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

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

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Modern English

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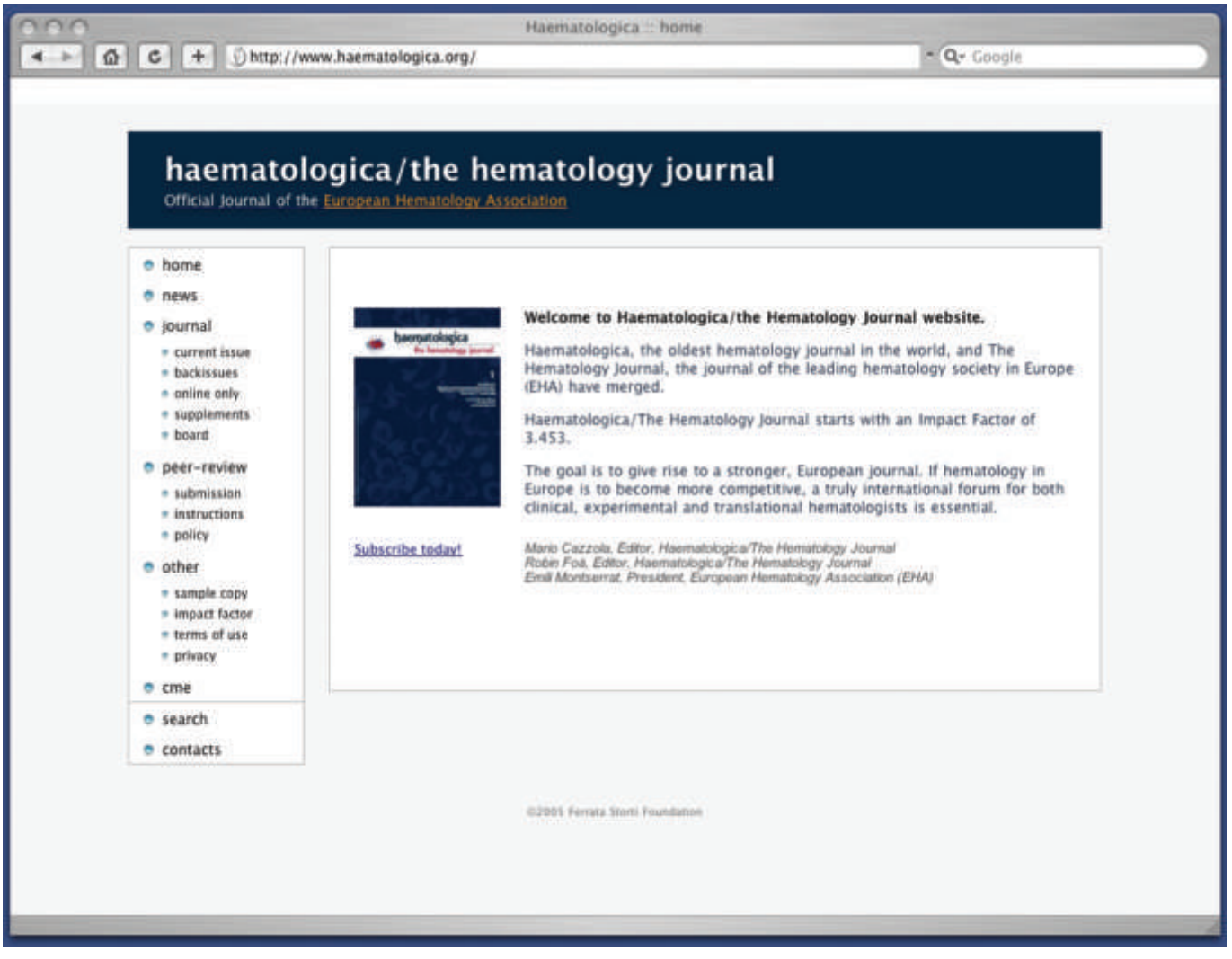

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10th International Workshop, Sydney, Australia, 10-14 April 2005

PLENARY SESSION 1: MALIGNANT CLONE

PL1.01

UNCONVENTIONAL SWITCHED BONE MARROW PLASMA CELLS WITH IG V-REGION MUTATIONS

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The neoplastic plasma cells in multiple myeloma are, by definition, located in bone marrow. Their Ig heavy chain genes have invariably undergone switch recombination and the rearranged Ig variable region genes have acquired somatic mutations. Physiologically plasma cells with these characteristics have been shown to arise in response to T-cell dependent antigens in the spleen or peripheral lymph nodes.[1,2] These plasma cells have the potential to be very long lived.[3,4] Vaccines that induce neutralizing antibodies against bacterial exotoxins or viral envelope proteins typically induce long-lived bone marrow plasma cells. Analysis of antibody responses induced by these vaccines shows that the B cells are induced to form germinal centers, where they undergo clonal expansion and affinity maturation through Ig-variable-region-directed hypermutation and selection.[5,6] This selection involves the uptake of the antigen that induced the response and its successful presentation toCD4 T cells within the germinal center.[7] While the cellular and molecular basis for the production of these bone marrow plasma cells are relatively well defined, recent data from a number of sources suggest that some bone marrow plasma cells may be produced in a different way. Understanding alternative pathways for bone marrow plasma cell production may alter our view of the pathogenesis of multiple myeloma and have implications for the management of this disease.

The first pointer to an alternative route to production of switched plasma cells with hypermutation comes from experience treating patients with autoimmune disease with B-cell depletion therapy. This uses anti-CD20 antibody (rituximab), which was first introduced for the treatment of B-cell neoplasia. This treatment is highly effective at depleting B-cells although these recover after several weeks. As expected, from the conventional view of IgG production in the marrow, serum IgG levels are little effected by the treatment, reflecting the longevity of most IgG-producing plasma cells.[8-12] Specific antibody titers against tetanus toxoid were also unaffected.[11] Conversely there was a significant fall in autoantibody levels over a period of 1 to 2 months. This finding has been reported in: systemic lupus erythematosus (IgG anti-double-stranded DNA antibodies);[8,9] autoimmune hemolytic anemia (IgG warm antibodies),[10] seropositive rheumatoid arthritis (IgM rheumatoid factor),[11] factor VIII deficiency (acquired anti-factor VIII antibodies).[12] It seems unlikely that the plasma cells producing autoantibodies in these patients are directly rituximab sensitive for B cells are depleted in less than a week while autoantibodies fall over 4-8 weeks. This points to these plasma cells being relatively short lived and that physiologically they are renewed from precursor CD20-expressing B cells that are depleted by rituximab.

Two independent recent studies in mice have reported the accumulation of Ig V region mutations in B cells proliferating outside germinal centers.[13,14] One of these related to autoantibody production[14] and the other responses to the T-cell independent type 2 antigen – NP-Ficoll, which cannot be processed and presented in a form visible to T cells. Recent unpublished studies will be reported that indicate that mature B-cell clones specific for NP-Ficoll expand in response to this antigen after transfer into mice that lack the capacity to produce B cells by primary B lymphopoiesis. These clones are long lived and undergo extensive proliferation and as such are targets for sequential somatic genetic change. While multiple myeloma may well develop in cells that have undergone affinity maturation in germinal centres the data that will be discussed indicate that the possibility for an alternative cellular origin for the neoplastic cells has not been excluded.

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PL1.02

CELL CYCLE CONTROL IN MULTIPLE MYELOMA

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Cell cycle control and apoptosis are two major determinants of homeostasis in plasma cell differentiation and tumorigenesis. Multiple myeloma (MM) cells are malignant plasmacytoid cells arrested at progressive stages of plasma cell differentiation. They accumulate in the bone marrow mainly due to impaired apoptosis. However, cell cycle dysregulation must also contribute to monoclonal expansion of

MM cells in the development of the disease and aggressive MM cell proliferation during relapse. MM, therefore, has lost both cell cycle and apoptotic controls at distinct stages of malignant transformation. The mechanism that underlies cell cycle dysregulation in MM is, however, unknown. Cell cycle progression is controlled by the aggregate balance between positive regulators, cyclins together with cyclin-dependent kinases (CDK), and negative regulators, the CDK inhibitors (CDKI). Normal plasma cells are permanently withdrawn from the cell cycle. One specific CDKI, $p18^{INK4c}$, is up-regulated by the cytokine interleukin-6 (IL-6) and required for cell cycle termination and the generation of G1 arrested, functional plasma cells (*Mores et al, 1997, Immunity 6: 47; Tourigny et al, 2002 Immunity 17: 179*). In the absence of $p18^{INK4c}$, $CD138^+$ plasmacytoid cells form, but they fail to terminate the cell cycle or differentiate to end-stage, antibody-secreting plasma cells. Although bone-homing is affected, these plasmacytoid cells are rapidly eliminated by apoptosis. This was the first demonstration of cell cycle control of B-cell immunity, and provided a functional link between CDKI-mediated cell cycle control and apoptosis in plasma cell differentiation. These findings further suggest that the loss of CDKI function may play a critical role in MM pathogenesis. However, this possibility has not been verified, despite the observations in MM cells of mutations in the $p18^{INK4c}$ gene and methylation inactivation of the related $p16^{INK4a}$ and $p15^{IN4b}$ gene promoters. Over-expression of cyclin D1 or D3 is frequently associated with MM, implying that gain of the cyclin D function may contribute to the loss of cell cycle control in MM cells. However, this notion lacks experimental support, In fact, we have found that the cyclin D1 and D3 protein levels bear no relationship to the cell cycle status in primary bone marrow MM cells.

Based on the requirement for $p18^{INK4c}$ in normal plasma cell differentiation and the principles of cell cycle control, we propose that the loss of the mid-G1cell cycle checkpoint, caused by an imbalance between positive and negative G1 cell cycle regulators, precedes proliferative expansion of MM cells and is an early event in MM pathogenesis. To test this hypothesis, we have developed an effective, tiered *functional cell cycle assay* to dissect cell cycle control *in vivo* in primary bone marrow MM cells, using normal bone marrow plasma cells as a control. By *in situ* immunohistochemistry (IHC), we determine activation-specific phosphorylation of G1 cell cycle regulatory proteins and cell proliferation by Ki67 expression in bone marrow $CD138^+$ MM cells, in conjunction with analysis of DNA replication by BrdU-uptake *ex vivo* whenever possible. To calibrate the IHC results, we quantify the levels of expression and phosphorylation of specific cell cycle proteins in purified, $CD138^+$ bone marrow MM cells by a sensitive immunoblotting assay. Together, these assays have pin-pointed the specific cyclins, CDKs and CDKIs that are dysregulated in primary MM cells and associated with tumor stages. Analysis of RNA levels by real-time RT-PCR assays and genomic analysis have further identified critical genetic lesions and suggest underlying mechanisms for cell cycle dysregulation in MM cells. Preliminary longitudinal, retrospective IHC analyses of MM patients before and after treatment by this *functional cell cycle assay* indicate that remission and relapse are associated with dysregulation of specific early and late G1 cell cycle regulators. Our findings suggest an effective means to determine the cell cycle genetic lesions in individual MM patients in the context of tumor staging and treatment. In combination with the existing prognostic indicators, this cell-based assay will improve the prediction of MM progression and the aggressiveness of the disease. It will also advance our understanding of the relationship between loss of cell cycle and apoptotic controls in MM cells in the bone marrow microenvironment, and the design of cell cycle inhibitors as a therapeutic strategy for myeloma treatment.

Supported by NIH grants and a Specialized Center of Research for Myeloma grant from the Leukemia and Lymphoma Society.

PL1.03

CROSS-TALK BETWEEN PLASMA CELLS AND THE MICROENVIRONMENT: TRIGGERING AND TARGETING

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In multiple myeloma (MM), despite the peripheral origin of the clonal founder cell, peripheral lymphoid organs are unaffected and the malignancy is restricted virtually only to the bone marrow (BM). The BM predestination clearly underlies the *specific* need of these malignant cells for an external support that cannot be apparently found elsewhere in the immune system. A key element in the natural history of multiple myeloma is the development of tight interactions between MM plasma cells and the BM microenvironment that includes stromal cells, endothelial cells and osteoclasts which deliver pro-survival and anti-apoptotic signals to the malignant clone. This situation brings in the possibility of developing new treatment modalities for the treatment of MM by targeting these triggering interactions. Surprisingly, T cells are sparse if at all present in the BM of MM patients. MM-specific $CD8^+$ T-lymphocytes that recognize naïve tumor cells can be generated *in vitro* by different technologies that aim at developing cells useful for immunotherapeutic approaches. Still, T-cell generation appears to be hampered in patients with high tumor burden implying that the efficacy of a tumor vaccine should be restricted to eradicating residual tumor cells. In contrast, it has been proposed that innate effector cells may behave as the earliest defenders against tumors when stressed cells fail to repair mutations. In this context, a subset of interest is represented by Vγ9/Vδ2 T lymphocytes that are activated by infectious agents and hematological tumors through the recognition of phosphorylated non-peptidic metabolites. Aminobisphosphonates are also recognized by this lymphocyte subset and this recognition favors killing of MM cells. TCR-mediated effector functions of γδ cells are enhanced upon triggering of the activating receptor NKG2D by MICA, a stress-inducible antigen expressed by a number of epithelial and hematopoietic tumors. We have observed that MM cell lines U266 and LP1 express surface MICA which was also expressed by 8/8 primary MM cells obtained from the BM of newly diagnosed, untreated patients. MICA expressed by U266 and LP1 cell lines was not *per se* sufficient to elicit killing or IFN-γ production by Vγ9/Vδ2 T cell clones. Treatment of MM cell lines with the bisphosphonate pamidronate, however, strongly up-regulated cytotoxicity and cytokine production; both functions mostly depended on TCR triggering, but also on MICA engagement, as demonstrated by inhibition experiments. Treatment of patients' MM cells with the proteasome inhibitor (PI) MG-132 significantly up-regulated MICA surface expression. These results suggest a dual interaction between Vγ9/Vδ2 lymphocytes and MM plasma cells, involving both TCR triggering and NKG2D-mediated signals; they also indicate that both pathways may be up-regulated by PI. It is of interest that PI also have a significant efficacy in inducing apoptosis of endothelial cells, particularly when they have received a pro-angiogenic stimulus via VEGF. In con-

trast, the interaction between plasma cells and stromal cells does not protect stromal cells from the PI-induced damaging effect.

These observations lead us to discuss how PI act within the BM microenvironment and whether endothelial and stromal cells behave differently in their promoting activity of plasma cell growth. One possibility is related to the role of hypoxia which transiently upregulates the expression of HIF-1α and HIF-1β in endothelial cells, but not in primary stromal cells (that already express both molecules). Endothelial cells (HUVEC) exposed to hypoxic conditions are induced to proliferate and migrate.

PL1.04

IMMUNOPHENOTYPE OF THE MALIGNANT CLONE. IMPLICATIONS FOR MANAGEMENT

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In multiple myeloma (MM) the use of multiparametric immunophenotyping by flow cytometry is mainly restricted to research purposes and differential diagnosis of unusual cases. Nevertheless, it would probably be more useful in clinical practice than is usually thought. Here, we will review some areas of clinical interest for MM patients.

The immunophenotypic characterization of PC requires the design of a minimum panel of monoclonal antibodies (i.e. CD19, CD20, CD28, CD33, CD45, CD56, CD117 and sIg together with CD38 y CD138), preferably in quadruple combinations. This type of panel allows us to identify the presence of phenotypic aberrancies in myelomatous PC and to discriminate between normal and malignant PC. In our experience in a series of 700 untreated MM patients, in >90% of cases the malignant PC expressed phenotypic aberrancies such as antigen under-expression (decreased CD38 intensity), over-expression (increased reactivity for CD28, CD33, CD56) or asynchronous antigen expression (presence of sIg, CD20, CD45, CD117). The distinction between normal and myelomatous PC is particularly useful in two areas: differential diagnosis between MM and MGUS, and the investigation of minimal residual disease (MRD). In the first area, we have observed that in MM, the proportion of normal PC within the total PC compartment is always <5% while in MGUS cases the percentage of normal PC is always >5%, and in many of them this cell subset predominated over myelomatous PC, and this criterion was independent in multivariate analysis.[1] Moreover, Rawston *et al.* have shown that a high percentage of abnormal PC is associated with a high risk of transformation in MGUS patients.[2] As far as MRD investigation is concerned, immunophenotypic criteria are applicable to >95% of the patients and it provides stronger predictive information of risk of relapse than conventional electrophoresis. In the evaluation of the BM in morphological and electrophoretic remission following ASCT, we found that MRD negative patients had significantly longer EFS (38 vs 23 months).[3,4] Accordingly, for a more appropriate definition of response criteria, the term immunophenotypic remission should be considered, as it is already used in other disorders such as B-CLL. Another area of interest derived from the characterization of the antigenic profile of PC is research into the potential association between the immunophenotypic profiles and disease outcome, and whether or not these profiles might eventually help to define different risk groups. Our data, based on a large series of >500 patients uniformly treated with high dose chemotherapy, show that the absence of markers such as CD56 and CD117 as well as the expression of CD28 and CD19[5,6] is associated with an adverse clinical outcome. Finally, it should be noted that there is increasing interest in the relationship between protein expression and gene markers. We have observed that expression of CD56, CD117 and CD33 antigens is more frequently associated with a hyperdiploid PC DNA-cell-content by flow cytometry. By contrast, the presence of CD19, CD20 and CD28 markers tend to be associated with a diploid DNA-cell content. Moreover, patients with deletion of 13q as well as those with IgH rearrangements were more frequently negative for CD56, CD45 and CD117 markers.

Finally, identification of PC by immunophenotyping will help to assess the degree of BM infiltration and contamination of apheresis as well as in the specific analysis of the DNA-cell content (DNA ploidy) and the cell cycle distribution (proportion of S-phase PC) of the myelomatous tumor clone. These parameters offer relevant prognostic information.[7,9] Altogether those data illustrate the potential clinical value of immunophenotypic studies in multiple myeloma.

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PLENARY SESSION 2
CYTOGENETICS & MOLECULAR PATHOGENESIS

PL2.01
INTEGRATION OF GENETICS IN A COMPREHENSIVE PATHOGENESIS MODEL FOR MYELOMA

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Two major subtypes of multiple myeloma (MM) can be differentiated; MM harboring IgH translocations (IgH-TRX) and having non-hyperdiploid (NH) karyotype and MM with hyperdiploidy (HYP). IgH-TRX result in upregulation of oncogenes by IgH enhancers. There are three major types of IgH-TRX; i) those that result in direct upregulation of either cyclin D1 or D3 [t(11;14) and t(6:14)]; ii) those that directly or indirectly result in upregulation of cyclin D2 [CCND2 translocations and t(4;14) and t(14;16)] and; iii) those that involve other chromosome partners and with unknown consequences. The first two groups are the so-called *primary IgH-TRX* and the last is the so-called *secondary IgH-TRX*. The primary IgH-TRX have a high degree of association with the NH karyotype.

MM with HYP and a low prevalence of IgH-TRX is characterized by the presence of multiple trisomies of the odd numbered chromosomes (except 13). These patients have a lower prevalence of IgH-TRX than patients with NH myeloma (20% vs. 80%). HYP MM is less aggressive and patients have an overall better prognosis. The causative oncogenic event of HYP MM is not known, although all have a low level of expression of cyclin D1.

The pathogenetic pathways that lead to the formation of plasma cell (PC) neoplasms are evident since MGUS. Patients with MGUS harbor the same chromosome abnormalities as in MM and with a similar prevalence; for instance all IgH-TRX observed in MM are also observed in MGUS. Uncertainty existed regarding the presence of t(4;14) in patients with MGUS, and the suggestion had been made that its presence would invariably lead to MM. We and others observed t(4;14) in patients with MGUS and smoldering MM at a similar prevalence as, and without progression to MM. Of note the proportion of t(11;14) MGUS is higher than in MM (25% vs. 16%) and even higher in patients with earlier PC proliferations such as light-chain amyloidosis (~50%). At this meeting we report that the differential pathway of pathogenesis based on ploidy (HYP vs. NH) is also seen in MGUS (WJ Chng *et al.*).

