction chemotherapy and the rest of the patients 80% (105/131) received at least one cycle of standard remission-inducing chemotherapy.

Results: In the analysed group of 105 patients, myeloblast expression of glycoprotein-P was confirmed in 39% of cases (41/105). Evaluation of different laboratory (leukocytes, blast percentage in bone marrow, leukemia immunological subtypes) and clinical parameters (age, gender, Karnofsky index) analysed during diagnosis did not yield a significant statistical difference. Not was it revealed that Pgp expression offers statistically significant influence on the percentage of those having achieved CR (CR in 28 Pgp+ patients; 68% versus CR in 49 Pgp- patients; 77%). Cumulative probability comparison of DFS and OS periods between AML-Pgp+ and Pgp- also did not reach significant statistical difference. Pgp expression was the most often observed among patients with AML-M1 (54%; 22/41). Next we analysed correlation between Pgp presence on myeloblasts and coexpression of lymphoid antigens. Atypical lymphoid antigens (from T- and B-lymphocyte

lineages) were observed in 42% cases of the analysed AML patients (44/105). A significant difference was confirmed while comparing the coexpression of lymphoid antigens on Pgp+ (56%; 23/41) and Pgp- (33%; 21/64) myeloblasts (with the chi2 test p=0.022). Comparison of the CRs percentage in 16 Pgp+/Ly- group patients (78% CR) and 20 Pgp+/Ly+ (72% CR) no difference was found between both groups. Cumulative probability comparison of DFS and OS periods between Pgp+/Ly+ and Pgp+/Ly- showed a longer OS period in Pgp+/Ly+ patients group, but not significant difference (p=0.07). **Conclusions:** Subsequent investigations of the AML diagnosed group confirmed lack of correlation between Pgp expression and percentage of patients achieving haematological remission or longer DFS and OS periods. Thus, the myeloblast expression of Pgp in patients diagnosed with AML should not be treated as a significant prognostic factor. A simultaneous higher probability of longer DFS and OS periods in Pgp+/Ly+ patients group (vs Pgp+/Ly-) suggests that lymphoid antigen expression on myeloblasts can be acknowledged as a positive prognostic factor in AML.

906 PRIMARY HEPATIC LYMPHOPLASMOCYTOID LYMPHOMA HEPATITIS C VIRUS (HCV) RELATED RESPONDING TO PEGYLATED-INTERFERON PLUS RIBAVIRIN THERAPY

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Background. Lymphoplasmacytoid non-Hodgkins lymphoma is a B-cell malignancy with abnormal production of a monoclonal IgM protein. Generally the bone marrow biopsy is diffusely infiltrated with lymphocytes, plasmacytoid lymphocyte and some plasma cells. The lymphoproliferative disorder is rarely extramedullary with infrequent finding of solitary organ involvement. It is controversial whether HCV is involved in B-cell lymphoproliferative disorders, but some authors have associated the HCV infection with development of lymphoproliferative diseases such as Waldenstroms macroglobulinemia and some types of low-grade non-Hodgkin lymphoma, after resolution of the haematological malignancies with anti-viral treatment.

Aims. We describe the strict association between HCV infection and an unusual localisation of low-grade non-Hodgkin lymphoma well responding to therapy with pegylated-interferon plus ribavirin.

Methods. We observed a 66-year-old man with an asymptomatic ipergammaglobulinemia in HCV infection. Laboratory studies showed an IgM kappa monoclonal component (MC) of 5.4 g/dl, without Bence-Jones proteinuria, total protein of 15 g/dl, high beta-2-microglobulin (8.36 g/dl), Waaler Rose positive reaction (1:20480), high rheumatoid factor (81897 UI/ml). Transaminase level was normal, no signs of hepatic deficiency, with a high HCV copies titre (31825872 copies/ml). HCV genotype was 2a/2c. Objective examination was negative for pathological lymphnodes. Despite the high MC level, bone marrow biopsy showed a limited plasmacellular infiltrate (about 15-20%), without a monoclonal lymphoid infiltrate. Molecular biology of bone marrow aspirate resulted negative for heavy chain rearrangement (frame region 3 and 2) and for bcl-2 rearrangement. No osteo-lysis in skeletal radiography. The abdomen ultrasonically (US) examination showed two hepatic hypoechogenic lesions localized at V-VII liver segment (10,6x5cm) and at VIII liver segment (3cm), without splenomegaly. A nuclear magnetic resonance confirmed the presence of the two lesions, with infiltrating attitude. An US-guided fine needle biopsy (with Surecut 21G) of one focal liver lesion showed the presence of diffuse infiltrate by small size B cell with lymphoplasmacytoid features resulted to be diagnostic for lymphoplasmacytoid non-Hodgkins lymphoma (stage IE). We started to treat the patient with combined antiviral therapy: pegylated-interferon and ribavirin.

Results. After 1 year of treatment the monoclonal component is decreased from initial level of 5.4 to 0.6 g/dl, with improvement of other previously altered parameters such as beta2-microglobulin (from 8.36 to 4.67 mg/L) and total proteins (from 15 to 8 gr/dl); . Transaminases level remains in normal range. The high HCV viral load became undetectable. Hepatic involvement shows a significant improvement: the smaller lesion disappeared and the other is reduced about 50% (from 10.6x5 to 5x3 cm). The therapy is well tolerated with grade 2 anemia and periodical dose reduction of ribavirin.

Conclusions. These findings suggest a response to antiviral therapy by lymphoproliferative disorder. In this case, the pathogenesis of lymphoma is probably related to HCV infection and the response to antiviral therapy is a confirm of that. This case shows a strict association between some infection such as HCV and lymphoproliferative disorders, especially in case of atypical localization and confirms the utility of strategy of a first-line therapy with anti-viral agents.

907 PRESENCE OF LYMPHOID ASSOCIATED ANTIGENS ON ADULT ACUTE MYELOID LEUKEMIA MYELOBLASTS A POSITIVE PREDICTIVE FACTOR FOR OUTCOME

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Background: The incidence of lymphoid co-expression in AML varies considerably between studies. In most studies, expression of lymphoid associated antigens on AML blasts lacked prognostic significance. We have performed a study in a group of 195 de novo diagnosed AML patients determining whether lymphoid immunophenotype had a significant impact on clinical characteristics, complete remission (CR) and disease-free survival time (DFS).

Methods: These cases of untreated adult patients with de novo acute myeloid leukemia (AML) were diagnosed according to the French-American-British guidelines governing criteria and classification of leukemic blast immunophenotype. Each patient received at least one cycle of standard remission-inducing chemotherapy. Material for this study consisted of bone marrow harvested through routine biopsy aspiration. Using double and triple staining by specific monoclonal antibodies, immunophenotype was evaluated using flow cytometry for particular CD determinants. Positive results were defined as leukemic blast fluorescence levels higher than 20% as compared to control group levels.

Results: Immunophenotypes atypical to AML were observed in 41% of cases (88/195). Most often observed were co-expression changes of lymphoid antigens in myeloblasts (AML+Ly in 60% of cases, 53/88). Myeloblasts of diagnosed patients expressed five determinants of T-lineage (CD2, CD3, CD4, CD5, CD7) and three determinants of B-lineage (CD10, CD19, CD22). Among 53 pts, lymphoid T-lineage AML+Ly co-expression was evident in 41 cases. CD7 lymphoid antigen was most frequently observed (41%). These patients were also evaluated basing on other laboratory and clinical parameters. Statistical analysis calculated the mean age of AML+Ly patients as lower than the AML-Ly group (41.0 versus 47.8 years, respectively; $p=0.042$). Essentially, the percentage of myeloblasts in bone marrow of the AML group was higher ($p=0.007$). Substantially higher determinants characteristic for the precursors CD34, HLA DR and Ttf were also observed in the AML+Ly group. Analysis as to the efficacy of induction therapy in both groups yielded that CR was more frequently achieved in the AML+Ly group (79% versus 58% in AML without coexpression; $p=0.0042$). 5yr DFS for all patients was higher in AML +Ly group (DFS 34%), compared to AML-Ly (DFS 23%) ($p=0.046$). For patients who only received chemotherapy in AML+ Ly and AML-Ly group DFS was 15 % and 12 % respectively ($p=0.065$). No difference was found between AML+Ly and AML-Ly groups restricted to patients who received bone marrow transplantation (BMT): DFS 73% and 53% respectively ($p=0.243$).

Conclusions: It was subsequently found that co-expression of lymphoid antigens in the AML group correlated with a higher frequency of remission and longer DFS period. This study demonstrates that immunophenotyping seems to be additional prognostic factor and provides important information for identifying subsets of patients with different clinical features.

908 SAFETY PROFILE OF IMATINIB IN A PATIENT IN DIALYSIS TREATMENT AFFECTED BY CHRONIC MYELOID LEUKEMIA (CML) PHILADELPHIA POSITIVE

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Background. In the safety profile of imatinib the most frequently reported adverse reactions are haematological events, gastrointestinal disorders as nausea and vomiting, edema, muscle cramps, diarrhea and dermatologic events. Fluid retention events occur in about 50 percent of patients treated with daily dose of 400 mg, with prevalence of edema involving some districts such as periorbital area and lower extremities. Rarely occurs grade 3-4 edema with pleural or peritoneal effusion. Fluid retention is not related to renal function, and creatinine clearance is not significantly improved. Imatinib metabolism is predominantly hepatic, with only 13 percent of renal involvement.

Aims. We present a case of imatinib therapy during dialytic treatment in absence of previous clinical trials about safety in a patient with chronic renal failure.

Methods. A man 60 years old referred to our Department with leucocytosis in chronic renal failure due to polycystic disease from 20 years. Renal transplant was performed on 1991; due to a systemic Sporothricosis the immunosuppressive therapy (methylprednisone, azathioprina, cyclosporina A) was reduced and the patient presented a graft reject with subsequent need of dialytic treatment. Laboratory findings showed leucocytosis (WBC 44 x10⁹/L), anemia (Hb 8.8 gr/dl), normal platelets count with high lactate dehydrogenase levels (941 UI/L). Bone marrow biopsy and cytogenetical analysis were conclusive for CML with chromosome Philadelphia in all analysed metaphases. Molecular biology was positive for both chimeric proteins p210 e p190. The spleen was enlarged at physical and ultrasonography abdomen examination (15 cm bipolar diameter). The calculated risk prognostic score (according to the Sokal or Hasford systems) was low. Despite dialytic treatment the patient was treated with imatinib at low dosage for the first two weeks (200 mg/die), than 300 mg/die, with allopurinol 300 mg/die. The patient was also affected by a dilated-hypertensive cardiomyopathy and he was in treatment with other drugs such as beta-adrenergic blocking agents (carvedilol), cardiac glycosides (digoxin), angiotensin-converting enzymes inhibitors (ramipril) and oral anticoagulants (warfarin).

Results. After 6 months of imatinib therapy the patient shows a complete haematological response, without worsening of renal function, at stable dose of imatinib 300 mg/die. Anemia was not responsive to eritropoietin treatment before and during first weeks of therapy. Now hemoglobin level is about 12 gr/dl with acquired response to eritropoietin treatment. We attend to do bone marrow and cytogenetical evaluation after 6 months of therapy.

Conclusions. This case results to be the first in literature for safety of imatinib in posttransplant, after suspension of immunosuppressive treatment, and during dialysis. Therapy is well tolerated, without toxicities. Despite the high frequency of fluid retention present in literature, the patient didn't show significant edema signs, although the high risk due to dialytic treatment.

909 ABNORMALITIES AT 14Q32 BREAKPOINT IN B-CLL ARE ASSOCIATED WITH UNMUTATED IGTVH GENES OR VH3-21 AND VH3-23 GENE USAGE.

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BACKGROUND. The introduction of BIOMED-2 primers and protocol have allowed routine analysis of mutational status in B-CLL patients. Unmutated IgVH genes, and VH3-21 or VH3-23 gene usage, whether with mutated or unmutated IgVH genes, have been associated with more aggressive disease and poorer survivals in B-CLL. Likewise, interphase FISH has enabled identification of recurring chromosomal abnormalities, including those associated with poorer outcomes, such as 17p13 abnormalities and 11q23 deletions. This information permits better informed decisions on treatment strategies at presentation.

AIMS. To investigate associations between mutational status, gene usage and recurring cytogenetic abnormalities

METHODS. We have tested one hundred B-CLL patients for IgVH mutational status and the presence of recurring cytogenetic abnormalities using CLL Set and t(11;14) probe (Abbott Diagnostics). Full immunophenotypic analysis was carried out on each patient and morphology assessed independently and reported as typical monomorphic B-CLL, or atypical, pleomorphic morphology.

RESULTS. Thirteen patients showed abnormalities at 14q32, detected by the t(11;14) probe. Of these, nine had unmutated IgVH genes and two of these had trisomy 12. Of the remaining four patients with mutated IgVH genes, three had either VH3-21 or VH3-23 gene usage. Immunophenotypic analysis was available on most patients and showed strong expression of CD79b, CD11a and CD22. Eleven out of thirteen patients had been reported independently as having atypical, pleomorphic morphology, before immunophenotyping, mutational status or FISH analysis results were available.

CONCLUSION. This study has demonstrated a strong association between abnormalities at 14q32 and poorer prognosis indicators including unmutated IgVH genes and VH3-21 or VH3-23 gene usage. Of particular interest is the association with strong expression of CD22 in all cases, suggesting the presence of t(14;19) (currently being tested), which juxtaposes the bcl-3 and CD22 genes to the IgH region of chromosome 14, leading to their overexpression. Ligands for CD22 include several molecules with tyrosine kinase activity, suggesting a possible biological explanation for increased proliferation in these B-CLL patients. Therapeutically, this may be an important subgroup of patients to identify as possible candidates for therapy with anti-CD22 or tyrosine kinase inhibitors.

910 NON HODGKIN'S LYMPHOMAS IN GREECE ACCORDING TO THE REVISED EUROPEAN-AMERICAN / WHO CLASSIFICATION OF LYMPHOID NEOPLASMS A RETROSPECTIVE ANALYSIS OF 810 CASES

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Background/Aim:The purpose of this retrospective study, the largest unselected series in our country, was to illustrate the clinicopathologic features of NHL classified according to the REAL/WHO classification of lymphoid neoplasms.

Methods:A retrospective analysis and clinical features of histologic subtypes was conducted in 810 patients (age ≥ 15 years) with NHL who were treated at 8 major centers representative of Greece.

Results:There were 435 males and 375 females with an age >30 years in 95% of them. B-symptoms presented in 34% of the patients, while 45.3% had stages I-II and 54.6% had stages III-IV. LDH was increased in 37% of the patients. B-cell lymphomas formed 88% of the cases whereas T-cell lymphomas formed 12% of the total. Low risk lymphomas accounted for 30.7%, aggressive for 55.8% and very aggressive for 15% of all NHLs.

Among low risk lymphomas extranodal (MALT-B-cell lymphoma) was the most common subset while follicular lymphoma grade I and II and small lymphocytic presented with equal frequency. Among aggressive lymphomas diffuse large cell lymphoma was the most common subtype; this entity along with large cell immunoblastic accounted for 45.2% of all B-cell lymphomas.

Among the T-cell lymphomas, peripheral T-cell lymphoma and anaplastic large cell lymphomas of T/null-cell type were the most common subtypes.

The most common extranodal presentation was gastrointestinal tract. Next in frequency was primary extranodal NHL of the head and neck region.

Conclusion:This is the first report of a large series of malignant lymphoma in Greece using the REAL/WHO classification. It appears that there are no significant differences between NHL in Greece and other large series as far as clinical and extranodal presentation. The frequency of follicular lymphoma in the current study is comparable to this reported from Asian countries and mainland Europe, but lower than that of U.S. and Northern European series. There were not important differences in the incidence of the remaining histologic subtypes between Greece and other European countries.

911 ANALYSIS OF THE BONE MARROW LYMPHOCYTES WITH FLOW CYTOMETRY IN MYELODYSPLASTIC SYNDROMES

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Introduction:Immunosuppressive therapy is known to be effective in some patients with myelodysplastic syndromes (MDS). It suggests that the immune system plays a role in the pathophysiology of MDS.

Aim: We studied the bone marrow (BM) lymphoid population and its subtypes (CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/16+/56+, CD19+) of patients with MDS and patients with normal BM (control group) by flow cytometry.

Methods: The patients with MDS were classified according to WHO classification on the basis of generally agreed criteria (bone marrow cytology, histology, karyotype and peripheral blood count). There were 30 pts with MDS: 4 patients with RA, 13RCMD, 1RAR, 1RCMD-RS, 25q(-), 1-MDS-U, 3-RAEBI, 3-RAEBII, 1-CMML with WBC <13 G/L and 1-CMML with WBC >13 G/L. 19 allogeneic BM transplantation donors served as controls.

Cell staining was performed on whole bone marrow during routine phenotyping of lymphocytes and analysed in FACS Calibur (BDIS) flow cytometer. All of the results have been statistically tested by using t-Student test for the independent groups and Pearsons correlation. For statistically significant results were $p<0.05$.

Results: There was a larger percentage of lymphocytes in MDS than in normal BM (median: 19% vs 12.5%; $p=0.017$) and following subtypes: CD3+ cells (median: 77% vs 56%; $p=0.0028$) and CD3+/8+ cells (median: 34.5% vs 25%; $p=0.007$). NK (CD3-/16+/56+) cells and B (CD19+) cells proportions were decreased (median: 13% vs 14%; $p=0.019$; median:

10% vs 24%; $p=0.01$). There wasnt statistically significant difference in proportion of CD3+/4+ cells between MDS and normal BM.

There wasnt correlation between the number of T (CD3+) cells and B (CD19+) cells ($p=0.00089$). Only between following subtypes of T lymphocytes: CD3+/8+, CD3-/16+/56+ and B lymphocytes (CD19+) was found a positive correlation ($p=0.0002$; $r=0.65$ and $p=0.00000$; $r=0.82$).

In particular subtypes of MDS in proportion to normal BM there were following differences: in group of MDS-RCMD (number of pts=13)-in percentage of lymphocytes (median: 15% vs 12.5%; $p=0.038$), T lymphocytesCD3+ (median: 75.5% vs 56%; $p=0.0004$) and B lymphocytesCD19+ (median: 10.5% vs 24%; $p=0.006$); in group of RAEBI and RAEBII (number of pts=6)in percentage of lymphocytes (median: 23% vs 12.5%; $p=0.005$) and CD3+/4+ lymphocytes (median: 39% vs 29%; $p=0.02$).

Remaining subtypes of MDS were too small to be analysed statistically.

Summary: Percentage of BM lymphocytes and its subtypes CD3+ and CD3+/8+ are increased, but CD3-/16+/56+ and CD 19+ - decreased in proportion to normal BM.

912 THE ROLE OF THE VERY LATE ANTIGEN-4 (VLA-4) INTEGRIN IN THE EOSINOPHIL MIGRATION IN CHRONIC EOSINOPHILIC LEUKAEMIA

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Background: An essential step for eosinophils (Eo) migration involves their constitutive expression of the very late antigen-4 (VLA-4; CD49d/CD29) cell surface integrin that binds to vascular cell adhesion molecule 1 (VCAM-1) on the endothelial cell layer and to extracellular matrix proteins. Their consequent transendothelial migration is then facilitated by the 2 integrins, CD11b/CD18

(Mac-1) and CD11a/CD18 (LFA-1). Migration of Eo into specific tissues is a hallmark of monoclonal disorders of Eo proliferation such as acute or chronic eosinophilic leukaemia (CEL), but the exact mechanism involved in cell migration is unclear. **Aims:** In this study, we compared the expression of the VLA-4, Mac-1 and LFA-1 integrin subunits on the Eo of a CEL patient and Eo of healthy subjects, as well as their chemotactic response. **Methods:** The patient was a 60-year-old female first referred to our unit due to skin lesions and leukocytosis with eosinophilia. The diagnosis of CEL was based on blood sample and bone marrow aspirate and biopsy analyses using conventional criteria. Karyotype showed 47,XX,16q+,+21. BCR-ABL transcript was negative. Infiltration by mature Eo was seen in histology of a skin lesion. Twelve blood donors were used as healthy subjects. Eo were isolated from peripheral blood using a percoll gradient followed by immunomagnetic cell sorting. The expressions of the LFA-1, Mac-1 and VLA-4 integrin subunits were analysed by flow cytometry using CD11a, CD11b and CD49d FITC-conjugated monoclonal antibodies, respectively, and results were expressed as percentage of gated Eo. Chemotaxis to eotaxin was performed in ChemoTx-5 chamber. Measurement of Eo peroxidase activity was used to quantify transmigrated Eo. Results were expressed as absorbance at 460nm (optical density; OD). **Results:** A discrete increase in the expressions of both CD11a and CD11b expressions, and a high increase in CD49d (the VLA-4 alpha integrin subunit) were observed on the Eo of the CEL patient compared to expressions on Eo of healthy subjects (99.8% vs 97.9%, 99.3% vs 97.8%, 77.6% vs 47.6%, respectively; $P<0.001$). The chemotaxis to eotaxin (100ng/ml) was significantly ($P<0.05$) potentiated in CEL Eo (OD = 0.70 0.04), compared to Eo from healthy subjects (OD = 0.30 0.07). **Conclusions:** Results suggest that an up-regulation of the expression of the VLA-4 integrin on the surface of Eo may be involved in the increased migration of these cells observed in CEL disease.

913 CLONAL EVOLUTION IN CHRONIC MYELOID LEUKEMIA DURING IMATINIB TREATMENT

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Background. The presence of cytogenetic abnormalities in addition to the Ph chromosome in chronic myeloid leukemia (CML) has been known for a long time and is associated with disease progression. Clonal evolution (CE) in Ph-negative cells is a novel phenomenon that has been observed in CML patients treated with imatinib mesylate. The long-term clinical

outcome in imatinib-treated CML patients with newly detected CE in Ph-cells is not yet known.

Aims. To detect cytogenetic evolution in patients treated with imatinib in chronic phase of CML in whom interferon treatment failed and to observe the outcome of patients with clonal evolution.

Methods: Conventional cytogenetics and interphase fluorescence in situ hybridization (FISH) were used to identify additional cytogenetic abnormalities in 48 patients treated with imatinib for the median time of 30 months (range 7-39 months). The outcome of all pts was assessed.

Results. Complete hematologic response was achieved in 47 of 48 pts (98%) on imatinib, total major cytogenetic response (complete plus major) in 38 of 48 pts (79%). Cytogenetic evolution was observed in 7 pts (14.6%). Median duration of imatinib treatment before the CE identification was 21 months (range 6-30 months). In 5 (10.4%) additional abnormalities were present in Ph+ clonus, in 2 (4%) pts in Ph- clonus. The most common additional abnormality was trisomy 8 (5 pts) and second Ph chromosome (3 pts). In one patient deletion of the long arm of chromosome 7 (q11.2) was found. Hematologic progression was observed in 3 of 7 pts (42.8%) with CE and in 4 of 42 pts (9.5%) without CE. Secondary malignancy was diagnosed in 1 patient. All 48 pts are alive, in 4 of them the treatment with imatinib was stopped due to progression or second malignancy.

Conclusions. Cytogenetic evolution in CML pts on imatinib was documented in 7 pts, in both Ph+ and Ph- cells. Suppression of Ph+ clonus with imatinib may enable the manifestation of Ph+ clonal disorders in CML pts. A longer follow-up time and systemic monitoring of cytogenetics is needed to establish the prognostic impact of CE in Ph- clonus.

914 POST RITUXIMAB THROMBOCYTOPENIA IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA (NHL)

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Background:

Rituximab is effectively used in therapy of autoimmune thrombocytopenia and it is widely used in therapy of NHL. Rituximab is generally well tolerated. Unexpectedly we observed 4 cases of thrombocytopenia after therapy of NHL with rituximab.

Aims:

The purpose of the study was the description of these rare cases of thrombocytopenia in the course of therapy of NHL with rituximab.

Methods:

Among 114 patients with NHL treated with rituximab with standard doses 4 cases of thrombocytopenia were observed. Since the year 2000 Rituximab was used with chemotherapy as the first or subsequent line treatment including in vivo purging before mobilization or after stem-cell transplant (SCT).

Results:

All 4 patients were male (age range 45-55 years old) diagnosed with Mantle-cell lymphoma (MCL) (case 1 & 3), Follicular lymphoma G1 (case 2) and Diffuse large B-cell lymphoma (DLBCL) (case 4) who in the course of treatment was re-diagnosed to MCL.

Clinical stage: III and IV according to Ann Arbor

In all cases thrombocytopenia grade 4 (WHO) was observed.

Case 1: Two doses of rituximab were used before mobilization of stem cells after third line therapy (6xCOP, 7xCHOP, 3xESHAP). Thrombocytopenia after chemotherapy (COP, CHOP, ESHAP) was not observed.

Due to thrombocytopenia after rituximab mobilization was delayed.

Case 2: After 4 cycles of R-COP as first line therapy thrombocytopenia was observed. It disappeared after subsequent line therapy (2xCHOP).

Case 3: Rituximab was used after first line therapy (3xCOP + 5xCHOP) before stem-cell mobilization (dose 1) and after SCT (dose 2). Thrombocytopenia was observed for six months until patient's death.

In case 1 and 3 prednisone was used as therapy of thrombocytopenia without significant effect.

Case 4: Rituximab was used after second line therapy (6xCHOP, 2xESHAP) before stem-cell mobilization (dose 1) and after weekly (dose 2,3) for two weeks. Thrombocytopenia was observed after dose 2 of Rituximab. EPOCH therapy was used as the third line therapy after which thrombocytopenia was not observed any more.

In three patients bone marrow was not involved. Suppression of thrombopoiesis or damaged megakaryocytes were observed.

Conclusions:

Thrombocytopenia after rituximab was not related to the number of prior therapies. In two cases thrombocytopenia resolved in the course of

subsequent chemotherapy. Autoimmune mechanism may be considered and needs further investigation.

915 SAFETY AND EFFICACY OF AMBISOME IN CHILDREN AND NEONATES IN TURKEY. S. ANAK* AND THE PMS STUDY GROUP

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Methods: From October 1997 through January 2003, 285 case report forms on children and neonates treated with AmBisome were collected.

The objective was a prospective evaluation of the safety and efficacy of AmBisome in children with suspected fungal infections where toxicity precludes the use of conventional amphotericin B (CAB). Twenty sites actively participated. Information on demographics, underlying disease, indications for use of AmBisome together with the dose, duration of therapy, evaluation of safety and treatment outcome were collected.