The patterns of progression from MGUS to MM are likely to follow the specific pathways that are preferred based on the unique cytogenetic background of the MGUS cells. For instance we have recently been able to show that while mutations of ras are seen in ~30% of patients with MM, they are far more common in patients with t(11;14) (~50%) than in other cytogenetic categories (e.g. t(4;14) at <5%). Thus it is only logical to postulate that the study of progression from MGUS to MM should be done at the level of the individual cytogenetic subtype. We have also found that some of the perceived genetic alterations hold little relationship to the baseline genetic category and have no significant impact on clinical outcome (e.g. p16 methylation). Based on these observations a model can be put together that explains disease pathogenesis and that also discusses possibility for progression from the earlier stages. The following principles and hypothesis need to be tested:

1. Genetic events leading to progression are likely different and based on the underlying cytogenetic category; those leading to progression for t(4;14) & t(14;16) are unknown.
2. The t(11;14) is likely negatively selected by progression as its prevalence drops with advancing stage of the disease. Accordingly it is likely that in the very early stages of late B-cell neoplasms a much larger proportion of cases are indeed t(11;14) but only a fraction of them progress to detectable MGUS and myeloma. This notion would imply that if we had more sensitive methods of detection of pre-malignant stages than those capable of detecting MGUS or AL, one would see a much larger fraction of individuals with sub-clinical t(11;14).
3. We believe the strong association with pseudodiploid karyotypes for t(11;14) MM is because of the synchronized and coordinated activity of centrosomal cycle and the mitotic cycle as dictated by cyclin D1. It is also tantalizing to speculate that this added stability is an evolutionary disadvantage to the clone, explaining the overall less aggressive clinical outcome.
4. While cyclin D1 levels are increased in most (all?) cases of hyperdiploidy MM, the causative genetic events need to be defined. It is possible that the gene dosage effects of trisomies is sufficient for the cells to become transformed but their presence in MGUS suggest they are, again as IgH-TRX, not sufficient for evolution to MM.
5. Several observations have been made that describe a lower prevalence of bone disease in patients with aggressive cytogenetic categories. In particular bone lesions appear to be less common among individuals with either the t(4;14) or the t(14;16). Given that these two IgH-TRX are also observed in the pre-malignant stages but at reduced prevalence, it is also possible that; i) the evolution to MM from MGUS is faster in these categories (although this needs to be formally proven and is being studied), and; ii) that bone disease develops as a consequence of all the important local and paracrine cytokine interactions, but that it is also dependent on the duration of the contact between the bone stroma/osteoclast/obsteoblast with the malignant PC. The relatively shorter time of close interaction between PC cells in a case that progresses rapidly to MM does not allow for the long term bone destruction consequences. This would also be consistent with the group of younger patients with IgA MM, no bone lesions, aggressive clinical features and t(4;14).
6. We have recently shown that more than half of patients with PC leukemia have deletions of the p53 locus and an additional fraction of patients also have mutations of p53. In most cases of MM there is retention of the normal p53 pathway, mandating residence of the MM cells to the bone marrow microenvironment, and associated apoptosis when the leave this nurturing site. The loss of p53 activity lessens normal apoptotic signaling and allows for cells to emancipate from the bone marrow with the resulting phenotype of PC leukemia and extramedullary MM. The association we reported between p53 deletions and plasmacytoma further supports this. This would also suggest the relapse pattern of multiple plasmacytoma as arising from clonal evolution to p53 independence.
7. Little is known regarding baseline susceptibility for development of MGUS and MM. Racial differences can be thought to arise because of differential efficiency in repair of dsDNA breaks or other genes associated with genomic stability. As such it is possible that the actual composition of MM (NH vs. HYP) in the different race categories will be different from that now frequently reported for "Caucasian MM."
8. Gene expression profiling of MM needs to be done with con-

sideration of these defined categories. Accordingly the study of MM progression by gene expression profiling should only be done by directly comparing similar subgroups of the disease (e.g. t(11;14) MGUS compared to t(11;14) MM, and not all cases at large given the "pollution effects" of all other progression pathways. While unbiased analysis is still needed, the creation of artificial clustering of groups of patients based on algorithms and disregard for underlying fundamental oncogenic lesions is likely to do little to further our understanding of MM.

9. In addition to the development of targeted therapeutics, genetics must now be incorporated into risk stratified and predictive therapeutic approaches. We need to know the impact of cytogenetic category in responsiveness to novel therapeutics, on high dose therapy outcome, duration of response, bone disease, need for allogeneic strategies, etc.

We thank all patients who have kindly and altruistically provided research samples without which these studies would not be possible.

PL2.02

EARLY PATHOGENIC EVENTS IDENTIFIED BY CHROMOSOME TRANSLOCATION AND CYCLIN D EXPRESSION DETERMINE MYELOMA BIOLOGY AND CLINICAL COURSE

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Recurrent Ig translocations mediated by errors in switch recombination and somatic hypermutation. Multiple myeloma is a tumor of plasma cells (PC) with immunoglobulin (Ig) genes that have undergone somatic hypermutation and isotype switch recombination. Based on the hypothesis that a critical event in the pathogenesis of MM is mediated by errors in these germinal center processes, we identified recurrent Ig gene translocations in MM cell lines and patient samples. There are five recurrent loci: 15% 11q13 CCND1, 4% 6p21 CCND3, 15% 4p16 FGFR3/ MMSET, 7% 16q23 c-MAF, 4% 20q11 MAFB. Approximately 15% have an IgH translocation with an unidentified partner, including c-myc, which is infrequent at diagnosis (3%), but increases with relapse (40%) and is nearly universal in cell lines (>90%). Several groups have shown that these recurrent translocations identify important prognostic groups with reproducibly poor survival for patients with 4p16 and 16q23 translocations, and generally good survival for 11q13.

CCND dysregulation in patients with and without Ig gene translocations. Analysis of gene expression profiles generated at UAMS has revealed that a CCND gene is dysregulated in all patients, not just those with a recurrent Ig translocation and has allowed the 60% of patients lacking a recurrent IgH translocation to be divided into 4 groups: those with elevated CCND1 (D1, 34%), CCND1 and CCND2 (D1+D2, 6%), CCND2 (D2, 17%) and no CCND (NONE, 2%). CCND1 is not normally expressed by B cells or plasma cells. We have found that in contrast to patients with 11q13 translocations, it is bi-allelically dysregulated in the D1 and D1+D2 groups. These patients have frequent hyperdiploidy (90%) with multiple trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21. In fact CCND1 is the single gene that provides the greatest discrimination between patients with and without hyperdiploidy (excluding those patients with 11q13 translocations). The D1+D2 patients are over-represented in the relapsed population (6% vs 17%) and have a significantly higher gene expression proliferative index (PI>0.2 in 43% vs 5% for D1 and 16% for D2). Despite the fact that they are identified by the expression of a single gene, the D1 group is characterized by the shared expression of a large number of genes that result from not only the chromosomes involved in trisomies, but also genes involved in the interferon response pathway (TRAIL, pkr, etc). The D2 group has no obvious distinguishing characteristics, while the NONE group contains several patients with macrofocal BM disease lacking large numbers of malignant plasma cells in the sample submitted for microarray analysis.

Identification of translocation and cyclin D (TC) groups. The translocation and cyclin D (TC) groups can be easily identified by examining the expression level of six genes (CCND1, CCND2, CCND3, c-MAF, MAFB and MMSET) in purified PC. This has been done successfully using several different expression platforms (Affymetrix HuFL, Hu95Av2, Hu133, QPCR), with samples generated in different institutions, and using different mechanisms of PC purification (positive vs negative selection). This analysis can identify patients with different genetics, biology, clinical features (90% incidence of bone disease in 11q13 and D1 groups, vs 55% in 4p16 and maf groups), prognosis and response to therapy. The TC groups form the basis for one molecular classification of MM that can be easily reproduced and applied to variously generated clinical data.

Targeted therapy for the MM. Regardless of the method used to identify 4p16 patients (FISH, RT-PCR, microarray) they have a poor survival, whether or not they express FGFR3. This applies to both patients treated with conventional or high-dose therapy, suggesting that novel treatment approaches are required. We have shown that in two cell lines with activating mutations of FGFR3, a selective tyrosine kinase inhibitor of FGFR3 induces cell cycle arrest, differentiation and apotosis, providing the basis for proceeding with a clinical trial of FGFR3 inhibitors in t(4;14) MM. Animal models have shown that activated FGFR3 is transforming in pre-B cells, although it has not yet been shown to be transforming in PC. We have generated transgenic mice in which expression of activated FGFR3 is targeted to cells undergoing switch recombination to IgG1. Although we have very high levels of expression of FGFR3 in PC, the mice have not developed gammopathy or lymphomas. In contrast mice with expression of MMSET type I and type II in lymphocytes develop late-onset B cell lymphomas.

Germinal center events determine MM biology and clinical course. An important implication of these observations is that despite the genomic complexity and multiple pathways of tumor progression, apparently early pathogenic events determine the ultimate biology and clinical course. To test this hypothesis we have sought to model these events in mice, starting with two extreme examples. Using identical transgenic constructs we have generated mice in which either BCL-6 or c-MYC is sporadically activated in the germinal center by the process of somatic hypermutation. These mice developed diffuse large B-cell lymphoma or MGUS/MM, respectively. We suggest that germinal center activation of CCND, FGFR3/MMSET, c-MAF or MAFB results in a similar (though obviously more narrow) spectrum of tumor phenotype, with important biologic and clinical implications.

PL2.03

PATHOGENESIS OF MYELOMA: IG TRANSLOCATIONS, CYCLIN D DYSREGULATION, OTHER EVENTS

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Immunoglobulin translocations(TLC). There appear to be two pathways involved in the pathogenesis of MGUS and MM. Nearly half of tumors are non-hyperdiploid (NHRD), and mostly have IgH TLC that involve 5 recurrent loci: mainly 11q13(CYCLIN D1) and 4p16(FGFR3 & MMSET), but less often 6p21(CYCLIN D3), 16q23(c-MAF), and 20q12(MAF B). The 5 recurrent TLC are mediated mostly by errors in IgH switch recombination as B cells pass through a germinal center. The remaining tumors are hyperdiploid (HRD), with multiple trisomies of chromosomes 3,5,7,9,11,15,19, and 21, and infrequently have IgH TLC involving these 5 recurrent loci. TLC involving a MYC gene (c- >> N- > L-) are absent in MGUS but occur in 3% of MM tumors, 45% of advanced MM tumors, and >90% of HMCL. MYC TLC are a very late progression event, occurring at a time when MM is becoming more proliferative and less stromal cell-dependent. They provide a paradigm for secondary (Ig) TLC, which typically are karyotypically complex, do not involve B-cell specific DNA recombination mechanisms, and can occur at anytime during tumor progression, including MGUS. Secondary TLC, which include all MYC TLC, most IgL TLC (λ>>κ), and IgH TLC not involving the 5 recurrent partners, have a similar prevalence in HRD and NHRD tumors. Surprisingly, two independent Ig TLC are found in 58% of HMCL, 25% of advanced MM tumors, and 5% of MGUS tumors.

Dysregulation of a CYCLIN D gene: an early, unifying event in MGUS and MM. Despite the low proliferative index of MM tumors, there is a dysregulated and/or increased expression of a CYCLIN D gene in virtually all MM and MGUS tumors. The level of CYCLIN D expression is comparable to that in highly proliferating plasmablasts and in contrast to quiescent, terminally differentiated normal plasma cells. About 25% of MGUS or MM tumors have an IgH TLC that directly dysregulates CYCLIN D1(11q13), CYCLIN D3 (6p21), or a MAF gene (c-MAF, 16q23 or MAF B, 20q11) encoding a transcription factor that targets CYCLIN D2. About 40% of MM tumors do not have a t(11;14), but bi-allelically express CYCLIN D1 (not expressed in normal B cells or PC); virtually all of these tumors are HRD. Most other tumors, including those with a t(4;14) translocation, have increased expression of CYCLIN D2. The HRD tumors that bi-allelically express CYCLIN D1 but not CYCLIN D2 are not represented among the 50 HMCL, suggesting that these tumors are particularly dependent on interactions with bone marrow stromal cells, and that primary TLC provide one step towards stromal independence.

Several oncogenic events can disrupt the RB pathway in the same MM tumor. Despite the dysregulation of a CYCLIN D gene in virtually all MM tumors, other components of the RB pathway also can be disrupted, The p16 promoter is methylated in about 25% of either MGUS or MM tumors, and in 90% of HMCL. Methylation of the p16 promoter is uniformly associated with lack of p16 expression, but nearly 50% of MM tumors express little or no p16 despite the absence of methylation of the p16 promoter. Although low or no p16 expression may be important early in pathogenesis, it is unclear if this is an active oncogenic event. By contrast, bi-allelic deletion of p18INK4c is a late tumor progression event, occurring in 30% of HMCL, about 2% of all MM, and nearly 10% of the most proliferative MM. Curiously, we find increased expression of p18INK4c in most HMCL and proliferative MM tumors that have not bi-allelically deleted p18. Thus these tumors have become insensitive to increased expression of p18 despite only rare inactivation of RB *per se* in MM. In support of these observations, retroviral-mediated expression of exogenous p18 inhibits the growth of HMCL that express little or no p18, but has no effect on growth of an HMCL that already expresses a high level of p18.

Early events critical in determining tumor phenotype: is MM several different diseases? On the basis of 5 recurrent TLC and CYCLIN D expression, MM can be assigned to 7 translocation/ CYCLIN D expression (TC) groups (4p, maf, 6p, 11q, D1, D1+D2, and D2) that have an increased expression of at least one CYCLIN D gene compared to normal bone marrow PC; an eighth NONE group includes about 1% of MM tumors that do not express an increased level of a CYCLIN D gene. We think that dysregulation of a CYCLIN D gene is an early – perhaps initiating – event in the pathogenesis of MGUS and MM. Other oncogenic events (additional numeric and structural chromosome changes, further disruption of the RB pathway, MYC TLC, and mutations of RAS, p53, and PTEN) mostly occur during progression as tumors increase in bulk and become more proliferative and less stromal cell-dependent. However, the TC groups have differing gene expression profiles and biology, including significant differences in the prevalence of bone disease, frequency at relapse, and progression to extramedullary tumor. This suggests that MGUS/MM may represent several different diseases that continue to have distinct phenotypes despite overlapping progression events. The TC groups may provide one basis for stratifying tumors for response to different therapeutic regimens. However, regardless of this possibility, the identification of TC groups that are based on early pathogenic events, which are shared by MGUS and MM, should continue to provide a foundation for biologically and clinically relevant insights.

PL2.04

EPIGENETIC CHANGES IN THE MOLECULAR PATHOLOGY OF MULTIPLE MYELOMA

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Aberrant methylation of the 5´ gene promotor regions is an epigenetic phenomenon representing a major mechanism for silencing of tumor suppressor genes in many cancer types. In our studies, we compared the aberrant promotor methylation profile of known or suspected tumor suppressor genes in monoclonal gammopathy of undetermined significance (MGUS) with that of multiple myeloma (MM). Several genes were found to be frequently methylated in MM including p16, p15, E-cadherin (CDH1), DAPK, and DcRI, whereas methylation of other genes was infrequent (RASSF1A, MGMT) or completely absent (RARβ). In MGUS, abnormal methylation was observed as well, and the percentage of MGUS cases with at least one gene methylated was similar to that of MM (80%). However, the mean methylation-index (a reflection of the methylation status of all of the genes tested) of MGUS cases was lower than that of MM (0.15 versus 0.30; $p< 0.001$). For most genes, the

methylation frequencies were higher in MM cases compared to MGUS, which was particularly evident for CDH1. Whereas critical chromosomal abnormalities (14q translocations, deletion of 13q) are identical in MGUS and MM, methylation of certain genes may be associated with transition from MGUS to MM.

PL2.05

ALTERNATIVE GENE SPLICING IN MYELOMA: ABERRANT SPLICING OF HYALURONAN SYNTHASE 1 PREDICTS FOR POOR SURVIVAL

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Overexpression of genes with dysregulated cellular function promotes cancer. However in addition to increased levels of a particular protein, abnormalities in pre-RNA splicing patterns can result in abnormally regulated splicing or mutationally stimulated alterations in cis-splicing elements and use of otherwise cryptic splice sites. Abnormal regulation of splicing is expected to lead to increased levels of particular splice variants. Aberrant splicing itself may result in abnormal proteins not made in normal cells. Abnormal splicing events may be among the first steps in the transformation process as well as promoting disease progression. Here we describe the impact on MM of aberrant splicing in hyaluronan synthase 1 (HAS1) and abnormal regulation of alternative splicing in RHAMM, two proteins functionally linked as, respectively, ligand and receptor. HAS1, a plasma membrane protein, synthesizes hyaluronan (HA), an extracellular matrix molecule which is biologically active in malignant spread and signaling. HA is also a ligand for RHAMM a centrosomal protein that plays a central role in malignant cell motility. Abnormal expression of RHAMM in model systems results in mitotic abnormalities that may lead to chromosomal missegregation in multiple myeloma (MM).[1] The expression patterns of HAS1 and RHAMM have been characterized in patients with MM. HAS1 and HAS2, but not HAS3, are overexpressed in malignant cells from MM and WM. Only malignant cells that express HAS1 are able to synthesize extracellular HA, and expression of HAS1 is correlated with an ability to undergo RHAMM-dependent motility. Further, we have identified a family of novel splice variants of HAS1 that are overexpressed in MM, but are absent from non-malignant lymphocytes.[2] Overexpression of HAS1 or the novel HAS1 splice variants correlates with reduced survival in MM. HAS1 gene products are prognostic only when expressed by the circulating components of the MM clone, providing a relatively non-intrusive means of monitoring the progression of malignant disease. In MM, expression of the HAS1 family is downregulated as malignant cells differentiate to the plasma cell stage, to be replaced by upregulation of HAS2, likely reflecting different biological imperatives for MM B and plasma cells. To date, this is the only instance of a marker for blood-borne malignant cells that predicts for disease outcome. The correlation between poor survival and the expression of HAS1 and its splice variants by circulating B cells is indicative of a key role for expression of HAS by *progenitor-like* compartments of the MM clone that circulate in the blood and mediate malignant spread to distant bone marrow sites. In MM patients we detected overexpression of HAS1 transcripts and identified three aberrantly spliced variants of HAS1 designated as HAS1Va, Vb, and Vc. All HAS1 variants are truncated as the result of short exon skipping and/or partial retention of intronic sequences. Bioinformatic analysis predicts appropriate folding of HAS1 variant proteins and retention of HA synthase activity. Protein expression of HAS1 variants was confirmed by Western blotting using MM cell lines. Production of extracellular and intracellular HA by MM B cells was verified by a particle exclusion assay (PEA) and HA staining. The expression of HAS1Vb either alone or in combination with HAS1 and its variants in MM B cells from peripheral blood strongly correlates with poor survival (p=0.001). Our analysis of HAS1 and variants expression suggests that HAS1 family members, particularly HAS1Vb, may contribute to early myelomagenesis since these transcripts are detected individually or in combination with other HAS1 variants' in MM and MGUS patients at the time of diagnosis. We speculate that HAS and RHAMM, a predictor of poor outcome in MM, play key roles in chromosomal instability in MM. HAS1 is aberrantly spliced in MM, potentially leading to intracellular expression of HAS1 and synthesis of intracellular HA that may bind to RHAMM. RHAMM overexpression and/or abnormal isoform splicing correlates with poor outcome in MM.[3] HA binding and centrosomal targeting domains of RHAMM overlap,[4] suggesting HA and centrosomal sites may compete for RHAMM. Both over- and under-expression of RHAMM have severe consequences for cell division. Consistent with the results in transfectants, MM is characterized by centrosomal abnormalities and extensive chromosomal instability suggestive of a mechanistic relationship with overexpressed RHAMM. Dysregulated RHAMM, in the context of aberrant intronic HAS1 splicing and HA synthesis, may be a strong contributor to the extensive, complex and progressively increasing extent of chromosomal abnormalities in MM. HAS1Vb, the only variant with a strong clinical impact, also appears to be the only splice variant that produces intracellular HA, a form of HA that may modulate RHAMM associations with the mitotic spindle. MM B cells have intracellular HA. We speculate that at least part of the strong clinical impact of aberrant HAS1Vb intronic splicing reflects the predicted ability of intracellular HA synthesized by HAS1Vb to modulate the function of RHAMM by inhibiting centrosomal targeting, thereby promoting aberrant mitosis. HAS1 and its variants, particularly HAS1Vb, in concert with RHAMM may be key contributors to chromosomal instability in MM. This work highlights a previously unrecognized role for abnormal gene splicing (HAS1) and for the dysregulation of alternative splicing to favor particular isoforms (RHAMM) in the pathogenesis of MM. Abnormalities in HAS1 and RHAMM may be very early events in myelomagenesis, as well as contributing to the emergence of increasingly aggressive disease over time.

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FOCUS SESSION 2
INNOVATIONS IN STANDARD AND SUPPORTIVE THERAPY

F2.01
IS THALIDOMIDE FIRST LINE THERAPY?