Results: Two hundred forty two children (≤ 15 years), and 43 neonates (< 2 months) were included, 43.7% girls and 56.3% boys. The median age of the children was 6.34 yrs (0.3-15.0), and neonates 0.5 mts (0.03-1.9). The median weight was 21.5 kg for children (3.1-100.0), and 2 kg for neonates (1.0-4.0). In 80.6% of the children (excluding neonates), haematological malignancy was the underlying disease with BMT in 13 of these patients (6.7%).

The baseline clinical diagnosis (children population) indicated: 64 patients (26.3%) had FUO, 67 (27.6%) had pulmonary symptoms, 33 (13.6%) had symptoms of haematological disease, 15 (6.2%) had signs of hepatic disease and 5 (2.1%) had symptoms of CNS disease. Antifungal treatments were applied to 123 (43.2%) patients before administration of AmBisome, mainly fluconazole (74 patients) and CAB (60 patients).

For 166 patients (36 neonates) information on microbiologic diagnosis was available. In 81 of the suspected fungal infection the diagnosis was considered confirmed: 77 patients with Candida (21 were neonates), 6 with Aspergillus.

Thirty seven Candida infections were identified as *C. albicans*, in 10 patients a non-*albicans* species was identified. In 36 patients no species was mentioned. Aspergillus was mentioned for 6 patients: *A. flavus* for 1 patient, and 5 Aspergillus infections without specification.

The AmBisome median daily dose was 1.62 mg/kg, median duration of 15 days (2-147). The median highest dose was 1.84 mg/kg/day and given for 13 days. The median dose in neonates was 2.0 mg/kg/day (0.1-21.4) given for a median of 16 days (3-28), in children 1.5 mg/kg/day (0.1-21.4) for 13 days (2-122).

Twenty seven adverse events (AE) were described. The most frequent AE was hypokalaemia in 14 patients (4.9%). Fever was reported in 5 patients (1.8%). The investigators considered 25 AEs as 'mild' or 'moderate', 3 AEs were described as 'severe' and 3 as 'life-threatening'. For 7 AEs no severity was described.

Treatment outcome: 62.7% were cured. Clinical improvement was reported for 16.1% of patients (CR + PR = 78.8%). Forty nine patients (17.6%) expired; in 37 (13.3%) of these, the underlying disease was the cause of death. In 12 patients (4.3%) fungal disease was cause of death. Treatment failure was reported for 5 patients.

Conclusion: AmBisome can be considered as an effective and safe treatment in Children including neonates, with few side effects, similar to the existing literature.

916 TREATMENT OUTCOME OF ADOLESCENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA TREATED WITH PAEDIATRIC PROTOCOL OF EORTC

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Despite intensified chemotherapy, adolescents and young adults with acute lymphoblastic leukemia (ALL) still have lower rates of survival than younger children.

The purpose of our study was to evaluate the outcome of adolescents and young adults with newly diagnosed ALL and treated in the 58881 trial of the EORTC. Between January 1995 and December 2000, 55 patients (10-21 years of age) were entered in this study. The median of age was 14 years. Increased WBC $\geq 30,000/\text{mm}^3$ was noted in 42% of patients. Sixty eight per cent of these adolescents were included in the very high group.

The results of this study noted a poor response to corticosteroids in 29% of patients aged 15 years and more vs 13% for those aged 10-14 years.

The complete remission rate after induction was 85 % in the group 10 -14 years vs 81 % for those aged 15 years and more. Bone marrow transplantation in first complete remission was achieved for four adolescents, 3 of them are still alive in first complete remission and one is dead after 13 months of graft.

The overall survival (OS) and the events free survival (EFS) at 5 years for the patients aged between 10 - 14 years and those who were 15 years and more, were 54 % vs 59,5 % and 44 % vs 55,75 %, respectively.

Univariate analysis showed that unfavourable prognostic factors for EFS were as follows: WBC $\geq 30\,000/\text{mm}^3$ ($p=0,04$), and a total dose of L-asparaginase lower than 80000 U/mm ($p=0,03$).

Comparative data from other studies and data from this study indicates that OS and EFS can be improved, especially for adolescents aged 15 years and more when treated with intensive paediatric protocols.

917 PRAME AND BCR/ABL GENE EXPRESSION ANALYSIS FOR QUANTITATIVE MONITORING OF MRD IN CML BY MEANS OF REAL TIME PCR

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Background: PRAME gene expression was established in great variety of different malignancies including leukemias. In the case of healthy donors it was only shown to be expressed in testis, ovary, endometrium and in adrenal glands. It was also shown that the level of PRAME gene expression depended on leukemia stages and could serve as an indicator of minimal residual disease (MRD) in different types of leukemias. Lately we have found out by means of nested PCR that PRAME gene is expressed in different phases of chronic myelogenous leukemia (CML): in chronic stage – 34,4% of total cases, in acceleration stage – 80%, in blastic crisis – 95%.

Aims: To evaluate and to compare expression levels of PRAME gene and chimeric BCR/ABL oncogene as markers of minimal residual disease in CML.

Methods: CML patients were treated by glivec or interferon α and at the moment of investigation were in the first remission with full cytogenetic response. Total RNA was extracted from blood and bone marrow samples of 34 CML patients using acid guanidine thiocyanate for cell lysis and CsCl gradient purification. RNA samples were reverse transcribed into cDNAs which were then used in Real Time PCR evaluation of PRAME and BCR/ABL gene expression against expression of β -2-microglobulin. K562 cell line cDNA was used to plot a calibration curve. All samples with Ct for PRAME and BCR/ABL genes exceeding 40 cycles were considered to be negative.

Results: Among 34 patient tested 7 were PRAME and BCR/ABL negative, 7 showed both PRAME and BCR/ABL expression, 10 were BCR/ABL+ and PRAME-, and the other 10 were BCR/ABL- and PRAME+. Relative gene expression for PRAME gene was in range of 1,12 to 5,3x10-4 and for BCR/ABL gene 3,1x10-1 to 5,01-4.

Conclusion: Real Time PCR quantitation of BCR/ABL gene expression is considered to be a valuable tool for MRD monitoring in CML. Our data suggest that about 30% of CML patients in remission demonstrate PRAME gene expression positivity being at the same time BCR/ABL-negative. In such cases the PRAME gene expression is the only marker of MRD. We propose here that to use PRAME marker in addition to BCR/ABL one in Real Time PCR assays provides some extra valuable information about behaviour of leukemic clone in CML. and@blood.ru

918 NHL DIAGNOSED AS AN ORBITAL TUMOR.

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6 cases of NHL were diagnosed among 93 orbital tumors operated in Dept of Otolaryngology, CMUJ, during the last 5 years. At diagnosis, the presenting symptom was exophthalmos, usually slowly progressive during 4-6 months. Histopathology revealed 3 MALT lymphomas, 2 MCL (mantle cell lymphoma) and 1 FL (follicular lymphoma).

Low grade lymphomas, are usually diagnosed in an advanced clinical stage, however in our patients only 1 case (FL) had a disseminated disease confirmed by bone marrow trephine biopsy. In 1 of the MCL cases, the local spread of the disease was confirmed in parotid salivary gland, the following 4 cases were diagnosed in I-st clinical stage. Its speculative whether localized disease at presentation is secondary to early diagnosis or is a characteristic feature of lymphomas growing in this localization.

These histopathology subtypes are different from primary CNS (central nervous system) lymphomas (usually being DLBCL - diffuse large B-cell lymphoma) and secondary involvement of CNS by 'high grade' lymphomas (DLBCL, lymphoblastic lymphoma, Burkitt Lymphoma). None of the patients had a CNS involvement despite large local tumor mass (3-8 cm diameter).

The response to chemotherapy, followed in some cases by involved field radiotherapy (IFRT), was generally good all patients are alive (medium follow-up is so far 3 years). There was one relapse in a patient with localized disease, not subjected to consolidation IFRT. We would therefore recommend the combined treatment strategy. Our data suggest that there is however no need for prophylactic radiotherapy of CNS.

919 AUDIT OF SKIN TUNNELLED CENTRAL VENOUS CATHETERS INSERTED IN A SINGLE INSTITUTION OVER A SIX YEAR PERIOD

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Background: Skin tunnelled central venous catheters (STCVC) are a vital component of care for many patients with haematological conditions. Complications such as infection can be associated with significant morbidity and even mortality. In our institution STCVC are inserted by three designated experienced operators in a ward setting. This allows flexibility in timing of the procedure and reduces inconvenience for the patient.

Aims: To ensure that our practice is safe compared to previously published data, and to assess the pattern of bacterial infections necessitating catheter removal.

Methods: Data relating to all STCVC inserted in our unit was prospectively collected between 01/06/1997 and 31/12/2003.

Results: 186 catheters were inserted into 132 patients. 165 were inserted via a subclavian approach and 21 via the femoral vein. Immediate complications occurred in 13 patients (7%), including haematoma (4 patients), pneumothorax (3), acute chest pain (3), malposition of catheter (2) and extravasation (1). The catheters remained in place for an average of 102 days (range 0-465). The outcome of the catheters was as follows:

Catheter removed due to complications 71 (38%)

Removed due to completion of treatment 65 (35%)

Patient died with catheter in situ 40 (22%)

Follow up lost 7 (4%)

Catheter still in use 3 (2%)

Infectious complications were the commonest reason for catheter removal. Infection not responding to antibiotics accounted for 38/71 (54%) catheter removals. No bacterial growth was found in 10 cases. Stenotrophomonas maltophilia was by far the most common isolate, accounting for 14 cases.

Conclusion: STCVC insertion by a designated experienced team is safe in the ward setting. Complication rates are lower than previously published figures. Infection is the commonest reason for catheter removal with Stenotrophomonas maltophilia the commonest causative organism.

920 HYDROXYUREA IN THE TREATMENT OF PRIMARY THROMBOCYTOSIS: A RETROSPECTIVE STUDY OF A SINGLE INSTITUTION.

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Background: Over the last 20 years a vast array of data have been accumulated on the efficacy of hydroxyurea (HU) in patients with Philadelphia-negative myeloproliferative disorders (MPD). However, several side effects have been described as well.

Aims: Besides many anecdotal reports, no evaluation of their prevalence and type exists in large series of treated patients.

Patients: We report the side effects of HU in a retrospective, single institution, cohort study of 120 patients (49 males and 71 females, mean age 59.1115.01 years, median follow-up 7.58 years), 50 with polycythemia vera (PV) and 70 with essential thrombocythemia (ET), diagnosed in agreement with Polycythemia Vera Study Group criteria.

Results: In 14 cases (11.6%, 9 ET and 5 PV) therapy had to be withdrawn for unwanted side-effects (2 symptomatic macrocytic anemia with Hb less than 85 gr/L and MPV over 130fL, 2 fever reactions, 2 allergic reactions, 4 cases each of leg painful ulcers, 3 acute leukemia or myelodysplasia and 1 pancreatic cancer), representing a rate of 1.3 case/100 patient/year. Two cases of nail pigmentation were observed.

Conclusion: In our experience, HU showed to be an effective and safe drug in most patients with MPD. Prompt recognition of side effects, which have been mostly minor and rapidly subsiding on drug withdrawal, is in any case crucial to avoid more severe complications.

921 EUROPEAN EXPERIENCE WITH ZEVALIN: SUCCESSFUL IMPLEMENTATION OF RADIOIMMUNOTHERAPY

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Background: Yttrium-90 ibritumomab tiuxetan (Zevalin®) is a first-in-class radioimmunotherapy that targets tumor cells with an anti-CD20 monoclonal antibody and induces cytotoxicity with yttrium-90 radiation. Zevalin is approved in Europe for adults with rituximab relapsed/refractory CD20-positive follicular lymphoma. A model for implementation of Zevalin in a US hospital was presented by Vansant and colleagues (ASH 2003, abstract 4952). The model presented here is based on our university hospital experience.

Aims: To define hospital departmental roles for the implementation of Zevalin therapy based on procedures we established to accommodate 2 Zevalin clinical studies: a phase II study in patients >60 years with refractory/relapsed diffuse large B-cell lymphoma not eligible for peripheral SCT, and a phase III study of Zevalin versus no further treatment in follicular lymphoma having responded to first-line chemotherapy.

Methods: In our hospital, the local principal investigator (hematologist) and patient meet for initial consultation; the patient is evaluated for study eligibility, informed consent is obtained, and Zevalin treatment issues are discussed. Following consultation, a pretreatment evaluation including CT-scan, bone marrow biopsy, and complete blood counts as specified by study protocols are scheduled. FDG-PET scan is also scheduled according to local policy. The hematologist then suggests to the nuclear medicine physician (NMP) a theoretical agenda based on the following schedule: day 0, predose rituximab followed by optional 111-Indium Zevalin for imaging, and Day 8, second rituximab predose followed by 90-Yttrium Zevalin. The NMP checks this agenda with the radiopharmacist and accordingly orders one or both radioisotopes at least a week before Zevalin administration. On treatment day, once labeling has succeeded, the radiopharmacist calls the nurse-in-charge in the hematology department to start the predose rituximab. Within 2 hours post-rituximab infusion, the nurse-in-charge transports the patient to the NM department for Zevalin administration. The patient returns to the hematology department within 30 minutes and is discharged 2 hours later. Follow-up evaluations with the hematologist occur weekly for 12 weeks; patient response is evaluated 6 weeks, 3 months, and 6 months post-treatment.

Results: Since October 2001, 29 patients, of whom 11 were referred by their local hematologist, were screened. Median duration of initial consultation was 1 hour (range 12). Informed consent was readily obtained for all patients, except 1, at end of first consultation. Baseline requirements were obtained within a median of 10 days (range 7-15) of initial consultation. Twenty of 29 patients were scheduled to receive Zevalin. All received Zevalin within 3 weeks following the initial consultation. There were no logistical problems with the delivery and reconstitution of either yttrium-90 or indium-111, and there was no delay in rituximab or Zevalin administration.

Summary/Conclusions: Clinical trials with Zevalin for NHL identified critical points that require cooperation between all caregivers involved in RIT. The hematologist was the most appropriate person to coordinate the RIT process, while collaboration between the hematology department and the NM department was crucial to seamless Zevalin administration.

922 GAMMA-DELTA T-CELL RECEPTOR (TCR GD) EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTE POPULATION OF MULTIPLE MYELOMA (MM) PATIENTS

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Background: Gamma-delta T lymphocytes appear to possess intrinsic cytolytic activity against tumour cells in carcinomas, sarcomas and lymphomas. Activated gd T cells express antigens CD 25+ (late activator) and CD69+ (early activator) on their surface. **Aim:** determine a mean percentage (%) of gd T cells in peripheral blood of untreated MM patients and verify the impact of gd T cells presence in peripheral blood on the MM clinical outcomes. **Material and Methods:** 30 newly patients (pts) with diagnosis of MM, admitted to Department of Hematology,

Blood Cancers and Bone Marrow Transplantation of Wroclaw Medical University between 2002-2004 and 14 healthy controls were included. 5 pts were of stage I, 1 pt of I/II, 3 of II and 21 of III stages (Durie-Salomon classification). Samples of blood were taken at the moment of MM diagnosis. TCRgd 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, CD16-PE, CD56-PE) and CD25-PE, CD69-PE for identify activated Tgd cells. **Results:** In peripheral blood of all MM pts mean % of total Tgd lymphocytes (4,28 %) was lower than in control group (5,63%) ($p>0,05$); in I+II stages was 5,48% and in III stage 3,41%. Mean % of gd T cell in MM pts with $Kr\geq 2mg\%$ was 2,75%, LDH ≥ 500 2,11%, $b2M\geq 2,5ng/ml$ 2,6%, $plz >30\%$ 3,05%, $Hb<10g\%$ 3,33% and with hyperviscosity syndrome 2,26%. Mean % of gd T cell in MM pts with $Kr<2 mg\%$ was 4,43%, LDH <500 4,25 %, $b2M<2,5ng/ml$ 4,59, $plz <30\%$ 4,8%, $Hb>10g\%$ 4,6%, without hyperviscosity syndrome 4,7%. ($p>0,05$) In advanced MM at diagnosis also mean % of gd T cells was lower than in control group (5,63%) ($p<0,05$). Mean % of activated Tgd cells CD25+ and CD69+ was higher in bone stages 0+I+II, than in bone stage III (% gdT CD25+ in 0+I+II was 0,28%; in III 0,067% ($p<0,05$) and % gdT CD69+ in 0+I+II was 0,87%; in III 0,3% ($p>0,05$)). **Conclusions:** In MM pts, with advanced disease in diagnosis mean % of gd T cells was lower than in MM pts with less aggressive disease. Despite the absence of statistical significance, there is a favorable trend, that MM patients with higher mean percentage of Tgd cells in peripheral blood at diagnosis have better clinical outcomes and are classified into lower advanced stages of disease. Simultaneous activated Tgd CD25+ cells mean percentage elevation in peripheral blood of less advanced MM patients was observed (statistically significant). This correlation indicates, that higher percentage of activated Tgd CD25+ lymphocytes may be considered as a marker of better clinical outcomes in MM patients.

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923 RITUXIMAB IN COMBINATION WITH CHOP REGIMEN IN T-CELL ANGIOIMMUNOBLASTIC LYMPHOMA (AILD-TL) RICH IN LARGE B CELLS. FAVOURABLE RESULTS IN FOUR PATIENTS.

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BACKGROUND: B-cell clonal population is detected in 20% to 40% of AILD-TL. Moreover a subgroup of AILD-TL has been shown to display substantial number of CD20+ large B cells. This subgroup shares the poor prognosis of the classical AILD-TL with a 2-year survival of 40% (Lome-Maldonado, Leukemia 2002). We had postulated that this AILD-TL subgroup might benefit from a treatment with anti-CD20 monoclonal antibody (rituximab) combined to the classical CHOP regimen.

METHODS: We report four cases of untreated AILD-TL rich in large B cells treated with rituximab (375mg/m given on day 1 of each cycle) in combination with CHOP (cyclophosphamide, doxorubicine, vincristine, prednisone) chemotherapy (recycling at d21). Patients were planned to receive 8 cycles, if a good response (at least partial response) was observed after 4 cycles.

RESULTS: All the patients presented classical characteristics of AILD-TL with generalized lymphadenopathy, poor performance status, elevated LDH. 2 out of 4 patients had a serum M- component. Auto-immune hemolytic anemia occurred in 2 out of 4 patients. Histologic examinations revealed classical features of AILD-TL with more than 25% of large CD20+ cells with LMP-1 positivity in 3 cases. PCR analysis of tumoral lymph node showed a dominant T-cell clone in 2 cases, one of them also with a dominant B-cell clone. The two remaining cases had oligoclonal T-cell populations with polyclonal B-cell repertoire. A complete remission was achieved in 4 cases after 4 cycles and at the end of treatment for the 3 evaluable patients (treatment is still ongoing in one patient). One patient relapsed at 13 months with a low tumor burden and was treated with cyclophosphamide alone. At the present time, all the patients are alive without evolutive disease at 5, 23, 30 and 37 months.

CONCLUSION : These results led us to consider that rituximab adjonction to CHOP could improve the prognosis of AILD-TL rich in large B cells. This combination warrants further evaluation in a larger phase II study.

924 DOES GRAFT SOURCE HAVE IMPACT ON THE OUTCOME FOLLOWING HLA IDENTICAL SIBLING HEMATOPOIETIC STEM CELL TRANSPLANTATION ?

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Introduction: Peripheral blood is used more increasingly as an alternative to allogeneic bone marrow as source of stem cells in allogeneic stem cell transplantation in patients (pts) with hematological malignancies. Definite efficacy of peripheral blood stem cell transplantation (PBSCT) in the respect of all the aspects was not established yet. Aims: We have compared the use of allogeneic marrow with use of the peripheral blood cells from HLA identical sibling with respect to the engraftment, transfusions, transplant related mortality (TRM), acute and chronic GvHD, relapse and overall survival. Methods: We have analyzed 72 patients (pts) with different hematological malignancies (14 with sAA, 23- CML, 17- AML, 17- ALL and 1 pt- MM) treated in our Center with allogeneic SCT. Median age was 29 (9-52 years), M/F- 49/23. Pts were divided into two groups depending on the stem cell source- bone marrow transplantation (BMT)- 50 pts and peripheral blood SCT (PBSCT)- 22 pts. All pts have received unmanipulated suspension of mononuclear cells (MNC) from HLA identical sibling. Conditioning regimens were adjusted to the primary disease and GvHD prophylaxis was the combination of CsA+ Mtx+ MPDN. Results: Considering the number of MNC, pts with PBSCT have received much bigger sample (2,46 vs 8,7x 10⁸/kg BW, p<0,001). Recovery of neutrophils >0,5x 10⁹/l and platelets >20x 10⁹/l were faster (p<0,01) in the group with PBSCT in compare with the group with BMT for 3, ie 4 days respectively. Requirements for transfusions of either RBC units or Plt units were much higher in the group with BMT (p<0,01). Those pts also had mucositis gr 2-3 more often (33,5% vs 10,7%, p<0,05). There was no significant difference between the groups considering the frequency of aGvHD (70,6% vs 81,2%, ns), but cGvHD was more frequent in pts with the allogeneic PBSCT (41,2% vs 79,6%, p<0,05). Stem cell source did not have significant influence on TRM (15,21% vs 35,02%, ns). Also, groups did not differ significantly in the relapses (20,8% vs 10%, ns). Pts with BMT had better overall survival comparing with the other group (log- rank test 2,1), in due to fatal form of atypical cGvHD that lot of pts with PBSCT have developed. Conclusion: Engraftment is faster when PB is the source of stem cells and thus, consecutively the minority of early complications are related to PBSCT. Frequency of relapses and total survival depending on the stem cell source should be estimated at the larger, homologous groups considering the primary disease.

925 CIRCULATING VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTORS (VEGFR-1 AND VEGFR-2) IN UNTREATED PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Vascular endothelial growth factor (VEGF) transduction pathway may be very active in B-cell chronic lymphocytic leukemia (B-CLL) cells and contributes to their enhanced survival. Vascular endothelial growth factor receptor-1 (VEGFR-1) and receptor-2 (VEGFR-2), are the high-affinity VEGF receptors, playing an important role in de novo blood vessel formation and hematopoietic cell development. The aim of our study was to compare the concentration of VEGFR-1 and VEGFR-2 in the serum of 70 never treated B-CLL patients in different stage of disease according to Rai classification and 20 healthy volunteers. The receptors concentration was assessed using an enzyme linked immunosorbent assay (ELISA). We observed significantly higher concentrations of VEGFR-2 in the B-CLL group than in control group (median 2975.4 ng/ml and 2245.3 ng/ml, respectively (p= 0.004). We also found that the levels of VEGFR-2 in Rai stage III and IV patients were significantly higher (median 3589.9 ng/ml) than in patients in Rai stage 0-II (median 2977.8 ng/ml) (p=0.04). The levels of VEGFR-2 were also significantly higher in the group of B-CLL patients with progressive disease (median 3220.0 ng/ml) as compare to patients with stable disease (median 2689.5 ng/ml) (p=0.02). In contrast, the levels of VEGFR-1 were not significantly different in B-CLL and control group (median 47.9 ng/ml and 48.7 ng/ml respectively, p=0.89). We also did not find any correlations of the level of VEGFR-1 and the stage of B-CLL. In conclusion: VEGFR-2 but not VEGFR-1 may have an important influence on the course of B-CLL.

926 RESULTS OF FIRST LINE TREATMENT IN ADVANCED HODGKINS LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background: Advanced Hodgkin s Lymphoma (HL) is a curable malignancy since MOPP regimen was introduced. In an attempt of greater efficacy and reduce toxicity, others combination chemotherapy regimens and/or radiation therapy has been introduced.

Aim: We reported our experience in the treatment of intermediate/ advanced HL ($\geq$ stage IIB) with two different first line therapy regimens (6-9 cycles) and involved field radiation therapy (IFRT) if bulky disease and/or involvement of the spleen were present at diagnosis Patients and Methods: in this study, 111 HL patients treated and followed in our hospital were retrospectively analysed: 51 patients received ABVD (adriamycin, bleomycin, vinblastine and dacarbazine) and 60 patients MOPP/ABV (mechlorethamine, vincristine, procarbazine, prednisone, adriamycin, bleomycin and vinblastine) regimens. In the ABVD group, 57% of the patients were female with a median age of 28 years (14-74) and 80% of them did pathological staging (PS): stage IIB-45%, stage III-33%, stage IV-22% and bulky disease-47%. In 63% of the patients the histology was nodular sclerosis and the median IPI was 2. The median of cycles administrated was 6 and 45% of the patients received radiation. One patient did splenectomy. In the MOPP/ABV group, 38% of the patients were female with a median age of 33 years (14-77) and 68% did PS: stage IIB-43%, stage III-32%, stage IV-25% and bulky disease-42%. In 50% of the patients the histology was nodular sclerosis and the median IPI was 2. The median of cycles was 6 and 37% of the patients received radiation. Two patients refused went on the chemotherapy.

Results: In the ABVD group, complete remission was achieved in 74% of patients and partial remission in 16%. Response was not achieved in 6% and 4% of the patients had disease progression. Relapsed was observed in 22% of patients. There were not secondary malignancies. Ten patients had died (20%): 1 patient of pulmonary infection, 8 patients of disease progression and 1 patient of cardiac toxicity. Five-year overall survival (OS) and disease free survival (DFS) rate was found as 70,3% and 70,5% of 50 valuable patients, respectively. Median survival time was not achieved. In the MOPP/ABV group, 58 patients were valuable for response: complete remission was achieved in 84% of patients and partial remission in 10%. Response was not obtained in 3% and 2% of the patients had disease progression. Relapses occurred in 17% of patients. Two patients had secondary malignancies. Eleven patients had died (19%): 4 patients of infection, 4 patients of progression disease, 1 patients of hepatic toxicity and 2 patients of others causes. Five-year OS and DFS rate was 81,1% and 72,2%, respectively. Median survival time was not achieved.

Conclusions: In our experience, long term survival and toxicity his higher in the MOPP/ABV group, nevertheless this is a retrospective study and all we can conclude is that our results showed that HL is a curable malignancy with both regimens.