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F2.02
THALIDOMIDE AND CARDIOVASCULAR COMPLICATIONS IN MULTIPLE MYELOMA

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Thalidomide (T), a synthetic derivative of glutamic acid, was originally marketed as a sedative and anti-emetic agent for pregnant women. Not approved by FDA because of concern of neuropathy with long-term administration, it was withdrawn from other markets in 1961 when its potent teratogenic properties became apparent. However, during that time the anti-inflammatory activity of the drug in the treatment of erythema nodosum leprosum (Sheskin, 1965) was noted and T was ultimately approved by the FDA in 1998 for this indication. Based on its antiangiogenic activity (D'Amato *et al.,* 1994), clinical trials in oncology were initiated. Case reports in dermatological literature (Flageul et al, 2000) described a possible association between T and venous thromboembolism (VTE), but this observation was not confirmed in our original study of 169 extensively pretreated MM patients. The incidence of VTE was modest (<5%) (Singhal *et al.,* 1999) and was similar in subsequent studies (Tosi *et al.,* 2002; Kumar *et al.,* 2003). The virtual lack of myelosuppression made T an ideal agent in combination with corticosteroids and cytotoxic chemotherapy. In two trials conducted at the Memorial Sloan Kettering Cancer Center, VTE occurred in 27% of newly diagnosed MM patients treated with T, dexamethasone (D) and doxorubicin (dox) and in 7% of those treated with T and D (Osman et al, 2001). The role of T as a prothrombotic agent was clearly defined when a phase III trial with upfront randomization to thalidomide showed a significantly higher incidence of VTE in the T arm (Zangari *et al.,* 2001). The incidence of VTE when dexamethasone was used with T in relapsed MM patients was <10% (Palumbo *et al.,* 2004; Dimopoulos *et al.,* 2001), but in the newly diagnosed patients, an incidence of 26% was reported (Cavo *et al.,* 2002). Concomitant use of chemotherapy can affect the hypercoagulability state (Table 1). Among 232 MM patients who received a combination of chemotherapy and T on two protocols that differed only by the inclusion of dox in one, VTE incidence was significantly different (dox 16% vs. 3.5%, p= 0.02; Zangari *et al.,* 2002). A synergistic prothrombotic effect of dox and T was also confirmed in an experimental model of thrombosis in rabbits (Biemond *et al.,* 2003). Endothelial cell dysfunction has been observed *in vitro* after incubation with dox and T (Kaushal *et al.,* 2004). Preliminary data indicate that Velcade, a new proteasome inhibitor, when used in dox-T containing regimens, can significantly reduce the incidence of VTE (Zangari *et al.,* 2004). Thrombosis appears early in the course of T treatment (50% within 2 months) (Cavo *et al.,* 2004) and it has a higher incidence in newly diagnosed patients compared to in relapsed patients. In a multivariate analysis of 535 patients treated with T in various combinations, disease stage failed to show a significant impact on thrombosis; newly diagnosed status, T and dox combination and presence of chromosome 11 abnormalities were the only independent risk factors. Among the 535 patients analyzed, 82 developed VTE: thromboses were catheter-related in about 1/3 of patients, localized in the lower extremities in 2/3, and associated with pulmonary embolism in <5%. VTE development did not affect the overall survival (Zangari *et al.,* 2003) MM is a risk factor for VTE. In a recently reported study (Srkalovic et al, 2003) the incidence of thrombosis was 7.5% in patients with MGUS and 10% in treated MM patients). Specific factors may promote hypercoagulability in MM. High IL-6 levels are known to increase fibrinogen, tissue factor, factor VIII, von Willebrand factor, and to reduce antithrombin and protein S (Kerr et al, 2001). Fibrin assembly and fibrinolysis can also be influenced by high immunoglobulin levels (Gabriel *et al.,* 1992; Carr *et al.,* 1996). As observed in other malignancies, the function of the protein C system is altered in MM. Decreased APC resistance in the absence of factor V Leiden mutation was described as an independent risk factor for VTE development (Zangari *et al.,* 2002).

In T and D combination trials (Table 1), prophylactic low dose warfarin or aspirin modestly reduced the incidence of VTE (Cavo et al, 2004; Baz *et al.,* 2004). When therapeutic anticoagulation with warfarin was instituted, VTE was observed in 7% of patients (Weber *et al.,* 2002) The prothrombotic effect of the dox-T combination (Table 1) was completely abrogated by the prophylactic use of enoxaparin (40 mg/d), given during the first three months of treatment. In T-responsive patients who developed VTE, our experience suggests that it is reasonable to resume the treatment with T if full anticoagulation has been established and continued for the total duration of therapy (Zangari *et al.,* 2004). The rate of VTE recurrence was 13.8% overall, not significantly different from the rate observed in other cancers (9-17%; Lee *et al.,* 2003).

Table 1. Incidence of VTE with prophylactic anticoagulation in thalidomide-treated patients.

Therapy	*No prophylaxis*	*Warfarin 1 mg/daily*	*Warfarin (INR 2-3)*	*Enoxaparin (40mg/d)*	*Aspirin (81 mg/d)*
T+D in newly diagnosed patients	25% Weber, 2002 (24 pts) 18% Rajkumar, 2004 (102 pts)	13% Cavo, 2004 (52 pts)	7% Weber, 2002 (46 pts)		
T+D at relapse	1.6% Palumbo, 2004 (120 pts) 7% Dimopoulos, 2001 (44 pts)				
T+dox in newly diagnosed patients	34.5% Zangari, 2004 (87 pts)	31.4% Zangari, 2004 (35 pts)		14.7% Zangari, 2004 (68 pts)	17,8% Baz, 2004 (103 pts)
T+dox at relapse	16% Zangari, 2002 (192 pts)				

Among the other cardiovascular complications during T treatment, bradycardia and syncope are noteworthy. In 660

patients randomly assigned to receive combination chemotherapy with or without T, the only cardiovascular toxicities (grade ≥3) significantly increased in the T arm were sinus bradycardia, syncope and VTE. In a study of 96 patients treated with T and chemotherapy, 53% developed a heart rate <60/min, which was symptomatic in 10 patients (19%) (Fahdi *et al.,* 2004).

In the management of cardiovascular complications, available data suggest that VTE prophylaxis should be applied for at least the first three months of therapy to all newly diagnosed patients treated with T in combination with D or dox. In the relapse setting, prophylaxis is clearly indicated for patients treated with T/dox regimens. Prophylactic low molecular weight heparin and full anticoagulation with warfarin currenthy appear the most effective strategies.

F2.03

NEW ADVANCES IN THE MANAGEMENT OF MYELOMA BONE DISEASE

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Skeletal complications commonly occur among myeloma patients as a result of the enhanced bone loss associated with this B-cell malignancy. Commonly patients develop pathologic fractures of long bones as well as compression fractures of vertebral bodies. These fractures may or may not be associated with lytic lesions since patients also frequently show generalized bone loss. Often these patients require radiotherapy to relieve bone pain or treat actual or impending pathologic fractures. Less commonly patients develop spinal cord compression or hypercalcemia. These and other skeletal complications contribute to the deterioration in quality of life and independence of many myeloma patients. Patients with myeloma experience an average of two or more of these complications per year.

Intravenous chronic bisphosphonate therapy has emerged as an important component of the overall management strategy for most patients with multiple myeloma. These agents, particularly zoledronic acid and pamidronate, have been shown to effectively reduce skeletal complications for patients with multiple myeloma. First, a randomized, placebo-controlled trial showed that when IV pamidronate 90 mg every 4 weeks was administered in conjunction with anti-tumor therapy for myeloma patients with osteolytic lesions, the incidence of skeletal complications was significantly reduced, and the number of patients developing these complications was decreased. Importantly, this bisphosphonate showed positive benefits on the patients' pain, reduced their analgesic requirements, and also prevented the deterioration in the quality of life and performance status observed in the placebo group. Attempts to increase the dose or reduce the infusion time have been met with renal dysfunction from a specific glomerular lesion.

Recently, a more potent bisphosphonate, zoledronic acid, has shown efficacy for patients with multiple myeloma. A large randomized clinical trial comparing this newer agent to pamidronate among myeloma and breast cancer patients with metastatic bone disease has been completed. Zoledronic acid was administered every 3 to 4 weeks at 4 or 8 mg over 5 minutes initially. Because of more patients showing increases in creatinine in the zoldedronic acid groups, the infusion time was increased to 15 minutes. This resolved the renal issue for patients receiving the 4 mg dose but those receiving the 8 mg dose had the dose changed to 4 mg because of the continuing risk of renal problems. The results from this trial demonstrated that zoledronic acid (4 mg in a 15-minute infusion) was at least as effective as pamidronate (90 mg via a 2-hour infusion) in the overall efficacy analysis, with a similar safety profile. However, a multiple event analysis showed that zoledronic acid significantly reduced the risk of developing skeletal events by an additional 16% compared with pamidronate during the 24 months of treatment. Long-term follow-up shows similar renal safety for zoledronic acid administered over 15 minutes compared to the pamidronate given over 2 hours.

It is important to monitor serum creatinine among patients with myeloma receiving these drugs. However, there are a multitude of possible causes for rises in creatinine among these patients besides bisphosphonates. Uncommonly patients may show osteonecrosis of the jaw with chronic bisphosphonate use but it is unclear that discontinuation of these agents changes the course of this disorder. Maintaining excellent oral hygiene is increasingly recognized as an important part of the care of patients receiving these drugs. Also it is important to be aware that many myeloma patients show low levels of vitamin D as well as poor oral calcium intake. Oral supplements of these compounds are useful to optimize bone health in many patients with myeloma. Performing exercise, especially of weight bearing type, also helps maintain bone strength.

A variety of new approaches are entering clinical trials including interrupting the RANK-RANKL signaling pathways with antibodies and other inhibitors. Whether these ultimately prove useful clinically either alone or added to bisphosphonate therapy remains unclear. Pre-clinical studies also show anti-myeloma effects of bisphosphonates both *in vitro* and *in vivo*. Recent by completed clinical studies are evaluating the safety of higher doses of zoledronic acid so that these higher doses may be evaluated in future trials attempting to define the anti-myeloma effects of this drug clinically.

F2.04

PLASMAPHERESIS IN PATIENTS WITH MULTIPLE MYELOMA AND RENAL FAILURE

J Behrens, G Gaskin, N Iggo, D Samson, G Eddison, G Jackson

On behalf of the UK Myeloma Forum and the UK National Cancer Research Network

Renal failure in patients with multiple myeloma is often due to a number of factors including dehydration, amyloidosis, hypercalcemia, non-steroidal anti-inflammatory agent damage, hyperuricemia and infection. The most frequent cause of renal damage is tubular damage induced by Bence-Jones proteinuria. Careful attention to fluid and electrolyte imbalance together with removal of nephrotoxic drugs and treatment of the underlying myeloma can reduce the degree of renal damage and improve renal function. Apart from direct myeloma therapy, there has been a lot of interest in plasmapheresis as a means of reducing paraprotein and particularly light chain load. The data on plasmapheresis in myeloma and renal failure will be discussed but these are mainly small trials which have generally been inconclusive. Plasmapheresis is, however, widely used as part of treatment for patients with myeloma and renal failure. The UK MRC and the UK Myeloma forum have launched a large randomized phase 3 study (the MERIT study) to study renal outcome in patients with multiple myeloma who present with renal failure. Patients are randomized to receive myeloma therapy and either best renal supportive care or the same supportive care with 7 plasmaphereses within the first 14

days of presentation (with the aim of giving 4 plasmapheresis in the first 7 days). The study will be presented in detail and the numbers of patients randomized will be discussed and international participation encouraged for this very important study.

F2.05

PREVALENCE, PATHOGENESIS AND TREATMENT OF ANEMIA WITH ERYTHROPOIETIC AGENTS IN MULTIPLE MYELOMA

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Prevalence of anemia. Anemia is a common complication of myeloma that may already manifest at the time of diagnosis. Early studies[1] found hemoglobin levels < 12.0 g/dL in 62% of patients. Although today many patients are diagnosed earlier in the course of their disease, the prevalence of anemia has not changed substantially. In our recent study[2] in elderly patients (median age: 68 years) prevalence of anemia (Hb ≥12.0 g/dL) was 72%. In a recent large European Cancer Anemia Survey (ECAS) the prevalence of anemia (Hb <12.0 g/dL) was 53% in patients with myeloma and lymphomas. The incidence rate in patients who were non-anemic at enrolment and followed up for 6 months under chemotherapy was 56%.[3] Anemia usually normalizes in patients, who achieve complete remission after chemotherapy, but persists in patients who are unresponsive and recurs in those with relapsing disease. Anemia is more frequent in patients with long-standing disease, when toxicity of long-term treatment, impairment of renal function and heavy tumor load contribute to its induction and aggravation.

Pathogenesis. Anemia in myeloma is often multi-factorial with inadequate erythropoietin production, decreased numbers of erythroid precursors and reduced responsiveness of the erythron to erythropoietin and impaired iron utilization as well as shortened life span of red blood cells being the most important pathogenetic factors. In addition, dilutional anemia due to paraprotein-induced expansion of the plasma volume and a direct pro-apoptotic effect of aggressive myeloma cells on erythroid precursors[4] as well as myeloma specific tumor treatment may contribute and/or aggravate anemia.

Indications for treatment. Many myeloma patients present at age 65 or older with co-morbidities. These facts and the possible impairment of organ function by myeloma and amyloidosis and the side effects of myeloma treatment make myeloma patients particularly vulnerable to suffer from even mild anemia. Hence, the indication for treatment should mainly be based on the individual patient's anemia-related symptoms and less on the actual hemoglobin level, as sometimes is recommended in anemia treatment guidelines.

Erythropoietic agents. Erythropoietin is the most active stimulator of erythropoiesis and active in multiple myeloma yielding response rates (increase in Hb level > 2.0 g/dL) in 60-80% of patients. Treatment should be started with 30-40,000 IU erythropoietin per week or with 2.25 µg/kg darbopoetin per week. In case of no response after 4 weeks (increase in Hb < 1g/dL) the dose of erythropoietic agents should be increased by 50%. Is this does not result in significant improvement after 8 weeks, treatment should be discontinued. Patients with stable or responding disease and without severe infections or a very recent episode of anesthesia and surgery are most likely to respond. Response rates are higher in patients with low endogenous erythropoietin levels and in those with preserved bone marrow function (platelet counts >100,000/µL) whereas patients with heavy transfusion dependence (> 2 transfusions/month) are less likely to respond. Treatment should be continued until optimal improvement of symptoms and hemoglobin levels should be maintained at the level needed to alleviate the consequences of anemia. The treatment may be continued for several months and even for years if required. Often, dose reductions or an increase of the treatment interval is necessary to prevent overshooting of hemoglobin levels.

Iron supplementation. Parenteral or oral iron supplementation must be given in patients with concomitant iron deficiency and should be considered in patients with the 'chronic inflammation' type of anemia. A recent publication showed a significantly faster time to response and higher response rates with parenteral iron supplementation.[5]

Tolerance of erythropoietin treatment. Erythropoietin is very well tolerated in myeloma. So far, more than 900 patients have been enrolled into prospective randomized trials comparing erythropoietin or darbopoetin with placebo or with an untreated control group.[6-9] None of these showed a significant increase in adverse effects; particularly, there was no significant increase in hypertensive episodes or thromboembolic complications, which are slightly increased in anemic patients with other malignant diseases during erythropoietin treatment. Analysis of survival of treated and untreated groups did not reveal a survival benefit for erythropoietin. According to a recent report[10] treatment with erythropoietin in patients on thalidomide therapy is also safe and not associated with an increased risk of thromboembolic complications.

Benefits of erythropoietin treatment. The most important benefits of erythropoietin treatment comprise a significant improvement in overall quality of life, exercise capacity, increase in hemoglobin levels and reduction in transfusion needs. Most trials have, however, analyzed only the functional aspects of quality of life, for instance the performance status. Despite this limitation it is noteworthy that practically all published reports on erythropoietin treatment in anemic cancer patients showed a positive relationship between erythropoietin induced correction of anemia and quality of life related parameters. A recent phase IV study revealed quality of life benefits in anemic cancer patients responding to erythropoietin even in case of tumor progression.[11] A further interesting study revealed that cognitive impairment induced by chemotherapy (*chemo-brain*) can be prevented by erythropoietin treatment of anemic cancer patients.[12] Erythropoietin treatment was also reported to stimulate appetite and mood and was able to maintain baseline body weight in comparison to untreated controls with progressive cancer, who lost weight during further cancer treatment.

Conclusions. Anemia is the most frequent hematologic complication in patients with multiple myeloma and needs careful assessment of possible clinical symptoms. In case of significant anemia related side effects, treatment with erythropoietic agents should be considered.

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PLENARY SESSION 3: EVOLVING MYELOMA

PL3.01

EPIDEMIOLOGY OF MULTIPLE MYELOMA

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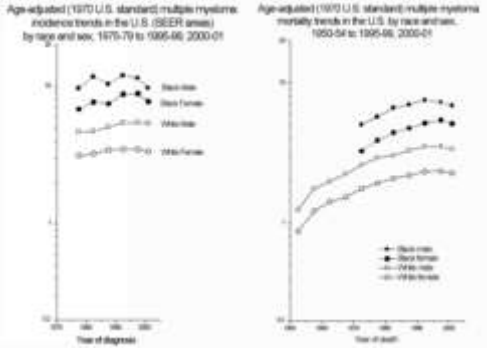
The incidence rates of myeloma show over a 10-fold difference around the world with highest rates reported among African Americans in the United States (U.S.), and lowest rates reported among Asians. According to the American Cancer Society, there will be about 15,270 new cases of multiple myeloma and 11,070 deaths in the U.S. in 2004. The U.S. annual age-adjusted incidence and mortality rates for multiple myeloma rose from the 1950s to the 1990s and then leveled off; nevertheless, rates remain two-fold higher among blacks than whites, and higher among men than women (see Figures). Increase in myeloma incidence rates up to the 1990s may be partially explained by the improvements in case ascertainment and reporting because of the availability of more accurate diagnostic tools and better access to medical care overtime.



The causes of multiple myeloma and the reasons for the racial difference in incidence are unclear. The suggestive associations between the risk of multiple myeloma and exposure to ionizing radiation, solvents, agricultural and farming occupations, including those with exposure to pesticides and farm animals, have previously been reported. Recent studies suggested that certain lifestyle and genetic factors, particularly low socioeconomic status (SES), obesity, and familial aggregation may also be linked to the excess risk of myeloma.

We present here data from our recent population-based case-control study conducted among women in Connecticut to examine the impact of environmental, lifestyle and genetic factors. This study included 179 myeloma cases (87.2% whites and 12.8% blacks) aged 21-84 years, diagnosed between 1996 and 2002, and 691 population controls (96.4% whites and 3.6% blacks). Information on education, income, smoking, alcohol consumption, diet, use of hair coloring products, occupation and family history of cancer was obtained by personal interviews. Blood and buccal swabs were collected, DNA was extracted, and genotyping for selected immune single nucleotide polymorphisms (SNP)

was carried out for 148 cases and 590 controls. Genotyping was conducted on the Taqman platform at one laboratory and validated with the conditions posted on the SNP500Cancer database website (http://snp500cancer.nci.nih.gov). Odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression. For statistical analyses of the SNP we used subjects who were homozygous for the most prevalent genotype as the reference group. Educational attainment was inversely associated with risk, adjusted for age and race (p trend=0.004). Risk was significantly elevated for subjects with less than a high school education compared to individuals with graduate or professional degree (OR=2.1, 95% CI=1.1-4.3). Similarly, an increased risk was observed for those in the lowest annual income category (<$10,000) (OR=2.1, 95% CI=0.9-4.4) when compared to subjects with an income of >$50,000. There was a suggestion of increasing risk with increasing body-mass index (BMI) for weight measured one year before diagnosis with an OR of 1.3 for the obese category (95% CI=0.7-2.4). However; the dose-response relationship was not statistically significant. We found no evidence of increased risk of myeloma for smoking and alcohol use. Compared to women who *never* used hair coloring products, there was no elevated risk of myeloma among women who used any hair coloring product, or used blonde colors or dark colors. Our findings support the previously observed association that the risk of multiple myeloma increases with decreasing SES. Low social class may be a surrogate for a set of negative environmental characteristics such as poor housing, dangerous jobs that may result in differential exposure to occupational carcinogens, unemployment, limited access to medical care, stressful home or work environments, poor nutrition or exposure to infectious agents. However, the SES-related exposures that may contribute to multiple myeloma risk remain to be determined. Interleukin-6 (IL-6) is a key moderator for Th2 response with a major role in the differentiation of B cells to plasma cells and survival of multiple myeloma cells. A common SNP (-174 G/C, rs1800795) in the IL-6 gene promoter region is associated with decreased production of IL-6. In our study, the OR for risk of myeloma with the C/G and C/C genotype were 1.2 and 1.6, respectively, suggesting a trend, but the 95% CI included the null value.

Table 1. OR* and 95% CI for multiple myeloma risk associated with IL-6 promoter genotype.

IL-6-174 genotype	Cases (%)	Controls (%)	OR (95% CIs)
G/G	40 (32.5)	206 (38.3)	1.0
C/G	58 (47.2)	252 (46.8)	1.2 (0.8-1.8)
C/C	25 (20.3)	80 (14.9)	1.6 (0.9-2.8)

** For non-Hispanic Caucasians only, adjusted for age*

It appears that myeloma may have a substantial environmental etiologic component. This component however, is still not completely understood. Evidence is now emerging from the literature of complex interactions between genes and environment in the pathogenesis of cancer. The future challenge is to determine which environmental agents and genetic factors influence myeloma risk, both as independent risk factors and as modifiers of each other.

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PL3.02

UPDATE ON SMOLDERING MULTIPLE MYELOMA

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Smoldering multiple myeloma (SMM) has been characterized by a serum M-protein level of 3 g/dL or higher or 10% or more plasma cells in the bone marrow. Frequently, a reduction of uninvolved immunoglobulins in the serum and a small amount of M-protein (light chains) in the urine are found. To be classified as SMM, these patients must have no evidence of related organ or tissue impairment (end organ damage) such as hypercalcemia, renal insufficiency, anemia, or bone lesions (CRAB) related to the plasma cell proliferation. In addition, the plasma cell labeling index is low. Biologically, these patients have a benign monoclonal gammopathy or monoclonal gammopathy of undetermined significance (MGUS) but this is not apparent when the patient is seen initially because the size of the serum M-protein and the number of the bone marrow plasma cells exceed those allowed for a diagnosis of MGUS.

The definition of SMM is not standardized. It is not know whether both serum protein >3 g/dL or bone marrow plasmacytosis >10% are necessary for diagnosis or whether only one parameter is adequate.

Many patients with SMM progress to symptomatic multiple myeloma (MM). Risk factors predicting progression to multiple myeloma have not been carefully studied in a well defined group of SMM with long-term follow-up.

We are reviewing the medical records and bone marrows of all patients seen at the Mayo Clinic within 30 days of recognition of an IgG or IgA M-protein >3g/dL or bone marrow containing >10% plasma cells from 1960-1995. This allows for a minimum potential follow-up of 10 years. Patients are categorized into 3 groups: 1) serum M-protein > 3 g/dL, but bone marrow plasma cells <10%; 2) bone marrow plasma cells >10% but serum M-protein <3 g/dL; 3) serum M-protein >3 g/dL and bone marrow containing >10% plasma cells. Patients with primary amyloidosis, MM or those who have received chemotherapy are excluded. The probability of progression will be ascertained in each group and thus provide a more accurate classification of SMM. Risk factors for progression including age, gender, hepatosplenomegaly, hemoglobin, calcium, creatinine, albumin, presence of bone lesions, size and type of serum M-protein, number of bone marrow plasma cells, reduction of uninvolved serum immunoglobulins, and presence, type and amount of monoclonal light chain in the urine will be evaluated.

PL3.03

THE EVOLVING VERSUS THE NON-EVOLVING NATURE OF ASYMPTOMATIC MONOCLONAL GAMMOPATHIES: MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING MULTIPLE MYELOMA

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The term monoclonal gammopathy of undetermined significance (MGUS) indicates the presence of a serum monoclonal protein lower than 30 g/L and less than 10% bone marrow plasma cells (BMPC) in the absence of clinical manifestations attributable to the monoclonal gammopathy. In

MGUS, the rate of malignant transformation is 1% per year with, in our experience, the plasma cell mass measured by the M-protein size and the proportion of bone marrow plasma cells being the key factors for malignant transformation. Kyle & Greipp coined the term smoldering multiple myeloma (SMM) for patients with an M-protein size higher than 30 g/L and more than 10% BMPC but with no organ-related or tissue impairment. We have recently reported the natural history of 53 patients with SMM diagnosed according to the stringent criteria defined by Kyle & Greipp and we recognized the so-called evolving type. Patients with non-evolving or classic type had a long-lasting stable M-protein until the onset of symptomatic disease. In contrast, patients with the evolving type showed a progressive increase in the M-protein size until symptomatic myeloma developed. In the overall series, the median time to progression to symptomatic MM was 3.2 years. However, the median time to progression was significantly shorter in the evolving type (1.6 vs 4.2 yrs; p=0.007). Interestingly, 59% of patients with the evolving type had a previously recognized MGUS that also had an evolving pattern while a previous MGUS was only seen in 4% of patients with the non-evolving type. Despite the limited number of patients, there was a trend towards a higher response rate to therapy after progression in the evolving type. We hypothesized that eventually all patients with an evolving pattern had previous MGUS, evolving SMM being a transient step between MGUS and symptomatic MM. We also hypothesize that non-evolving MM is comparable to MGUS but with higher a probability of progression due to its higher plasma cell mass. In fact, we found a different pattern of genetic abnormalities by comparative genomic hybridization depending on the SMM type. Thus, all patients in the evolving type had abnormalities (chromosomal losses, 1q gains) while chromosomal losses were uncommon in the non-evolving type. In addition, we found no 1q gains in the non-evolving SMM. The CGH pattern of our patients with evolving SMM is very similar to the CGH changes reported in symptomatic MM. In summary, we identified two variants of SMM: 1) the evolving variant with a progressive increase in the M-protein size, a previous MGUS and a short time to progression and 2) the non-evolving variant with long-lasting stable M-protein, no previous MGUS and a longer time to transformation to symptomatic MM. Concerning the transformation rate in our MGUS patients we found that the plasma cell mass was predictive for transformation for the first 10 years and that after this period the frequency of malignant transformation seems to be independent of the initial plasma cell mass. This suggested that there are two MGUS populations: the evolving and the non-evolving. On the basis of our observations in SMM and in MGUS we are now investigating the possible existence of two types of MGUS. One evolving and one non-evolving with different pathogenetic mechanisms. To ascertain whether these conditions are pathogenetically different we are performing the immunophenotype of BMPC, the measurement of serum angiogenic factors (VEGF, bFGF, HGF, IL-6 and TNF-α) and gene expression profiling. In summary, our hypothesis is that there are two types of MGUS, an evolving and a non-evolving type, with different pathogenetic mechanisms, malignant transformation being a constant in the evolving type, irrespective of the initial M-protein size. Thus, the evolving MGUS could be viewed as an early myeloma from the beginning while the non-evolving type would be a true long-lasting MGUS requiring a second trigger to initiate malignant transformation. The preliminary analysis of our MGUS series which includes 359 patients with enough follow-up to determine the evolving or the non-evolving type according to the M-protein measurements shows an actuarial malignant transformation probability at 12 years of follow-up of 87% for the evolving type versus 13% in the non-evolving type ($p < 0.001$).

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PL3.04

THE INTERNATIONAL STAGING SYSTEM FOR MYELOMA: AN UPDATE

P Greipp

Mayo Myeloma Amyloid Dysproteinemia Disease Oriented Group

PL3.05

ADVANCES IN DIAGNOSIS AND IMAGING IN MULTIPLE MYELOMA

B Durie

International Myeloma Foundation

FOCUS SESSION 3: THE MALIGNANT CLONE

F3.01

NORMAL PLASMA CELL DEVELOPMENT AND PATHOGENESIS OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE/MULTIPLE MYELOMA

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Defining the cell of origin and its clonal history remains a central question in understanding the biology and behavior of multiple myeloma (MM). Several interdigitating approaches will be required to fully address this question. Our focus has been on utilizing Ig variable (V) region gene analysis to probe tumor origins in MM, and its pre-malignant counterpart, monoclonal gammopathy of undetermined significance (MGUS).

V gene analysis tracks critical phases of normal B-cell development. A functional rearrangement of V_H and V_L genes allows the healthy B-cell to leave the bone marrow (BM) and circulate in the periphery. V genes define the domains recognizing specific antigen, and this encounter in secondary lymphoid organs can trigger the germinal center (GC) reaction, where somatic mutation actively targets V genes. Isotype switch can also occur here, and for both mechanisms, the inducible enzyme activation induced cytidine deaminase (AID) is essential. AID is an early component of the machinery which generates double strand DNA breaks, a feature of both somatic mutation and class switch recombination (CSR). There has been interest in whether these mechanisms of DNA modification, in particular dysregulated AID activity, may be implicated in the development of B-cell tumors as it can target several genetic loci. Mutational and switch activity, however are by no means restricted to the GC, as ectopic events can occur.

Normal maturation of B-cells to plasma cells (PC) *in-vivo* leads to the accumulation of the largest pool of terminally differentiated cells in the BM. These have been poorly described at the immunogenetic level in the human setting. Here, we utilized V genes to analyze normal PC at this site. We used the V_H gene, $V_{4\text{-}34}$ to track features of this population. Isotype-switched $V_{4\text{-}34}$ sequences were readily detected in whole populations of PC. $V_{4\text{-}34}$ sequences were invariably somatically mutated, a feature shared by genes derived from the V_{H1} and V_{H3} families, indicating a common characteristic. Switched transcripts were more frequent than Cμ sequences. In $V_{4\text{-}34}$ transcripts, clonal expansions of PC were apparent. Interestingly, these displayed intraclonal heterogeneity, indicative of localized mutational events. This also suggests that mutational heterogeneity in V_H genes in some cases of MGUS could derive from such events in the BM. These MGUS can subsequently transform to MM to display a homogeneous pattern. This is consistent with the proposed stage of neoplastic arrest in MM occurring at a stage when mutational activity has been silenced. These observations invoke a pathway of local B-cell maturation in the BM which may be subverted to give rise to MGUS and MM.

To model B-cell maturation events further, we examined plasmablasts generated to >99% purity *in vitro* from healthy blood B lymphocytes. Polyclonality was evident by diverse CDR3, and V_H genes were invariably mutated, consistent with derivation from memory B-cells. These cells displayed μ and γ VH functional transcripts, with sterile germline transcripts and switch circle transcripts revealing on-going deletional recombination events. Notably, AID expression was also observed at a high level, associated with continual isotype switch events. These plasmablasts derive from post-follicular B cells undergoing isotype switch, and this is likely to be a point of transformation in MM. CSR appears feasible at the plasmablast level, and alters the perception that critical B-cell-associated mechanisms do not persist to late stages.

Persisting AID and switch activity at the plasmablast stage has relevance in understanding origins of MM. To date, the differentiation status of the cell of origin in MM is not fully delineated. Tumor-derived Cμ transcripts in some typical MM cases implicate isotype switch events in tumor origins, and suggest a less-mature cell of origin, as do $CD19^+$ B-cells bearing clonotypic markers in a few studies. However, the frequency, differentiation status and malignant potential of these cells is uncertain. Frequent chromosomal aberrations in switch loci at 14q32 in MM again substantiate a critical role for switch events, possibly as a primary neoplastic occurrence. These 14q32 translocation are more frequent in non-hyperdiploid than hyperdiploid myeloma, and arise early in disease, as observed in the pre-malignant MGUS condition.

For on-going somatic mutation to occur in the BM, it will most likely require initiating signals via sIg in B-cells, with AID active. Post-follicular $sIgM^+$ B-cells can normally be found here, capable of maturing further to secrete Ig, conceivably also capable of switching. Intriguing recent findings reveal a potentially critical role for AID in neoplastic transformation. In AID-deficient mice injected with pristane, frequent aberrant translocations of Myc into the switch (S) region were surprisingly observed, indicating that the initial lesions leading to double strand DNA breaks are independent of AID (*Unniraman et al. Nat Immunol 2004;11:1117-23*). However, subsequent experiments showed that AID was required for the out-growth of translocation-positive cells, suggesting that AID plays a pivotal role in tumur progression, possibly due to its mutagenic activities. Clearly, both mutational and switch events in the normal BM, and especially the role of AID, even during the plasmablast phase will have to be mapped further to identify tumur origins of MGUS and MM.

F3.02

A CELLULAR MODEL FOR MYELOMA CELL GROWTH AND MATURATION BASED ON AN INTRACLONAL CD45 HIERARCHY

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Multiple Myeloma (MM) is a plasma-cell malignancy mainly characterized by the accumulation of malignant plasma cells within the bone marrow. This review shows that the biology of CD45 illuminates that of MM and, more specifically, provides a better delineation of a tumor cell *hierarchy* of clinical interest. We show that in MM, as in normal plasma cell differentiation, there is an intraclonal CD45 hierarchy that is a gradient of CD45 expression on myeloma cells directly related to their proliferation rate and differentiation status. This CD45 hierarchy allows for the design of a cellular model for MM-cell growth and maturation in which CD45 bright myeloma cells represent the proliferating compartment and CD45 low myeloma cells the quiescent compartment. This model includes an aberrant phenotype that is a lack rather than a decline of CD45, lack reflecting the terminal phase of the disease and/or an aggressive presentation of MM. Data from the litterature suggest that CD45 bright myeloma cells are targeted by interleukin (IL)-

6, whereas CD45 negative myeloma cells with a high clonogenic capacity are targeted by insulin/insulin-like growth factor 1 (IGF-1). This model will be useful for both a better understanding of the basic biology of MM and a better stratification and therapeutic approach of the patients. Finally, this model presents MM as a self-renewing plasma cell disease, although the first oncogenic events such as 14q32 translocations clearly occur earlier in a B-cell.

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F3.03

IDENTIFICATION OF INTERCELLULAR COMMUNICATION SIGNALS INVOLVED IN MULTIPLE MYELOMA WITH MICROARRAYS

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Survival and proliferation of multiple myeloma cells (MMC) involve complex interactions of MMC with their bone-marrow environment, in particular with bone cells. To elucidate some of these intercellular communication signals, we have determined the gene expression profile (GEP) of MMC and of the major components of the bone-marrow environment using Affymetrix microarrays. These GEP were compared to those of normal bone marrow plasma cells or of plasmablastic cells.

A hallmark of plasma cells is the expression of syndecan-1, which plays major roles in epithelial cells, in particular as the co-receptor of heparin-binding growth factors. We found that heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a growth factor for malignant plasma cells. HB-EGF gene is mainly expressed by the bone marrow environment. Amphiregulin (AREG) and neuregulins (NEU1-4) are other heparin-binding factors of the EGF family. Using Affymetrix DNA microarrays, we showed that the AREG, NEU2 and NEU3 genes were expressed by purified primary myeloma cells from 65 patients and that the expression was higher than in normal bone-marrow plasma cells or plasmablastic cells. NEU2 or NEU3 recombinant proteins were not available for biological study. AREG stimulated IL-6 production and growth of bone-marrow stromal cells. Using real-time RT-PCR, we found that MM cells expressed ErbB receptors and that AREG could promote their growth. EGF or TGFα, that did not bind syndecan-1 have no myeloma cell growth activity. Furthermore, PD169540 (a pan-ErbB inhibitor) and IRESSA (an ErbB1-specific inhibitor) induced apoptosis of primary myeloma cells from 10/14 and 4/14 patients, respectively, and there was a synergistic effect with dexamethasone. Altogether, these data provide strong evidence for an important role of EGF family members able to bind syndecan-1 in the biology of multiple myeloma and emphasize the advantages of using ErbB inhibitors that might target myeloma cells as well as the tumor environment.

We also found that B-cell activating factor (BAFF) and A Proliferation-Inducing Ligand (APRIL) promote multiple myeloma cell survival and proliferation. The main site of production for BAFF and APRIL is the bone marrow microenvironment. MM cells express the BAFF/APRIL receptor transmembrane activator and CAML interactor (TACI) at varying levels and TACI expression is associated with the presence of a functional BAFF receptor. Affymetrix HG-U133 DNA-microarrays expression data of CD138-purified MMC from 65 newly-diagnosed patients were analyzed by two-sided supervised clustering of groups with higher ($TACI^{high}$) vs. lower TACI ($TACI^{low}$) expression levels. Patients in the $TACI^{low}$ group had worst clinical parameters. A set of 659 genes was differentially expressed between $TACI^{high}$ and $TACI^{low}$ myeloma cells. $TACI^{high}$ myeloma cells displayed a mature plasma cell gene signature, indicating dependence on the bone-marrow environment. Functional categories of genes overexpressed in the $TACI^{high}$ group included chemokine receptor, adhesion molecule, growth factor and transduction signal. In contrast, the $TACI^{low}$ group had a gene signature of plasmablasts suggesting an attenuated dependence on interactions with bone marrow stroma cells. Taken together, our findings foster a clinical use of inhibitors interfering with the BAFF/APRIL-TACI interaction such us the TACI-Fc fusion protein, and at the same time suggest the use of gene expression profiling to identify the group of patients which might benefit most from this kind of treatment.

F3.04

THE FUNCTION AND MOVEMENT OF CD45 MOLECULE IN INTERLEUKIN-6--INDUCED MYELOMA CELL PROLIFERATION

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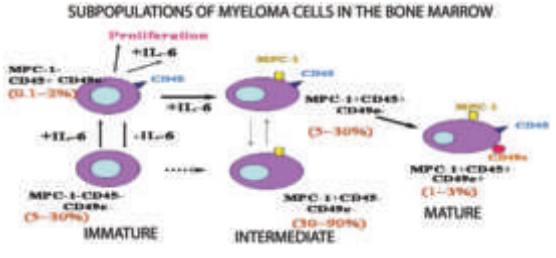
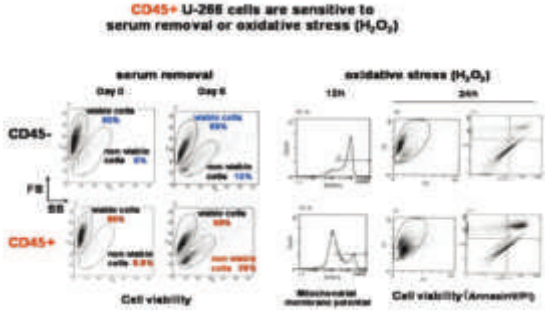
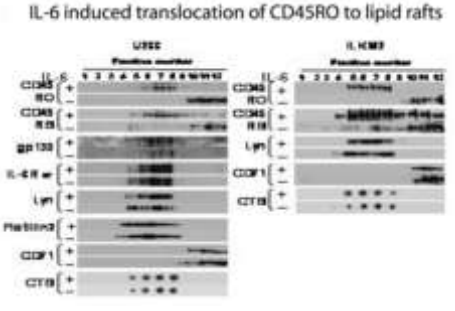
There is marked heterogeneity in the morphology, cytogenetics and phenotype of human myeloma cells. Myeloma cells can be classified into at least 5 subpopulations with respect to the expression of MPC-1, CD49e(VLA-5) and CD45; $MPC\text{-}1^{-}CD45^{+}CD49e^{-}$, $MPC\text{-}1^{-}CD45^{-}CD49e^{-}$ immature myeloma cells, $MPC\text{-}1^{+}CD45^{-}CD49e^{-}$, $MPC\text{-}1^{+}CD45^{+}$ $CD49e^{-}$ intermediate myeloma cells and $MPC\text{-}1^{+}CD45^{+}$ $CD49e^{+}$ mature myeloma cells are found in the bone marrow of most myeloma patients, roughly at 0.1~2%, 5-30%, 30-90%, 5-30%, and 1-3%, respectively. Only $CD45^{+}$ immature myeloma cells can respond directly to IL-6 to proliferate in primary myeloma cells as well as myeloma cell lines. Also, $MPC\text{-}1^{-}CD45^{-}CD49e^{-}$ immature myeloma cells sorted from bone marrow samples as well as $CD45^{-}$ U-266 cell lines can be changed to $CD45^{+}$ cells by addition of IL-6 *in vitro*. In both $CD45^{-}$ and $CD45^{+}$ U-266 cells, STAT3 and MAPK (ERK1/2) can be activated in response to IL-6 equally between them, but src family kinases such as Lyn and Fyn can be activated only in $CD45^{+}$ U-266 cells. Thus, the activation of the src family kinases associated with CD45 expression is a prerequisite for the proliferation of myeloma cells. From PCR-based cDNA subtraction assay and microarray screening, we found that the expression of VDAC(voltage dependent anion channel)-1 gene was increased in $CD45^{+}$ U266 cells. $CD45^{-}$ U266 cells are more sensitive to stress stimuli such as H_2O_2 and thapsigargin treatment through more increased release of cytochrome C and augmented activity of caspases 3 and 9. Furthermore, the transfectants of VDAC-1 gene showed more sensitivity to these

stress stimulations than mock transfectants in U-266 cells. Therefore, we suppose that CD45^{+} immature myeloma cells can proliferate with the increased amount of IL-6 in the bone marrow of human myelomas, but the amount of increased IL-6 is not usually enough for the expanded immature myeloma cells to proliferate further and survive. CD45^{+} immature cells might be converted into CD45^{-} myeloma cells, because CD45^{-} cells are more resistant to stress conditions, escaping from apoptosis. The expression of CD45 molecule on the myeloma cells could contribute to the expansion of population size of myeloma cells along with the amount of IL-6. CD45, a receptor-type tyrosine phosphatase, is essentially required for IL-6-induced proliferation in human myeloma cells, which express CD45RO, but not CD45RA. We found that IL-6-induced CD45 translocation to lipid rafts in isoform different manners by sucrose density gradient centrifugation and confocal microscopy. In myeloma cells, CD45RO could translocate to lipid rafts more rapidly than CD45RB, but exogenously expressed CD45RA was unable to translocate. When the IL-6Rα-transfected B-cell line was sitmulated with IL-6, CD45RA could not translocate despite CD45RB being able to do so.