927 SUPPRESSIVE EFFECT OF HIGH-DOSE CHEMOTHERAPY (HDC) ON THE CLONAL GROWTH OF GRAFTED PERIPHERAL BLOOD GRANULOCYTE PROGENITORS IS REDUCED 72 HOURS AFTER COMPLETION OF HDC

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Hematopoietic stem cells (HSC) transplantations are usually performed 24-48 hours after the end of high-dose chemotherapy (HDC). It is possible, however, that at that time the transplanted HSC can be affected by the administered conditioning regimens. We report the results obtained in 30 patients (pts) with hematological malignancies (myeloma multiplex -14, Hodgkins disease -6, non-Hodgkins lymphomas -4, chronic myeloid leukemia -6) treated with HDC with peripheral blood stem cell transplantation (PBSCT). The following HDC regimens were applied: melphalan -14, DEXA-BEAM (dexamethasone, BCNU, etoposide, cytarabine, melphalan) - 10 and BuCy2 (busulfan, cyclophosphamide) - 6. To evaluate the impact of HDC on the growth ability of less /14/ and more /7/ mature CFU-GM, sera were taken directly before cytoreductive chemotherapy, on the last day of HDC (0 day) and on days 1, 2, 3, 4 and 5 after the end of HDC. After melphalan, for CFU-GM/14/ the following results were received: days 0 - 549, 1- 559, 2 - 6812, 3 - 8210, 4 958, 5 - 1008% and for CFU-GM/7/: days 0 - 6011, 1 - 598, 2 7211, 3 807, 4 909, 5 1057%, determined as a percentage of those obtained before

HDC (100%). After Dexa-BEAM, for CFU-GM/14: days 0 - 4513, 1 - 498, 2 - 5912, 3 - 8515, 4 - 969, 5 - 10611%, and for CFU-GM/7: 0 - 4315, 1 - 509, 2 - 6311, 3 - 8911, 4 - 9811, 5 - 1108%. In case of BuCy2, for CFU-GM/14: days 0 - 5513, 1 - 677, 2 - 6915, 3 - 8510, 4 - 10312, 5 - 1087% and for CFU-GM/7: days 0 - 5012, 1 - 716, 2 - 7616, 3 - 989, 4 - 10511, 5 - 1018%. PBSC were infused 72 hours after HDC. The median time to reach granulocytes > 0.5 G/L and >1.0 G/L was 12 (8-17) and 13 (9-18) days, respectively. The recovery of platelets >20.0 G/L and >50.0 G/L occurred after 17 (9-48) and 25 (11-54) days, respectively. We conclude that the suppressive effect of the sera taken on days 0-2 on the CFU-GM growth depends on the HDC regimen. We have also shown that third day after completion of HDC is the optimal time for the infusion of HSC. The time of hematological reconstitution is not correlated with the HDC-program ($p > 0.05$).

928 A-FETOPROTEIN (A-FP) IN THE PROGNOSIS OF HEPATITIS C S. Fragatou

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Background: HCV is responsible for the majority of post transfusion hepatitis. In Greece, about 65% of transfusion dependent thalassaemics are seropositive for anti-HCV. Of these patients 30% have chronic active hepatitis and half of them are viraemic requiring anti-retroviral therapy. The aim of this study is to investigate the value of a-FP in the prognosis of the disease and its correlation with liver fibrosis and siderosis in thalassaemia.

Methods: We studied 12 adult seropositive patients with thalassaemia major mean age 35 years. All patients are on regular iron chelation therapy with desferrioxamine (7) and deferiprone (5). Seven of the patients, had chronic active hepatitis and tested HCV-RNA positive. Two of these patients responded to therapy with Interferon and have remained HCV-RNA negative for a period of 4 years thereafter.

Parameters investigated are serum ferritin, urinary iron excretion, (UIE) a-FP, ALT, liver iron (SQUID Susceptometry and T2 Magnetic Resonance Imaging), liver fibrosis and siderosis (biopsy).

Results: Table shows laboratory parameters for all 12 patients.

(TABLE 1)

In one female patient 50 years of age, a-FP was abnormal. From her history, anti-HCV was detected positive since 1990 when HCV testing became available in Greece. Chronic active hepatitis was diagnosed 3 years after the detection of anti-HCV. She became HCV-RNA positive (genotype 3a) and did not respond to anti-viral treatment. Progression to cirrhosis and carcinoma (HCC) of the liver was diagnosed after 6 and 13 years respectively.

Iron stores have been low (ferritin 600 mg/dl, UIE 22mg/24hours, liver iron 215 µg.griv) during the last three years prior to HCC diagnosis.

Increasing levels of a-FP were observed during the last six months before HCC diagnosis. The patient died of hepatic coma. No cardiac or other complications were observed.

Conclusions: In these study, thalassaemic patients infected with HCV have normal values of a-FP. Since increased levels of a-FP were observed prior to HCC diagnosis, this may be a useful prognostic marker for the clinical process of the infection.

No correlation was observed between a-FP levels and liver fibrosis or siderosis.

Patients	HCV-RNA	a-FP ng/ml	Fibrosis (stage I-IV)	Siderosis (stage I-IV)	Ferritin mg/L (mean values)	ALT u/L (mean values)
5	+	3.32	2-5	I-IV	3.800	91
5	-	2.66	1-6	II-IV	431.75	35
2	{-}	2.25	4	II,IV	1.848	42.5
after therapy						

Caption 1: TABLE 1

929 CTL PEPTIDES FOLLOWING BY T CELL REPERTOIRE AS A PREDICTIVE FACTOR OF GVT EFFECT AFTER REDUCED INTENSITY STEM CELL TRANSPLANTATION

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[Background] An alloresponse of donor T cell is mainly the cause of GVHD as well as the graft-versus-tumor(GVT) effect after RIST for solid tumor such as RCC, colon cancer and so on. Some of papers have been reported that it has been shown clinical GVT effect clearly, but it is needed to know the mechanism of GVT effect after RIST more. Cytotoxic T lymphocytes (CTLs) can recognize the matched HLA peptides or their binding motif and also tumor antigen, too. As far as we know, these are

enough evidences in GVT effect after RIST for solid tumor **[Aims]** The aim is to examine whether we can know that GVT effect as a predictive factor and donor specific lymphocyte such as CTLs work as the GVT effect following allogeneic hematopoietic stem cell transplantation can recognize target tumor antigen or how long it will continue after GVHD have been shown. We examined the possibility of the induction of effective CTLs patient with HLA A 24 or HLA A2. **[Methods]** Peripheral blood mononuclear cells (PBMCs) were obtained at 1-2 week intervals in GVHD phases following after RIST for metastatic colon cancer. We examined complementarity determining region(CDR3) size spectratyping of T cell receptor β chain subfamilies using donor sorted CD8 positive cells which have been shown some peaks and the PCR products which have clonal peaks were sequenced. The amino acid sequence of CDR3 region was deduced. In the other hand, we examined whether some clones of which obtained from T cell repertoire can recognize to which part of patients HLA A2 or A 24 peptides as well as patients tumor antigen. **[Results]** The analysis of repertoire in Vb4 subfamily was shown the only strong clonal peak in acute GVHD which was placed in 358bp. And that peak decreased according to the improvement of acute GVHD. There was no other clonal peak in acute GVHD. In chronic GVHD, the single peak of vb4 repertoire was completely decreased and the new single peak that placed in 339bp has been shown. And in vb14 or vb15 subfamilies, there were same strong peaks in repertoire analysis 376bp, 200bp, respectively. These three peaks have been shown continuously during in chronic GVHD, not acute GVHD. CEA is the marker of this patient clinical effectiveness after RIST. Therefore, we examined some binding motifs in patients HLA A2 and A24 and these motifs can recognize tumor peptides in CEA whole sequences. In four of 19 clones, we found the matched binding motif between in vb4 of acute GVHD phase and in HLA A2. In twelve of thirteen clones, we got same binding motif between in vb14, vb15 subfamilies and in HLA A 24 peptide in chronic GVHD, also, not HLA A2. **[Conclusion]** In summary, we found that there are some different CTL clones between acute GVHD and chronic GVHD in repertoire analysis. In terms of different GVHD phase, we got different HLA restricted clones of 9-mer CEA peptides.

930 ANTIBODY IDENTIFICATION IN NEWBORNS FOUND WITH DIRECT ANTIGLOBULIN TEST (DAT) POSITIVE. A SIX YEARS STUDY G. Bolas

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Aim of present work is the identification of antibodies in newborns with direct antiglobulin test (DAT) positive.

Material - Method: We studied retrospectively from 1/1/1998 up to 12/31/2003, 1997 newborns and their mothers. Each newborn was checked for group ABO, Rhesus with phenotype, Kell and DAT. Each mother was checked for group ABO, Rhesus with phenotype, Kell and indirect antiglobulin test (IAT). In each DAT positive, the study was continued (elution) to identify the antibody. For the laboratorial analysis of newborns the sample used was cord blood and in certain cases venous blood.

Results: At the particular time interval 1997 newborns in total were studied and 100 of them (percentage 5%) had a DAT positive and the antibodies found were IgG type immunoglobulin. Anti-A antibody was identified in 67 newborns (67%) (newborns of group A with mothers of group O), anti-B antibody in 15 newborns (15%) (newborns of group B with mothers of group O), anti-D antibody in 2 newborns (2%) (newborns D+ from mothers D-), anti-E antibody in one newborn and anti-Jka antibody in an other one. The eluates, in 3 newborns were found negative and in the remaining 11 no special antibody could be identified. The results are presented in the following table.

Conclusion: We observe that the majority of antibodies that cause positive DAT in the newborns belong in ABO blood group system and are due to ABO incompatibility (the mother belonging to group O and the newborn to either A or B).

ANTIBODY	NEWBORNS	PERCENTAGE
anti - A	67	67%
anti - B	15	15%
anti - D	2	2%
anti - E	1	1%
anti - Jk ^a	1	1%
Not identified	14	14%
TOTAL	100	100%

Caption 1: Table

931 HEMOPHAGOCYTIC SYNDROME IN CHILDREN SUFFERING FROM LEISHMANIA DONOVANI

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Objective. The aim of the present study is to determine the cases of 'reactive' hemophagocytic syndrome in young children infected by 'leishmania donovani' parasite and to contribute to the differential diagnosis from other causes of hemophagocytic syndrome.

Cases-Methodology. We studied retrospectively the files and bone marrow (BM) smears of all patients suffering from leishmaniasis over a 5.5 year period (1997-2002).

Results. The patients comprise 41 children infected by leishmania D., giving an incidence of 8 cases per year, with an average age of 4.6 years (SD+3.4). Male to female ratio was 1.1/1. In all cases, clinical symptoms included fever and hepatosplenomegaly, while laboratory testing revealed increased ESR and CRP, anemia, thrombocytopenia and neutropenia. Almost all cases exhibited liver disorders with an increase of SGOT, SGPT and γ -GT, while hypofibrinogenemia was confirmed in one case (6.25%) and hypertriglyceridemia in 7 of 16 cases examined (43%). A review of the BM smears was carried out on 37 of 41 cases. Apart from the parasites, obvious hemophagocytosis was observed in 33 of 37 cases (89.2%) and was related with cells of erythroid, lymphoid, and myeloid series. Serological testing for autoimmune diseases for RF, anti-DNA, ANA, Ra-test and complement was negative, save for one case with a positive Coombs in C3d. The leishmania antibody titer varied from 1/400 to 1/1600 in the 30 cases reviewed. No active infection was found in 30 out of 41 cases, for viruses such as EBV, CMV, HBV, HCV, HAV, adenoviruses, as well as, for leptospirosis, toxoplasmosis, and fungi, which are potential causes of hemophagocytic syndrome. There were no serological evidence for the remaining 11 cases. Treatment with antimony salts, or with liposomal amphotericin, led to full recovery of patients and restoration of affected clinical and laboratory parameters.

Conclusions. Although the number of cases with leishmaniasis and hemophagocytosis we present is significant, the lack of data relevant to fibrinogen, cholesterol, and triglycerides deprives us of the ability to refer to statistical data concerning 'reactive' hemophagocytic syndrome because of leishmaniasis in Greece. Despite this, all cases of young patients with high fever, hepatosplenomegaly, cytopenia, and hemophagocytosis, especially in cases with hypofibrinogenemia and hypertriglyceridemia, elements that contribute to the diagnosis of hemophagocytic syndrome, should be tested for L. Donovanii, before the disease is attributed to other causes, such as other histiocytic disorders.

932 HYPERLEUKOCYTOSIS IN ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS INELIGIBLE FOR STANDARD INDUCTION CHEMOTHERAPY DRAMATICALLY REDUCES OVERALL SURVIVAL

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Background. Undertreatment of elderly patients (age > 60 years) with acute myeloid leukaemia (AML) can explain their lower complete remission (CR) rate than that of younger patients. To date, elderly AML patients have been considered ineligible for standard induction treatment because of host-related factors, age-related organ dysfunction, poor performance status, increased frequency of co-morbidity and limited tolerance of the toxicity of intensive chemotherapies. Therefore, for many elderly AML patients the final goal has been to provide supportive care and achieve a partial cytorreduction of the leukemia. In these patients, median survival is very low compared to that of elderly patients treated with standard induction therapy, and CR is rare.

Aims. The aim of this study was to verify whether hyperleukocytosis (> 50.000 mm³) at diagnosis can modify the overall survival (OS) rate in this subset of AML patients.

Methods. In this study we considered 86 elderly AML patients ineligible for standard treatment. They were candidates for palliative treatment only due to performance status (PS) >3 (31%), age > 77 y.rs (28%) and age-related organ dysfunctions (41%). A cytorreductive agent alone or in combination (Ara-C, Etoposide, Thioguanine, Hydroxyurea) was given to 65 (76%) patients whereas the remaining 21 (24%) were treated only with supportive therapy (ST). They were compared with 96 elderly patients eligible for standard treatment, to verify the effect of hyperleukocytosis on OS.

Results. Among the patients ineligible for standard induction therapy, 25 (29%) patients had hyperleukocytosis; median survival was lower in this subgroup (0.3 mo vs 1.3 mo, p<0.0001). There were no significant differences between the two subgroups in terms of PS, treatment (ST vs other treatment), age, FAB subtype and CD34 expression whereas the presence of hyperpyrexia (> 38C) was associated with the subgroup without hyperleukocytosis (p = 0.002). Notably, within the group eligible for standard induction therapy, the hyperleukocytosis factor yielded no difference in terms of OS (5.7 months without vs 7.5 months with hyperleukocytosis; p=0.7).

Conclusion. Our data suggest that hyperleukocytosis could be an important factor in determining treatment for elderly AML patients eligible only for palliative treatment options. The choice of more 'aggressive' chemotherapy should be considered in this category of patients.

933 CHRONIC MYELOGENOUS LEUKEMIA WITH E19A2 AND E18A2 BCR-ABL TRANSCRIPTS

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Background: An infrequent variant of the Philadelphia chromosome, the BCR-ABL e19a2, which encodes the chimeric fusion protein p230, was previously related to an infrequent type of mild chronic myeloproliferative syndrome: neutrophilic-chronic myeloid leukemia (CML-N). CML-N is characterized by mild splenomegaly, moderate leukocytosis with expansion of mature neutrophils and thrombocytosis. Recently several report indicated, that e19a2 may associate with the classical CML phenotype and even accelerated and blastic phases can occur within a relatively short time period. We report on a patient with a presenting diagnosis of chronic phase CML, which showed a blastic transformation within six months. **Methods:** Initially cytogenetic test showed Philadelphia positivity with 40% duplication. RT-PCR analyses for BCR-ABL translocation indicated two aberrant specific transcripts (810 and 949 bp instead of 417 bp for b3a2). Based on size information, both bands were likely to originate from a micro-type break i.e. the e19a2 fusion gene. Complementary DNA sequencing analysis confirmed the presence of e19a2 fusion transcript together with a previously not described transcript that lacked exon 19 of the BCR gene (e18a2). However, the novel transcript was not in frame, which is unlikely to code for a functional protein. **Conclusion:** The co-existence of this novel transcript may be explained either by a parallel presence of a novel chromosomal breakpoint or by alternative splicing mechanisms. Our case further supports that BCR-ABL micro transcript may not always be associated with a mild form of CML.

934 TREATMENT OF HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) WITH RITUXIMAB, HIGH-DOSE CHOP, ESHAP AND AUTOLOGOUS TRANSPLANT (ASCT)

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This work is sponsored by a grant from the Ministry of Public Health of the Czech Republic No. XC-8231/3

Background. Several studies have shown that consolidation therapy with autologous transplant (ASCT) after full-length induction could be beneficial to patients with DLBCL and age-adjusted IPI (aIPI) 2 or 3. Rituximab was recently found to improve outcome both in young and elderly patients with DLBCL.

Aim. To increase long-term survival of DLBCL with aIPI 2 or 3, Czech Lymphoma Study Group have explored several regimens with high-dose CHOP induction and ASCT consolidation. Results of the last modification, which adds Rituximab and ESHAP to the above mentioned schedule, are presented.

Methods. From May 2002 to January 2004, 32 patients with high-risk DLBCL (19 men, 13 women, median age 46 years, range 19 to 60) were treated with 3 cycles of MegaCHOP (Cyclophosphamide, 3 g/m² d 1, Vincristin 2 mg d 1, Doxorubicin 75 mg/m² d 1, Prednisolone 60 mg/m² d 1-5, G-CSF 5 ug/kg from day 6) followed by 3 cycles of ESHAP (Etoposide, 60 mg/m² d 1-4, Methylprednisolone 500 mg d 1-4, Cisplatin, 25 mg/m² d 1-4, AraC 2 g/m² d 4) and BEAM (BCNU, 300 mg/m², AraC, 1600 mg/m², Etoposide, 800 mg/m², Melphalan, 140 mg/m²) with autologous transplant. Stem cells were collected after 1st cycle of ESHAP. Four doses of Rituximab 375 mg/m² were given at the first day of each cycle

beginning from 1st or 2nd MegaCHOP. Patients with bulky disease or residual mass after transplant were further consolidated with involved-field radiotherapy.

Results. 21 pts (66%) had aalPI 2 and 10 pts (34%) aalPI 3. 21 of 25 mobilized patients had successful stem cell harvest (84%), with median 10.82×10^6 CD34 cells/kg collected (range, 5.05 to 42.7×10^6 /kg). So far 17 patients were transplanted and radiotherapy was given to 13 patients. 27 pts are evaluable for response with 21 CRs and 6 PRs (overall response rate, 100 %). There was no progression on treatment, but four patients (13%) have died of treatment toxicity: one patient died suddenly after 1st MegaCHOP and three patients have died of sepsis while on-treatment. Three other grade 4 infectious episodes were noted and the only relapse off-treatment occurred in patient who did not finish the schedule because of infectious toxicity. Overall survival is 87% and failure-free survival 83% after median of 9 months follow-up (2-20).

Conclusion. The described regimen offers excellent short and possibly good long-term control of disease in high-risk DLBCL. However, the number of life-threatening events is much higher than observed previously with the same regimen without Rituximab. Therefore, it cannot be excluded that Rituximab added some extra toxicity to this very intensive treatment schedule.

935 MANNOSE BINDING LECTIN POLYMORPHISMS IN MULTIPLE MYELOMA

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Mannose binding lectin (MBL) is a calcium dependent lectin that plays an important role in innate immunity by activating the classical complement pathway and phagocytosis. MBL inactivates complement via MBL associated serine protease (MASP). There are wide variations in serum MBL level in human due to genomic polymorphisms in the MBL gene. 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.

Codon 54 and codon 57 polymorphisms in the exon 1 of the MBL gene were investigated in the DNA samples from 50 controls and 40 patients diagnosed as Multiple Myeloma (MM). Distributions of allele A and allele B were compared and statistical analysis was performed.

As a consequence distributions of AB genotype and allele B which causes predisposition to infections were significantly high in MM patients in comparison to control group ($p=0,011-0,014$).

Multiple myeloma patients (n=40)			Control (n=50)	Chi-Square p
MBL Genotypes			n	
AA	30	0.75	47	0.94
AB	10	0.25	3	0.06
Alleles				0.011
A	70	0.875	97	0.97
B	10	0.125	3	0.03
				0.014

Caption 1:

936 CYTOTOXIC T-LYMPHOCYTE ANTIGEN-4 MICROSATELLITE POLYMORPHISM IS ASSOCIATED WITH MULTIPLE MYELOMA IN TURKISH POPULATION

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Multiple myeloma (MM) is a B-cell malignancy characterized by an accumulation of malignant plasma cells in the bone marrow. It is unclear whether genetic background could have an etiological impact on MM or influence on the course of the disease. The cytotoxic T-lymphocyte-associated antigen 4 gene (CTLA-4) which is in region on 2q33 encodes T-cell receptor involved in control of T-cell proliferation and mediates T-cell apoptosis. The receptor protein is a specific T-lymphocyte surface antigen that is detected on cells only after antigen presentation. Thus

CTLA-4 is directly involved in both immune and autoimmune responses. In this study, the microsatellite polymorphism in the exon 3 of the cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene was studied in patients with MM (23 patients) and healthy controls (100 samples).

As a result of the study, amplification products of CTLA-4 were determined that the allele size changed between 64 bp and 134 bp in Turkish population. This is the first report that A11 (64bp) allele was determined in MM patients (2/23). The frequency of A2 (128bp) allele was significantly increased ($p=0.016$), however, the frequency of A6 (112bp) allele was decreased ($p=0.013$) in MM patients as a whole, compared with control subjects.

Our results suggest that there may be an association between the A2 (128bp), A11 (64bp) alleles and MM.

937 USEFULNESS OF PROCALCITONIN MEASUREMENT IN THE DIAGNOSIS OF FEBRILE NEUTROPENIA. STUDY OF 33 PATIENTS

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Background. The diagnosis of infection in patients with neutropenia secondary to chemotherapy is difficult due to the fact that symptoms are unspecific, being fever the only manifestation of sepsis in most cases. Besides, fever can be due to other non-infectious causes. Procalcitonin (PCT), a calcitonine precursor peptide probably synthesized in mononuclear phagocyte cells, has been described as a specific marker of systemic non-viral infection.

Aims. 1. To study the usefulness of the PCT measurement for the diagnosis of systemic bacterial infection in neutropenic ($ANC < 0,5 \times 10^9/L$) patients with fever. 2. To establish baseline values of PCT in patients with haematological malignancies at the time of the admission and at the onset of neutropenia.

Patients and Methods. Thirty three patients with diagnosis of haematological malignancies treated on induction chemotherapy or on conditioning regimens for stem cell transplantation were included. Sera samples on the day of admission, at the beginning of neutropenia, the first day of fever and daily for six days were collected. Immunoluminometric assay was used for PCT measurement. Episodes of fever were classified according to the clinical and microbiological diagnosis in three groups: (1) documented bacteraemia; (2) bacterial isolation without bacteraemia; and (3) fever with negative cultures.

Results. PCT mean (SD) levels at the time of admission and at the onset of neutropenia were 0.337 (0.143) and 0.33 (0.22) ng/ml, respectively (normal level < 1 ng/mL). Group 1: 10 patients with bacteraemia, *Escherichia coli* (4 patients), *Klebsiella pneumoniae* (1), coagulase-negative *Staphylococcus* (3), *Streptococcus mitis* (1) and *Lactobacillus* sp (1). PCT mean levels at 24-48 hours from the beginning of fever were 3.681 (4.33) ng/ml in gram-negative and 1.76 (3.26) ng/ml in gram-positive infections. Group 2 (bacterial isolation without bacteraemia): 8 patients (urine culture in 3, sputum in 4 and catheter-related in one) with PCT mean level of 1.67 (2.24) ng/ml. Group 3 (fever with negative cultures): 15 patients, 6 of them related with mucositis, with PCT mean level of 0.64 (0.55) ng/ml. Significant differences were only observed between patients with gram-negative bacteraemia infection and patients with fever but negative cultures ($p < 0.05$).

Conclusions. PCT levels remain at normal values in patients with haematological malignancies at the time of the admission and at the beginning of neutropenia. Determination of PCT is an useful marker in the neutropenic fever from infectious origin specially in those with gram-negative bacteraemia.

938 PREVALENCE OF METHYLTETRAHYDROFOLATE REDUCTASE (MTHFR) C677T VARIANT IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES. PRELIMINARY RESULTS

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Background: The enzyme 5,10-methyltetrahydrofolate reductase (MTHFR) catalyzes the synthesis of 5-methyltetrahydrofolate, the methyl donor for methionine synthesis and the precursor of the S-adenosylmethionine. S-adenosylmethionine is the universal methyl donor for methylation reactions, including that of DNA methylation. The

common C677T mutation in MTHFR gene results in an alanine-to-valine substitution in the MTHFR protein, in thermolability and reduced enzyme activity in vitro and affects DNA methylation. On the other hand, recent studies displayed that thermolabile MTHFR variant leads to enhanced thymidine pools and better quality DNA synthesis that could offer some protection from the development of colon cancer and leukemias (particularly those with acute lymphoblastic leukemia or leukemias with translocations).

Aim of this study was to assess the prevalence of the C677T MTHFR genotype and the contribution of this genotype to predisposition to leukemia in patients with various types of chronic hematologic malignancies.

Methods: Two hundred and four patients with hematologic malignancies (aged 21-83, mean 65,3611,26) and 122 healthy controls were studied. Particularly, 75 patients suffered from myeloproliferative disorders (16 from CML, 4 with myelofibrosis, 36 from essential thrombocythemia, 18 from polycythemia vera), 40 from myelodysplastic syndromes (17 from RA, 2 from RARS, 12 from RAEB, 3 from REAbt, 6 from CMML), 36 from B-CLL, 14 patients from low-grade Non-Hodgkins lymphoma (including Waldenström Macroglobulinemia, follicular, marginal zone and MALT lymphomas) and 39 patients from multiple myeloma. DNA samples were genotyped for the MTHFR C677T mutation by a PCR-based RFLP assay. Samples were categorized as homozygous for the thermolabile variant (TT), heterozygous for wild type and variant (CT), or homozygous for the wild type (CC).

Results: Twenty-two out 204 patients (10,78%) were homozygous for the C677T variant, 111 (54,41%) were heterozygous, and 91 (44,60%) were homozygous for the wild type (compared with 12,29%, 40,16% and 47,54% of controls, respectively). However, no statistical significance was established between the total number of patients and normal controls ($p=0,4269$). In terms of specific types of malignancy, it was interesting that in patients with CML a slight higher incidence of wild type MTHFR was displayed (10 of 16 patients, 62.5%); similar results were displayed in patients with lymphoma (wild type incidence 71,42%), while patients with MDS exhibited a higher incidence of thermolabile variant (57,50% heterozygotes and 10% homozygotes), but without statistical significance ($p=0,110$, $p=0,067$ and $p=0,150$, respectively).

Conclusions: In this forerunner study, we did not find any association between the MTHFR C677T variant and risk for specific types of chronic hematologic malignancies. The possible role of the presence of homozygosity (TT) and predisposition for hypomethylation of specific tumor-suppressor genes in patients with malignancy remains to be determined.