We further confirmed that the translocated CD45 bound to IL-6Rα, Lyn and flotillin-2, followed by dephosphotrylating negative regulatory Tyr507 of Lyn. Therefore, these indicate that a rapid translocation of CD45RO to lipid rafts may be responsible for IL-6-induced proliferation, and that the isoform change from CD45RA to CD45RO gives human myeloma cells the ability to respond to IL-6.

In conclusion, CD45 molecule is essentially required for IL-6-induced proliferation of myeloma cells, and isoform change of CD45 molecule is also critical to IL-6 signaling; a rapid translocation of CD45RO, but not CD45RA to lipid rafts is important for IL-6 signal transduction. In view of the therapeutic strategy in multiple myeloma, it may be useful to prevent translocation of CD45, especially CD45RO, to lipid rafts in IL-6 stimulation of myeloma cells.

F3.05

IN MULTIPLE MYELOMA 14q32 TRANSLOCATIONS ARE PRESENT IN MEMORY B CELLS BUT RAS MUTATIONS ARE RESTRICTED TO THE PLASMA CELL COMPARTMENT

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The target cell that accumulates the genetic events necessary for the pathogenesis of multiple myeloma (MM) is, at present, unknown. The aim of this study was to investigate whether clonotypic memory B-cells have accumulated genetic alterations identical to their corresponding MM plasma cells (PC). We isolated cells with a PC phenotype (CD38^{++} CD19^{-}CD45^{-}/iCD56$^{-/+/++}$) or a memory B-cell phenotype (CD38-19^{+}27^{+}) from patients with MM or MGUS. We confirmed that the memory B-cell population was not contaminated with PC. As a result, we were able to identify memory B cells expressing MM specific oncogenes associated with 14q32 translocations (FGFR3; IgH-MMSET; cyclin D1) identical to the autologous MM PC in 4/4 myeloma and 2/3 MGUS (monoclonal gammopathy of undetermined significance) patients. For most patients, the oncogene expressing memory B cells comprised <1% of the memory B-cell compartment. To investigate the presence of RAS mutations in memory B-cells we FACS-sorted a total of 22,800 memory B cells from 6 MM patients all having K-RAS61 mutations in their PC. All cells were analyzed using a sensitive allele-specific competitive blocker-PCR assay for detection of K-RAS61 mutations. RAS mutations were absent in memory B-cells from all six MM patients.

These results show that the clonal hierarchy in MM includes memory B cells harboring 14q32 translocations. However, the memory B cells lack transforming RAS mutations, showing that these cells are premalignant remnants of the clone giving rise to MM. Thus, it seems unlikely that cells in the memory B-cell compartment are directly involved in maintaining the malignant MM PC clone.

PLENARY SESSION 4
AUTOLOGOUS TRANSPLANTATION

PL4.01

MAINTENANCE TREATMENT WITH THALIDOMIDE AND PAMIDRONATE AFTER AUTOLOGOUS TRANSPLANTATION FOR MYELOMA: SECOND ANALYSIS OF A PROSPECTIVE RANDOMIZED STUDY OF THE *INTERGROUPE FRANCOPHONE DU MYELOME*

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High dose therapy (HDT) supported with autologous stem cell transplantation has been introduced in the management of aggressive myeloma. However, after a single or a double transplantation, almost all patients ultimately relapse. New strategies are required to control the residual disease after HDT. The *Intergroupe Françophone du Myélome* (IFM) initiated a trial designed to evaluate the impact of maintenance treatment with thalidomide and pamidronate after HDT. From April 2000 to October 2003, 1019 myeloma patients at diagnosis under the age of 65 years were enrolled in the IFM 99 protocol; 780 of them, without or with only one adverse prognostic factor (beta-2 microglobulin ≥3 mg/L or deletion of chromosome 13), were enrolled in the IFM 99 02 protocol. They received the following treatment: 1) 3-4 cycles of the VAD regimen; 2) a first autologous transplant prepared with melphalan 140 mg/m^2; 3) a second autologous transplant prepared with melphalan 200 mg/m^2. Patients without progressive disease 2 months after the second transplant were randomized to receive: no maintenance treatment (arm A), maintenance treatment with pamidronate (arm B) or maintenance treatment with thalidomide and pamidronate (arm C). As of October 2004, 588 (75%) have been randomized: 197 in arm A, 194 in arm B and 197 in arm C. thalidomide was found to improve the progression-freesurvival (PFS). Indeed, the 3-year post-randomization probability of PFS was 56% in the thalidomide arm versus 34% in arm A, and 37% in arm B (p<0.01). Pamidronate was found to decrease the incidence of bone events post-transplantation. Indeed, the 3-year cumulative risk of bone events post-randomization was 65% in arm A versus 26% in arm B, and 24% in arm C (p<0.04). With a median follow-up of 3 years from diagnosis, the 3-year overall survival was similar in the 3 treatment groups: 83% in arm A, 78% in arm B, and 78% in arm C (NS). Among the 588 randomized patients, 4 factors were associated with a longer EFS in the multivariate analysis: low beta-2-microglobulin at diagnosis (p<0.001), deletion of chromosome 13 (p<0.02), response at time of randomization (p<0.001), and treatment arm (p<0.02).

In conclusion, the second analysis of the IFM 99 02 trial strongly suggests that: 1) thalidomide is an effective maintenance treatment to prolong the duration after high dose therapy and 2) pamidronate is an effective maintenance therapy to decrease the incidence of bone events after transplantation.

PL4.02

WHAT HAVE THE TRANSPLANT REGISTRIES TAUGHT US?

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The myeloma registry of the European Group for Blood and Marrow Transplantation (EBMT) has collected and registered data on allogeneic and autologous transplants consecutively since 1983. The registry now contains information on more than 15,000 autologous and 1,500 allogeneic transplants in patients with multiple myeloma (MM), performed in more than 500 centers. Time for follow-up is very long, up to more than 17 years. For autologous transplants, overall survival (OS) and progression-free survival (PFS) is only about 5%, 15 years post transplant. At 9-11 years follow-up, there is a tendency for a plateau in PFS at the 10%-level, but this later drops again. Thus, there is no evidence for cure of disease in the whole cohort of autotransplant patients, although results might be better in patients transplanted as part of first-line therapy, which requires further analysis. In the allogeneic transplant group, OS and PFS is 15% and 10%, respectively, at 17 years post-transplant. The curves plateau from 13 years and onwards, indicating that a small proportion of patients might be cured. In the annual updates on registry data performed since 1994, favorable prognostic factors for autologous transplantation have repeatedly been lower age, response to chemotherapy, one line of primary induction treatment, stage I-II at diagnosis and low beta-2-microglobulin at diagnosis. The same factors are associated with a better prognosis also in allogeneic transplantation, with the addition of female recipient gender. Separate registry studies with respect to the importance of donor gender have recently been undertaken.

The registry is now internet-based, and the individual centers are encouraged to enter their data directly into the database. Data can be reported in two different forms: one simpler form – medA – including a limited number of parameters, which allows the analysis of crude survival, transplant-related mortality (TRM) and disease evolution, and one expanded form – medB – which comprises a large number of disease- and transplant-related parameters, enabling detailed analysis with respect to e.g. prognostic and procedural factors. The validity of data is checked by the EBMT Statistics department in Leiden, under the supervision of the registry head-physician. The computerized medB-form can also serve as a standard CRF for prospective study purposes.

In spite of the obvious major drawback of registry studies, i.e patient selection in individual centers, the large EBMTdatabase can serve several purposes:

Continuous monitoring of the transplant activity, including trends in the use of new methods. The increasing use of autologous PBSC grafts during the 1990s, which has now essentially replaced bone marrow in this setting, has been evident in registry data. Furthermore, analysis of allogeneic transplantation with PBSCgrafts has not demonstrated any advantage with respect to survival, TRM or transplant-related complications as compared with bone marrow transplantation. Results from retrospective registry studies, sometimes using case-matching for prognostic factors, have preceded and matched the data from later prospective randomized trials addressing the same issues. Some examples on this are: i) The use of TBI in autologous transplantation, which is associated with poorer survival compared with high-dose

melphalan alone; ii) autologous graft *purging* with positive CD34-selection does not improve survival or freedom from progression compared with unpurged autografts; iii) tandem autologous transplantation improves OS and PFS compared with single transplantation. The benefit and timing of second autotransplants has been further studied in a large registry analysis of 7,452 patients described as being either in a tandem graft program or not, and the conclusion was that in order to induce an improved outcome, the second transplant should be performed before disease progression and within 6-12 months of the first transplant. In a recent retrospective registry the use of allogeneic transplantation with reduced intensity conditioning (RIC) has been studied in 229 patients. OS and PFS at 3 years were 41% and 21%, respectively. TRM at 1 year was 23%. Limited chronic GvHD was associated with better survival compared with no or extensive cGvHD. In 98 patients with no major risk factors (chemoresistant disease, >1 prior transplant and female donor-male recipient) OS at 3 years reached 70%.

Retrospective studies using case matching or other statistical methods to obtain comparable groups. Allogeneic transplantation with full-dose conditioning in 189 patients was compared with an equal number of case-matched autotransplant controls. TRM was significantly higher after allogeneic transplantation – 41% – vs 13% after autografting, while median survival was signficantly poorer, 18 and 34 months in the allo- and autotransplant groups, respectively. In a later study, 339 allogeneic bone marrow transplants performed from 1983 to 1993 were compared with 225 patients allografted during the years 1994 to 1998. TRM was significantly reduced in the latter group, 30% vs 50% in the previously transplanted cohort, which resulted in an improved survival of about 50% at 4 years, vs 30% in the previous group. The impact of interferon maintenance treatment after autologous transplantation was investigated in another study: 473 interferon-treated patients were compared with 419 untreated controls. Specific inclusion criteria were applied, and the results were statistically corrected for some differences in the distribution of prognostic factors. The results demonstrated that OS and PFS were significantly better in the interferon-treated group.

Studies of "rare events". It is essentially not possible to make large prospective studies of rare occurrences because the patient numbers are too limited, and in this setting, analysis of data from a large database has an important function. In a case-matched registry study, 25 myeloma patients transplanted with an identical twin donor was compared to 125 autologous and 125 allogeneic transplants. TRM after syngeneic transplantation was low compared with allografting, relapse rate was significantly lower than in the autotransplant group, and OS and PFS were significantly superior to allogeneic transplantation and tended to be better than after autografting. In a study of autologous transplantation of rare isotype MM, 149 patients with IgD- and 415 with non-secretory (NS) myeloma were analyzed. These patients presented with a higher frequency of stage III disease at diagnosis compared with *common* type myeloma. For IgD myeloma OS was significantly poorer than in *common* type, while there was no difference for NS myeloma. Another recent study addressed the outcome after autologous transplantation in plasma cell leukemia (PLC): 135 patients with PLC were compared with 9,887 patients with MM. The groups did not differ with respect to demographic and prognostic factors, except age where PLC were slightly younger. Survival was significantly inferior in the PLC group, 22 months, compared with 55 months for the MM patients.

To conclude, retrospective registry data have been valuable as a source for several studies, and some results have been later confirmed in prospective randomized trials. The registry investigations of *rare events* would hardly be possible to undertake prospectively due to small patient numbers. Many study results serve as a basis of clinical importance in the daily decision-making for MM patients.

PL4.03

HIGH DOSE THERAPY OPTIONS FOR ELDERLY MYELOMA PATIENTS: RESULTS OF A RANDOMIZED CONTROLLED TRIAL

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Background. Is well established that high-dose therapy is an effective standard treatment for multiple myeloma patients. However, the standard conditioning regimen, melphalan 200 mg/m^2, may be too toxic in elderly patients. At diagnosis, about 70% of patients are older than 65. Evidence that intermediate-dose therapy improves survival is limited, but is very well tolerated in older patients. In a case-matched control analysis, we have shown that melphalan 100 mg/m^2 (MEL100) with ASCT is superior to standard oral melphalan and prednisone (MP) as frontline therapy in patients aged 63-64 years.

Methods. In a multicenter study, 194 newly diagnosed myeloma patients, aged 50-70 were randomized to receive at diagnosis either conventional chemotherapy (6 courses of oral melphalan and prednisone [MP]) or intermediate-dose therapy (2 courses of melphalan at 100 mg/m^2 [MEL100]) with stem cell support. The response was evaluated according to the European Group for Blood and Marrow Transplantation. In the MP arm 99 patients, were enrolled median age 63 (range: 52-70), female sex (45%), stage IIA (32%), IIIA (58%), IIIB (3%); myeloma protein IgG isotype (60%), IgA (27%), Bence Jones proteinuria (11%); median serum β2-microglobulin 2.9 mg/L. In the MEL100 arm 95 patients were enrolled, median age 65 (range: 51-70), female sex (46%), stage IIA (36%), IIIA (56%), IIIB (5%); myeloma protein IgG isotype (67%), IgA (23%), Bence Jones proteinuria (9%); median serum β2-microglobulin 2.9 mg/L.

Results. Response rate was higher after MEL100. The frequency of near complete remission (nCR) was 6% after MP and 25% after MEL100 (p=0.0002). The number of patients who did not respond or with progressive disease was 55%, in the MP group and significantly lower, 26%, in the MEL100 group ($p < 0.05$). At 3 years, MEL100 increased event-free survival (EFS) from 16% to 37% and overall survival (OS) from 62% to 77% ($p < 0.001$). Clinical outcomes were thus stratified by age groups. In patients aged 65 to 70: nCR was 8% after MP and 25% after MEL100 (p=0.05); at 3 years, MEL100 improved EFS from 18% to 31% (p=0 .01) and OS from 58% to 73% (p= 0 .01), Patients aged 65 to 70 had a median OS of 37.2 months (MP) versus 58 months (MEL100). Hematologic toxicity was significantly shorter in the MP group in terms of incidence of neutropenia and thrombocytopenia and transfusion requirements (p<0.0001). Alower incidence of unknown origin fevers, mucositis and at least one organ toxicity was observed in MP group. In patients

aged ≥65 of both groups the incidence of hematologic and extra-hematologic toxicity was similar to that in the entire population.

Conclusions. Intermediate-dose melphalan constitutes a more effective first-line regimen than standard treatment, specifically in those aged ≥65, improving response rate, EFS and OS.

PL4.04

TOTAL THERAPY FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: THE ARKANSAS EXPERIENCE WITH 1000 PATIENTS TREATED WITH TOTAL THERAPY 1, 2 AND 3

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The total therapy (TT) concept was developed in 1989 to advance therapy for multiple myeloma (MM) by systematically incorporating all available active agents into patients' front-line management. With a median follow-up of 11 yrs for TT1 (N=231), ~40% remain alive and ~30% event-free at 10 yr; ~30% of the 40% initially achieving CR remain in continuous CR at 10 yr. An update of a previously published pair-mate comparison with standard SWOG chemotherapy revealed 10 yr rates of event-free survival (EFS) of 24% vs. 7% ($p < 0.0001$) and overall survival (OS) of 35% vs. 13% ($p < 0.0001$). TT2 (N=668) employed intensified induction prior to and introduced consolidation chemotherapy after melphalan 200 mg/m^2 (MEL 200) – based tandem autotransplants, followed by interferon maintenance with added DEX pulsing during the first year. Patients were randomized up-front to receive ± thalidomide (THAL) throughout – those results remain blinded and the reported data concern the entire TT2 population. With a median follow-up of 2.5 yr of alive patients, TT2 produced superior EFS and OS in comparison to TT1 with 5 yr rates of EFS of ~50% vs. ~25% ($p < 0.0001$) and OS of ~70% vs. 60% ($p<0.06$); the 5 yr continuous CR rate was ~60% vs. ~30% ($p<0.0001$). The benefit of TT2 was particularly apparent among the 2/3 of patients lacking cytogenetic abnormalities (CA). Achieving CR at the completion of induction and conversion from CA prior to therapy to a No CA status prior to transplant both extended the post-transplant survival. According to CDR3 PCR analysis, ~80% of those in clinical CR but only ~10% of patients in PR achieved molecular CR. MRI was performed at baseline and serially during the successive phases of TT2: resolution of focal lesions lagged behind M-protein response by ~18 mos; the presence of > 5 focal lesions at baseline was an adverse variable for both EFS and OS, especially in the presence of CA ($p<0.0001$); normalization of MRI abnormalities ("MRI-CR") was an independent favorable parameter for EFS and OS ($p<0.01$). Gene expression profiling (GEP, Affymetrix) was available in 351 patients at baseline: a model was developed that distinguished risk groups with markedly different MM-related death events – 12% among 316 good risk vs. 63% among 35 high risk patients (mainly related to altered expression of chromosome 1 genes and in particular overexpression of the cell cycle promoter gene, CKS1B), accounting for superior 3 yr OS of 85% vs. < 20% ($p<0.000001$. TT3 (N=108) incorporates Velcade (VEL) into the front-line management of MM together with DEX + THAL plus PACE chemotherapy ("VDT-PACE"); 2 such cycles are applied as induction prior to MEL 200 tandem transplant and 2 cycles are administered as consolidation, followed by VDT maintenance; THAL + DEX is also applied in peri-transplant and peri-induction and –consolidation to suppress potential MM survival/proliferation signals produced during hematopoietic recovery. Median times to achieving M-protein reduction by at least 50%, 75%, 99% were 0.5 mos, 1.6 mos and 5 mos and thus significantly shorter than with TT2; the projected incidence of near-CR (only IFE positive) with TT3 is > 80% vs. ~65% with TT2 ($p=0.02$). Thus, these preliminary results suggest that our objective of increasing the frequency CR by assuring a higher tandem transplant completion rate through shortening induction from 4 to 2 cycles of more effective VDT-PACE appears to be attainable. CD34 yields were usually in excess of 20 million cells/kg. Two patients have died so far – 1 from disease progression prior to 1st transplant and 1 from sepsis during THAL + DEX post-transplant #1. Results of ancillary imaging studies (MRI, PET-CT) and of GEP of purified plasma cells and whole biopsies at diagnosis and after a test dose of single agent VEL and 48 hr post VDT-PACE will also be presented. These studies are aimed at predicting clinical outcome and delineate molecular pathways affected and how therapies affect the molecular signaling in the MM-interactive/-growth and survival-supportive bone marrow micro-environment.

PL4.05

IS DOUBLE AUTOGRAFT THE STANDARD OF CARE?

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Eight years ago, the randomized IFM90 trial first demonstrated the superiority of autologous stem cell transplantation (ASCT) compared to conventional chemotherapy in terms of complete remission (CR) rate, event-free survival (EFS) and overall survival (OS).[1] Two recently published randomized studies fully confirm the IFM data[2,3] and ASCT is currently considered as the standard of care at least up to the age of 65. However, with ASCT, median EFS is only 25-30 months and median OS only 55-58 months. Long term remissions are observed only in patients with favorable prognostic characteristics (low β2 microglobulin level and no adverse cytogenetic abnormalities). In order to improve these results, Barlogie *et al.* developped the concept of double ASCT.[4] In newly diagnosed patient, CR rate was 40% and median EFS and OS were respectively 43 months and 68 months. The IFM was again the first to conduct a randomized trial (IFM94) comparing single and double ASCT.[5] In this study, 399 previously untreated patients under the age of 60 years were randomly assigned to receive either a single ASCT prepared by melphalan 140 mg/m^2 (Mel 140) plus total body irradiation (TBI) or a double ASCT, the first being prepared by Mel 140 and the second by Mel 140 plus TBI. On an intention to treat basis, CR or very good partial remission (VGPR) was achieved by 42% of patients in the single ASCT group versus 50% in the double ASCT group ($p=0.1$). The probability of 7-year EFS was 10% versus 20% ($p=0.03$) and the probability of 7-year OS 21% versus 42%.

Four other randomized trials have addressed this issue, but have not yet been fully published.[6-9] The current results

of these 5 trials are presented in the Table below.

			CR rate (%)		EFS (months)		OS (months)	
	nb pts	age	single vs double	*p*	single vs double	*p*	single vs double	*p*
IFM94 (5)	399	< 61	42 vs 50	0.1	25 vs 30	0.03	48 vs 58	0.01
MAG95 (6)	227	< 56	39 vs 37	NS	31 vs 33	NS	49 vs 73	0.14
Bologna (7)	220	< 61	31 vs 43	NS	21 vs 31	0.02	56 vs 60	NS
GMMG (8)	261	< 66	-	-	23 vs NR	0.03	--	
Hovon (9)	303	< 66	13 vs 28	0.002	20 vs 22	0.01	55 vs 50	NS

Thus, in 4 out of 5 randomized trials median EFS was significantly longer after double ASCT. The only published study and the available data from other studies are in favor of double ASCT. However, several issues remain to be addressed.

1) Should double ASCT be offered to all patients ? The use of initial prognostic factors could help in determining which patients benefit more from double ASCT. In the IFM94 trial, double ASCT was superior to single ASCT in prognostic subgroups defined by β2 microglobulin or LDH but, due to a small number of patients in each group, the difference was not statistically significant. The superiority of double ASCT was significant only in patients failing to achieve at least VGPR within 3 months after one ASCT. Therefore in our experience double ASCT should be offered to all patients up to the age of 65 with the exception of patients achieving at least VGPR after the first one.

2) Can results of double ASCT be further improved? In the double ASCT arm of the IFM94 trial, median EFS was only 30 months and the probability of 7-year EFS was only 20%. Moreover, patients with poor initial prognosis (high β2 microglobulin and adverse cytogenetic abnormalities) have a poor outcome even after double ASCT. Strategies to further improve results of double ASCT are clearly warranted. One way is to further increase dose intensity. Barlogie and colleagues have reported impressive results with a more dose-intensive regimen termed *Total Therapy II*.[10] A recent analysis of 462 patients included in the Total Therapy II program compared to 231 patients treated with the previous *Total Therapy I* protocol showed a higher CR rate (66% versus 43%) and an increased 4-year EFS rate. This improvement was more marked for patients without cytogenetic abnormalities (4-year EFS 70% vs 37%). In the IFM 99-04 for poor prognosis patients (high β2 microglobulin level and chromosome 13 deletion by FISH), the first ASCT was prepared by melphalan 200 mg/m^2 and the dose of melphalan was escalated to 220 mg/m^2 before the second ASCT. The preliminary results are very encouraging: CR + VGPR 59%, median EFS 30 months. The introduction of novel agents is another attractive option and we already have results with Thalidomide given as maintenance therapy after double ASCT. The IFM99-02 trial shows a significant prolongation of EFS in patients with standard risk with a combination of thalidomide plus pamidronate (median EFS not reached at 3 years) compared to observation or Pamidronate only (< 30% EFS at 3 years).

3) Could treatments including novel agents replace double ASCT? The IFM99-06 compares MP, MP plus thalidomide (MPT) and double ASCT prepared by intermediate dose melphalan (100 mg/m^2) in patients aged 65-75. Preliminary results show no difference in the response rate between MPT and double ASCT (CR + VGPR 65% vs 57%, CR 14% vs 18%).

However, until now there is no available comparaison of conventional chemotherapy plus novel agents and double ASCT with high-dose melphalan.

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FOCUS SESSION 4
BONE DISEASE IN MYELOMA

F4.01
PATHOGENESIS OF MYELOMA BONE DISEASE

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Bone destruction is a frequent and major source of morbidity in patients with myeloma (MM). Bone lesions occur in up to 80% of patients. Symptoms due to bone destruction in MM include bone pain, pathologic fractures and hypercalcemia.[1] In advanced MM, normal bone remodelling is uncoupled with bone destruction no longer linked to new bone formation.[2] Even in the early stages of MM, the increase in osteoblast numbers that occurs in response to the increased bone resorption is not associated with a relative increase in osteoblast activity leading to net bone loss. In advanced MM, osteoblast function and number are markedly decreased or absent. Studies over the last several years have identified several factors that play a critical role in the bone destruction process. These include RANKL, MIP-1α, and IL-3,[1] which are stimulators of osteoclast (OCL) formation. RANK ligand is produced by marrow stromal cells when myeloma cells find them. RANKL is a potent inducer of OCL formation. In addition, levels of osteoprotegrin, a decoy receptor that inhibits the effects of RANKL, are also decreased in MM. We, and others, have found that MIP-1α and IL-3 levels are increased in marrow plasma of 70% of MM patients. MIP-1α and IL-3 can directly induce OCL formation and can also enhance the effects of RANKL. In contrast, the basis for the decreased osteoblast activity in MM has not been clearly defined. Tian and coworkers[3] found that MM cells produced DKK1, an inhibitor of the WNT signaling pathway that is critical to osteoblast differentiation. DKK1 levels in the marrow correlated with the extent of bone disease in MM patients and DKK1 inhibited osteoblast differentiation *in vitro*. In addition, Oyajobi *et al.* (*American Society of Bone and Mineral Research, Seattle, WA, 2004*) have reported that MM cells increase DDK1 production by marrow stromal cells. These results suggest that MM cells can both produce and induce an inhibitior of osteoblast differentiation. Our laboratory has recently shown that IL-3 can also inhibit osteoblast differentiation as well as induce OCL formation in MM.[5] IL-3 acts indirectly via CD45$^+$ CD11b$^+$ monocyte-macrophages to suppress osteoblast differentiation. Thus, there are multiple stimulators of OCL activity and inhibitors of osteoblast differentiation in MM. These factors in combination result in the purely lytic process that occurs in MM with no new bone formation to repair the

destructive lesions. These combined effects are responsible for the extreme severity of the bone disease in myeloma.

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F4.02

OSTEOBLAST FUNCTION IN MYELOMA (ENDOTHELIN 1, OSTEOBLAST PROTEASOME)

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Our understanding of the interactions that occur between myeloma cells and the bone marrow microenvironment has improved dramatically in recent years. Myeloma cells thrive in bone for reasons that are not entirely clear, but probably involve direct cell-cell interactions that occur in that site between myeloma cells and other cells, including stromal cells, bone cells, and osteoclasts. As a consequence of these interactions, myeloma cells change their gene expression patterns - in fact, their phenotype - and express proteins that increase bone resorption and inhibit bone formation. These dual effects are responsible for the subsequent characteristic bone disease. Much attention has been focused on identification of the factors involved. These have proven elusive, in part because they are dependent on the cell-cell interactions that occur at the bone-marrow interface: these are not easily replicated *in vitro.* Recent data suggest several conclusions:[1] cell-cell interactions involving VCAM-1 and $\alpha 4\beta 1$ integrin are important,[2] myeloma cells and stromal cells in bone express RANKL and this is an essential component for subsequent osteoclast activation,[3] myeloma cells also produce the chemokine MIP1 which stimulates osteoclasts to resorb bone[4] stromal cells, in the presence of myeloma cells, produce the endogenous inhibitor of the WNT/β-catenin pathway DKK-1. DKK-1 is a powerful inhibitor of osteoblast differentiation and thus bone formation. The proteasome inhibitor Velcade is having striking success in the treatment of myeloma. It causes apoptosis in myeloma cells, presumably because of its effects of inhibitiny NF-κB. However, it likely has additional effects not previously anticipated. Velcade, like other proteasome inhibitors, stimulates osteoblast differentiation and bone formation. It does this by increasing transcription of the bone growth regulatory factor BMP-2, which causes osteoblast differentiation and bone formation. Proteasome inhibitors have this effect in part because they alter expression levels of the hedgehog signaling molecules Gli2 and Gli3, causing impaired degradation of Gli2 and blocking formation of a truncated form of Gli3 which acts as a transcriptional repressor of BMP-2.

These findings have important implications for the treatment of myeloma and its associated bone disease. They raise the possibility that a single treatment affects both neoplastic cells and host cells to decrease tumor burden and repair the associated osteolysis. Experiments to test this notion are currently in progress.

F4.03

BISPHOSPHONATES AND THE BONE MARROW MICROENVIRONMENT

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Multiple myeloma is associated with the development of a devastating bone disease characterized by bone pain and an increased likelihood of fracture. Bone destruction is mediated by increased osteoclastic bone resorption, which results from increased osteoclast recruitment and differentiation, and/or activity. Inhibiting osteoclastic bone resorption represents an important approach to managing the bone disease associated with multiple myeloma. Currently the only established approach to inhibiting bone resorption in multiple myeloma is with bisphosphonates. These compounds inhibit farnesyl diphosphate synthase, which prevents protein prenylation and causes osteoclast apoptosis. These compounds have been shown to be effective in treating myeloma bone disease and in certain studies it has been suggested they may be associated with an increase in survival. These observations have stimulated considerable interest in bisphosphonates and raised the possibility that they may have an additional anti-myeloma effect beyond their effect on osteoclasts. The mechanism for any anti-myeloma is unclear but may reflect a direct effect on the biology of the myeloma cells or an indirect effect either via the inhibition of osteoclastic bone resorption or via alternative cell types or pathways in the bone marrow microenvironment. *In vitro,* studies have demonstrated that bisphosphonates can inhibit myeloma cell proliferation and promote apoptosis directly via an inhibition of the mevalonate pathway. Studies have also demonstrated that these compounds can inhibit tumor cell adhesion and invasion and block proteinase activity. However, it remains unclear whether these direct effects are important *in vivo.*

In order to begin to address these issues a number of studies have now been performed in murine models of myeloma bone disease. For example zoledronic acid has been studied in both the 5T2MM murine model of myeloma and the SCID/Hu model.[1,2] In each case zoledronic acid administration, initiated at the time of myeloma cell injection or following myeloma cell establishment, resulted in a profound inhibition of myeloma bone disease. In these studies treatment was also associated with a reduction in serum paraprotein and tumor burden, and an increase in survival. Pamidronate has also been shown to prevent the development of bone disease and reduce serum paraprotein in the SCID/Hu model. In contrast, studies have demonstrated that while ibandronate is effective in preventing the development of bone disease in the ARH-77 and 5TGM1 models it has little effect on serum paraprotein.[3,4] The reasons for the differences between studies are unclear but may reflect subtle differences in the mechanism of action of these bisphosphonates. Alternatively, this may reflect the choice of animal models. The 5T2MM and SCID/Hu models are microenvironment dependent models in that myeloma cells are largely confined to bone, not found in other organs and do not grow *in vitro.* In contrast the ARH-77 and 5TGM1 models are microenvironment independent models in that

although they also grow in bone they grow *in vitro* and hence are not necessarily dependent upon the bone, marrow microenvironment for their survival. This may suggest that by inhibiting osteclastic bone resorption bisphosphonates remove an important source of local growth factors and cytokines that support the growth and survival of myeloma cells. In support of this, other agents that target osteoclastic bone resorption, such as Fc.OPG are also able to slow myeloma growth in bone.[5,6]

An alternative explanation is that bisphosphonates affect other cells in the bone marrow microenvironment which then indirectly effect the growth and survival of myeloma cells. Treatment of mice bearing 5T2MM cells was associated with a decrease in micro-vessel density suggesting that zoledronate influences angiogenesis. In support of this zoledronate has been shown to inhibit endothelial cells proliferation and inhibits angiogenesis in other *in vivo* models.[7] However, in the 5T2MM it is not clear whether this is the result of a direct effect on angiogenesis or via an effect on factors released during bone resorption.

In addition, studies have shown that bisphophosphonates can promote an increase in γδT cell population, which may promote an anti-myeloma effect.[8] It was proposed that this may be via a direct effect of nitrogen-containing bisphosphonates on T cells. However, there are alternative explanations such as the inhibition of FPP synthase in peripheral blood mononuclear cells or tumor cells,[9,10] which would result in accumulation of upstream components of the pathway such as isopenteny diphosphate. This molecule has also been shown to promote expansion of γδT cells *in vitro.*

Taken together there are increasing data to suggest that bisphophonates can alter the bone marrow microevironment making it less favorable for the growth and survival of myeloma cells. Whether this is through the result of inhibition of bone resorption and/or effects on angiogenesis and γδT cells is unclear. The challenge will be to dissect these mechanisms and exploit this understanding to increase the anti-tumor effect of bisphosphonates.

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F4.04

MOUSE MODELS OF TUMOR MICROENVIRONMENT AND BONE DISEASE

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Myeloma is characterized by the clonal expansion of plasma cells, whose growth and dissemination is typically restricted to the bone marrow. Myeloma is frequently (in about 80% of patients) associated with osteolysis, resulting from increased osteoclast activity and decreased osteoblast activity. Change in the rate of bone turnover precedes progression from MGUS to symptomatic myeloma.[1] Myeloma is also associated with neo-angiogenesis, and higher microvessel density (MVD) is associated with poor prognosis.[2] The dependence of myeloma on the bone marrow microenvironment, initially gleaned form the growth and dissemination of the disease, finds support from clinical observations: treatment of myeloma patients with inhibitors of osteoclast activity (e.g., pamidronate)[3,4] and anti-angiogenic agents (e.g., thalidomide)[5-7] often has clear anti-myeloma activity. Investigators have used several mouse models to study the relationship between myeloma and the bone marrow microenvironment: The 5TMM murine myeloma model, originally developed by Radl,[8] and its variants,[9–11] and models in which human myeloma cells or cell lines grow in immune-deficient mouse strains (SCID and others).[12-16] The SCID-hu model in which myeloma cell lines[17] or primary myeloma cells[18] grow in a human bone implanted into a SCID mouse is unique among the SCID mouse-base models. Although several of the mentioned models reflect the complete dependence of myeloma cells on the bone marrow microenvironment for survival and growth, this discussion will concentrate on observations from the SCID-hu model for primary myeloma and their confirmation *in vitro*; this is the only model that can captures the heterogeneity of myeloma cells from patient to patient, and hence identify differences as well as common features in the requirements of myeloma cells for survival and growth. In this model, growth of myeloma cells from all patients depended absolutely on the human bone microenvironment.[19] However, in contrast to cells from patients with classical myeloma that grew and disseminated exclusively in the human bone marrow, cells from patients with extramedullary disease grew also on the outer surfaces of the human bone. Growth of myeloma was associated with the typical changes in the bone marrow microenvironment; increased microvessel density (MVD), loss of osteoblast activity and increased osteoclast activity, which resulted in osteolysis that was restricted to the human bones. We examined whether myeloma cells depended on the changes they induced in the bone microenvironment, by measuring the effects of anti-angiogenic and anti-osteoclastic agents on their respective targets and on the myeloma cells. Thalidomide, when activated by human liver implants, had profound anti-myeloma activity in >85% of experiments, with corresponding reductions in MVD.[20] The more specific anti-angiogenic agent endostatin had anti-myeloma activity in 50% of experiments, also with corresponding reductions in MVD.[21] In both cases, it was not possible to discern whether the anti-angiogenic effect was the cause for reduction in tumor burden or its result, although endostatin not directly affecting myeloma cell survival *ex-vivo* would indicate that in responding experiments the myeloma cell required angiogenesis for survival. Inhibitors of osteoclast activity (bisphosphonates, RANK-Fc, OPG) effectively prevented oste-

olysis. Inhibition of osteoclast activity and formation was associated with profound anti-myeloma effects, but only in experiments in which myeloma cells from patients with classical myeloma that grow only in the bone marrow were used. Inhibition of osteoclast activity had no effect on the growth of extramedullary myeloma.[22,23] These and similar observations in the 5T2 model[24,25] indicate that medullary myeloma requires osteoclast activity for survival, in contrast to extramedullary myeloma that is biologically different and is not affected by inhibition of osteoclasts. The relationship between myeloma cells and osteoclasts was further investigated *in vitro*: freshly purified primary myeloma cells attracted osteoclast precursors and induced their differentiation to mature, functional osteoclasts, in a RANKL-dependent process. Mature osteoclasts, in turn, supported long term survival of myeloma cells, and allowed them to maintain their low proliferative rate.[22,26] Both myeloma cell-induced osteoclast differentiation and osteoclast support of myeloma cell survival required contact between myeloma cells and the osteoclasts or precursors. This apparent interdependence of myeloma cells and osteoclasts may explain the large number of osteoclasts present around foci of myeloma cells and the associated lytic bone lesions. Changes in myeloma cell gene expression as a consequence of interaction with osteoclasts divided myeloma cells into 2 groups; one group with many genes commonly changed, resembling changes observed in normal plasma cells, and the other containing cells from extramedullary disease and myeloma cell lines with only a few genes commonly changed, indicating their reduced dependence on osteoclasts for survival ref 27. Proteomics analysis identified that osteoclast mediated survival of myeloma cells was associated with upregulation of soluble chondroitin synthase-1. Notch2 activation and upregulation of serglycin were identified as potential targets for chondroitin synthase activity. The interactions between myeloma cells and osteoblasts appear more complex. Whereas in patients and in the models, growth of myeloma is associated with elimination of mature osteoblasts, myeloma cells do not affect osteoblast differentiation or survival *in vitro*. Osteoblasts had varied effects on survival and proliferation of myeloma cells.

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PLENARY SESSION 5
NEW THERAPEUTIC AGENTS

PL5.01

BORTEZOMIB – FIRST IN THE CLASS OF PROTEASOME INHIBITORS AND AN IMPORTANT ADVANCE IN THE TREATMENT OF MULTIPLE MYELOMA

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Bortezomib (VELCADE®, formerly known as PS-341) is a selective, reversible proteasome inhibitor that represents a significant advance in the treatment of multiple myeloma (MM). The proteasome is a multicatalytic complex responsible for the regulated degradation of ubiquitinated intracellular proteins. With proteasome inhibition, targeted proteolysis is disrupted, resulting in downregulation of survival signaling pathways. The effects of bortezomib have been most extensively studied in MM. Following *in vitro* exposure of MM cells to bortezomib, the pro-apoptotic molecules caspase-3, -8, and -9 are activated, DNA repair is inhibited, and the tumor suppressor protein p53 and inhibitor-κB (I-κB) are stabilized. Persistence of I-κB prevents activation of nuclear factor (NF)-κB, a transcription factor critical to MM cell survival. NF-κB increases MM cell survival by conferring chemoresistance and promoting cell-stromal interactions such as regulation of MM cell adhesion–induced IL-6 transcription. Expression of VCAM1 and ICAM1 is decreased with bortezomib, resulting in aberrant stromal interactions and altered signaling cascades. The end result of bortezomib exposure is increased chemosensitivity, decreased chemoresistance, and apoptosis. Importantly, *in vitro* studies demonstrated additive antimyeloma activity with the combination of bortezomib plus dexamethasone (dex), and the ability of bortezomib to overcome chemoresistance to doxorubicin, melphalan, and dex.

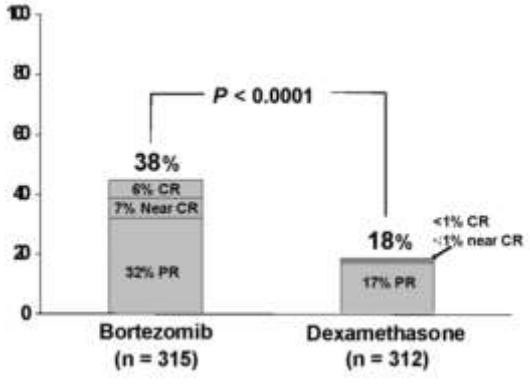
These preclinical data provided the rationale for the clinical development of bortezomib. In a phase 1 dose-escalation study in patients (pts) with advanced hematologic malignancies, antitumor activity was observed in 9 of 9 pts with plasma cell dyscrasias, including 1 CR. Toxicities were manageable, and phase 2 development with an initial focus on MM proceeded.

SUMMIT was a phase 2 study conducted in 202 heavily pretreated pts with relapsed and refractory MM. All pts received bortezomib 1.3 mg/m^2 by IV bolus on days 1, 4, 8, and 11 of a 21-day cycle for up to 8 cycles. Dex 20 mg po

was administered to pts with progressive disease after 2 cycles or stable disease after 4 cycles. Responses were graded according to the criteria of the European Group for Blood and Marrow Transplantation and confirmed by an Independent Review Committee. The response rate to bortezomib alone was 35% (CR + PR + MR), and an additional 24% responded with stable disease. The median number of prior lines of therapy was 6, and importantly, 89% of pts with CR had been refractory to their prior treatment. Responses were durable: a median duration of response of 12.7 mo was observed after extended follow-up of 23 mo (median).

In a second phase 2 study, CREST, 54 pts with MM who had relapsed or been refractory to front-line therapy were randomized to receive bortezomib 1.0 or 1.3 mg/m^2, using the same regimen as SUMMIT with the same dex option and response criteria. In the 1.0 mg/m^2 dose group, the rate of response (CR + PR + MR) to bortezomib alone was 33%, and it was 44% for bortezomib ± dex. In the higher-dose group, the response rates were 50% and 62%, respectively. Responses were also durable: 9.5 mo in the lower-dose group and 13.7 mo in the higher-dose group after treatment with bortezomib ± dex.