939 CLINICAL VALUE OF ASSESSMENT THE CHANGES OF P-GLYCOPROTEIN-RELATED MULTIDRUG RESISTANCE IN CML PATIENTS UNDERGOING IMATINIB MESYLATE THERAPY

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BACKGROUND: Development of imatinib mesylate (Glivec, Novartis, Basel, Switzerland) is an important advance in the management of chronic myeloid leukemia (CML). However, some patients become resistant during the treatment and some are even inherently resistant to imatinib. Its long-term unsuccessful use may ruin chances of resistant patients to benefit from alternative therapies. Therefore it is important to predict the responsiveness among patients thus allowing them to get the most adequate treatment. AIM: To assess in a long-term follow-up study the changes in P-glycoprotein (Pgp) related multidrug resistance (MDR) in CML patients undergoing imatinib therapy, to estimate the occurrence of MDR in CML patients, and to determine the correlation of Pgp expression and activity to the treatment outcome. METHODS: Twenty-eight adult patients with advanced CML (5 in blast crisis (BC), 10 in accelerated phase (AP) and 13 in chronic phase (CP)) were monitored in 3 months intervals starting with July 2001. Previous treatment included mostly HU and IFN alpha; 6 patients received MDR-related therapy. Imatinib was administered as oral therapy: 600 mg/day for BC and AP and 400 mg/day for CP, respectively. Bone marrow (BM) and peripheral blood (PB) cells were examined using flow cytometry. Pgp phenotype was analysed in anti HLA-DR/Pgp combination and Pgp activity was measured in Rhodamine dye (Rh123) functional efflux assay. RESULTS: Among 28 patients, 9 died, 8 relapsed and 2 were refractory. Central and peripheral myelogenous cells confirmed similar MDR status;

Pgp-associated resistant cells were found both in BM and PB. Most of the patients achieving hematologic remission showed stable Pgp activity, whereas in those who underwent clinical relapse and died the tendency of Pgp activity increase was observed. CONCLUSION: In the absence of BM aspirate it is possible to assess Pgp phenotype and transport activity only on PB samples. Our results showed that longitudinal follow up of Pgp-related MDR status could detect resistant patients which might be crucial for their further treatment. As increased Pgp-related MDR activity can be associated with chemoresistance to imatinib therapy, Pgp-related MDR screening may become a prognostic tool of important clinical value for detecting imatinib-resistant patients.

940 NON-HODGKIN'S LYMPHOMA EPIDEMIOLOGY IN A SINGLE CENTER.

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Background

In underdeveloped countries, is difficult but so important to know more precisely the epidemiology of certain more frequent diseases in order to plan effective treatments and use resources adequately.

In Mexico, Non Hodgkins Lymphoma (NHL) comprises one of the 5 more frequent causes of cancer deaths and the perception is that our patients arrive the most, with advanced disease.

AIMS: To know the incidence and characteristics of NHL patients received at one of the most important reference hematology centers in the country.

Methods: During a 24 month period, all patients with a confirmed diagnosis of NHL were recorded including: Age, sex, number of physicians and time to diagnosis, histopathological and immunophenotyping diagnosis according to REAL classification, Ann Arbor staging, extranodal disease, and IPI prognostic features.

Results:

394 patients were recruited with NHL diagnosis As an adult hematology department those with less than 15 years were excluded.

Age: median 54.9 years (15 - 87), > 60 yrs: 165 (41%), < 60 yrs: 229 (59%)

Sex: 54% male, 46% female.

Median time for diagnosis confirmation (from the first symptom): 7.9 months (2- 18 months)

Median number of physicians visited to diagnosis: 3.3

T Lymphomas: 93(23%), B Lymphomas 301 (77%)

from which 289 were CD20 positive (96%)

Indolent: 27%, Aggressive and very aggressive: 73%

Ann Arbor staging: Early disease (I-II): 14%, Advanced disease (III-IV): 86%.

84% had B symptoms.

IPI risk:

Low: 8%

Intermediate low: 12%

Intermediate high: 53%

High: 27%

From extranodal disease we found 189 patients (47%) infiltrating breast, lung, bladder, liver, pericardium, blood vessels, lips, eye, CNS, among others.

The most frequent histologies:

Diffuse large cell lymphoma: 41%

Small cell: 8%

Follicular (I, II, III): 11%

T cell peripheral: 53%

Conclusions: In our experience, LNH is one of the most frequent hematological malignancy. These results show a very high incidence of advanced disease in a younger population. Other important issue shows that the median time to diagnosis represent a risk factor for advanced disease and more complicated approaches. This must allow us to start a campaign for early derivation to specialized centers. Our center has a total population of near 4 000 000 people, that lead us to an incidence of 5 x 100 000 inhabitants per year.

941 ALEMTUZUMAB IN ROUTINE USE IN PATIENTS WITH ADVANCED, HEAVILY PRETREATED CLL IS AS EFFECTIVE AS REPORTED IN PHASE II TRIALS

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Background: There are few published reports on alemtuzumab treatment in heavily pretreated patients, with response rates ranging between 33% and 52%.

Aims: To determine the efficacy of alemtuzumab in patients with advanced, heavily pretreated CLL.

Methods: This is a phase I pilot trial conducted on a compassionate use basis to determine the efficacy of alemtuzumab in 37 patients (23 male/14 female) with CLL (n=33), Richters transformation (1 each with DLBCL and B-PLL), and PLL (n=2). Alemtuzumab was given alone (n=31) and in combination with fludarabine phosphate (n=6, of whom 2 patients received the combination subsequent to alemtuzumab monotherapy). After dose escalation, alemtuzumab was administered at 30 mg 3 times weekly for up to 30 weeks.

Results: The median age was 66 years (range 4980); 1 patient had Rai stage 0 (T-PLL), 13 patients had Rai stage II, 4 Rai stage III, and 19 Rai stage IV disease. All patients had been heavily pretreated (median 4 prior regimens, range 17). All but 1 patient had previously received fludarabine phosphate, and 17 were considered fludarabine phosphate-refractory. Using National Cancer Institute (NCI) Workshop criteria, 13/34 patients (38.2%) responded to alemtuzumab treatment. There was complete remission (CR) in 5.9% of patients, partial responses (PR) in 32.4%, and stable disease (SD, >2 months) in 41.2% of patients. Immediate progressive disease was observed in 20.6% of patients. Peripheral blood responses included 41.2% CRs, 44.1% PRs, and 5.9% SD. Bone marrow responses were available in 14 cases, with 28.6% CRs, 21.4% PRs, and 50% SDs; nodal responses included 3.3% CRs, 33.3% PRs, and 43.3% SDs. The overall response rate in fludarabine phosphate-refractory disease was 42.1% (8/19). The fludarabine phosphate/alemtuzumab combination stabilized disease in 5/6 patients treated; the sixth patient died early during therapy from septicemia. Thus far, 11 patients died (117 months after alemtuzumab initiation), and 26 patients are alive (after 124 months, median 8.5). There were 4 CMV and 2 tuberculosis reactivations; 8 grade III/IV infections (including the 2 tuberculosis cases), of which, 1 was fatal; and 2 hypersensitivity reactions >grade 2. Interestingly, one second primary tumor potentially resulting from immunosuppression, namely Hodgkins lymphoma, developed. Alternatively, this could be considered as a rare example of Richters transformation manifesting as Hodgkins lymphoma.

Summary/Conclusions: We conclude that alemtuzumab is effective in these usually heavily pretreated CLL patients, and that results in this group of patients are comparable to those reported in clinical trials.

942 BONE MINERAL DENSITY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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BACKGROUND : Osteoporosis is currently receiving increasing attention as an important late effect in survivors of childhood cancer and its treatment because of their quality of life and its negative effect on the survivors ability to perform developmentally appropriate activities .

Aims : study was carried out to clarify the problem of bone disease and metabolism in acute lymphoblastic leukemia (ALL) .

Methods : The study was conducted on thirty children with ALL (15 newly diagnosed and 15 after two years of chemotherapy) and 10 age and sex matched healthy children as a control group .The studied children were subjected to full history taking , thorough clinical examination and laboratory investigations was performed as complete blood picture, bone marrow examination , serum calcium , phosphorus, parathormone

hormone , alkaline phosphatase and osteocalcin as a markers of bone formation and serum type 1 collagen telopeptide as a marker of bone resorption . Furthermore, bone density (BMD) was measured by dual energy x-ray absorptiometry on lumbar spine and femoral neck bones.

Results : Our results demonstrated that bone formation markers were significantly reduced in newly diagnosed cases of ALL while bone resorption markers showed non significant decrease when compared to control group . In cases of ALL after two years of starting chemotherapy bone formation markers were normalized while bone resorption markers were significantly increased compared to control group . BMD was significantly reduced at diagnosis and remained low after chemotherapy . Also, we found significant negative correlation between BMD and years elapsed since the beginning of chemotherapy .

Conclusions: Children with ALL are at risk for osteoporosis and diminished skeletal resistance to fracture later in life because of the disease itself and chemotherapy .

943 HEMATOLOGIC, CYTOGENETIC AND MOLECULAR RESPONSE TO IMATINIB MESYLATE IN 26 PATIENTS AFFECTED BY CML

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Background: Imatinib-Mesylate (STI-571) induces high rates of cytogenetic and hematologic response in CML patients (pts), whereas molecular responses are poor.

Aims: to evaluate the rate of hematologic, cytogenetic and molecular response and the outcome after Imatinib in our Division.

Methods: we analysed 26 pts (18 M, 8 F), with a median age at diagnosis of 49.5 y (range 19-63 y) affected by CML, diagnosed between 1991 and 2003 and treated with Imatinib from August 2000.

Sokal score was calculated in 18 pts and was low, intermediate and high in 9, 5, 4 pts respectively. Only 5 pts received Imatinib as first line therapy, whereas 21 were initially treated with different treatment options (Interferon only, Interferon and Cytarabine, Hydroxyurea, Busulfan, 6-Mercaptopurine, Autologous Bone Marrow Transplantation or other chemotherapy regimens). 2 pts were eligible for related sibling BMT.

At the beginning of Imatinib treatment, 22 pts were in chronic phase and 2 in accelerate phase, whereas 2 pts were in blastic evolution. They had been treated with Imatinib for a median of 22.5 months.

Results: complete cytogenetic response (RCC: Ph+ 0 %), partial cytogenetic response (Ph+: 1-35 %), minor cytogenetic response (Ph+: 35-95 %) and no response were: at 6 months: 11/19 (57.8%), 2/19 (10.5%), 2/19 (10.5%), 4/19 (21 %); at 12 months: 9/18 (50%), 5/18 (27%), 0/18, 4/18 (22.2%); at 24 months: 5/9 (55.5%), 2/9 (22.2%), 0/9, 2/9 (22.2%). Molecular response at PCR analysis was demonstrated in 1 patient (low Sokal score at diagnosis) after 12 months of first line therapy with STI. Molecular analysis at 6 months, performed in 14 cases, was positive in all. At 30 months first PCR was positive in 4/8 pts and nested PCR in 4/8 pts.

Complete hematologic response was achieved in 24/26 pts (92.3 %).

7/26 pts presented mild-moderate adverse events related to Imatinib: thrombocytopenia, flu-like syndrome, hepatic alteration, erithematous rash, fever and peri-orbital edema, but no pts discontinued treatment definitively.

22 pts are now alive; 2 pts, who were treated in blastic evolution, died after 9 and 23 months of therapy respectively; 1 patient evolved in extramedullary blast crisis and died after 7 years from diagnosis and 30 months of STI administration; 1 patient, diagnosed in accelerate phase, who underwent allogeneic sibling transplant after 3 months of STI therapy, relapsed and died after 6 months from BMT.

Summary/Conclusions: our data confirm the favourable impact of Imatinib in the treatment of CML, but a longer follow-up is needed for a complete evaluation of these results.

944 FACTOR V CAMBRIDGE MUTATION IN HEALTHY AND THROMBOPHILIC SERBIAN POPULATION

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The most frequent inherited thrombophilic state, found in 20-60% of the patients, is the resistance to activated protein C (APC). In at least 95% of these cases, resistance to APC is caused by the FV Leiden

mutation. Recently, two novel mutations affecting another cleavage site for activated protein C in factor V (at position 306) have been described. These mutations, the Factor V G1091C (FV Cambridge) and Factor V A1090G (FV Hong Kong), are relatively rare and their association with venous thrombosis is not clearly defined.

The aim of the study was to investigate the incidence of FV Cambridge mutation in Serbian subjects.

A study was carried out a group of 120 unrelated healthy blood donors and in 42 patients with deep venous thrombosis and with activated protein C ratio value below 2. In all patients response to the APC was measured using standard test. APC ratio below 2 was considered as resistance to APC. Mutations were detected by polymerase chain reaction, followed by digestion with specific restriction enzymes (BstNI) as previously described. Normal and mutated alleles were distinguished by the size of the restriction fragments, separated by electrophoresis on 10% polyacrylamide gels and visualised by silver staining.

FV Cambridge mutation was found neither in the 120 control subjects, nor in the group of patients with activated protein C ratio value below 2. Absence of FV Cambridge mutation in our study is in concordance with previously reported low frequency of this mutation. Based on our findings, we suggest that FV Cambridge mutation should not be included in a routine screening genetic test for the thrombophilic Serbian population.

945 MONITORING OF MINIMAL RESIDUAL DISEASE FOR THE PATIENTS WITH CML RECEIVING INTERFERON-ALPHA AND GLIVEC THERAPY.

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Background. With the usage of alpha-interferon and especially Glivec for treatment of patients with CML (chronic myeloid leukemia) an unprecedented percent of complete cytogenetic remissions has become the reality. A highly-sensitive PCR-monitoring nowadays is a method of choice of studying minimal residual disease in cases when BCR-ABL-positive cells can not be detected by routine cytogenetic methods.

Aims. The comparison of the results of cytogenetic and molecular methods for the CML patients with complete cytogenetic remission (CCR). The main aim is to estimate the effect of the treatment and to recognize the early signs of probable relapse.

Methods. The nested PCR (qualitative analysis) was used to value the minimal residual disease of 37 patients with CCR of CML chronic phase. Both bone marrow (22 samples) and peripheral blood (47 samples) were used. The comparison of the results of cytogenetic and molecular monitoring was held. In 1st group of patients there were 16 patients receiving interferon-alpha therapy, in the 2nd group there were 21 patients receiving Glivec therapy (after failing interferon therapy). The median age was 44 years. The median duration of treatment in 1st group was 48 months and 12 months in 2nd group.

Results. In the 1st group 6 patients were BCR-ABL negative, 4 patients were BCR-ABL positive and 6 patients had unstable molecular remission (3 of them had positive results before cytogenetic relapse). In the 2nd group 12 patients showed complete molecular remission and were BCR-ABL negative, 5 patients were BCR-ABL positive and 4 patients with unstable molecular remission (2 of them were positive results before cytogenetic relapse).

Summary. For the majority of CML patients with complete cytogenetic remission of CML receiving conservative therapy BCR-ABL transcript is detectable with molecular methods. Glivec therapy allows to reach comparatively higher ability to reach complete molecular remissions than interferon-alpha therapy. PCR is a highly sensitive method of detecting of minimal residual disease for the patients with complete cytogenetic remission. To get more extended information about the kinetics of minimal residual disease a quantitative molecular methods should be used.

946 COMPARISON BETWEEN NODAL AND EXTRANODAL PRESENTATION OF DIFFUSE LARGE B-CELL LYMPHOMAS

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Molecular differences have been found between nodal and extranodal diffuse large B-cell lymphomas (DLBCL), suggesting different genetic origin (Raghoebier et al 1991). We retrospectively analyzed and compared the characteristics of the patients with nodal or extranodal DLBCL who presented to the Haematology Department of our Hospital from 1989 till today.

Of 282 patients with DLBCL, 143 (50.7%) had nodal and 139 (49.3%) had extranodal lymphoma. The median age was 60 years old for patients with nodal and 58 years for patients with extranodal DLBCL. The majority of the patients with extranodal DLBCL had stage I and II (49.2% and 34.1% respectively), while patients with nodal DLBCL were equally distributed in all four stages.

No statistical difference was found between the two groups when they were compared for the age at diagnosis, the gender and the existence of anaemia at presentation. On the contrary nodal DLBCL was associated with higher tumor burden (LDH, $p=0.002$), better performance status (ECOG, $p<0.001$) and worse response to treatment ($p<0.001$) than extranodal DLBCL.

We conclude that there are clinical differences between nodal and extranodal DLBCL lymphomas. This observation combined with the reported differences in genetic origin suggests that nodal and extranodal DLBCL lymphomas may be regarded as separate entities.

947 EFFECTS OF THROMBOCYTAPHERESIS ON DONOR'S PLATELET FUNCTION AND COAGULATION SYSTEM

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Background :

The exact causes of thromboembolic phenomena following thrombocytapheresis is still undefined. In addition to increased blood viscosity due to hemoconcentration, possible changes in coagulation and fibrinolytic system during or immediately after apheresis are thought to be responsible.

Aim :

To determine the effects of thrombocytapheresis on platelet aggregation-activation, coagulation and fibrinolytic system.

Methods :

A total of 20 healthy donors without any history of haemorrhagic diathesis or drug ingestion were enrolled the study. Full blood count, PT, aPTT, INR, fibrinogen, ADP and collagen induced platelet aggregation, ristocetin cofactor activity, protein C-S, AT III, D-dimer, FVIIIa, tPA, PAI and p-selectin were performed before and after thrombocytapheresis.

Results :

After thrombocytapheresis, there was a statistically significant decrease in hemoglobin ($p<0.001$), white blood count ($p<0.001$), platelet count ($p<0.001$), fibrinogen ($p<0.05$), protein S ($p<0.01$), AT III ($p<0.01$) and FVIIIa ($p<0.05$) while INR ($p<0.001$), PT ($p<0.001$), and aPTT ($p<0.001$) showed a significant increase. Platelet aggregation, RCoF, protein C, D-dimer, tPA, PAI and p-selectin results were not affected.

Conclusion :

Thrombocytapheresis affects coagulation protein levels which may contribute to thrombotic episodes in donors but does not cause any change either in platelet function or fibrinolytic system.

948 CLINICAL OUTCOMES OF CHEMOTHERAPY USING FLUDARABINE-BASED REGIMENS IN 31 PATIENTS WITH LYMPHOMA

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Background: Fludarabine (Fludara®) is one of the most effective agents in the treatment of indolent lymphoma and chronic lymphocytic leukemia (CLL) in Caucasians. Treatment with fludarabine as a single agent resulted in response rates of 40%84% in chemotherapy-naïve patients, with an overall response rate of 30%70%, and a complete response rate of 10%38% in pretreated patients with indolent lymphoma. Combination chemotherapy regimens containing fludarabine, such as FN, FMD, and FC have demonstrated clinical efficacy.

Aims: To investigate the efficacy and tolerability of FMD and other fludarabine-based regimens in the treatment of Chinese patients with lymphoma.

Methods: Thirty-one lymphoma patients (18 male, 13 female) were enrolled during April 2001 to February 2004. Median age was 53 years (range 27.9-90 years). Twenty-four patients were diagnosed with low-grade lymphoma, 7 patients with medium-grade lymphoma, and 9 patients had B-symptoms. Nineteen patients were treated with a regimen of FMD (fludarabine 25 mg/m²/day Days 1-3, mitoxantrone 8 mg/m²/day

Day 1, and dexamethasone 20 mg/day Days 14), 3 patients with R-FMD (rituximab + FMD), and 9 patients with other regimens containing fludarabine. Fludarabine combination therapy was administered for a median of 3 cycles (range 16).

Results: Twenty-one of the original 31 patients completed fludarabine combination therapy and were evaluated for objective response. In patients with indolent lymphoma receiving FMD, the overall response rate was 86% (18/21), and 8/21 (38%) patients achieved a complete response. No response was observed in the 4 patients with medium-grade lymphoma. Myelosuppression and mild GI reactions were common adverse events. Hematologic toxicities included leukopenia (61%, grade III/IV, 8%), thrombocytopenia (18%, grade III/IV, 4%) and anemia (26%). G-CSF infusion was required for only 5% of patients, and transfusion of red blood cells or platelets was not required. Mild nausea and vomiting occurred in 20% of patients. Rare adverse events included febrile neutropenia (5 patients; all were controlled by IV infusion of antibiotics and supportive measures), transient abnormal liver function and jaundice (1 patient), and diffuse skin rash (1 patient).

Summary/Conclusion: Fludarabine-based regimens, including FMD, for the treatment of indolent lymphoma in Chinese patients resulted in an overall response rate of 86% and a complete response rate of 38%. Further investigation is warranted to explore potential application of chemotherapy using fludarabine for low-grade lymphomas.

949 LOW-DOSE THALIDOMIDE AND CORTICOSTEROIDS FOR THE TREATMENT OF CHRONIC IDIOPATHIC MYELOFIBROSIS

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BACKGROUND: Chronic idiopathic myelofibrosis (CIM) is a myeloproliferative disease characterized by excessive bone marrow fibrosis, osteosclerosis and angiogenesis leading to progressive anemia, leukoerythroblastosis, extramedullary hematopoiesis, splenomegaly and constitutional symptoms. Allogeneic hematopoietic stem cell transplantation is the only potentially curative option but is limited in the small subset of younger patients. Conventional therapeutic approaches such as androgens, corticosteroids, erythropoietin, hydroxyurea and splenectomy ameliorate disease related anemia and symptoms without a clear survival benefit. Thalidomide exhibits potent cytokine modulation and antiangiogenic properties and has been proposed as a novel treatment for CIM.

AIM: Low dose thalidomide in combination with methylprednisolone was given in CIM patients to treat disease related cytopenias.

METHODS: Five CIM patients, two male and three female, aged 66 to 84 years old were given thalidomide 50mg and methylprednisolone 0.1-0.2mg/Kg daily. CIM had been diagnosed 4 to 68 months before entry to study. All patients had adverse prognostic features, which included at least three of: anemia (hemoglobin <100gr/L), age >64 years, leukopenia (<4x10⁹/L), circulating blasts >1% and presence of hypercatabolic symptoms. Two patients had thrombocytopenia (platelets <150x10⁹/L), two patients had white blood count <4x10⁹/L and all patients had anemia (hemoglobin range: 71 to 84gr/L) that demanded regular red blood cell transfusions. Cytopenias did not respond to previous treatments, including erythropoietin, corticosteroids, androgens, hydroxyurea or combination of the above. None of the patients had undergone splenectomy.

RESULTS: Four out of five patients presented significant increase of hemoglobin values after a period of treatment ranging from 3 to 8 weeks. Median pre-treatment hemoglobin value was 75gr/L and increased post-treatment to 105gr/L. Hemoglobin values increase ranged from 15 to 50gr/L (median 30gr/L) and red blood cell transfusions were discontinued in all four patients. The fifth patient did not presented any increase of hemoglobin after treatment for three months. Platelets in the two patients with thrombocytopenia, increased from 49x10⁹/L and 108x10⁹/L to 140x10⁹/L and 216x10⁹/L respectively. White blood counts in the two patients with leukopenia increased from 2.95x10⁹/L and 2.52x10⁹/L to 5.96x10⁹/L and 12.76x10⁹/L respectively. Any patient did not experience thalidomide related toxicity.

CONCLUSIONS: Combination of low dose thalidomide and methylprednisolone may be effective for the treatment of cytopenias in the majority of CIM patients, despite failure of previous conventional treatments. Most patients present a clinically relevant improvement of anemia and reduction of transfusion requirements associated with a low toxicity profile.

950 INTERMEDIATE-DOSE AMPHOTERICIN B LIPID COMPLEX (ABELCET, 3 MG/KG/DAY) FOR THE TREATMENT OF INVASIVE FUNGAL INFECTIONS IN ADULTS WITH HEMATOLOGICAL MALIGNANCIES: RESULTS FROM A PROSPECTIVE STUDY

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BACKGROUND: Amphotericin B lipid complex (ABLC) was the first lipid complex to be developed; it was designed to provide a less toxic alternative to conventional Amphotericin B (AmB) without compromising efficacy. Most studies are made with 5 mg/kg/day doses for both proved and suspected invasive fungal infections (IFI).

AIM: In this study, we report a prospective single-center experience about the use of ABLC at intermediate doses (3 mg/kg/day) in patients with proven or suspected IFI.

METHODS: 93 patients were treated for more than 3 consecutive days with ABLC at 3 mg/kg/day between August 1997 and September 2003. Full data has been analysed to date for 76 patients. There were 42 men and thirty-four women, median age 48 years old (range: 20-72) receiving ABLC at three mg/kg/day at a median dose of 192 mg/day (range: 150-250 mg/day) during a median of 10 days (range: 4-72).

RESULTS: IFI (EORTC/MSG criteria) included 31 proven or probable IFI (45.1% pulmonary aspergillosis (n=14), 1% sinus aspergillosis (n=1), 29% systemic candidosis (n=9), 19.3% fungemia (n=6), 3.2% others (n=1)), 27 cases of possible IFI, 18 cases of a pulmonary infection in patients at very high risk for aspergillosis but who did not meet the radiological criteria for possible IFI. Sixty-one patients were neutropenic (<500 neutrophils) before clinical signs of infection appeared and 8 patients were not neutropenic but were receiving immunosuppressive therapy, especially for acute/chronic graft versus host disease.

Before ABLC treatment started, 2 patients had been under prophylactic treatment with azoles (2.6%), 13 patients with conventional AmB (17.4%), 28 patients with ABLC (37.3%) and 1 patient with Ambisome (1.3%). In 28 cases, patients received other antifungal therapies at therapeutic doses before ABLC administration, which was shifted to ABLC for toxicity in 11 of cases or disease progression in 9 of cases.

To avoid secondary effects related with abelcet administration, pre-medication was given to all patients, and it consisted on a combination of prednisone+antihistamines (43.4%), prednisone+paracetamol (17%), paracetamol+antihistamines (32.1%), paracetamol alone (1.9%) or the three drugs together (3.8%). Ionogram, hepatic and renal function was monitored during treatment.

Complete clinical response and partial clinical response was 74.2%. For aspergillosis, corresponding rates were 78% and in candidosis 73%. 39 courses were complicated by one or more side effects: fever (9.2 %), chills (17.1%) and cutaneous rash (1.3%). For the whole group, serum creatinine level remained stable before treatment: medium creatinine serum level at start of treatment was 89 µmol/l (range: 43-541) and medium creatinine serum level after treatment was 95 µmol/l (range: 40-520).

ABLC at median doses is a therapeutic option for proven or suspected fungal infections, potentially having lower toxicity than standard doses and less toxicity than conventional amphotericin-B. Moreover, it enabled us to treat patients who were unable to tolerate an efficacious dose of Am B. Larger randomized studies using this dose as monotherapy or in combination with echinocandins appear warranted based on our results.