Toxicities with bortezomib in the phase 2 studies were manageable. Peripheral neuropathy was the most clinically significant event (grade 3 in 12% of pts in SUMMIT[9]); however, ≥ grade 3 neuropathic symptoms and those resulting in discontinuation resolved or improved during the follow-up period in the majority (71%) of pts in phase 2 trials. Thrombocytopenia was the most common ≥ grade 3 event in phase 2 trials; it was cyclical, with platelet counts decreasing during the treatment phase and recovering toward baseline during the rest phase of each cycle. Continuation or retreatment with bortezomib was offered in phase 2 trials to pts with the potential to benefit from extended therapy. A median of 7 additional cycles was received by 63 pts. The safety profile was similar to that of the phase 2 trials, with no evidence of cumulative toxicity.



APEX, a pivotal phase 3 international trial, randomized 669 pts with relapsed MM to either bortezomib or high-dose dex. Bortezomib was administered for the first 8 cycles using the same dose and schedule as SUMMIT, followed by once-weekly treatment for the first 4 wks of a 5-wk cycle ≥ 3 cycles. Dex 40 mg po was administered on days 1-4, 9-12, and 17-20 for four 5-wk cycles, then on days 1-4 for five 4-wk cycles. At the preplanned interim analysis, bortezomib demonstrated a statistically significant benefit in time to progression (TTP) and survival versus dex, and the data monitoring committee decided to allow all pts in the dex arm to receive bortezomib. At the final analysis, bortezomib provided a significant 78% improvement in median TTP (6.2 mo vs 3.5 mo) and significantly higher response rates (Figure) and survival benefits. Importantly, the subset of pts receiving bortezomib as second-line treatment received significant benefit as well; thus, treatment of MM at first relapse with bortezomib may be considered an effective therapeutic option.

Clinical trials assessing the benefit of bortezomib as front-line therapy and in combination with other therapeutic agents are ongoing in pts with MM. Proteasome inhibition, unknown as a therapeutic intervention only 5 years ago, has emerged as a novel approach to treating MM and has demonstrated promising activity in other tumor types

PL5.02

LENALIDOMIDE (CC-5013, REVLIMID™) AND OTHER IMIDS

D Weber

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Based on the encouraging activity of thalidomide in multiple myeloma,[1] immunomodulatory derivatives (ImiDs) have been developed in an attempt to avoid troubling side effects of thalidomide such as neuropathy and thrombosis. *In vitro*, the immunomodulatory derivatives inhibit angiogenesis, activate caspase 8, enhance Fas-induced apoptosis and may down-regulate NFK-B activity.[2,3] They also increase natural killer and T-cell numbers, block secretion of cell growth and migration factors like TNF-α, IL-6, and VEGF and have effects on adhesion molecules.[2,3]

Based on this promising activity of the ImiDs *in vitro*, phase I and II studies of CC-5013 (Revlimid) and phase I studies of CC-4047 (Actimid) have been initiated and subsequently reported, confirming the promising activity of these agents in patients with relapsed or refractory myeloma.

Richardson *et al.* treated 26 patients with relapsing or refractory myeloma in a phase I study of CC-5013 at 4 dose levels (5 mg, 10 mg, 25, and 50 mg). Of 24 patients considered eligible for response, a ≥ 50% reduction in paraprotein was noted in 7 patients (29%), and 17 (71%) patients had a ≥ 25% reduction in paraprotein.[4]Responses were noted at all dose levels and grade 3 hematologic toxicity was noted in 3 patients resulting in study termination. No dose limiting toxicity was noted in the first 28 days. An additional 10 patients were treated with the 50 mg/d dose and among 12 of 13 patients who continued at this dose, grade 3-4 myelosuppression was noted in all beyond 28 days. No recurrence was noted after dose reduction to 25 mg/d, thus it was concluded that the MTD was 25 mg/d.

A similar schedule of CC-5013 has been investigated in another phase I study where a ≥50% reduction in paraprotein was noted in 3 patients (30%), all treated at doses of 25 mg or above[5]. A 50% reduction of platelet count was noted in 5 of 6 patients treated for a prolonged period of time and thromboembolism was noted in 1 patient. Neither study revealed any significant somnolence, constipation or neuropathy.

On the basis of these results a randomized phase II study of CC-5013 at a dose of 30 mg po daily or 15 mg po bid was performed. Patients were treated on day 1-21, and subsequent courses began on day 28[6]. After 60 patients were enrolled, a preliminary analysis showed increased myelosuppression, despite similar response rates, and the 15mg bid arm was closed and an additional 30 patients were added

to the 30 mg qd arm to further define the efficacy of daily dosing. Among 83 patients evaluable for response, CR was noted in 6%, 50-99% M protein reduction (PR) in 18%, 25-49% M protein reduction in 14%, while 47% had disease that remained stable and 14% demonstrated disease progression (PD). Dexamethasone was added at 4 weeks for patients with PD and at 8 weeks for SD. Among these 30 patients, 33% achieved at least a PR.[6]

Based on these encouraging results a phase II trial of CC-5013 with dexamethasone for previously untreated patients with multiple myeloma has been initiated.[7] Preliminary results in 13 patients reveal at least PR (50% reduction of serum M-protein, 90% reduction in urine BJP) in 85% of patients. Accrual of 31 patients is planned.

Results of a phase I study with another immunomodulatory derivative of thalidomide, CC-4047 (Actimid) have also been recently reported.[8] Patients were treated with daily oral doses of either 1, 2, 5, or 10 mg for 4 weeks. Neutropenia was the major dose limiting toxicity (14/24 patients with grade 3-4 neutropenia) and the maximum tolerated dose was 2 mg. Deep venous thrombosis was noted in 4 patients (most likely unrelated in 1 patient). CR was noted in 16%, PR in 38%, MR in 17%, stable disease in 25% and the median event free overall survivals were 28 and 90 weeks, respectively.[8]

The ImiDs appear to have significant activity in myeloma and reduced non-hematologic toxicity compared with thalidomide. Hematologic toxicity appears more severe than with thalidomide, although it also appears easily manageable. Although these drugs, activity as single agents is promising, these agents are likely to be most useful in combination in the future and are important new additions to the list of active agents for the treatment of multiple myeloma.

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PL5.03

PEGYLATED DOXORUBICIN IN COMBINATION WITH IMMUNE-MODULATORS AND ARSENIC-CONTAINING REGIMENS FOR THE MANAGEMENT OF MULTIPLE MYELOMA

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Plasma cell dyscrasia is a spectrum of diseases that is centered on a malignant clone of plasma cells. Multiple myeloma is one of the variants where the malignant clone results in end organ damage and eventually the patient succumbs to the complications of the disease. The bone marrow of multiple myeloma patients usually shows high number of angiogenic vessels. Pegylated doxorubicin has the advantage of being able to extravasate through abnormal bone marrow angiogenic vessels thus exposing the malignant cells to high concentrations of the anthracyclines. Another advantage of the compound is its long half life which exposes the malignant plasma cells to high concentrations of the anthracycline for a lengthier period of time as compared to the non pegylated compounds resulting in a theoretical by higher kill. DVd (doxil, vincristine and reduced schedule decadron) is an effective and well-tolerated regimen in newly diagnosed MM pts with a response rate of 88%, yet CR and Near CR (NCR) rates are low at < 20%. In the (relapsed/refractory multiple myeloma) Rmm pts the overall response rate is 22% and NCR is <5%. DVd significantly reduces abnormal angiogenic activity in the treated patients; however this finding does not have an impact on PFS. Thalidomide (T) the first of the immunomodulatory class has shown activity in MM pts. Biologically T has a direct anti-myeloma effect in addition to its ability to modulate integrins, rendering the myeloma cell vulnerable and sensitized to different chemotherapeutic agents. T as a single agent or in combination with steroids results in CR+NCR rates of < 20%. We evaluated the role of T in combination with DVd in Nmm & Rmm pts with the primary objective of improving the response rate, quality of response and maintain the anti-angiogenic activity achieved with the DVd regimen. SWOG criteria were used to assess response, and NCR was defined as a decrease of the M-protein by >90%. Overall, both groups received a median of 6 cycles of therapy. Following an increased incidence of neutropenia, infections, paraesthesia and DVT in the first 11 Nmm and 20 Rmm pts; the protocol was amended to initiate pts on prophylactic amoxicillin 250 mg BID, acyclovir 400 mg BID ASA 81 mg/d. GM-CSF or G-CSF was given if the total WBC was less than 5000/L on day 1 of therapy and a more aggressive V dose reduction schema was adopted. Following these amendments the incidence of pneumonia dropped from 8 cases in the Rmm to none in the next 74 enrolled patients, and no neutropenia requiring therapy was reported in any of the groups. DVT was reduced from an overall rate of 56% to 13% (excluding the patients who discontinued aspirin). The overall CR/ NCR rates were virtually identical for both Nmm (46%) and Rmm, pts (47%), as was the time to best response (median of 4.2 months for both groups). Stable disease or better occurred in 84% and 89% of the Nmm and Rmm respectively. DVd in combination with T and the appropriate supportive care measures resulted in a high response rate as well as an improved quality of response similar to what is achieved with high dose therapy. With these results in mind we decided to pursue studying other immune-modulators with DVd. Revlimid (R), the second

generation of this group of agents, is 50 to 2000 times more potent than T in stimulating T-cell proliferation triggered via the T-cell receptor, and 50 to 100 times more potent than T in augmenting IL-2 and IFN-α production with a lesser side effect profile. We therefore initiated a phase I/II trial to define the MTD of R in combination with DVd, then we proceeded to expand the MTD dose level to evaluate the efficacy and safety of the combination in patients with Rmm. DVd was administered in the same fashion as previously described, with a standard phase 1 dose escalation of R to identify the MTD. All patients received amoxicillin, acyclovir and aspirin 81mg prophylactically. Twenty-five pts Rmm pts are enrolled with 75% of those refractory to active therapy. The DLT was sepsis/septic shock that occurred at dose level 3 (R 15mg) with two of the patients developing non neutropenic sepsis. The MTD for R was defined at 10mg. Three patients started therapy with a neutrophil count < 500/ÌL and or platelet counts < 50k/μL; all 3 patients were responders. There was one grade 4 hyper-coagulation event in the form of a PE that has recovered. In the expanded cohort there were 2 grade 3 neutropenias & one grade 3 neuropathy requiring dose reductions of R and D in each pt with resolution of the neutropenia and neuropathy. Three of 25 (12%) patients achieved CR, 5/25 (25%) NCR. All CR+NCR patients (33%) are refractory patients. All patients except for 4 achieved > 25% reduction of the M-protein after one cycle of therapy and 3/4 after 2 cycles. R at 10 mg is the MTD in combination with the DVd in RMM. DVd-R is an extremely effective regimen with a SWOG response rate >66%, CR+NCR of >33% in refractory stage 3 patients with minimal toxicity.

In view of the complex support system for MM the development of agents interfering with different aspects of the tumor microenvironment is critical in the management of the disease. Arsenic trioxide, an anti-tumor agent with a multifaceted mechanism of action, induces apoptosis in *vitro* in MM cell lines and freshly isolated cells from MM patients and, in phase 2 clinical trials as a single agent displays clinical activity in patients with advanced stage MM. *In vitro* studies have shown that ATO sensitizes myeloma cells to Dex, and AA potentiates the effect of ATO, a phase II trial combining ATO with Dex and AA was initiated to investigate short and long-term responses as well as tolerance to therapy. Twenty evaluable RMM pts were treated with the combination of ATO, Dex and AA. Following induction therapy patients achieving SD or better and entering a plateau phase, patients were maintained on 5 weeks of ATO-AA followed by a 2 months break with steroids given 4 days of each month. Six pts achieved NCR+PR (by SWOG criteria), 10 pts stable disease and 4 experienced progressive disease. Median survival for the 14 patients alive is 18.3 months (2.5-24.2). The regimen was generally well tolerated with one patient each experiencing grade 3 hyperglycemia, headaches, burning at IV site, neutropenia, dehydration, syncope, or fatigue. One patient experienced a grade 4 painful neuropathy and was taken off study with the event resolving in 4 weeks without any specific therapy. The long-term duration of the responses as well as the ability of most of the patients to tolerate this long-term therapy regimen (1.9 years) is encouraging. Currently several groups are investigating the role of ATO in combination therapy as a chemotherapeutic sensitizer as well as an agent that could be used in combination with immunomodulatory agents to take advantage of the non-overlapping mechanisms of action allowing for a better quality response and more importantly prolonged plateau phase.

In summary the availability of new chemotherapeutic delivery systems allowing for exposing the tumor cells to high concentrations of the active agent for a lengthier period of time could result in a higher tumor kill. The addition of immunomodulatory agents that modulates the multiple myeloma microenvironment thus sensitizing the malignant cells to chemotherapy and maintaining the biological effects achieved by the chemotherapy has resulted in a significant improvement in patients outcome.

PL5.04

PHASE I STUDY OF THE SAFETY AND EFFICACY OF BORTEZOMIB (VELCADE) IN COMBINATION WITH CC-5013 (REVLIMID) IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA (MM): THE REVVEL STUDY

PG Richardson,[1] R Schlossman,[1] N Munshi,[1] D Avigan,[1] S Jagannath,[2] M Alsina,[3] D Doss,[1] K Colson,[1] M McKenney,[1] K Hande,[1] S Gorelik,[1] C McAlister,[1] A Freeman,[1] D Warren,[1] C Mitsiades,[1] T Hideshima,[1] RR Michelle,[1] D Collins,[4] K Balinski,[5] C Byrne,[5] C Chrystal,[5] T Myers,[4] E Trehu,[4] R Knight,[5] D Schenkein[4]

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Introduction. Bortezomib and CC-5013 are novel agents with activity in relapsed and refractory multiple myeloma (MM). Both agents have unique mechanisms of action and directly promote apoptosis of MM cells, inhibit adhesion of MM to bone marrow stromal cells, inhibit cytokines important for MM cell growth and survival, are antiangiogenic, and have immunomodulatory effects. Bortezomib has achieved response rates of 35% in relapsed and refractory patients (pts), with a median duration of response and overall survival of 14 and 18 mo, respectively. CC-5013 has induced durable responses with >50% reductions in M-protein seen in about 30% of pts with relapsed and relapsed refractory MM, including pts who had received prior bortezomib, in phase 1 and 2 clinical trials. *In vitro,* CC-5013 potentiates the apoptotic effect of bortezomib in MM.1S cells but clinically is not associated with significant peripheral neuropathy, which is dose limiting for bortezomib. These data suggest that combination treatment may result in greater efficacy in MM.

Methods. Pts are being enrolled in cohorts of three to one of eight dose levels to determine a maximal tolerated dose (MTD) of the combination. At the MTD, an additional cohort of 10 pts will be enrolled to further evaluate response to treatment. Doses of bortezomib are 1.0 or 1.3 mg/m^2 on days 1, 4, 8, and 11, with a 10-day rest. CC-5013 doses range from 5-20 mg/day po on days 1-14. Each cycle is 21 days. Toxicity is assessed according to NCI CTC criteria version 3.0. Dose-limiting toxicity (DLT) is defined as any grade (G) 3 or greater non hematologic toxicity and any G4 hematologic toxicity, with G4 neutropenia lasting > 5 days and/or neutropenic fever and platelets <10k on more than one occasion despite transfusion. Efficacy assessments are performed at the end of cycle 2 and at each subsequent cycle. Response is defined according to the Bladé criteria.

Results. Enrollment is proceeding in the dose-escalation portion of the study. Nine pts with relapsed and refractory MM have been enrolled and 6 treated to date. Of these 6 pts, 2 were relapsed and 4 were relapsed and refractory, with a median number of 3 prior therapies (range, 1-6). Three pts had undergone prior SCT; 5 had received prior thalidomide, and 2 prior bortezomib. With a median of 2 cycles com-

pleted, pts have tolerated bortezomib at 1.0 mg/m^2 and 1.3 mg/m^2 and 5 mg/day of CC-5013 without DLT and only one episode of G3 neutropenia, but no other significant toxicities have been reported. Six pts are currently evaluable for response, with stable disease observed in 4 pts and minor response in 2 pts.

Conclusions. Bortezomib and CC-5013 are novel agents that can be combined at active doses. Further investigation is ongoing to determine MTD and activity in relapsed and relapsed, refractory MM.

PL5.05

OVERVIEW OF NEW THERAPIES AND FUTURE DIRECTIONS

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We are now poised to use genomics and proteomics to characterize progression from normal to MGUS to MM, to identify novel treatment targets, to classify MM, to identify targets in individual patients, and to design clinical protocols of targeted therapies. First, gene profiling demonstrates modulations of transcripts associated with MGUS and MM versus normal individuals, as well as correlating with progression from MGUS to MM.[1] Second, array CGH identifies unique amplifications (i.e., chromosome 1q21) and deletions in the MM genome and then validated novel genetic targets.[2] Third, genetic profiling provides the basis for a novel molecular classification of MM,[3] Fourth, genomics and proteomic studies can identify unique targets for combination therapies of individual patients.[4] Fifth, genomics and proteomics can provide the framework for designing clinical protocols to enhance cytotoxicity and avoid the emergence of drug resistance. The agents to be combined include: 1. drugs targeting the MM cell and its BM microenvironment (proteasome inhibitors and immunomodulatory drugs,[5,6] histone deacetylase inhibitors,[7] and VEGF inhibitors;[8,9] 2. drugs targeting MM cells at the cell surface (IGF-1R tyrosine kinase inhibitor and CD40 antibody)[10,11] cytoplasm (hsp 90 inhibitors),[12] mitochondria,[13] and nucleus (telomerase inhibitor);[14] and 3. drugs targeting the BM microenvironment (p38MAPK and IKK inhibitors).[15,16] For example, gene profiling has shown that bortezomib induces hsp 90, and that combined therapy with bortezomib and hsp 90 inhibitor 17AAG markedly augments cytotoxicity.[17] Gene profiling showed that hsp 27 transcripts to be induced when MM patients become resistant to bortezomib; this observation, coupled with the known role for upstream p38MAPK regulating hsp 27, provided the framework for using inhibitors of p38MAPK to abrogate hsp 27 expression and thereby restore sensitivity of MM cells to bortezomib *in vitro*.[18] Proteomic studies show that bortezomib inhibits DNA repair,[19] providing the preclinical rationale for trials combining bortezomib with Doxil or melphalan. Our cell signaling data shows that thalidomide and revlimid trigger caspase 8, and dexamethasone triggers caspase 9 mediated apoptosis,[20] providing the basis for combining these agents to activate dual apoptotic signaling. bortezomib (caspase 9 and 8) has similarly been coupled with revlimid (caspase 8).[20] Early studies are rationally combining three novel agents. For example, we have combined proteasome inhibitor Bortezomib with hsp 90 inhibitor 17AAG, since hsp 90 is required for the unfolding of misfolded proteins and their subsequent binding to the 20S proteasome core and degradation. The histone deacetylase 6 inhibitor tubacin binds polyubiquinated misfolded proteins and facilitates their transport to aggresomes, another mechanism for their degradation.[21] Our preliminary *in vitro* studies therefore have combined bortezomib, hsp 90 inhibitor 17AAG, and tubacin together to target and inhibit breakdown of misfolded proteins at multiple levels, thereby markedly enhancing MM cell cytotoxicity *in vitro.* Since it is not possible to evaluate active agents in all combinations, these studies are central and required if we are to translate science to the bedside and rapidly identify the most clinically active combined regimens.

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PLENARY SESSION 6
TUMOR MICROENVIRONMENT AND ANGIOGENESIS

PL6.01

INFLUENCE OF THE TUMOR MICROENVIRONMENT ON DRUG RESPONSE AND DRUG RESISTANCE IN MULTIPLE MYELOMA

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The acquisition of drug resistance continues to limit the clinical success of treatment for multiple myeloma.[1] Often, acquired drug resistance is manifested by multi-factorial resistant mechanisms and reversal is very difficult.[2,3] Because of the complexity of acquired drug resistance, we and others, have explored mechanisms contributing to *de novo* resistance. By definition de-novo resistance is present prior to drug exposure and selection for drug resistance. Mechanisms associated with *de novo* drug resistance may contribute to the failure to eliminate minimal residual disease and facilitate the emergence of acquired drug resistance. We propose that targeting *de novo* resistance could enhance the efficacy of currently used drugs and reduce the probability of the emergence of acquired clinical drug resistance. Specifically, we have shown that cell adhesion of myeloma cell lines, as well as primary patient specimens, via β1 integrins causes resistance to a wide variety of cytotoxics including alkylating agents.[3-8] We have referred to the phenomenon as cell adhesion mediated drug resistance or CAM-DR.