951 SERUM CYTOKINE PROFILES IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES IN RELATION TO THE FAB, WHO AND IPSS CLASSIFICATION

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Background:

Myelodysplastic syndromes (MDS) are characterized by peripheral trilineage cytopenias in generally hypercellular bone marrows. The increased proliferation is associated with increased cell apoptosis and a potential to evolve towards acute leukemia. Various classifications have been implemented for MDS taking into consideration the blastic counterpart in the FAB classification, the presence of cytopenias and genetic aberrations in the IPSS and WHO classification. Various cytokines are implicated in the pathogenesis and progression of MDS characteristics. TNF-α and TGF are implicated in apoptosis, IL-1b in the inflammatory response and endothelial activation, VEGF in angiogenesis and IL3 in erythroid precursor maturation.

Aim:

The aim of the current study was to assess the levels of the aforementioned cytokines in a group of MDS patients and examine the relation with the standard MDS classifications.

Methods:

106 patients with MDS were included in the study. No therapeutic manipulations had proceeded serum measurements apart from occasional blood transfusion. ELISA measurements were performed using commercial kits (R & D Systems Inc, Minn USA for IL-3, IL-1b, TNF-a, b2M and Bender MedSystem, Vienna, Austria for VEGF and TGF). Bone marrow smears, biopsies and karyotype definition were performed in all patients.

Results:

In the overall group of MDS patients the meansd levels were 10,294.37 pg/ml for IL3, 13,4919.05 pg/ml for VEGF, 6,377.62 pg/ml for TNF-a, 2,92.11 mg/ml for b2M, 0,910.84 pg/ml for IL-1b and 80,0340.02 ng/ml for TGF. According to Kruskal Wallis analysis for the FAB grouping IL-b was significantly increased in the RAEB, RAEBt and CMML subtypes in comparison to the RA and RARS subtypes to ($p < 0.05$). The same applied for VEGF ($p < 0.05$). In the IPSS scoring system IL-1b showed increasing levels with increasing score which marginally failed to reach significance ($p = 0.06$) whereas for VEGF the difference was non significant. In the WHO classification IL-1b was significantly increased in the mixed myeloproliferative /myelodysplastic group in comparison to the other groups ($p < 0.01$). The rest of the measured cytokines IL-3, TGF-a, TNF-a and b2M showed no differences among the subtypes in any of the classification.

Conclusion:

With the exception of IL-1b it seems that the MDS grouping with the current classifications does not reflect cytokine activity.

952 CLINICAL STUDY OF FLUDARABINE, MITOXANTRONE, AND DEXAMETHASONE (FMD) IN THE TREATMENT OF REFRACTORY/RELAPSED MULTIPLE MYELOMA

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Background: Multiple myeloma is a malignancy of terminally differentiated B-lymphocytes. This systemic malignancy is treatable, but rarely curable. With conventional therapy, only 5% of patients achieve complete remission. Salvage therapy is needed for patients who have either relapsed or have primary refractory disease. VAD (vincristine + doxorubicin + dexamethasone) benefits 40%–50% of relapsing patients and about 30% of those with primary unresponsive disease. Fludarabine has demonstrated efficacy in chronic lymphocytic leukemia (CLL) and low-grade lymphoma, and pilot studies suggest activity in the treatment of multiple myeloma (MM).

Aims: The purpose of this trial is to compare the safety and efficacy of FMD with VAD for refractory/relapsed MM.

Methods: A total of 27 Chinese patients with refractory/relapsed MM have been treated since March 2003: 5 patients treated with FMD (fludarabine 35 mg/m²/d IV Days 1–3, mitoxantrone 8 mg/m²/d IV Day 1, and dexamethasone 20–40 mg/m²/d Days 1–4 repeated every 28 days), and 22 patients treated with VAD (vincristine 0.5 mg/m²/d IV Days 1–4, pirarubicin 10 mg/m²/d IV Day 1, and dexamethasone 20–40 mg/m²/d repeated every 28 days).

Results: A partial response (PR) rate of 40.0% and an overall remission rate of 60.0% (2 patients PR, 1 patient minimal response) were observed in patients treated with FMD compared to 22.7% and 31.8%, respectively, in patients treated with VAD ($P < .05$). Median time to achieve PR was 88 days in the FMD group versus 68 days in the VAD group ($P < .05$). Approximately 40.0% of patients in the FMD group had >50% decrease in M-protein or >20 g/L rise in hemoglobin, compared to 22.7% and 18.2%, respectively, in the VAD group ($P < .05$). The median time to achievement of >50% decrease in M-protein, decline of 80% of myeloma cells in bone marrow (BM) or <5% of myeloma cells in BM, and >20 g/L rise in hemoglobin was 62 days, 57 days, and 70 days, respectively. There was no significant difference between groups in serum calcium, creatinine, and GPT pre- and post-treatment. The level of serum β_2 -microglobulin was 1042.8 72.3 μ g/L post-treatment in the FMD group, which was lower than that observed pre-treatment (2350.2 184.0 μ g/L; $P < .05$). The median nadir white blood cell count was 0.9 0.46 10^9 /L in the FMD group versus 2.09 0.6 10^9 /L in the VAD group ($P < .05$), and the incidence of fever and cough was higher with FMD compared to VAD ($P < .05$).

Summary/Conclusions: FMD was an effective and safe regimen in the treatment of refractory/relapsed MM. The PR rate and overall remission rate were significantly higher in FMD-treated patients with refractory/relapsed MM compared to those treated with VAD.

953 POOR STEM CELL MOBILIZATION CORRELATES WITH DELAYED SHORT TERM ENGRAFTMENT IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES SUBJECTED TO HDT+AUTO-SCT

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Background: Between March 1998 and August 2003 50 acute leukaemia pts and 93 lymphoma pts underwent 154 procedures of high dose therapy supported by unmanipulated autologous stem cell transplantation (HDT+autoSCT). In 11 lymphoma pts double HDT were carried out. **Aims:** The aim of the study was to determine the influence of poor or ineffective mobilization on kinetics of short term engraftment in this cohort of pts. **Methods:** Stem cells derived from BM in 37 pts (24 leukaemia pts and 13 lymphoma pts) and from PB - in 106 pts (26 leukaemia pts and 80 lymphoma pts). BM was an elective source of the graft in 10 pts, in subsequent 27 pts BM was harvested after 1 (17 pts) or 2 ineffective mobilizations (10 pts). In 98 pts stem cells were obtained after 1 mobilization course and in 8 pts after the second one. These data were analysed to determine kinetics of short term engraftment in the groups of pts according to the stem cells source PBSC and BM. **Correlations** between age, previous chemotherapy, stem cell source, number of infused CD34+ cells, MNC, CFU-GM and parameters of short term recovery were evaluated. **Results:** In BMT cohort - short term haematopoietic recovery was delayed in the group of patients failing 2 mobilization cycles. Comparing to the pts with BM harvested up front, we observed significant differences in the duration of neutropenia and granulocytes recovery ($p = 0.02$ and $p = 0.048$). Similar results comparing to the pts subjected to BM harvested after 1 ineffective mobilization course ($p = 0.03$ and $p = 0.048$) were assessed. Analysis of the age, previous therapy duration, number of chemotherapy cycles, radiotherapy use and number of infused CD34+, MNC, CFU-GM did not reveal any significant differences between these 3 observed groups of pts. In PBSC group short term neutrophil and platelet engraftment was significantly delayed in patients subjected to 2 mobilization cycles comparing to the patients after 1st successful mobilization ($p = 0.03$ and $p = 0.01$). Analysis of the age and previous therapy did not reveal any significant differences between two observed groups of pts. Number of infused CD34+ and MNC were similar in both. The only difference found was a significantly higher number of CFU-GM in the group of pts with first successful mobilization. In BM and PBSC groups of pts - significant correlations between the number of infused CD34+ cells and kinetics of short term engraftment were found in patients subjected to BM with 1 or without prior mobilization course, but not in the patients subjected to 2 mobilization procedures. **Conclusions:** These results suggest that poor or ineffective mobilization indicates delayed short term recovery after HDT supported by autologous stem cell transplantation in pts with haematological malignancies. However the quantity and quality of the graft (CD34+, MNC and CFU-GM) have no impact on short term engraftment kinetics in this group of pts.

954 SHORT COURSES OF BUSULFAN IS MORE EFFECTIVE AND SAFER THAN HYDROXYUREA IN THE MANAGEMENT OF ESSENTIAL THROMBOCYTHEMIA: A RETROSPECTIVE STUDY OF 143 PATIENTS

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Cytoreductive treatment usually decreases the platelet count as well the incidence of thrombotic events in ET. Treatment with alkylating agents (mainly busulfan) and less Hydroxyurea (Hu) is known to increase the risk of leukemia. Therefore the positive clinical effect of cytotoxic treatment should be evaluated against the leukemogenic effect of the drug given. Our study included 143 patients, median age 63 years (range 15-94), 73% of them have found to have one or more risk factors for thrombosis. The median platelet count at diagnosis was 922×10^9 /l. Busulfan was administered in 40 patients as a short course including: 4 mg/day first week, 2 mg/day next 3 weeks and 2 mg every other day until achievement of response (usually for 3 months). Hu was given continuously in dose 500-2000 mg/day (46 patients). Recently, anagrelide was initiated in

7 patients and as additional treatment in 14 patients; 50 patients did not require cytotoxic therapy. All patients treated with busulfan achieved complete hematological response. Median cumulative dose per course was 124 mg. The median follow-up was 86 months. Twelve were retreated with busulfan for relapse. Overall, 53 courses were given. The median duration of response was 30 months. No toxicity was observed during the treatment, no leukemic transformation was seen, but 2 patients who received Hu for relapse developed myelofibrosis. In the Hydrea-treated patients, the median follow-up was 80 months: 4 cases of leukemia developed, one of them following myelofibrosis. Four additional patients developed myelofibrosis 6-8 years on Hu treatment. The new widely used non-cytotoxic drug anagrelide, although effective in reducing platelet counts, requires prolonged continuous therapy and often is associated with clinically significant adverse effects, which prevent the use in elderly and women planning future pregnancies. Our study showing effectiveness and safety of short courses of busulfan with no leukemogenic effect compared to Hu may 'rehabilitate' this old drug as a good alternative management in high-risk ET.

955 ANTI-ANGIOGENIC PROPERTIES OF LOW-DOSE 5-AZA-2'-DEOXYCYTIDINE (DECITABINE) IN THE TREATMENT OF HIGH-RISK MYELODYSPLASTIC SYNDROMES

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BACKGROUND

The myelodysplastic syndromes (MDS) are clonal disorders characterized by dysplasia and/or cytopenia in one or more cell lines, and maturation defects. Low-dose decitabine, a DNA-hypomethylating agent, has shown activity in two previously published phase II trials in the treatment of high-risk MDS. Because of the revival of thalidomide, used as an anti-angiogenic agent in the treatment of MDS and multiple myeloma, we wondered if low-dose decitabine also had anti-angiogenic properties. As human angiogenin is one of the most potent inducers of angiogenesis, we measured the levels of angiogenin in the third phase II study. Previous studies reported that angiogenic factors are increased in MDS and may play a role in the leukemogenic process.

AIMS

The aim of our side-study was to determine the possible anti-angiogenic properties of low-dose decitabine in the treatment of high-risk MDS. Therefore we would expect a decline in the level of human angiogenin. The overall response rates, the correction of cytogenetic abnormalities and the rise in platelet count are yet previously published or accepted for publication.

METHODS

Of the last 13 patients in the third phase II study we collected plasma samples at baseline, after the first and after the second course of decitabine, that means on day 0, day 43 and day 85. Samples were stored and frozen at minus 20 degrees Celsius. We used the Human ANG Immunoassay for the quantitative determination of human angiogenin (Quantikine®, R&D Systems, Minneapolis, USA). Each sample was measured twice and the mean number was determined. Because of a 200-fold dilution the samples had to be multiplied by 200.

RESULTS

The mean baseline value was 1733 pg/ml (range 1084 - 2509). After the first course this was 1752 pg/ml (range 1139 - 2254). After the second course the mean value was 1692 (range 1111 - 2253). We could not find a relationship between the level of human angiogenin baseline characteristics, response rates, time to progression and overall survival.

CONCLUSION

It appeared that the mean values of human angiogenin were not significantly less after one or two courses of low-dose decitabine in the treatment of high-risk MDS. Therefore we concluded that low-dose decitabine has no anti-angiogenic properties in the treatment of high-risk MDS, at least it did not lower the level of one of the strongest inducers of angiogenesis, human angiogenin. We are currently investigating the levels of other angiogenic factors. Unfortunately, we were not able to complete the measurements of the microvessel density in the bone marrow smears of the patients prior to exposure to decitabine and after the second course.

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956 RAPAMYCIN HAS STRONG ANTI-PLASMACYTOMA ACTIVITY IN VITRO AND IN VIVO

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Background:

Recent reports have shown that phosphatidylinositol 3-kinase (PI3-K) signaling plays an important role for the growth and survival of malignant plasma cells. One of the downstream effectors of the PI3-K is the mammalian target of rapamycin (mTOR). It has been shown that, under serum free conditions, inhibition of mTOR has only a modest effect on malignant plasma cell growth. However, as an important regulator of cell growth, mTOR activation relies not only on the presence of a signal from PI3-K/Akt, but also requires the presence of sufficiently high levels of amino acids and ATP.

Aims:

We studied the effects of rapamycin, a potent and highly selective inhibitor of mTOR, on malignant plasma cell growth in the presence of nutrients.

Methods:

The effect of fetal calf serum (FCS) and rapamycin on activation of mTOR downstream targets was studied by Western blotting. The influence of rapamycin on malignant plasma cell growth was studied in vitro by [³H]-thymidine incorporation and staining of cells with PI-triton, and in vivo in our SCID mouse model for human plasmacytoma. Finally, we started treatment of a myeloma patient who had been refractory to former treatments with chemotherapy, autologous tandem transplantation, thalidomide and bortezomib.

Results:

We could show that, in malignant plasma cells, the presence of nutrients is essential for the activation of mTOR. Also, the activation of mTOR by FCS could be abolished by pre-treatment with rapamycin. In the presence of serum, rapamycin was highly effective at inhibiting malignant plasma cell growth and induced apoptosis and/or G1 cell cycle arrest in all plasmacytoma cell lines tested. Furthermore, treatment with low doses of rapamycin significantly prolonged survival compared to untreated controls (median survival: 108 days vs. 58 days) in an in vivo SCID mouse xenograft model for human plasmacytoma. In the myeloma patient, treatment with relatively low doses of rapamycin (2-5 mg/day p.o.) alone was able to stabilize the patient's clinical condition and previously rising level of M-protein for a period of 20+ weeks.

Summary/conclusion:

The in vitro and in vivo efficacy of rapamycin indicates that inhibition of mTOR may provide an interesting treatment option for patients with malignant plasma cell tumors.

957 EFFICACY OF COMBINATION CHEMOTHERAPY WITH FLUDARABINE AND CYTARABINE IN THE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA: A CLINICAL REPORT OF 19 PATIENTS

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Background: Fludarabine-based regimens are effective in a variety of hematologic malignancies.

Aims: The primary objective of this study was to evaluate the efficacy and toxicities of fludarabine (flu) in combination with cytarabine (ara-c) in the treatment of relapsed/refractory acute lymphoblastic leukemia (ALL).

Methods: Between March 2000 and February 2004, 19 Chinese patients with relapsed/refractory ALL were treated with flu- and ara-c-based chemotherapy regimens. Fifteen were male and 4 female, with a median age of 19 years (range 11-54 years). Fifteen patients had B-cell ALL (including 6 with Philadelphia chromosome positive ALL), 3 had T-cell ALL, and 1 had acute mixed lineage leukemia. Among these patients, 10 had refractory ALL, and 9 had relapsed ALL (2 presented in first relapse, 4 in second relapse, 1 in third relapse, and 2 relapsed after autologous stem cell transplantation). Fifteen patients were treated with flu (30 mg/m² IV over 30 minutes) and ara-c (1000 mg/m² every 12 hours IV for 610 doses) for 4 or 5 days. The ara-c infusions commenced 4 hours after the start of the fludarabine infusions. Four patients were treated with 3 drugs: flu (same regimen as above), ara-c (100 mg/day every 12 hours for 610 doses), and mitoxantrone (4 mg/d IV Days 14).

Results: Eight of the 18 patients (44.44%) with ALL achieved a complete response (CR), and 2 patients (11.11%) achieved a partial response (PR), for an overall response rate of 55.55%. In the 9 patients with Philadelphia chromosome negative ALL, 4 (44.44%) achieved a CR. Of the 6 patients with Philadelphia chromosome positive ALL, 3 achieved a CR, 2 a PR, and 1 was non-responsive. Of the 3 patients with T-cell ALL, 1 achieved a CR and 2 had no response. The patient with acute mixed lineage leukemia achieved a CR. All patients had hematologic toxicity. White blood cell (WBC) counts were $<1 \times 10^9/L$ in 18 patients, and the median time to WBC $<1 \times 10^9/L$ was 10 days (range 5-12 days). The lowest neutrophil count was observed 3 to 12 days after the end of treatment. Eighteen patients had platelet counts $<20 \times 10^9/L$, for a median duration of 5 days (range 1-20 days). The lowest platelet count was seen 3 to 12 days after the end of treatment. Three patients experienced pneumonia as a result of myelosuppression. Five patients experienced gastrointestinal symptoms, including nausea/vomiting, diarrhea, and anorexia; these symptoms resolved soon after treatment was completed. Two patients experienced palpitations; sinus tachycardia without other cardiac arrhythmia or myocardial ischemia was observed on ECG, and resolved after treatment was completed.

Summary/Conclusions: Promising results were observed with the treatment of fludarabine and cytarabine in Chinese patients with relapsed/refractory ALL. The toxicity of the regimen was acceptable. Larger studies are warranted to evaluate the efficacy and toxicity of this regimen in a larger patient population.

958 RECURRENT MISCARRIAGE SYNDROME AND INFERTILITY IN VENEZUELAN PATIENTS: ACQUIRED AND HEREDITARY COAGULANT DEFECTS

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The thrombotic defects associated with fetal wastage are thought to result from thrombosis of early placental vessels; peak fetal wastage occurs mainly in the first trimester, but some cases also occur in the second and third trimesters. The earlier the pregnancy the smaller the placental and uterine vessels and greater the propensity to undergo partial or total occlusion by thrombus formation. Thrombotic occlusion of placental vessels, both venous and arterial, affects fetal nutrition and fetal viability. Different thrombotic hemostasis defects associated with recurrent miscarriage syndrome have been reported. The antiphospholipid syndrome is the most common prothrombotic disorder causing recurrent miscarriage syndrome. The object of this paper is to present the study of the congenital deficiencies of some blood coagulation protein and the detection of antiphospholipid syndromes associated with recurrent miscarriage syndrome in pregnant women with history of two fetal miscarriages. **Materials and Methods:** Fifty five (55) pregnant women with diagnosis of recurrent miscarriage were studied. The patients were followed with daily fetal activity chart starting at 28 weeks, biophysical profile and Doppler flow of umbilical artery at 32, 34, 36, 38 weeks, delivery at discretion of the obstetrician. The following tests were done: hematology, prothrombin time, PTT, thrombin time, fibrinogen level, lupus anticoagulant determination, antinuclear antibodies, anti-DNA antibodies, VDRL, anticardiolipin antibody (IgG and IgM), antiphospholipid antibodies (antiphosphatidylserine, antiphosphatidylinositol, antiphosphatidylethanol amide, antiphosphatidic acid, antiphosphatidylcholine, determination of protein S and protein C antigenic and coagulant, plasminogen levels, antithrombin levels. The patients were treated with aspirin (100mg daily), low weight heparin (Clexane, 40 mg subcutaneously per day), prenatal vitamins, iron, folic acid. **Results:** the defects found were: 2 patients positive for antinuclear antibodies and anti-DNA antibodies, 1 patient positive only for antinuclear antibodies, 2 patients with antinuclear antibodies and antiphospholipid antibodies, 2 patients with antinuclear antibodies and anticardiolipins, 3 patients with protein C deficiency, one patient with proteins C and S deficiency. The patients had a viable fetus except one patient with associated HELLP syndrome, one patient who received treatment only for 2 months, and the other only for one month. **Conclusion:** This study shows that the recurrent miscarriages can be associated with acquired or congenital hemostatic defects and the use of low molecular heparin and aspirin during pregnancy in women with history of at least two miscarriages prevent thrombosis in placental vessels and fetal wastage.

959 G-CSF AS TREATMENT FOR ACUTE HYPOPLASTIC/ POSTMYELODYSPLASTIC MYELOID LEUKEMIA OF THE ELDERLY. A STUDY OF 25 CASES

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Background: Acute myeloid leukemia (AML) in the elderly has a poor prognosis, with a median survival of few months and less than 10% of long-term survivors. Standard chemotherapy has not proven superior to supportive care. Some literature reports showed antileukemic activity of G-CSF in hypoplastic acute leukemia and in relapsed AML after allogeneic stem cell transplantation, suggesting that stimulation of normal residual marrow cells may be therapeutic.

Aim: To analyse the tolerability and efficacy of G-CSF treatment in selected elderly patients with AML (no blastosis, no favourable cytogenetics, residual normal marrow cell metaphases, age > 70 or unfit for standard chemotherapy)

Patients and methods: Twenty-five patients with newly diagnosed AML were treated. Median age was 70 (range 60-86) and M/F ratio 7. All had cytopenia at presentation, with 0-26% peripheral blood (PB) blast cells (median 2%) and marrow myelodysplasia (23/25: 92%) or hypoplasia (< 20% cellularity: 4/24: 16.7%). Median marrow blast percentage was 34% (range 10-71%). Karyotype showed normal metaphases in 4 (19%), intermediate-risk abnormalities in 7 (33%) or high-risk abnormalities in 10/21 evaluable cases (48%), with normal metaphases ranging from 6 to 90%. G-CSF was given sc, at 300 mcg/daily. Dosage was subsequently tapered to maintain the absolute neutrophil count (ANC) > 1.500 x 10³/cmm. No additional treatment was given except low-dose corticosteroids in 8 patients. Treatment was stopped in patients who had no response after 1-2 months. **Results:** Median treatment duration was 3 months (0.5-18) with a mean weekly dosage of 1309 mcg. Compared to baseline, three of 12 patients lost transfusion dependence. Mean ANC increased from 0.6 x 10⁹/L to 4.8 x 10⁹/L (P<0.0001) (best result) and mean platelet count from 101.7x10⁹/L to 140.5x10⁹/L (P=0.019). Peripheral blast cell percentage did not change significantly. Seven patients showed a complete PB response lasting a median of 11 months (3-91+) and 16 showed at least a partial PB response, according to the criteria of Cheson (2000), lasting a median of 3 months (1-12), for an overall PB response rate of 92%. Posttreatment marrow analysis was available in 17 cases. Median marrow blast percentage and median normal metaphases did not change significantly. Four cases showed a morphological complete remission (16%), with persisting myelodysplasia and cytogenetic abnormalities. Treatment was well tolerated; five patients had infections and only two required hospitalization. After a median of 7.5 months, 3 patients are lost to follow-up. G-CSF was stopped in one patient after 3 months because of WHO grade 3 osteomyalgia, in two because of lack of response, in 16 responding patients because of progression after 3-17 months (median 9), and in one after being in persistent CR for 12 months. Two responding patients are still on G-CSF after 6-18 months. Median actuarial survival of the entire group is 10 months (range 2-93) and 13 months for the 7 responders.

Conclusion: These data suggest that G-CSF may be an effective and non toxic way to slow the progression of acute leukemia in selected elderly patients with AML.

960 ANGIOGENESIS IN CHRONIC MYELOPROLIFERATIVE DISEASES DETECTED BY CD34 EXPRESSION

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Background: Increased bone marrow angiogenesis estimated as bone marrow microvessel density (MVD), or as serum angiogenic factors levels and or immunohistochemical expression of these factors in bone marrow biopsy has been demonstrated in a variety of hematological disorders including chronic myeloproliferative diseases (MPDs).

Aim of this study was to investigate the MVD in 25 cases of myelofibrosis with myeloid metaplasia (MMM).

Patients and methods: MVD was estimated by CD34 immunohistochemical expression in bone marrow biopsies. A control group of 27 patients without bone marrow disease, 8 cases of polycythemia vera (PV), 41 cases of essential thrombocythemia (ET) and 9 cases of chronic myeloid

leukemia (CML) were also studied. Moreover, in MMM cases MVD were correlated with clinical, laboratory, histological parameters and the outcome of the patients.

Results: Our study confirmed a significantly higher degree of angiogenesis in MMM, PV, ET and CML compared with controls ($p < 0.001$, $p = 0.0007$, $p < 0.001$ and $p = 0.0008$ respectively). Angiogenesis was higher in MMM than PV, ET and CML cases ($p < 0.001$, $p < 0.001$ and $p = 0.008$). Increased angiogenesis was correlated with hypercatabolic symptoms in MMM patients ($p = 0.009$). No correlation with other clinicopathological parameters or clinical outcome was found. However, definitive conclusions regarding the prognostic value of increased angiogenesis may require additional follow up and a larger group of patients.

Conclusion: our results confirm the increases of angiogenesis in MPDs. Of course, MVD reflects the result of angiogenic activity, which is the number of bone marrow vessels. Detection of angiogenic factors immunohistochemical expression in the tissue, as well as quantification of angiogenic factors producing cells would show more insight into mechanisms involved in the pathogenesis of those diseases and might help in expanding new therapeutic strategies.

961 VITAMIN B12 AND FOLATE DEFICIENCY IN THE ELDERLY PATIENTS

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Aim: We try to examine the prevalence of vitamin B12 deficiency and folate deficiency in the elderly in a sample of the Hospitalized patients in our Department.

Methods: We study during the last 10 years 5.812 elderly people aged 65 years or older who hospitalize for various diseases.

We estimated the age-specific prevalence of vitamin B12 and of folate deficiency. Vitamin B12 deficiency is conventionally diagnosed if serum vitamin B12 was < 150 pmol/l. We defined metabolically significant vitamin B12 deficiency as vitamin B12 measured < 200 pmol/l. Folate deficiency, which usually refers to serum folate < 5 nmol/l, was defined as metabolically significant if serum folate was < 7 nmol/l.

Results: We found that the prevalence of vitamin B12 deficiency whether defined as low vitamin B12 or metabolically significant vitamin B12 deficiency, increased with age in all three studies, from about 1 in 20 among patients aged 65-74 years to 1 in 10 or even greater among people aged 75 years or greater. The prevalence of folate deficiency also increased with age, and was similar to that for vitamin B12 deficiencies, but only about 10% of people with low vitamin B12 levels also had low folate levels.

Conclusions: The high prevalence of vitamin B12 and folate deficiency observed in older people indicates a particular need for vigilance for deficiency of these vitamins. Reliable detection and treatment of vitamin deficiency could reduce the risk of deficiency-related disability in old age.

962 EFFECTS OF CLODRONATE (BONEFOS) ON SERUM CONCENTRATIONS OF CARBOXYTERMINAL TELEPEPTIDE OF TYPE I COLLAGEN AND AMINOTERMINAL PROPEPTIDE OF TYPE I PROCOLLAGEN IN CHEMOTHERAPY OF MULTIPLE MYELOMA

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Background: Disodium clodronate (Bonefos) is a bisphosphonate that has been prescribed as an adjuvant agent in the treatment and prevention of bone metastases in cancer patients. Clinical studies have confirmed that the beneficial effects of clodronate are related to its ability to inhibit bone resorption, thereby countering the osteolysis associated with local metastases and strengthening the bones of multiple myeloma patients.

Aims: The purpose of this study is to determine the effects of clodronate on biological markers of bone metabolism (carboxyterminal telopeptide of type I collagen [ICTP], aminoterminal propeptide of type I procollagen [PINP]) before and after treatment of patients with multiple myeloma.

Methods: Twenty-four patients with de novo multiple myeloma were recruited into 2 groups and treated with chemotherapy (MP or VAD regimen) with or without clodronate 300 mg/day intravenously x 5 days

for 2 cycles. ICTP and PINP were evaluated by enzymatic immunoassay and radioimmunoassay, respectively, in serum collected from patients before and after treatment.

Results: Before treatment, serum concentrations of ICTP were 7.08 ± 3.05 $\mu\text{g/L}$ in the clodronate-treated group ($n = 11$) and 6.87 ± 2.00 $\mu\text{g/L}$ in the chemotherapy alone group ($n = 13$) ($P > .05$). Detected serum concentrations of PINP were 54.20 ± 12.00 $\mu\text{g/L}$ in the clodronate-treated group and 62.32 ± 16.11 $\mu\text{g/L}$ in the chemotherapy alone group ($P > .05$). There was no significant difference between groups in either ICTP or PINP serum concentrations prior to treatment. Following treatment, serum concentration of ICTP (4.38 ± 1.78 $\mu\text{g/L}$) in the clodronate-treated group decreased by 31.8% compared to the pre-treatment level ($P < .05$). In contrast, serum concentration of ICTP was 6.69 ± 2.31 $\mu\text{g/L}$ after treatment with chemotherapy alone, which was similar to that observed before treatment ($P > .05$). Serum concentration of PINP after treatment was 86.32 ± 21.29 $\mu\text{g/L}$ in the clodronate-treated group, and 67.04 ± 28.59 $\mu\text{g/L}$ in the chemotherapy alone group ($P < .01$). Serum concentration of PINP (86.32 ± 21.29 $\mu\text{g/L}$) in the clodronate-treated group increased by 59% compared to the pre-treatment level ($P < .05$). However, in the group of patients that received chemotherapy alone, no change was observed in the serum concentration of PINP (67.04 ± 28.59 $\mu\text{g/L}$) after treatment ($P > .05$). Serum concentration of PINP after treatment in the clodronate-treated group was higher than that in the chemotherapy alone group ($P < .05$).

Summary/Conclusions: Clodronate significantly decreased serum levels of ICTP and increased serum levels of PINP in patients with multiple myeloma. The results suggest that clodronate may reduce osteoclast activity through the process of collagen synthesis in the bone, and therefore may be useful as an adjuvant agent to chemotherapy in multiple myeloma.

963 CHELATION THERAPY WITH DEFERIPRONE IN B-THALASSEMIA PATIENTS, THREE YEARS EXPERIENCE

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The purpose of the study was the evaluation of safety and efficacy of the new oral chelator - deferiprone (DFP) (Ferriprox ®, Apotex Inc) in b- thalassemia patients with severe hemosiderosis and poor compliance to desferioxamine (DFX) (Desferal ®, Novartis).

PATIENTS-METHODS: We examined 42 patients (18 males/24 females, aged 12-34 y.) who received DFP 75 mg / kg 3 t.i.d for 6-35 months. The inclusion criteria were ferritin > 2500 ng/ml, allergy to DFX and myocardial hemosiderosis (MRI measured T2, T2*). All patients underwent weekly monitoring of any adverse effect and close measuring of absolute neutrophil count (ANC). Serum ALT levels were obtained at baseline and monthly. Serum ferritin was monitored at baseline and every 3 months. We also determined Zn levels in serum and anti nuclear antibodies (ANA). Patients were divided in two groups: Group I (N=12) : Patients who received DFP < 12 months and Group II (N=18) : Patients who received DFP > 12 months (See Table 1- Results)

30 patients (71,5%) maintained normal levels of WBC ANC. 4 patients expressed neutropenic events ANC < 1500 /mm³, one received growth factor therapy. 8 patients (19%) presented WBC < 3000 /mm³ and ANC < 2000 /mm³. All patients except two had normal levels of Zn and in one detected positive ANA.

CONCLUSIONS:

- Deferiprone acts as an efficient iron chelate.
- There is a decrease (statistically significant) of serum ferritin levels and an amelioration of liver enzymes in both group of the study.
- Close monitoring for neutropenia is necessary during treatment with deferiprone

RESULTS:

GROUP I	N	Mean $\pm$ SEM	RANGE
Duration of therapy (Tx)	12	7,33 $\pm$ 0,59	7
Ferritin (baseline)	12	4072 $\pm$ 748	8653
Ferritin(after Tx)	12	2931 $\pm$ 749	7454
ALT (baseline)	12	69,41 $\pm$ 8,52	91
ALT(after Tx)	12	63,41 $\pm$ 2,2	128
Paired t-test $p < 0,003$ (Ferritin)			
Paired t-test $p < 0,039$ (ALT)			

GROUP I	N	Mean $\pm$ SEM	RANGE
Duration of therapy (Tx)	18	27,35 $\pm$ 4,78	81
Ferritin (baseline)	18	3698 $\pm$ 305	4292
Ferritin(after Tx)	18	2656 $\pm$ 406	5748
ALT (baseline)	18	79,71 $\pm$ 13,9	243
ALT(after Tx)	18	65,06 $\pm$ 9,51	155
Paired t-test $p < 0,003$ (Ferritin)			
Paired t-test $p < 0,472$ (ALT)			

Caption 1: Table 1- Results

964 A STUDY OF FLT3 ACTIVATING MUTATIONS AND EXPRESSION OF SURVIVIN MRNA IN PATIENTS WITH L-TYPE PML/RARA ACUTE PROMYELOCYTIC LEUKEMIA AT DIAGNOSIS AND MOLECULAR REMISSION

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The clinical relevance of FLT3 activating mutations in Acute Promyelocytic Leukemia (APL) and its molecular subtype is not clear. Survivin, an inhibitor of apoptosis protein is known to be expressed in leukemic cell lines and cancer cells but not in adult tissue except thymus and placenta. It has also been proposed as a useful biological marker for circulating neoplastic cells. The aim of this study was to screen 16 bone marrow APL samples at diagnosis, all expressing L-type PML/RARA for Internal Tandem Duplications (ITDs) and Asp835 mutations of the FLT3 gene and survivin expression as well as to examine survivin expression during PML/RARA molecular remission long after completing chemotherapy in patients with full haematopoietic recovery. For FLT3 activating mutations and detection of survivin we used specific PCR assay for cDNA and digestion with EcoRV for Asp835 mutations. Sixteen patients (11 female, 5 male) with median age 52.5 (15-75) years and median follow up 37 (8-115) months were examined. Only one patient relapsed 30 months after diagnosis, who became molecular negative after treatment with arsenic trioxide, one patient died during induction therapy and for 2 patients no samples were available during follow up. Thirteen patients could be evaluated for survivin mRNA expression during molecular remission. All patients were treated according to the APL protocol of the Greek AML Study Group. In 3 out of 16 patients (18.7%) ITDs were detected but no Asp835 mutations were found. The nly patients with haematological

relapse was negative for activating mutations both at diagnosis and relapse. In 4 out of 13 (33.3%) evaluable patients in molecular remission for L-PML/RARA with median follow up of 43 (15-88) months survivin could not be detected. ITDs were detected at diagnosis, in 2 out of 4 (50%) survivin negative patients tested in molecular remission. In this group of patients with L-type PML/RARA APL, FLT3 mutation status and detection of survivin during molecular remission had no statistically significant difference in age, gender, leukocyte number, platelet count, percentage of leukemic blasts in bone marrow and in the course of disease. At diagnosis, 3 patients who were found ITD negative had additional cytogenetic abnormalities, two involving chromosome 8 and one chromosome 10. No correlation was found with survivin expression during molecular remission, with median follow up 38 months. We conclude that the detection of survivin which cannot be detected by RT-PCR in normal bone marrows, could reflect a persistent clone with unknown significance for the clinical outcome of the patients. Further evaluation of the above described needs to be carried out.

965 PRIMARY MYELODYSPLASTIC SYNDROMES (MDS): SCORING PROGNOSTIC SYSTEMS IN 167 CASES

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AIM: To evaluate the prognostic value of scoring systems (IPSS vs Spanish Scoring System or SSS) in MDS.

PATIENTS AND METHODS: 167 patients were diagnosed primary MDS with an evaluable karyotype, but we excluded 18 CMML with leukocytes $>12 \times 10^9/L$ for univariate and multivariate analysis. 67 females and 100 males with median age 71 years (1-92) but only 30.5% were <65 years. FAB subtypes: 41 RA, 26 RARS, 36 RAEB, 30 t-RAEB, 16 dysplastic CMML. According to WHO classification were: 11 RA, 9 RARS, 19 RCMD, 18 RCMD-RS, 25 RAEB-1, 22 RAEB-2, 5 MDS-U, and 11 MDS associated with 5q- (13 cases were AML). Karyotypes were assigned according to ISCN 1995. The prognostic significance of initial clinical, pathologic and cytogenetic variables were evaluated by Kaplan-Meier estimate and compared by log-rank, Breslow and Tarone test, for overall survival (OS). Comparisons of remission rates were done by Chi-square analysis. The impact of prognostic factors was studied using Cox regression model. $p < 0.01$, M=median, m= months.

RESULTS: Median haemoglobin, leukocytes, neutrophil, platelets and bone marrow blasts percentage were: 97g/L, $4.7 \times 10^9/L$, $1.98 \times 10^9/L$, $115 \times 10^9/L$, 4.6%. Two or three cytopenias were present in 55.1%. 52.7% had trilineage myelodysplasia (TMDS) and 76.7% multilineage dysplasia (MD). 74/149 (46.7%) showed clonal abnormalities including: 25(15%) complex karyotype (80% involving 5/7 chromosomes), 12 trisomy 8, 11 del(5q), four 7/del(7q), 2 trisomy 21, 3 loss of Y chromosome, two involvement on 11q, two 3q21q26 rearrangement, one del (6q), and 12 with other abnormalities. Only 21 patients received intensive chemotherapy (47.6% with FLAG-IDA protocol) and 13/21(65%) achieved CR. 29.5% developed acute myeloid leukaemia (AML) during follow-up (48% for complex karyotypes, 41.6% for trisomy 8, 27.3% for del(5q) and 22.6% for normal karyotypes). AML evolution was related with high risk or int-2 groups of IPSS ($p=0.024$), high risk group of SSS ($p=0.016$) and RAEB / t-RAEB subtypes ($p < 0.001$). The 5-year OS probability was: 21.7% (M 14m). Univariate analysis showed poorer prognoses for OS in patients with: haemoglobin $<100g/L$ ($p < 0.0001$), platelets $<100 \times 10^9/L$ ($p < 0.0001$) blasts percentage $>5\%$ ($p < 0.0001$), two or three cytopenias ($p < 0.0001$), with TMDS ($p=0.001$) or MD ($p=0.002$), diagnosed RAEB/t-RAEB ($p < 0.0001$) or RAEB-1/ RAEB-2 ($p < 0.0001$), and complex abnormalities or involving 7 chromosome ($p < 0.0001$). Moreover, high risk or int-2 groups of IPSS ($p < 0.0001$), high risk group of SSS ($p < 0.0001$) showed the lower probability for OS. Multivariate analysis confirm that haemoglobin, International Prognosis Scoring Systems and Spanish Scoring System are independent factors for OS. The higher risk of dead is for patients with haemoglobin $<100g/L$ (OR 2.38) or high risk or int-2 groups of IPSS (OR 3.15) or int-1of IPSS (OR 2.01) or high risk group of SSS (OR 2.31)

CONCLUSIONS: In comparison with Spanish Scoring System, the International Prognosis Scoring Systems showed to have better discriminatory power regarding the patients clinical outcomes. Its likely that, FAB subtypes predict better risk of AML evolution

966 SENSITIVITY OF PCR IN DETECTING MONOCLONAL B-CELL POPULATIONS AND MONITORING OF PATIENTS WITH B-CELL MALIGNANCIES

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Background: PCR-based amplification of the IgH CDRIII is a very useful technique for the demonstration of a clonal B-cell population, especially when limited by a small amount of biopsy tissue.

Aim of this study was the detection of various forms of monoclonal B cell populations by using PCR to identify clonal immunoglobulin heavy chain genomic rearrangements, and the possible usefulness of this assay to monitor patients with B-cell malignancies.

Methods: Seventy five patients with B-cell malignancies (33 patients with B-chronic lymphocytic leukemia [B-CLL], 20 patients with low-grade lymphomas [including Waldenstrom Macroglobulinemia, hairy cell leukemia, follicular, marginal zone and MALT lymphomas], and 22 patients with multiple myeloma) were studied. DNA from bone marrow and/or peripheral blood was extracted using a standard protocol. PCR was performed according to the method described by Sykes et al (Leukemia 1997;11:153-158). In this protocol we used a seminested PCR protocol with a second amplification using a JH primer mostly internal to the one used in a preceding round of PCR.

Results: Monoclonal rearrangements were identified in 31 of 33 patients (93,93%) with B-CLL, in 16 of 20 of patients (80%) with low-grade Non-Hodgkin lymphoma, and in 14 of 22 patients (63,63%) with multiple myeloma. It is obvious that the method is more sensitive in patients with B-CLL than other types of B-cell malignancies. Seven patients (5 with B-CLL, 1 with hairy cell leukemia and 1 with myeloma) displayed two clones at diagnosis. Interestingly, a restoration of a polyclonal pattern was established in a patient with B-CLL, after treatment and achievement of complete remission. Moreover, in another patient with B-CLL and fever of unknown origin, a fine needle biopsy of liver revealed the presence of the same monoclonal band as in the peripheral blood, in conjunction with the demonstration by PCR of tuberculosis infection.

Conclusions: PCR analysis of immunoglobulin gene rearrangements detects about 63-93% of clonal lymphoproliferative proliferations with increased sensitivity for B-CLL. When this high sensitivity is concerned, this testing strategy can be especially useful in diagnostically problematic cases, when standard morphological and immunophenotypic analysis is used and could be the first step for more specific strategies for the detection of minimal residual disease.

967 THE RELATION BETWEEN BODY MASS INDEX AND THE OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT)

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Background: AHSCT outcome can be affected by numerous risk factors such as advanced disease, type of conditioning regimen, older age, and sex mismatch. Both obesity and undernourishment have been considered risk factors for morbidity and non-relapse mortality after AHSCT. Significantly worse survival has been demonstrated in severely underweight AHSCT recipients (Deeg HJ, BMT, 1995; Fleming HR, Am J Med, 1997; LeBlanc K, Haematologica, 2003). Different transplantation centers have calculated chemotherapeutic doses at conditioning regimen based on either ideal body weight or actual body weight. Therefore, we aimed to investigate retrospectively the relationship between recipient's body mass index on admission and transplant related morbidity, mortality and long-term outcome.

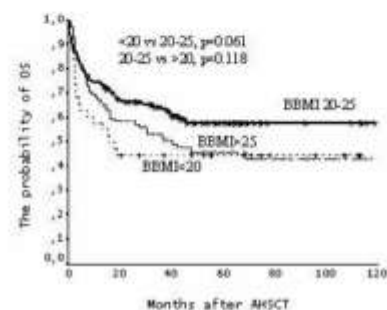
Patients and Methods: Between March 1994 and February 2003, 253 patients with a median age 31 year (range, 14-58) have undergone AHSCT at our stem cell transplantation unit form their HLA-identical siblings. The patients were classified in three groups according to their basal body mass index (BMI): underweight (BMI<20); normal weight (BMI 20-25), and overweight (BMI>25) patients. The majority of (%77) patients received busulfan and cyclophosphamide as a conditioning regimen and cyclosporine plus short-term methotrexate for graft versus host disease (GVHD) prophylaxis. We have usually calculated busulfan dose based on adjusted ideal body weight.

Results: The details of the patient's characteristics are shown in Table. Patients with low BMI were younger ($p<0.0001$). In low BMI group, the

majority of the patients' diagnosis was acute leukemia. The longest period from diagnosis to transplantation was observed in underweight patients ($p=0.013$). The incidences of acute (grade II-IV) and chronic GVHD were not affected by BMI. Early transplant related mortality was similar at each group. At median 69 months follow-up period in whole patients, 5-year- probability of disease free survival (DFS) and overall survival (OS) were 45.1%3.47% and 49.9%3.54%, respectively. Although we did not find any significant differences at 5-year cumulative probability of DFS and OS between three groups, normal weight patients had a tendency for higher OS rate than low BMI group ($p=0.06$) (figure).

In conclusion, the AML patients have less BMI than the CML group but lower BBMI did not correlate to any harm or benefit. Our analysis did not show any effect of BBMI on short and long term transplant related outcome. The relative homogeneity of BBMI and absence of patients with morbid obesity should have an impact on our analysis. We were not able to demonstrate any devastating effect of BBMI in our single center transplant cohort.

	BBMI<20 (n=28)	BBMI 20-25 (n=128)	BBMI>25 (n=96)	P
Median age (range)	22 (14-45)	30 (15-58)	36 (14-58)	<0.0001*
Diagnosis				
Acute leukemia (n=110)	20 (52.6%)	47 (59.2%)	43 (45.3%)	0.0001*
CML (n=110)	14 (36.8%)	39 (49.2%)	40 (42.1%)	<0.0001*
Other (n=20)	4 (10.5%)	14 (11.7%)	13 (12.6%)	0.0001*
Gender, M/F	16/12	72/56	43/54	0.131
Stem cell source: BM/ PB	16/12	41/79	32/63	0.621
Engraftment kinetics, median (range)				
Neutrophil > 0.5x10 ⁹ /L	15 (9.32)	15 (5.39)	14 (7.50)	0.699
Platelet > 20x10 ⁹ /L	17 (10.54)	15 (14.0)	15 (8.59)	0.280
Co-infection regimen				
Ablative vs Reduced Intensive TBI vs TBI	3/26 6/82	10/116 8/112	0/11 5/60	0.007 0.102
Median time between diagnosis to transplantation (months)	10.8 (3.3-126)	7.9 (2.4-120)	9.4 (3.3-69)	0.021*
Grade II-IV acute GVHD	13 (36.1%)	37 (33.3%)	33 (35.3%)	0.920
Chronic GVHD	12 (44.4%)	66 (64.1%)	52 (63.8%)	0.121
Early TRM	8 (21.1%)	21 (17.5%)	14 (14.7%)	0.687
5-year DFS	39.3 %	51.1 %	43.4 %	0.298
5-year OS	44.7%	57.3%	45.6%	0.117



Caption 1: table and figure

968 LARGE GRANULAR LYMPHOCYTE PROLIFERATIONS AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Background - Large granular lymphocyte (LGL) expansions were separated into leukaemia and reactive LGL proliferations. LGL proliferations may be reactive to different conditions, mainly viral infectious. Transient LGL proliferations are common after allogeneic hematopoietic transplantation. However few post autologous stem cell transplantation LGL proliferations have been published.

Aims - We reported four cases of polyclonal LGL proliferations after autologous peripheral blood stem cell transplantation.

Methods - There were 2 men and 2 women, the median age was 48 years. Autologous peripheral blood stem cell transplantation was realized for acute leukaemia in two cases and for solid tumours in two cases. Immunophenotype and clonality of LGL were studied by cytometry.

Results - Expansion of LGL could be documented between 1 and 34 months following autologous peripheral blood stem cell transplantation. LGL cells display a T-cell phenotype with expression of CD3, TCR $\alpha\beta$, CD8 and CD57. Clonality analysis showed a polyclonal T-cell expansion. Duration of the excess of circulating LGL may be chronic in some patients.

Conclusions - Our findings suggest that LGL proliferations may be observed after autologous peripheral blood stem cell transplantation. Some data have suggested that these lymphocytes might have an anti-tumour activity. Autologous transplantation may create favourable conditions for the 'spontaneous' development of an anti-tumour response mediated by LGL.

969 EXPRESSION OF KI-67, P53 AND BCL-2 ANTIGENS IN ACUTE LYMPHOBLASTIC LEUKEMIA: AN IMMUNOHISTOCHEMICAL STUDY
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Proliferative activity and deregulation of apoptosis are important features of the malignant clone, so the expression of antigens who are including in these processes may have some prognostic implications for the further course of the disease. In conformity with these facts we have analysed Ki-67, p53 and bcl-2 antigens expression in 52 newly diagnosed patients (pts) with acute lymphoblastic leukaemia (ALL). Diagnosis of ALL was based on the morphological and cytochemical criteria and on the immunophenotype features of leukemic cells. The expression of Ki-67, p53 and bcl-2 was evaluated by immunohistochemistry on the bone marrow biopsy specimens. The expression of these antigens was evaluated as % of positive cells in all bone marrow blast cells. The median age of this group patients was 35.5 yrs, overall survival (OS) 14.52 months, leukemia free survival (LFS) 10.47 months, and CR rate 71.15%. Mean level of Ki-67 expression was 16.19 $\pm$ 8.91, while the mean levels of p53 and bcl-2 were 11.21 $\pm$ 13.67 and 14.48 $\pm$ 28.85, respectively. Also, we found positive linear correlation between p53 and bcl-2 expression ($r=0.449>0.443$, $df=50$, $p<0.001$). Proliferative antigen Ki-67 had significant influence on OS and LFS ($p=0.0032$ and $p=0.0031$ respectively, by multivariate Cox analysis), while anti-apoptotic bcl-2 antigen had significant influence on OS and LFS ($p=0.0280$ and $p=0.0322$ respectively) as well as on achieving CR ($p=0.027$ by multivariate regression analysis). We have found that low Ki-67 expression and high bcl-2 expression on presentation had negative prognostic impact on the survival in our group of pts. Our results demonstrate that these pts who have the low proliferative and high anti-apoptotic activity on the presentation have 'high risk' disease and have to be observed as candidates for the treatment intensification.

970 CAMPATH-1H IN THE TREATMENT OF POOR RISK CHRONIC LYMPHOPROLIFERATIVE DISORDERS. SINGLE CENTER EXPERIENCE

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Campath-1H is a humanized monoclonal antibody anti-CD52, active in various malignant peripheral lymphoproliferative disorders. The drug is now available on the market, licenced mostly for 3. line of treatment in chronic lymphocytic leukemia after failure of alkylating agents and purine analogs.

Since January 2002 we treated 21 patient with following diagnosis with Campath-1H (MabCampath, Schering AG, Germany) recording toxicity and effect of the therapy: B-CLL: 11, B-SLL/CLL: 3, Mycosis fungoides: 3, B-PLL: one, T-PLL: one. Majority of patients received Campath-1H as their 3. line of treatment; median 3 (1-6). The patient with T-PLL was treated with Campath-1H in the first line. Planned dose of Campath-1H was 24 x 30mg in standard schedule 3 x 30mg/week. Median number of finally delivered doses was 21 (11-36). Fourteen patients were treated by intravenous 2 hours infusions, remaining 7 patients were given s.c. type of administration. For infection prophylaxis we used co-trimoxazole 960mg x 2/week, oral itraconazole (sol.) 2 x 100mg. Oral valacyclovir was used in different doses: in 10 patients were used very high doses (6g/day) in order to avoid CMV reactivation.

Median follow up is 12 months (2-20). Febrile reaction with chills in time of first infusion occurred on 18 patients (86%). Three patients without any reaction at the beginning of the treatment were those on s.c. administration. Six patients (29%) experienced either fever or chills during at least one of the following infusions. One patient developed Quinckes edema following first i.v. infusion, however, subsequent s.c. administration she tolerated very well and was able to finish the

entire course. No patient on s.c. administration developed late febrile reaction. Community acquired bacterial pulmonary infection developed in two patients during the treatment. The infection required in both cases combined antibiotic treatment including aminoglycosides on which both developed acute renal insufficiency grade II (NCI).

Reactivation of CMV was assessed by PCR each week of the treatment. Seven patients (33%) became PCR positive, 3 of them (14%) experienced CMV disease. All of these patients responded to gancyclovir and finally were able to finish the treatment with Campath-1H. From the cohort using the valacyclovir dose of 6g/day only one patient had the CMV reactivation.

All of 21 patients were assessed for the effect of the treatment. Complete remission was achieved in 6 (29%), partial response in 9 patients (43%). Overall response was 71%. Two patients progressed while on the therapy, 4 patients had stable disease (SD) after the treatment. Relaps after remission or progression of SD occurred in 11 patients (52%). Median time to relaps/progression was 6 months (2-14).

Conclusion: Treatment with Campath 1-H is associated with high response rate in poor risk malignant lymphoproliferative diseases. Toxicity is manageable, especially using subcutaneous administration and high-dose of valacyclovir in prevention of CMV reactivation. The response duration in very high risk patients should be improved.

971 EXPRESSION OF PROLIFERATION AND APOPTOTIC ANTIGENS IN NEWLY DIAGNOSED AND RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA

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The proliferation and apoptosis of the leukemic cells are very important indicators of biological features of leukemic clone. In this study we compared 52 newly diagnosed patients (pts) and 6 pts with relapse of acute lymphoblastic leukemia (ALL) with respect to proliferation and apoptosis. Diagnosis of (ALL) was established on the basis morphological and cytochemical criteria and on the basis immunophenotypic features of leukemic cells. The expression (%) of the proliferative antigens Ki-67 and PCNA, regulatory antigen p53, pro-apoptotic antigens CD95 and caspase-3, as well as anti-apoptotic antigen bcl-2 was analysed using standard immunohistochemical analysis of B5-fixed and routinely processed, paraffin-embedded bone marrow specimens. The median age of the our group patient was 35.5 yrs. The patients (pts) were treated with chemotherapy LALA-94 and remission was achieved in 71.15% cases. However, 50% of the pts relapsed. Both proliferative antigens Ki-67 and PCNA had significant higher expression on the presentation than in relapsed disease. Expression of Ki-67 was 25.00 $\pm$ 13.04 in newly diagnosed ALL, while in relapsed pts was 7.50 $\pm$ 4.18 ($temp=3.050>0.05=2.57$, $p<0.05$). On presentation antigen PCNA had value of expression 70.00 $\pm$ 25.90, and on relapse 30 $\pm$ 16.73 ($temp=4.969>0.05=2.57$, $p<0.05$). Regulatory antigen p53 had higher expression in relapsed pts (31.17 $\pm$ 29.05), than in newly diagnosed (6.83 $\pm$ 12.01), but this difference was not statistically significant ($temp=2.180<0.05=2.57$, $p>0.05$). Pro-apoptotic antigens CD95 and caspase-3 had significant higher values on the presentation than in relapsed disease. Initial expression of CD95 was 15.33 $\pm$ 13.55 and in relapse was 6.67 $\pm$ 10.33 ($temp=2.994>0.05=2.57$, $p<0.05$), while caspase-3 had values 11.33 $\pm$ 8.12 at diagnosis and 5.17 $\pm$ 6.18 on relapse ($temp=3.180>0.05=2.57$, $p<0.05$). Anti-apoptotic antigen bcl-2 had significant higher expression on relapse than on presentation (21.67 $\pm$ 22.28 and 11.67 $\pm$ 19.66, respectively) ($temp=3.180>0.05=2.57$, $p<0.05$). These data indicate that the expression of proliferative and proapoptotic antigens are higher at presentation, whilst the expression of anti-apoptotic antigen bcl-2 is higher in relapsed ALL, but these results deserve further examination in large cohort of pts needed.

972 INCIDENCE AND IMPACT OF FMS-LIKE TYROSINE KINASE (FLT3) GENE ITD MUTATION IN ACUTE MYELOGENOUS LEUKAEMIA

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Background: FLT3 is a class III receptor tyrosine kinase expressed in normal stem cells and blasts of myeloid leukaemia. Internal tandem duplication (ITD) of the FLT3 gene affecting the exons 14 and 15 leads to ligand-independent FLT3 dimerization and constitutive activation. This stimulates proliferation and induces inhibition of apoptosis which contributes to leukemogenesis.

Aims: We have screened a panel of acute myeloid leukaemia (AML) patients for the occurrence of FLT3/ITD mutation and correlated this mutation to patients' survival and basic hematological parameters.

Methods: RT-PCR for ITD in exons 14 and 15 of FLT3 gene was done on bone marrow samples of 67 AML patients at diagnosis.

Results: There was a 16,4% incidence of FLT3/ITD mutation in the cohort of examined patients. By cytogenetic subgroups there were 2/6 t(15;17) and 1 of 4 t(8;21) positive patients. The rest had normal and 2 had complex karyotype (both including trisomy 13, where FLT3 maps). Majority were of FAB M2 or M4 phenotype. For a subset of patients taken into comparative survival analysis there was a clear disadvantage for FLT3/ITD+ patients, their median survival was 3 vs. 16 months for FLT3wt patients ($p = 0,0431$). No difference was found for basic hematological parameters between two groups.

Conclusions: As it is evident today that FLT3/ITD is the single most common genetic abnormality in AML that also presents unfavorable clinical prognostic marker, it should be included in molecular diagnostic testing of myelogenous leukaemia.

973 RESULTS OF ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING REGIMEN AND HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH NON-HODGKIN LYMPHOMA

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Indolent and mantle cell lymphoma are not curable with standard chemotherapy. Allogeneic or autologous stem cell transplantation may be an effective treatment in patients with refractory or high risk disease. We present results of reduced intensity regimen (RIC) with allogeneic SCT (allo SCT) in 17 pts with primary refractory, relapsed or high-risk advanced NHL (12- CLL, 2- follicular, 3- mantle) and high-dose chemotherapy with autologous SCT (auto SCT) in 12 pts (1- CLL, 8- follicular, 3- mantle), treated between January 2000 and December 2003. Patients characteristic is shown in table. In the RIC allo SCT group the conditioning regimen consisted of fludarabine (150-180 mg/m²) plus busulfan (8mg/kg) plus ATG (40mg/kg) in 12 cases or fludarabine (150mg/m²) plus cyclophosphamide (2250mg/m²) in 5 cases. The patients received infusion of filgrastim-mobilized PBSC with a median of 4,6x10⁶ CD34+ cell/kg from HLA-matched sibling (15 pts), HLA-mismatched sibling (1patient) or unrelated HLA-matched donor (1 patient). Cyclosporine A was given as GVHD prophylaxis. All pts engrafted. Acute GVHD have been seen in 10/17 pts including 4 with grade>2. Chronic GVHD occurred in 7/17 pts. CMV infection was detected in 9/17 pts, including 1 pneumonitis and 1 hepatitis. With a median follow-up of 36 months OS and PFS are 41% and 30% respectively. Ten pts died: 3 of them in first 100 days after HSCT (aGVHD-1, CMV pneumonitis-1, sepsis-1), and 7 after 100 days (cGVHD-5, pneumonia-1, cerebral stroke-1). Patients treated with auto SCT received modified DEXA-BEAM (Dexamethason 184 mg/m², BCNU 300mg/m², Etoposide 800mg/m², Cytarabine 6000mg/m², Melphalan 140 mg/m²). Ten pts were transplanted with PBSC and 2 with BM stem cells; graft contained median 5,6x10⁶/kg CD34+ cells. All pts engrafted. With a median follow-up of 34 months OS and PFS are 75% and 42% respectively. No pts died in first 100 days after SCT, 3 died after 100 days (relapse-2, secondary myelodysplastic syndrome-1). Our findings on a limited number of pts suggest that auto SCT is a treatment with satisfactory PFS and low TRM for pts with mantle cell or refractory indolent NHL. Allo SCT with RIC regimen may be an effective salvage treatment for pts with refractory disease, but high incidence of GVHD is the main cause of mortality.

	RIC allo SCT	Auto SCT	p
Median age (range) yrs	52 (46-62)	46 (26-56)	<0.05
III/IV clinical stage	15/17 (88%)	9/12 (75%)	ns
N of prior chemotherapy regimens	2.6	2.6	ns

Caption 1: Patients characteristic

974 THROMBOEMBOLISM IN THROMBOCYTHEMIA PATIENTS WITH AN ADDITIONAL THROMBOPHILIC STATE

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Background: Myeloproliferative diseases (MPD) with thrombocythemia represent highly prothrombotic states. Evidence is already available that several factors may aggravate this thrombophilic state. Yet, there are no strict recommendations, as to which of these factors ought to be detected when considering management of MPD.

Aim: We assessed the risks of thrombosis and embolism (TE) in patients with MPD and thrombocythemia, in whom additional thrombophilic markers were detected.

Patients and Methods: A cohort of 43 MPD patients with thrombocythemia (34 with essential thrombocythemia, 4 with a prefibrotic stage of myelofibrosis, 4 with overlap-type MPD, 1 with polycythemia vera) has been prospectively studied. The mean age at diagnosis was 48 yrs (range 17-74), M:F ratio 17:26. In each patient, we detected the following hereditary thrombophilic markers: protein C (PC) and S (PS) heterozygous deficiencies, the mutation G-->A 20210 of the prothrombin gene, and the 'Leiden' mutation of factor V gene (fVL). In addition, response to activated PC (APC), antiphospholipid antibodies (APA), levels of factor VIII and antithrombin, homozygous methyltetrahydrofolate reductase (MTHFR) gene mutations C-->T 677 and A-->C 1298 and levels of homocysteine were analyzed as well. The incidence of the major (and potentially life-threatening) arterial and venous TE events has been documented.

Results: Nine patients out of 43 (21%) had a major TE event (6 venous, 2 arterial, 1 both venous and arterial); in 6/9 patients, TE was the first presentation of MPD. All patients had platelet counts between 400-800 x 10E9/l at the time of the event. In 10 patients, the presence of one of the hereditary thrombophilic markers was demonstrated. In these patients, TE events were far more frequent: 5/10 (50%) 'thrombophilia positive' patients suffered a major TE event (2 had PC deficiency, 2 had fVL mutations, and 1 a combination of PC deficiency and fVL), in contrast to only 4/34 (12%) patients in the 'thrombophilia negative' group ($P = 0.01$). Within the latter group, two patients with a TE event had decreased responses to APC (without having fVL mutation), another patient had elevated APA, but neither the APC-resistance without fVL, nor the APA increased the risk of TE significantly. We have not proven any impact of homocysteine levels, MTHFR mutations, or f. VIII levels on the frequency of TE. So far, 1 patient has died of a cancer unrelated to MPD, all other patients are alive (median follow-up 4.2 yrs), 37/42 of whom on thromboreductive therapy with anagrelide, hydroxyurea, interferon, or their combinations.

Conclusion: Detection of hereditary thrombophilia strongly increases the risks of a major TE event in MPD with thrombocythemia and should be performed in each patient. We suggest that the patients positively identified for hereditary thrombophilia (including those without any history of vascular events) should be considered as high risk and adequate treatments with thromboreductive and/or antiplatelet drugs should be administered with the aim to reduce the probability of TE events and their recurrences. We speculate that normalization of platelet counts in these high-risk patients may be mandatory.

976 EARLY NATURAL KILLER RECOVERY AND ENGRAFTMENT AFTER AUTOLOGOUS AND ALLOGENEIC HLA-IDENTICAL SIBLING HEMATOPOIETIC STEM CELLS TRANSPLANTATION. IS THERE A RELATIONSHIP?

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Background: An array of activating and inhibitory cell surface receptors regulates NK cell proliferation, cytotoxicity and cytokine release. Alloimmune reactions in hematopoietic stem cell transplantation (SCT) involve recipient residual and donor T, NK and stem cells playing a major role on engraftment, GVHD and GVL effect. NK cells are the first immune effectors cells to recover after SCT. While in the autologous setting cytokines may enhance immune recovery and NK cell activity might target minimal residual disease, in the allogeneic, NK cell recovery has been associated with a powerful GVL effect in HLA-haploidentical SCT. It has been difficult to identify a role for NK cells in HLA-identical siblings transplants. **Aims:** To determine the relation of NK cells number very early after autologous and allogeneic HLA-identical sibling SCT with engraftment. **Methods:** Immunophenotypic analyses of peripheral blood

were performed using two-colours cytometry with Mabs of Immunotech Co and analyzed by BD FACScan cytometer in accordance to the guidelines, on the day patients total leukocyte reached 500/mm (Day 1) and one week later (Day 2). The following pairs of Mabs were used: CD56/CD3; CD56/CD57; CD56/CD33; CD34/CD7; CD45/CD14. Absolute NK cells numbers ($\times 10^3$ cells/mm) were calculate from the percentage of total absolute lymphocyte counts. The day of engraftment was considered the first day of three consecutive days of a minimal of 500 neutrophils /mm and/or 20.000 platelets/mm. SPSS 10.0 software program was used for all statistical analyses. Mann-Whitney-U test was used to compare different subgroups of patients and Spearman correlation coefficient was calculated. A value of $p \leq 0,05$ was considered statistically significant. Results: 10 and 6 patients after autologous and HLA-identical sibling allogeneic SCT, respectively were studied. The mean number of NK cells were 119.89, 149.66 and 70.27/mm³ for Day 1 and 185.25, 209.70 and 156.73/mm³ on Day 2 for the entire group, autologous and allogeneic respectively. There werent statistically significant differences between the groups. In the whole group, the correlation coefficient between NK cell mean number and engraftment was $r = 0.649$ ($p=0.07$) for Day 1 and 0.415($p=0.158$) for Day 2; 0.491($p=0.150$) and 0.436($p=0.328$) for the autologous group; 0.926($p=0.08$) and 0.062($p=0.908$) for the allogeneic. There was no correlation between number of CD34 positive cells and number of NK cells ($r=0.013$, $p=0.961$ Day1 and $r=0.352$, $p=0.239$ Day 2), as well as for NK cell number, lymphocytes number and bacterial infections episodes. Conclusions: There is a tendency for a positive correlation between the number of NK cells on Day 1 and engraftment in the allogeneic SCT group. The presence of a tendency in such a small group might be significant in a higher number of patients.

977 IMERSLUND-GRASBECK SYNDROME: REPORT OF THREE PATIENTS

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Background: Selective malabsorption of vitamin B12, or Imerslund-Grasbeck syndrome (I-GS), is a rare hereditary autosomal recessive disease, characterized by a deficient ileal uptake of intrinsic-factor-bound vitamin B12. The ileal enterocytes and renal proximal tubule epithelia of I-GS patients contain few or no IF-Cbl receptors. Cbl malabsorption leads to severe megaloblastic anemia accompanied by failure to thrive, increased infection rates, neuropathy, usually accompanied by mild proteinuria. Age at presentation is variable: neonatal cases as well as patients in their early teens have been described. The typical patient is around age 5, time enough to deplete the two-year Cbl reserves acquired from the mother, and for symptoms to become apparent. Diagnosis relies on clinical data, morphological peripheral blood and bone marrow findings, and on evaluation of serum Cbl, methylmalonate and homocysteine. The Schilling test and the presence of proteinuria can help the diagnosis. The gene encoding cubilin (CUBN) has been recently identified and various mutations described in I-GS kindred. This disease has been reported in Middle East and Far East countries as well as in Northern Europe. This report presents three cases from Portugal.

Patients and methods: We report on 3 I-GS patients who presented with macrocytic anemia, hypersegmented neutrophils, megaloblastic hemopoiesis and undetectable serum Cbl. Physical examination revealed pallor and no hepatosplenomegaly. Failure to thrive affected 2 patients, one of which also had recurrent infections, and developmental anomalies (case 1). This patient received intramuscular Cbl once, before a correct diagnosis could be made, which delayed proper treatment, resulting in persistent developmental anomalies. The third patient presented with bruising associated with balance and motor coordination anomalies. Schilling test confirmed ileal Cbl malabsorption. Anti-IF antibodies, a specific feature of pernicious anemia, were detected in two cases, in spite of a normal gastric biopsy. Other causes of macrocytosis and digestive disorders with malabsorption were excluded. No patient had a history of known consanguinity.

Results: Table 1 ' Patient characteristics at presentation, and follow up. Conclusions The diagnosis of I-GS should be considered in a young child with Cbl deficiency for which no obvious etiology is found. The presence of megaloblastic anemia in children should be followed by investigation of proteinuria, due to the existence of this rare disorder. Prompt diagnosis and therapy are crucial because the risk of irreversible neurological damage has to be avoided. Treatment with Cbl should not be given to children with macrocytic anemia before vitamin B12 deficiency is clearly established, as it can misguide the diagnosis and delay treatment.

	Case 1	Case 2	Case 3
Age at presentation (months)	22	24	22
Failure to thrive	since age 5 months		Since age 15 months
Diagnosis delay	3 years	9 months	1.5 months
Developmental anomalies	x		
Neurological symptoms	x	x	x
Recurrent infections	x		
Hb (g/L)	69	103	77
MCV (fL)	100	111	108
Reticulocytes (%)	5	1.6	3.2
Leucocytes ($\times 10^9/L$)	2.6	7.9	6.2
Neutrophils ($\times 10^9/L$)	0.5	1.6	2.5
Platelets ($\times 10^9/L$)	675	123	168
Proteins (g/L)	0.73	0.3	0.3
Serum Cbl	undetectable	undetectable	undetectable

Caption 1:

978 NON-HODGIN LYMPHOMAS (NHL) OF WALDEYER'S RING - A SINGLE INSTITUTION 5 YEARS EXPERIENCE

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BACKGROUND: NHL of Waldeyer's ring (WR) accounts for more than half of all extranodal lymphomas of the head and neck. Most tumors are of diffuse, large B cell type. The prognostic factors: histologic type, tumour bulk and stage principally influenced overall survival, but the influence of International prognostic Index (IPI) is still controversial. The overall relapse rate after radiation therapy indicate the need for more effective combined modality approach

AIM: We analysed the influence of gender, age, hystopathological type, clinical stage (Ann Arbor), IPI and presence of anaemia, with therapeutical efficacy

METHODS: In the period January 1997- December 2002, 140 primary extranodal lymphomas were diagnosed at our Institute and twenty nine (20.7%) of them were primary NHL of Waldeyer's ring. They were treated with chemotherapy alone and on examination we assessed several prognostic parametres which we correlated with response rate.

RESULTS: Twenty one patients (72%) and 8 pts (28%) had primary tonsillar and nasopharyngeal lymphoma, respectively. Median age was 48 years (range 21-70). The hystopathological findings were as follow: 9 pts (31%) had MALT lymphomas, 10 pts (34.5%) had diffuse, large, B cell lymphoma, 4 pts (13.8%) had follicular lymphoma, 2 pts (7%) had peripheral T cell lymphoma, 3 patients (10.3%) had Burkitt lymphoma and 1 patient (3.4%) had lymphoplasmacytoid lymphoma. Only 1 patient had bone marrow involvement. Twenty three patients (79.3%) were presented with CS I and II. According to the IPI index most cases were in the low or low-intermediate risk groups. The majority of pts had (22/29) had lymph node involvement (mainly regional). Patients were treated with chemotherapy: antracycline combined therapy were given in 21 pts and 8 pts were treated without antracyclines. The overall survival (OS) was 57 months, while event-free survival (EFS) was 48 months.

CONCLUSIONS: Sex, age and histology did not significantly affect survival rate. Multivariate analysis showed that Ann Arbor stage, favourable IPI score, initially high value Hb (> 100 g/l) have significantly influence in better outcome. There were no difference in overall survival rate according to presence of antracyclines in chemotherapeutical regimens.

979 DISTINCT RETICULOCYTE FEATURES IN HEREDITARY SPHEROCYTOSIS AND AUTOIMMUNE HAEMOLYTIC ANAEMIA

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Background: Haemolytic anaemia, characterised by circulating spherocytic red cells, can stem from various etiologies, the principal ones being hereditary spherocytosis (HS), and an inherited disease, autoimmune haemolytic anaemia (AIHA). Although it has long been known that spherocytosis is a feature of mature red cells in both HS and AIHA, no sufficient information is currently available on the cellular characteristics of reticulocytes (RETIC) in these two disorders. Aims: In this study, we tested the usefulness of the RETIC parameters to distinguish SH from AIHA. Methods: We studied 32 EH patients and 19 AIHA patients attended at the Haematology and Haemotherapy Centre of State University of Campinas. The diagnosis of HS was confirmed

by familial studies, documenting the decreased membrane surface area by osmotic fragility curves and membrane proteins studies. The diagnosis of AIHA was established in patients who presented a positive direct antiglobulin test. The RETIC parameters absolute number (#) and percentage (%), # and % of low, median and high fractions (L-RETIC, M-RETIC and H-RETIC, respectively), immature RETIC fraction (IRF), mean cell volume (MCVr), cell haemoglobin concentration (CHCMr), haemoglobin content (CHr), red cell distribution width (RDWr), haemoglobin distribution width (HDWr), and cell haemoglobin distribution width (CHDWr) were evaluated in samples of peripheral blood, using an automated haematology analyser (Bayer ADVIAtm 120 Haematology System). The significance of differences between groups were determined by Wilcoxon and Fisher tests. Results: The mean values of # RETIC (361.42 164.59 vs 240.90 152.94, $P = 0.011$), CHCMr (34.31 2.10 vs 29.79 2.75, $P = 0.001$), RDWr (13.80 2.46 vs 11.21 3.93, $P < 0.001$) were higher in SH than in AIAH patients. The mean values of # L-RETIC (1,368.68 581.25 vs 2,042.36 830.39, $P = 0.002$) and MCVr (98.44 11.32 vs 115.07 10.55, $P < 0.001$) were lower in SH than in AIAH patients. No significant differences in % RETIC (9.22 4.93 vs 8.38 6.39, $P = 0.284$), % L-RETIC (92.49 133.18 vs 66.12 20.60, $P = 0.838$), % M-RETIC (20.78 11.90 vs 18.95 6.96, $P = 0.419$), # M-RETIC (715.78 458.93 vs 438.47 364.09, $P = 0.032$), % H-RETIC (9.18 7.15 vs 8.75 1.37, $P = 0.612$), # H-RETIC (363.62 435.34 vs 253.95 331.96, $P = 0.212$), IRF (9.06 7.26 vs 8.71 7.50, $P = 0.664$), CHr (33.10 3.22 vs 34.46 4.46, $P = 0.254$), HDWr (4.15 0.90 vs 3.64 0.92, $P = 0.055$), and CHDWr (3.99 0.58 vs 5.37 2.79, $P = 0.179$) were observed among SH and AIHA patients. Conclusions: These results indicate that # RETIC, # L-RETIC, MCVr, CHCMr and RDWr might be useful RETIC parameters in the initial screening of patients with SH and AIHA.

980 FAMILY DYNAMICS IN ACUTE LEUKEMIA

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AIM: The present study aimed to evaluate family relations, personal growth, family system maintenance in acute leukemia patients and their family.

METHOD: It was carried out during the years 2000 and 2002 at the Department of Hematology, University of Ioannina Hospital in co-ordination with the Laboratory of Medical Psychology, Medical School, University of Ioannina. 111 subjects participated voluntarily to the present study. Among them, 41 subjects were patients suffering from acute leukemia, who were initially hospitalised during the year 2001 at the Department of Hematology, while 70 subjects remaining were patients first-degree relatives, who were taking care of them during hospitalisation. The instrument adopted were: The Family Environment Scale (Form R-FES). Patients and their relatives were asked to complete these self-report instruments and a demographic questionnaire, while being at wards.

RESULTS: Results showed that relatives reported significantly greater conflict ($P = .016$), intellectual-cultural orientation ($P = .035$), active recreational orientation ($P = .043$), family organisation ($P = .001$). However, patients reported significantly greater moral-religious emphasis ($P = .001$). Logistic Regression was performed to account for risk factors. In particular, a significant positive correlation was obtained among independent risk factors, which were found to be cohesion ($OR = 2.27$, 95%CI 1.18-4.37), conflict ($OR = 1.61$ 95%CI 1.04-2.51). A significant negative correlation was found for the remaining risk factors, which were emotional expressiveness ($OR = .50$, 95%CI .26-.97), active recreational orientation ($OR = .57$, 95%CI .33-.97), moral-religious emphasis ($OR = .28$, 95%CI .13-.61).

CONCLUSIONS: According to the results, it could be suggested that patients suffering from acute leukemia and their relatives tend interact in terms of increased rigidity. Such interaction seems dysfunctional, since either an extremely high or an extremely low cohesion, conflict as well as intellectual-cultural and active-recreational orientation were observed. In addition, the degree of family organisation and control was higher for relatives than it was for patients. Psychological state of patients was correlated with the one of relatives. Despite the possible limitations of the present study, it could be suggested that family attitude towards a life threatening disease as well as psychosocial needs related to such a stressful situation should be reconsidered. In order to explore the effect of family relations on acute leukemia, a re-evaluation of the patients and their relatives participated to the present study should be attempted later in the course of illness. The involvement of family in the course of malignant disease as well as in coping with such crisis is considered as an important area of research.

981 CYTOKINES AND SOLUBLE ADHESION MOLECULES IN B-CLL: RELATION TO SERUM LEVELS OF SOLUBLE CD23 AND DISEASE PROGRESSION

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B-cell chronic lymphocytic leukaemia (B-CLL) cells are characterized by long survival and resistance to apoptosis in vivo. Several independent in vitro investigations pointed to the involvement of cytokines and soluble mediators in prevented apoptosis in these leukemic cells. However, few data are available on the in vivo relation between the levels of these mediators and disease progression.

In this study we measured the levels of circulating cytokines (IL-2, IL-6, IL-10, TNF- α , IFN- γ), chemokine (IL-8), soluble (s) adhesion molecules (sCD44 standard/sCD44st, sCD44 variant-6, and sL-Selectin) and soluble surface markers (sCD23, sCD40 ligand) in serum of 30 B-CLL patients and 10 healthy persons. All cytokines and soluble factors were measured in one institute using immunoassay Kits from the same source (Instant ELISA-Bender/MedSystems) to minimize the interassays variations.

Results demonstrated significantly higher serum levels of TNF- α , sCD44st and sL-selectin in serum of B-CLL patients as compared to serum of healthy individuals ($p < 0.05$, $p < 0.05$, $p < 0.005$ respectively). Serum level of IL-10 was particularly higher in the advanced stage of the disease as compared to healthy persons ($p < 0.001$). Since sCD23 is known as a marker for disease progression and reflects the tumour mass, we studied the relation between these cytokines and the levels of sCD23. B-CLL patients were classified into three groups on the basis of serum levels of sCD23 (group 1: 200-1500, group 2: 1500-4000 and group 3: more than 4000 U/ml). The results showed a significant correlation between the levels of sCD23, IL-10, sCD44st and sL-Selectin and the disease progression.

These data suggest that alteration in multiple cytokine networks and soluble factors in vivo might contribute to the expansion of the malignant clone and disease progression in B-CLL.

982 THERAPEUTICAL EFFECT OF RITUXIMAB-CHOP (R-CHOP) VS. RITUXIMAB-EPOCH (R-EPOCH) THERAPY IN PATIENTS WITH DIFFUSE LARGE - B -CELL LYMPHOMAS (DLCL)

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BACKGROUND: The long term results of R-CHOP combination in DLCL, demonstrate the high potential to be safe and effective as front -line therapy. Based on in vitro evidence that tumor cells are less resistant to prolonged exposure to low concentrations of the natural product class, compared with brief higher concentration exposure, EPOCH regimen was developed in which the natural products are administered as a continuous infusion.

AIM: to correlate therapeutic effects in pts with DLCL which were treated with R-CHOP or R-EPOCH chemotherapy.

METHODS: Patients were eligible if they were 18-65 years of age and had untreated DLCL. Pts treated with R-CHOP received the combination: rituximab 375mg/m² on day 1 of each of the 8 cycles; 750mg/m² of cyclophosphamide on day 1; 50mg/m² doxorubicin on day 1; 1.4 mg/m² of vincristine on day 1; 100 mg Prednisone per day for 5 days. EPOCH regimen was given as follow: Etoposide 50mg/m²/day + Doxorubicin 10mg/m²/day + vincristine 0.4mg/m²/day (CIV-94 hours continuous infusion) and cyclophosphamid 750mg/m²/day as bolus on day 5, and Prednisone 60mg/m² during 5 days. G-CSF was given as protocol required.

RESULTS: Thirty two pts were treated with immunochemotherapy: 12 pts were treated with R-CHOP and 20 pts were treated with R-EPOCH regimen. The characteristics of pts treated with R-CHOP were as follow: F/M ratio 6:6, median age: 46 (range 23-70); 5 pts had CSIIb, and 7 pts had CSIVb; 7/12 pts had bulky form of disease. The majority of pts had low IPI, and 5 had high intermediate or high IPI; LDH was elevated in 6/12 pts. In the R-EPOCH group, F/M ratio was 10/10, median age: 25 (range 19-63). Eight pts were in CS IIB, and 12 had advanced disease; 17/20 pts had bulky form of disease; the majority of pts had low IPI- 12/20; LDH was elevated in 9/20 pts. All pts treated with R-CHOP achieved CR of disease which lasted for 23 months; 2 patient revealed relapses: 1 patient died because of progression 8 month after CR, and the other relapsed 12 months after CR and is now treated with salvage regimen. 17/20 pts with R-EPOCH achieved CR lasted for 12 months; three pts died (all of them had SD or PR)

CONCLUSION: Our results suggested that both regimens are highly effective, especially R-EPOCH in the group of younger patients with advanced and bulky form of disease.

983 GENETIC CHARACTERIZATION OF THE POPULATION OF GRANDE COMORE ISLAND, ACCORDING TO THE MAJOR BLOOD GROUP SYSTEMS

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This study presents the first genetic data on Comorian population. The Comorian population is considered as a blend of influences from African Bantus, Arabs and probably Austronesians.

The aim of the present study is to assess the genetic structure of the current Comorian population according to the six major blood group systems, i.e., ABO, RH, KEL, FY, JK and MNS, which were analyzed by serological methods. In addition the Duffy genotypes were also determined by PCR using allele specific primers.

Blood samples were obtained from Comorian immigrants living in Marseilles, France. A total of 164 samples were obtained from unrelated adults of both sexes (sex ratio : 1/1). This sample can be considered as representative of Grande Comoro population based on three main reasons: i) the Comorian immigrant community in Marseille is nearly 70000 (10% of the total Comorian population), recent (since 1970), and almost exclusively from cities on the Grande Comoro Island (Direche-Slimani K 2002). The mother language of individuals was shingazidja in nearly most cases; ii) the community has preserved their Comorian heritage strictly with little intermixing with the local Marseille population (few interracial marriages); iii) criteria of inclusion in the study was limited to unrelated individuals whose mother, father, and all four grandparents had been born in the Comoros Islands. All individuals gave informed consent and completed a questionnaire assisted by the medical staff. The study was approved by the competent authorities in France.

Determination of ABO groups, RH (D, C, E, c, e) and KEL1 (K) antigens was performed using Olympus microplate technology on red blood cells (RBC) from two different sera for each antigen in accordance with the manufacturer instructions (Diagast, France). Detection of KEL2 (Cellano), KEL3 (Kpa), FY1 (Fya), FY2 (Fyb), JK1(Jka), JK2 (Jkb), MNS1 (M), MNS2 (N), MNS3 (S) and MNS4 (s) antigens was performed by tube tests or column agglutination (Scangel Biorad, France) using two different sera for each antigen. A total of 24 alleles of 6 genetic systems were explored. Unused samples were frozen and stored for further studies. Detection of FY*A, FY*B, FY*X and FY*Fy alleles was studied using a PCR-ASP-based technique developed for complete Duffy genotyping according to the previously described method (Olsson 1998a, 1998b).

The phenotypes frequencies analysis showed high level of Fy(a-b-) phenotype (86%), whose genetic background is exactly as the one observed in sub Saharan Africa. Findings based on analysis of genetic frequencies, distances and calculation of admixture with other populations indicated that African Bantus had made the main contribution to the gene pool (69.5%±5.2). The contribution from the Arabian Peninsula was smaller (29%±2.8) and the one from Indonesia was very low (1.6%±3.7%). Conclusion The major Bantu contribution was commensurate with the Bantu cultural influence. The contribution from Arabic peninsula seemed related to its permeating religious and linguistic influence. As for the language, the Indonesian impact on the Comorian gene pool was very small. This study confirms previous historical, sociological and linguistic data.

984 FEATURES OF MEGAKARIOPOESIS AT VARIOUS STAGES MYELOFIBROSIS WITH MYELOID METAPLASIA FOR PATIENTS SUFFERED FROM CHERNOBYL ACCIDENT IN REMOTE PERIOD

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Background Myelofibrosis with myeloid metaplasia (MMM) is an infrequent chronic myeloproliferative disorder defined by extramedullary haemopoiesis and bone marrow fibrosis that is a reactive phenomenon secondary to a clonal haemopoietic disorder

The purpose of this study was to quantify study the stage of differentiation of megakaryocytes at MMM patients with different stages affected from Chernobyl accident.

Material and methods The morphology of megakaryocytes study was performed on bone marrow aspirate in patients with MMM to compare

morphological and clinical features and patients without hematology disorders. We studied 15 patients MMM (median age, 541,9 years) and 11 patients without - control group (median age 505,8 years). All patients have suffered from Chernobyl accident and exposed low doses of irradiation in the range from 0,0071 to 1,9 Sv, in a control group - 0,006 to 0,5 Sv.

Results The definition of the disease MMM score was according to the criteria adopted by the Italian Consensus Conference on Diagnostic Criteria for MMM (Cervantes F., Barosi G. at all, 1998).

Compared with age- and sex-matched controls, the following cytomorphometric parameters of megakaryocytoграмма were significantly higher in MMM patients: megakaryoblasts (51,0% and 20,5%) (<0,01), number of naked nuclei (NN) (212,5% and 112,3%) and complete fragmentation nucleus megakaryocytes (4□1,7% and 0,30,2%). Simultaneously polychromatophile megakaryocytes were significantly decreased (312,7% and 413,3%) (P<0,05).

Median numbers and ranges of bone marrow functioning basophilic megakaryocytic cells were 15 2,5% in control subjects, 20 1,8% at MMM patients with II (unfolded) stage and 13 4,2% at patients with I (conceptual) stage. Median numbers and ranges of bone marrow functioning polychromatophilic megakaryocytic cells were 73 5,3% in control subjects, 75 9,4% at MMM patients with II (unfolded) stage and 66 11% at patients with I (conceptual) stage.

In MMM patients, the data bone marrow measurements revealed decreasing a rate of megakaryocytes with multilobulated (4-7 lobule) nuclei cells 28 3,2 % that was significantly less than in patients without mielofibrosis (59 2,9%). Simultaneously, three time increasing a rate of megakaryocytes with hyposegmented forms (less than 4-7 lobule) nuclei are observed at MMM patients that associated with megakaryocytic maturation.

A few of the most frequently found of megakaryocytic with mitoses, displastic forms, with vacuolization of cytoplasm as well as microforms among MMM patients.

In contrast to the control group, patients with MMM has 13 times increased quantity of cells of megakaryocyte raw with full fragmentation (p<0,05). The true increasing of the quantity of cells is noticed only for patients having II stage of MMM.

Conclusions The statistically significant (p<0.01) megakaryoblasts domination comparing with a control group is revealed in megakaryocytogram of patients with MMM megakaryoblasts as well as active platelet formation accompanied with pyknotic nuclei and naked nuclei. These changes are stipulated by presence of unknown factors affecting the megakariopoiesis and leading to a distortion of maturation with following exhaustion of it in a terminal stage.

985 HB ST. ANNA, [BETA 94 FG1 (ASP>TYR)], A NEW CLINICALLY SILENT G>T TRANSVERSION OBSERVED IN A DUTCH DIABETES MELLITUS PATIENT.

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Background: silent abnormal hemoglobins are often observed by chance during HbA1c monitoring.

Methods: an abnormal separation pattern was observed at the first HPLC HbA1c control for diabetes mellitus in a 56 years old Dutch woman of north European extraction. Her indices were showing high-normal Hb (14.7 g/dl) hyper-chromic (MCH 30.3 pg) macrocytic (MCV 99 fl) parameters with high normal PCV (0.48%) and RBC counts (4.84 1012/l). No history of anemia or hemolytic episodes was reported.

Results: the abnormal hemoglobin fraction was estimated at 42.5% and migrated as the HbA2 fraction on HPLC (Bio-Rad Variant-I b-short program). On starch gel electrophoresis at pH 8.7 and on the commercial SAS-MX alkaline gel (Helena Bioscience) the abnormal band was positioned between HbF and HbS and between HbA and HbS at acid pH (Titan Gel).

Conclusions: these data indicated a stable mutant on a beta-globin gene. Direct sequencing of the beta globin genes revealed a new GAC>TAC transversion at cd 94 of the beta globin gene in heterozygous form. This mutation, named Hb St. Anna after the name of the hospital where it was observed, is the fourth mutation involving the Asp residue at position 94. Hb Barcelona (Asp>His) and Hb Bunbury (Asp>Asn) were both associated with a higher O2 affinity in a Spanish and in an Italian and a Mexican family respectively. From the third case, Hb Chandigar (Asp>Gly), no physiological studies are reported and the mutation was

found in an Indian healthy father of a thalassemic child. Based on the hematological parameters we presume that Hb St. Anna is a stable Hb variant possibly associated with a slightly increase O2 affinity and decreased Bohr and cooperativity effect and we intend to verify this as soon as a fresh blood sample will become available.

986 DENDRITIC CELL HEMATOLOGIC NEOPLASIA: ONE CASE

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INTRODUCTION: Dendritic cell (DC) hematologic neoplasia is an unusual and recently described malignancy characterized by co-expression of CD123, HLADR, CD56 and CD45 dim in the absence of another lineage markers (B, T or myeloid), associated with a germ-line configuration of both the IgH and TCRgamma genes.

CASE REPORT: male, aged forty-eight, presenting with anemia, a three month evolution general syndrome, hepatomegaly, splenomegaly, inguinal and retroperitoneal adenopathies, with anatomo-pathologic diagnostic of lymphoblastic lymphoma.

Medullogram revealed 15% lymphoblastoid non-reactive to myeloperoxidase, esterase or PAS. Acid phosphatase was positive either in a multiple or single focal pattern.

Flow cytometric immunophenotypic profile showed that cells were of hematopoietic origin (CD45+ dim, CD117+), with a myeloid dendritic phenotype (CD123+dim, CD33+, HLADR+, HLADQ+, HLADP++, HLA-ABC+, CD36-/-, CD33+, CD16-, CD1a+d, CD87-/-, CD11c-/-), without highly specific markers of T lineage (surface and intracytoplasmic CD3), B lineage (cytoplasmic CD79A) or myeloid lineage (cytoplasmic MPO and lysozyme) with light scatter properties analogue to mature lymphocytes

As it has been described on DC neoplasia, aberrant expression of 7.1 and CD56 antigens was detected.

Molecular studies on separated cells (purity > 98%), revealed IgH and TCR gene polyclonal rearrangement; bone marrow karyotype was normal in the absence of 11q23 or t(2;5) abnormalities by fluorescence in situ hybridization.

CONCLUSIONS: we conclude that this case could be a DC hematologic neoplasia with two particular findings: 1) CD4 was negative and 2) immunophenotype could suggest a myeloid DC origin, while all DC malignancies reported to date belong to the lymphoplasmacytoid DC compartment.

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987 HYPERLIPIDEMIA INDUCED BY CORTICOSTEROID AND L-ASPARAGINASE THERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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BACKGROUND: Combination chemotherapy is being used in the treatment of childhood acute lymphoblastic leukemia (ALL). Corticosteroids and L-asparaginase are included in induction and reinduction therapies. Both drugs exert their effects through the inhibition of protein synthesis. Well recognized side effects of L-asparaginase are hyperglycemia, coagulopathy and pancreatitis; and there have been individual reports about lipid abnormalities. Combination of corticosteroids with L-asparaginase may contribute to the alterations in lipid synthesis and clearance. Corticosteroids impair lipid and lipoprotein metabolism by increasing hepatic cholesterol synthesis.

AIM: Here we report the lipid abnormalities in the 24 patients who received the combination treatment of corticosteroids and L-asparaginase.

METHODS: This study was conducted in the Department of Pediatrics in Sisli Etfal Training and Research Hospital from January 2000 to February 2003. 24 children who were arranged to receive CCG chemotherapy protocol for ALL were included. Three blood samples were obtained from each patient for biochemical analyses: 1) Following the interim maintenance, just before reinduction treatment, 2) During the reinduction treatment (at the 15th day), 3) A week after the end of reinduction treatment. The reinduction period in which corticosteroids and L-asparaginase were given together. The reinduction treatment included L-asparaginase and corticosteroids in addition to other chemotherapeutics

RESULTS: During the treatment cholesterol, triglyceride, VLDL and LDL levels increased significantly as compared with the pretreatment values. After the treatment these levels returned down to pretreatment

levels. Triglyceride levels were above 400mg/dl in 3 patients and above 1000mg/dl in one case. Of these four cases, two developed diabetes as well. Pancreatitis was not observed in any of the patients.

CONCLUSION: During the periods in which L-asparaginase is administered together with corticosteroids, the patients should be monitored for the development of hyperlipidemia. It should be kept in mind that, at levels above 1000mg/dl pancreatitis might be observed, therefore triglyceride lowering therapies should be initiated when necessary. Studies of larger size evaluating the incidence and progress of lipid abnormalities should be carried out in patients with ALL.

988 99MTC-MIBI SCINTIGRAPHY (MIBI-SC) AND 18F-DG POSITRON EMISSION TOMOGRAPHY (FDG-PET) IN MULTIPLE MYELOMA (MM) : A SINGLE CENTER SURVEY

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MIBI-Sc and FDG-PET are both useful in patients with MM. However, these imaging techniques investigate different pathophysiological mechanisms: cellular metabolism, difference of transmembrane potential and presence or not of functional PgP-170 and MRP1 for MIBI, cellular metabolism and presence of various membranar antigens (Glut-1,...) for FDG. In the present work-in-progress, MIBI-Sc and FDG-PET were compared in 7 patients: 2 with MM at diagnosis where MIBI-Sc and FDG-PET were matched (1 with increased bone marrow uptake and 1 with one focal lesion hyperactive with both tracers) and 5 with MM clinically, biologically and/or radiologically evolutive. In 2 of these last cases, FDG-PET was negative whereas MIBI-Sc showed one or more hyperactive foci (and abnormal bone marrow extent in one). In the 3rd patient, MIBI-Sc was negative but FDG-PET showed small hyperactive foci. In the 4th patient, MIBI-Sc showed increased bone marrow uptake with several hyperactive foci whereas bone marrow was quite replaced by hyperactive foci on FDG-PET. In the last patient, both tracer showed increased bone marrow uptake, abnormal bone marrow extent, extramedullary extent and hyperactive foci: most of the hyperactive lesions were matched but some FDG positive lesions were MIBI negative and inversely. Finally, increased clearance of MIBI was observed in 3 of the cases which suggest the presence of hyper-functional pgp-170 and/or MRP-1 mechanisms. We conclude that MIBI-Sc and FDG-PET represent complementary diagnostic tools with potentially different contributions with regard to the therapeutic management of the patients with MM.

989 CERA (CONTINUOUS ERYTHROPOIESIS RECEPTOR ACTIVATOR) PRODUCES A DOSE-RELATED ERYTHROPOIETIC EFFECT IN PATIENTS WITH MULTIPLE MYELOMA

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Background: CERA is an innovative, erythropoietic agent with distinct properties that point to a unique mode of action. Phase I studies have demonstrated that CERA provides potent, prolonged, dose-dependent erythropoietic activity, and may offer the opportunity for longer dosing intervals and thus greater flexibility and convenience than conventional epoetin therapy.

Aim: To identify a suitable CERA dose range for further clinical testing in patients with cancer-related anemia.

Methods: This was an open-label, exploratory, multicenter, two stage, dose-escalation study to investigate the hemoglobin (Hb) dose-response, pharmacokinetic and safety profile of CERA in patients with multiple myeloma-related anemia (Hb $\leq$ 11 g/dL) receiving chemotherapy. Patients (n=64, in randomized groups of 810) received CERA SC (2.0, 3.5 or 5.0 µg/kg) once every 3 weeks for 6 weeks (core period) with the option of extension for an additional period of up to 12 weeks. After a review of efficacy and safety data, extra patients were assigned sequentially to doses of 6.5 and 1.0 µg/kg, then 8.0 and 4.2 µg/kg. Demographics were similar in all patient subgroups. The primary efficacy variable was Hb change from baseline to end of initial treatment (EOIT, defined as completion of the core study period, transfusion or dose change) as assessed by regression analysis.

Results: CERA produced a rapid, statistically significant dose-related increase in Hb for doses up to 4.2 µg/kg, with a trend for a plateau in response at doses of 5.08.0 µg/kg. Mean Hb increased by 1.62.3 g/dL

with CERA 3.58.0 µg/kg and 5062.5% of patients had a response of ≥ 2 g/dL during the core period. Levels were maintained or improved from 612 weeks. Moreover, up to 90% of patients achieved an Hb response of 2 g/dL during the complete 18 week study period. Assessment of pharmacokinetic parameters documented a long elimination half-life of 6 to 10 days and this half-life was not dose dependent. CERA was generally well tolerated. AEs characteristic of the population under study and those attributed to CERA were rare (4 hypertension, 1 pyrexia) during the core period. There were no unexpected clinical and laboratory measurements, and no evidence of antibody development.

Conclusions: In anemic patients with multiple myeloma undergoing chemotherapy, CERA administered SC once every 3 weeks produced a rapid and sustained increase in Hb, consistent with its prolonged elimination half-life. The long dosing interval of CERA may enable administration on the same dosing schedule as chemotherapy agents; this may offer greater flexibility and convenience in the treatment of patients with cancer-related anemia.

990 ASTEREOLOGICAL ANALYSIS OF MEGAKARYOCYTES OF BONE MARROW IN PERNICIOUS ANEMIA

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Background: In pernicious anemia (PA), hematopoiesis is disturbed. That is most expressive on the level of erythrocyte lineage. Beside presence of megaloblasts in bone marrow, changes in myeloid and megakaryoblast series can be seen. The changes are the most evident among the megakaryocytes.

Aim : to perform the quantification of megakaryocytes changes in bone marrow in pernicious anemia.

Patients and methods: During the period between 1995 and 2003 in the Clinic of Hematology Ni, 118 patients with PA were examined so as and 30 patients with dyspeptic syndrome (control group). Group of patients with PA in relation to pathohistologic changes of gastric mucosa was divided in three subgroups.

Astereological examination of megakaryocytes was carried out by application of double lattice system - B100 which was placed on the plate with a smear of bone marrow. Referential space were megakaryocytes and field were selected systematically - zonal, until obtaining the adequate number of cells. As only a few megakaryocytes can be seen on microscopy fields it was hard to obtained their statistically adequate number for examination so every megakaryocyte which was seen was astereologically examined. The following parameters were used : relative surface and contour length, absolute surface of nucleus and cytoplasm (An-Ac), absolute contour nucleus and cytoplasm density (Bn-Bc), shaped factor of nucleus and cytoplasm (FOn-FOc) and nucleocytoplasmic (N/C) relation of megakaryocytes. Diameter of megakaryocytes was measured with ocular micrometer and calculated out of the absolute contour of cell density.

Results: Values of relative surface and contour length of megakaryocytes increase simultaneously with the degree of atrophic gastritis in PA according to control group and according to the severe atrophic gastritis. An and Ac of examined cells shows statistically significant growth in all three groups (p < 0.001). The most expressive growth An was in the third group of patients. Examined cells indicate growth of Bn and Bc, which were statistically significant for all three groups of patients in relation to the control group. There is excess of membrane in relation to the surface. FOn and FOc increase in all examined groups in relation to the control group and degree of atrophic gastritis. N/C relation increase in megakaryocytes as nucleus is much larger than cytoplasm. Examined megakaryocytes indicate their enlargement as a consequence of grow as can be seen in increasing of diameter (X 35,1 1,15 mikrometara).

Discussion : Increase of all these examined variables show the 'heavy' nature of cytoplasmatic and nuclear membrane. This can be explained as a try of a cell to promote its function with higher contour length and larger surface area. Increase of N/C relation is the result of slow maturation of nucleus and inadequate quantity of cytoplasm which is the result of unproductive megakaryocytes forming. Examined megakaryocytes indicate grow as can be seen in increasing of diameter.

Conclusion: There are megaloblastic changes not only of bone marrow erythroid elements, but also on the level of megakaryocytes lineage. The result of this is the phenomena of giant megakaryocytes presence

991 IN VITRO SENSITIVITY-BASED INTRATHECAL CYTOZAR THERAPY OF MULTIPLE MYELOMA COMPLICATED BY INTRASPINAL TUMOR GROWTH

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Tumoral lesion of nervous system in multiple myeloma (MM) is an urgent problem of the clinical hematology. The object of the study was comparing the in vitro chemosensitivity of tumor cells with the clinical data of MM patients with intraspinal tumor growth. Under the study there were 18 MM patients (III stage) with neurologic complications (radicular pain refractive to radial and polychemotherapy, lower paraparesis in 10 patients). MRI revealed vertebral lesions at different levels manifesting themselves in compression of subarachnoid area, roots, dural sac, or spinal cord due to intraspinal soft tissue neoplasm and cerebrospinal lumen stenosis. All patients underwent studying in vitro sensitivity to Cytozar of bone marrow tumor plasmocytes using DiSC-assay up to the lethal dose (LD70) which was reached at 0.6-3.0 mkg/ml preparation in the culture (1.8 mkg/ml, on the average). Cytozar dose administered intrathecally was 30 mg/m2. Therapy course included 1-3 injections in 2 days with a 4 week interval between the courses (3 courses, on the average). Estimation of the treatment efficacy was performed allowing for a pain syndrome severity, neurologic status, and moving activity according to Karnofsky scale. The positive dynamics of the moving activity became apparent, on the average, in 2 days (Karnofsky index 332.4 before intrathecal injection changed to 674.5 after it, P<0.001). The duration of this effect varied from 1 to 24 weeks (mean value 18). MRI of the vertebral column carried out in dynamics revealed the decrease in intraspinal tumor volume by 71.5% (20-100%). The outcome of the study showed that Cytozar intrathecal injection might be recommended for treatment of MM patients with intraspinal tumor growth if their bone marrow tumor plasmocytes demonstrated sensitivity to Cytozar in concentration of the latter not exceeding 3 mkg/ml.

992 ANALYSIS OF INTELLIGENCE QUOTIENT IN PATIENTS WITH HOMOZYGOUS BETA-THALASSEMIA IN SHIRAZ, IRAN

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Background: Thalassemia major is a relatively common hereditary haematological disorder leading to chronic and potentially lethal anaemia. With treatment, life expectancy has improved dramatically. However, the chronic condition challenges optimal physical and mental developmental.

Aim: our aim was to compare the Intelligence Quotients (IQ) of patient with Cooley's anaemia with the IQ of normal children.

Material and Methods: Two hundred and ninety four, 9 to 18 years old, randomly selected from 984 thalassemic patients (homozygote beta-thalassemia) and 294 age- and sex- matched control group, were studied. Intelligence Quotients (IQ) were computed with the Raven test.

Results: The mean IQ score $\pm$ standard deviation (SD) in the thalassemia group was 109.83 ± 15.94 . This score revealed no statistically significant difference with the control group's score (p value ≤ 0.079). A correlation existed between the thalassemia patients' IQ and their level of education (p < 0.049).

Conclusion: The IQ of thalassemia major patients does not differ significantly from the normal population.

993 INFECTIONS IN PATIENTS WITH ADVANCED HODGKIN'S DISEASE TREATED WITH ESCALATED BEACOPP

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Background: Intensive chemotherapy (CHT) escalated BEACOPP (BEACOPP esc.), introduced by the German Hodgkin's disease Study Group (GHSG), is one of the most effective CHT regimen in the therapy of patients (pts) with advanced stage (st.) of Hodgkin's lymphoma (HL). High intensity of therapy is associated with more frequent acute toxicity.

Aim: Here, we present incidence of infections during CHT.

Patients and methods: Since 2001 15 adult pts (10 men and 5 women) with median age 27 years (18-51) were treated as first line treatment

with CHT BEACOPP esc. All pts were treated with 4 cycles of BEACOPP esc + 4 cycles of basal BEACOPP (BEACOPP bas.) with facultative radiotherapy (RT). All pts received obligatory G-CSF 5ug/kg/day during BEACOPP esc. started from +9 day with median 5 (3-8) days of application /1 cycle of CHT. The administration of BEACOPPbas. was facultative supported by G-CSF in 4 pts for prolonged leukopenia before next cycle. Nodular sclerosis dominated in HL (n=11). 3 pts were in st. IIB with bulk or extranodal involvement, 8 pts were in st.III and 4 pts. in st. IV. 7 pts had bulky mass in mediastinum. The IPS was 0-2 in 7 pts and 3-6 in the rest of 8 pts with median 3 (0-7). Thirteen pts had 1-2 risk factors (RF), 2 pts 3 RF with median 2 (1-4 acc.to GHSG). Median follow up was 12.months (7-27). Significance was tested using Fisher exact test.

Results: 14 pts achieved complete remission after therapy, 1 is before final restaging. We found 15 infections with 2 febrile neutropenias in 10 pts during 8 cycles of CHT. Five pts (50%) had 2 different infections during CHT. Six infections (40%) were detected during BEACOPPesc., 9 (60%) infections in the second part of therapy during BEACOPPbas.. There were 1 parasitic, 8 bacterial, 2 viral, 3 fungal infections, 1 infection

without positive microbial cultivation (subcutaneous abscess). 4 pts had pneumonias, 3 tonsillitis, 2 subcutaneous abscesses, 2 cutaneous, 2 systemic and 2 dental infections. In two women therapy was complicated by febrile neutropenia between 2nd-4th cycle of BEACOPP bas. In 13 (86%) pts we had to postpone the start of next CHT with median 2 weeks (1-4) due to prolonged leukopenia and 3 (1-7) weeks due to leukopenia with infections.

Summary/conclusion: The regimen BEACOPP esc. is the most effective CHT for advanced st. of HL. The acute hematologic toxicity is high and therapy is often complicated by infection in immunosuppressive pts. We found 5 infections grade II, 10 grade III and febrile neutropenia grade III according to NCI/CTC scale. In our statistical analysis we found no risk factor for count and relevance of infection (age /p=0.2, p=0.06/, histology /p=1, p=1/, need of G-CSF /p=0.2, p=0.2/, st. of disease /p=0.2, p=0.3/, bulk in mediastinum /p=0.5, p=1/, RF acc. to DHSG /p=1, p=1/, IPS /p=0.6, p=0.09/, prolonged leukopenia /p=0.3, p=0.4/). MRSA is the most serious problem, so active surveillance and goal directed screening of infections is important prevention of the dissemination of infection.

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