We recently showed that the cell adhesion conferred resistance to melphalan-induced depolarization of mitochondria membrane potential despite similar numbers of melphalan induced interstrand crosslinks (ICL).[3] Together, these results suggests that adhesion increases the tolerance for melphalan induced ICL. The mitochondrial membrane potential is largely regulated by BCL-2 family members, and thus we asked whether changes in either the levels of pro- or anti- BCL-2 family members contributed to the resistance phenotype. Microarray analysis revealed a modest 1.4 fold reduction in the pro-apoptotic BH3 only BCL-2 family member Bim. We confirmed this finding at the protein level showing that adhesion of 8226 cells to FN resulted in a pronounced reduction in Bim protein levels.[3] We hypothesize that the reduction in Bim levels contributes to drug resistance, and we are currently validating this target in patients who are being treated with chemotherapy, including melphalan.

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PL6.02

ANGIOGENESIS IN MYELOMA - AN OVERVIEW

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Increased tumor angiogenesis has been associated with disease progression and poor prognosis in a variety of hematological malignancies and solid tumors. Angiogenesis is a striking characteristic of multiple myeloma (MM), both in focal osteolytic lesions and in diffusely infiltrated bone marrow. We and others have shown that the increased angiogenesis seen in MM has prognostic importance, and is correlated with disease activity, bone marrow plasma cell involvement, and plasma cell proliferative capacity. The formation of new blood vessels is induced by the malignant plasma cells and may contribute to disease progression in two ways: (i) by ensuring an adequate tumor nutrient supply and (ii) by paracrine stimulation of tumor growth. Myeloma angiogenesis is therefore an important subject for investigation and an appealing target for novel therapeutic agents. We have previously demonstrated a gradual increase in degree of bone marrow angiogenesis along the disease spectrum from monoclonal gammopathy of undetermined significance (MGUS) to smoldering myeloma (SMM) to newly diagnosed myeloma (NMM) and relapsed myeloma (RMM). In a study of 400 patients, the median microvessel density (MVD) was 1.3 (range, 0-11) in controls, 1.7 (0-10) in amyloidosis, 3 (0-23) in MGUS, 4 (1-30) in SMM, 11 (1-48) in NMM, and 20 (6-47) in RMM, $p<0.001$. Sixty-one per-

cent of NMM bone marrow plasma samples stimulated angiogenesis in an *in vitro* angiogenesis assay, compared to 0% of SMM and 75 of MGUS, $p<0.001$. Increased MVD in MM does not regress following conventional dose or high dose chemotherapy, but there is some evidence supporting regression in responders to thalidomide therapy. VEGF, bFGF, VEGFR1, VEGFR2, FGFR2 and FGFR3 are expressed by plasma cells in most patients with MGUS, SMM and MM. However, there was no significant difference in this expression between the 3 groups. More recently we have demonstrated using sensitive flow cytometric assays that cytoplasmic VEGF expression is significantly higher in MM compared to SMM/MGUS/AL. On the other hand, surface VEGF expression which is likely related to density of VEGF receptors on the cell surface was confined to the $CD45^+$ plasma cell compartment in MM, SMM and MGUS, with a trend to higher percent positive cells in the MM group compared to MGUS. Nevertheless VEGF/VEGFR expression alone does not seem to be the major explanation for differences in angiogenesis between MM and more indolent/benign plasma cell disorders such as MGUS and SMM. As in other malignancies, the induction of angiogenesis in MM involves alteration in the levels of pro- and anti-angiogenic stimuli. Recently, we have described loss of angiogenesis inhibitory activity with disease progression from MGUS to MM, which may in part account for the increase in angiogenesis in MM. In an *in vitro* human angiogenesis assay, 63% of MGUS bone marrow plasma samples inhibited angiogenesis, compared to SMM (43%) and NMM (4%), $p<0.001$. The inhibitory activity was heat stable, not overcome by addition of VEGF. We are presently conducting further studies to characterize the inhibitory activity.

PL6.03

NEW ANTI-ANGIOGENIC AGENTS

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Based on our observations that patients with increased micro-vessel density in the bone marrow had an inferior outcome, a trial with single agent thalidomide - the only commercially available agent at that time with proven anti-angiogenic activity - was started in 1998 and included 169 patients. The large majority of these patients had relapsed after autotransplantation and had abnormal metaphase cytogenetics. One third of such patients achieved a partial remission. Some patients had their best response ever on thalidomide. Not unexpectedly, the median event-free survival in this group of heavily pretreated patients was only 10 months, but surprisingly, at 4 years after the start of thalidomide, 20% of patients still had not shown any evidence of disease progression. Multivariate analysis showed that the presence of abnormal cytogenetics and high β2 microglobulin levels (>3 mg/L) prior to thalidomide were the most important factors associated with a poor outcome. These are the same factors also predicting for a poor outcome after high dose chemotherapy.

A thalidomide derivative IMID (revlimid) was shown to have much more potent anti-neoplastic activity *in vitro* and a more favorable toxicity profile in animals. In a dose escalating study we found that the maximal tolerated daily dose was 25 mg. The dose-limiting toxicity was thrombocytopenia. We then performed a randomized study comparing 25 mg/day x 20 days to 50mg/day x 10 days every 28 days. The majority of these patients (65%) were over the age of 60 and 90% had relapsed after autotransplantation. A total of 98 patients were enrolled. The event-free and overall survival at 12 months was 43% and 66%, respectively. No significant difference in outcome was observed with the two dose schedules. The presence of abnormal cytogenetics pre-study was not associated with inferior outcome. The most common toxicity was ≥ grade III thrombocytopenia, occurring in 77% of patients with a pre study platelet count < 100,000, vs. 43% in those with a pre study platelet count ≥ 100,000. Based on the apparent non-cross resistance of velcade and thalidomide, a phase I-II trial was initiated with velcade, thalidomide and dexamethasone (VTD). In the first cohort of patients a dose of 1 mg/m^2 of velcade was given on days 1, 4, 8 and 11 with a dose escalation of thalidomide from 50 to 100 to 150 to 200 mg daily. At least 10 patients were enrolled at each dose level of thalidomide. In the second cohort, velcade was given at a dose of 1.3 mg/m^2 with the same dose escalation of thalidomide. All patients had advanced and refractory myeloma; 80% had cytogenetic abnormalities and 80% had a prior autotransplant. A total of 79 patients have been enrolled so far. Maximal response was observed after 3 cycles of therapy: 40% achieved at least a PR with approximately half of these patients showing a ≥90% reduction in M protein. An additional 20% had a ≥25% M protein reduction. At this time no difference in outcome is observed according to velcade or thalidomide dose. The median event-free and overall survival is 7 and 21 months, respectively. On multivariate analysis a long interval (>5 years) between first treatment for myeloma and start of VTD was associated with a better outcome (HR 0.4; $p=0.03$), while treatment with thalidomide prior to VTD resulted in an inferior outcome (HR 4.1; $p=0.05$).

Gene expression profiling performed before and 48 hours after the combination of velcade with thalidomide showed an almost complete normalization of the micro-environment signature after 48 hours of treatment. This observation forms the basis for combining VTD with cytotoxic chemotherapy in our ongoing Total Therapy III trial for recently diagnosed patients, targeting not only myeloma cells, but also the micro-environment. An update of these studies will be provided during the meeting.

PL6.04

TARGETING OF VASCULAR ENDOTHELIAL AND PLATELET DERIVED GROWTH FACTOR SIGNALING BY RECEPTOR TYROSINE KINASE INHIBITORS IMPAIRS MYELOMA CELL-INDUCED NEOVASCULARIZATION IN THE 5TMM MOUSE MODEL

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A central issue in the biology of multiple myeloma (MM) is the complex interactive network between the tumor cells and the microenvironment, in which endothelial cells (EC) represent an important cellular component. Indeed MM-EC interactions do not only mediate the extravasation and homing of the tumor cells but result also in bone marrow neovascularization. We could demonstrate that MM cells are responsive to various chemotactic signals like IGF-1, laminin-1 and MCP-1, which are all produced by EC. Moreover EC have the potential to up-regulate the expression of receptors for some of these molecules (IGF-1R and 67 kD laminin receptor).[1, 2] We also found that EC can directly stimulate the transendothelial invasion capacity of MM cells

through up-regulation of the basement membrane degrading protease, MMP-9.[3,4] On the other hand, MM cells have the capacity to activate EC resulting in bone marrow neovascularisation. We used murine 5TMM models to study some aspects of myeloma cell induced-angiogenesis in an *in vivo* setting. We first showed that the murine 5T multiple myeloma models are able to induce angiogenesis by determining a tumur-related increase in bone marrow micro vessel density (MVD)[5]. Using the 5T2MM model we could analyze angiogenesis during preclinical myeloma stages. Histological analysis and assessment of MVD by CD31 staining demonstrated a preangiogenic stage of small tumour aggregates followed by an angiogenic switch and subsequently an angiogenic stage of progressive tumor growth and large, confluent tumor nodules. This angiogeneic switch was found to be associated with a change in the dominating tumor-phenotype, from $CD45^+$ MM to $CD45^-$ MM cells and an upregulation of VEGF production[6]. Different angiogeneic molecules, including VEGF, bFGF, angiopoitin-1 and HGF have been reported to be produced by MM cells. Among these factors, VEGF-A has been studied most in detail but it is not clear yet at which level this molecule is also the major mediator of MM cell induced angiogenesis *in vivo*. VEGF-A binds with high-affinity to its receptors, VEGFR-1 and VEGFR-2, which belong to a family of signaling molecules acting downstream have activated receptor tyrosine kinases (RTK). Another member of this family is the receptor for platelet-derived growth-factor (PDGF), a molecule that has also the potential to trigger EC activity. We could demonstrate that MM cells (human as well as murine 5TMM cells) express different PDGF transcripts. We performed a study in the 5T33MM model using different receptor tyrosine kinase (RTK) inhibitors to explore the functional involvement of the receptor tyrosine kinases VEGFR-1, VEGFR-2 and PDGFR-β in the myeloma cell induced neovascularization. Mice were injected with 5T33MM cells and received daily doses of one of the four compounds: SU10944 and STI-571 (Glivec) with selective activity against VEGF- and PDGFR-receptors, respectively, and the multi-targeted RTK inhibitors, SU6668 (against VEGF-, PDGF- and FGF-receptors) and SU11657 (against VEGF- and PDGF-receptors). We could conclude that specific blocking of VEGF- or PDGF-receptors induced a highly significant reduction in the 5T33MM-increased MVD ($p<0.001$ Mann-Whitney test). Treatment of 5T33MM bearing mice with the RTK inhibitor that targets simultaneously VEGF- and PDGF-receptors (SU11657) reduced the MVD to a level that was not significanty different from that observed in naïve mice. These data indicate that tumor cell- induced VEGF- and PDGF-receptor signaling in endothelial cells represent the most important mechanisms that underlie the angiogenesis process in MM. Using the 5T2MM model, a highly significant increase in survival ($p=0.0001$ log-ranck test) could be observed in animals that were treated with the same inhibitor, indicating that inhibiting the phosphorylation of these RTK might be an efficient tool to control tumour expansion in this malignancy.

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PL6.05

MOLECULAR MECHANISMS INVOLVED IN HOMING AND MIGRATION OF PLASMA CELLS IN RESPONSE TO CXCR4 STIMULATION AND DOWNSTREAM ACTIVATION OF THE PI3K PATHWAY

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Malignant plasma cells characteristically home to the bone marrow (BM). However, the mechanisms by which cells are recruited into and mobilized from the bone marrow into the peripheral blood (PB) are not well understood. In this study, we explored the molecular mechanisms involved in homing and migration of plasma cells in response to CXCR4 and investigated the role of the PI3K pathway in migration of MM cells in response to SDF-1. CXCR4 surface expression was determined by FACS analysis (PE CXCR4, Pharmingen) using samples from bone marrow and peripheral blood of patients diagnosed with multiple myeloma (MM), monoclonal gammopathy of undetermined significance (MGUS) and primary systemic amyloidosis (AL). All results were expressed as percent expression in gated $CD38^+$ and $CD45^+$ cells. Boyden chamber *in vitro* migration assays were performed using Kas6/1MM cells. In addition, live confocal microscopy was used to visualize changes in the subcellular location of YFP-fluorescent CXCR4 before and after SDF-1 stimulation. Cells were pretreated with 10mM LY294002 or 200nM rapamycin to assess the effect of inhibition of PI3K or mTOR on CXCR4 subcellular localization. Immunoblotting was performed to confirm inhibition of the PI3K pathway. Comparisons between groups was performed using Mann-Whitney testing. There was a significant difference between expression of BM CXCR4 in MM and MGUS ($p=0.02$). SDF-1 induced dose dependent migration of Kas 6/1 cells indicating a functional CXCR4 receptor. MM cells transfected with YFP-CXCR4 demonstrated surface localization on the cells. Three dimensional and continuous live imaging after SDF-1 stimulation for 30 minutes demonstrated alterations in the CXCR4-YFP leading to its capping, internalization subcellularly, and production of pseudopodia in response to SDF-1. This process was abrogated by pretreatment with LY294002 and rapamycin. These data demonstrate for the first time that the surface expression of CXCR4 is markedly elevated in the peripheral blood as compared to the bone marrow. Once in the bone marrow, and with the presence of excess SDF-1, the receptor becomes internalized and downregulated. In contrast to MM, CXCR4 expression on plasma cells in the bone marrow of normal and MGUS patients was higher. In addition, we demonstrate that the process of CXCR4 subcellular localization is abrogated by the administration of PI3K inhibitors LY294002 and rapamycin. These data suggest that CXCR4 expression is required for MM cells to circulate, and that downregulation occurs in the BM leading to immobilization of the cells. Future clinical trials using CXCR4 inhibitors, or inhibitors of PI3K and mTOR to prevent the homing and migration of MM cells into the bone marrow may be explored.

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FOCUS SESSION 6
WALDENSTRÖM'S SYMPOSIUM AND MYELOMA VARIANTS

F6.01

MECHANISMS OF ONCOGENESIS IN WALDENSTRÖM'S MACROGLOBULINEMIA

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Waldenström's macroglobulinemia (WM) is characterized by lymphoplasmacytic lymphoma and IgM monoclonal gammopathy. It accounts for 2% of hematologic malignancies with a median survival of five years. About 50% of patients have predominant, well differentiated lymphocytes while 40% of them have more plasmacytic morphology. The majority of malignant cells in WM express the B cell marker CD20, sIgM and sIgD. Our work shows that WM cells may arise from an unusual IgH VH3-expressing B cell population, usually but not always with a somatically mutated VH segment.[1] At diagnosis and in relapse, the frequency of clonotypic B cells is high in both BM and blood. In periods of disease characterized by low serum monoclonal IgM, the frequency of circulating clonotypic B cells is substantially decreased, even though the aggregate number of $CD20^+$ B cells, shown to be polyclonal, is often significantly elevated.[1]

Hyaluronan synthase1 (HAS1) synthesizes hyaluronan (HA). HA is known to be important in cancer for signaling, migration and spread. We have shown that HAS1 is overexpressed, and undergoes aberrant intronic splicing in WM. HAS1 gene products and altered localization of HA may lead to aggressive malignancy. We have recently identified three splice variants of HAS1 expressed in WM. For all three variants, the start codon and the entire sequence of the enzymatically active intracellular loop are present in the aligned cDNA sequences obtained from CD20+ B cells, suggesting that they retain the ability to synthesize HA. Although alternative splicing is a normal event contributing to protein diversity in humans, more than a dozen human cancers are associated with abnormalities in alternative splicing, particularly when intronic sequences are abnormally retained in the transcript. One cause of aberrant splicing is genetic variation (mutation and/or SNP) in or near splice donor and/or acceptor sites and cis-splicing elements (exonic and intronic splicing inhancer (ESE, ESS), splicing branch point and polypyrimidine tracts within introns, the consequences of which are exon skipping and/or intron retention in the transcript. In this context, we have identified germline SNP homozygosity in the HAS1 gene that characterizes >90% of WM patients, suggesting that SNP haplotype homozygosity in HAS1 may confer a predisposition to paraproteinemia. HAS1 homozygosity in WM (p=0.0001) is significantly more frequent than in healthy donors. We have sequenced selected HAS1 exons and introns from three WM patients to identify novel genetic variations. It is likely that aberrant splicing of HAS1 results from activation of cryptic splice sites, which lead to exon skipping and/or intron retention. The aberrant HAS1 splice variants identified here may promote malignant cell migration, enhance drug resistance and may contribute to genetic instability in WM.

Our working hypothesis predicts that in WM, genetic variation in the AS1 gene promotes alternative HAS1 splicing, leading to intronic HAS1 splice variants, and intracellular synthesis of HA by HAS1Vb. HAS1 and HAS1 variants may synergize with the RHAMM oncogene to promote the emergence of increasingly aggressive disease in WM. Overexpression of HAS1 and/or HAS1 variants may help to determine the ultimate balance between apoptosis/death or viability and clonal emergence. HAS1 thus represents a new type of marker that reflects biologically important properties of a malignant clone as it undergoes stepwise oncogenesis and/or disease progression. Since the HAS1 variants appear to be absent from healthy cells, they may present valuable clinical targets for development of new therapeutics that are highly selective for malignant cells.

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F6.02

NOVEL INSIGHTS INTO THE BIOLOGY AND THERAPY OF WALDENSTRÖM'S MACROGLOBULINEMIA

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In an effort to delineate mechanisms which permit the growth and survival of lymphoplasmacytic cells (LPC) in WM, we performed studies addressing the genetic basis, molecular pathogenesis and microenvironmental support in WM. A strong familial predisposition in WM was observed may among 257 consecutive patients with the consensus panel definition of WM, with 1:5 patients having a first degree relative with WM or a related B-cell disorder. Extensive cytogenetic analysis including BAC-FISH analysis demonstrated losses in 6q21-23 as the most commonly recurring cytogenetic abnormality, irrespective of familial predisposition. Moreover, by RT-PCR analysis we demonstrated loss of expression for BLIMP1, a master regulatory gene found on 6q21 which supports LPC differentiation. These studies also identified gross abnormalities in other genes (BCL6, PAX5, XBP1) regulating LPC differentiation. We therefore sought to clarify the role of bone marrow mast cells (BMMC), which are found in excess in patients with WM. These studies demonstrated that MC lines and MC from WM patients stimulated expansion of WM LPC. Importantly, MC widely expressed several ligands capable of supporting LPC expansion including CD40 ligand (CD40L), whose inhibition blocked in a dose dependent manner MC stimulated LPC expansion. These studies therefore demonstrate that BMMC support the growth of WM tumor cells, and therefore represent a novel therapeutic target for the treatment of WM.

F6.03

TREATMENT OF WALDENSTRÖM'S MACROGLOBULINEMIA

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Waldenström's macroglobulinemia (WM) is a distinct clinicopathological entity and its diagnosis is confined to patients with bone marrow infiltration by small lymphocytes showing evidence of plasmacytoid/plasma cell differentiation who also have serum IgM monoclonal protein. Most patients with WM present with clinical manifestations which are related to direct tumor infiltration of various organs and tissues and to the amount and specific properties of monoclonal IgM. However, some patients are being diagnosed by chance and do not have symptoms and signs attributable to the disease. Such asymptomatic patients should be followed without treatment until there is evidence of disease progression because they may remain stable for several months or years. The three main choices for frontline treatment of symptomatic patients with WM are alkylating agents, purine nucleoside analogs and the anti-CD20 monoclonal antibody rituximab. Despite the lack of prospective randomized trials, a rational selection of primary therapy for patients with WM can be made if one takes into consideration factors such as age, presence of co-morbid conditions, presence of cytopenias (thrombocytopenia in particular), need for rapid disease control and candidacy for high-dose therapy with autologous stem cell transplantation (ASCT).

The alkylating agent most commonly used has been oral chlorambucil. Approximately 50% of patients achieve a partial response, the time to response is slow and several months are needed to determine the chemosensitivity of the disease. The optimal duration of chlorambucil administration has not been defined and most clinicians give the drug for one or two years. Prolonged exposure to chlorambucil may impair stem cell collection. This agent may be the primary treatment of choice for elderly patients who do not require rapid disease control and who present without significant cytopenias.

The purine nucleoside analogs fludarabine and cladribine are active in WM. Responses occur in 40% to 80% of previously untreated patients and in most series the median time to response is 2 months. Thus, nucleoside analogs are particularly useful in patients requiring rapid disease control. The administration of these agents should be restricted to four courses or less in order to avoid stem cell toxicity and to reduce the incidence of opportunistic infections.

Rituximab is active in approximately one-third of previously untreated patients. Time to response is slow and exceeds 3 months on the average. Treatment with rituximab is well tolerated and myelosuppression is negligible. This agent may represent the treatment of choice for patients who present with significant cytopenia and for patients who are candidate for stem cell collection. Several phase II studies indicate that combinations of rituximab with nucleoside analogs and/or with alkylating agents may have a synergistic effect.

Data are accumulating which indicate that high-dose therapy with ASCT is feasible, safe and is associated with significant cytoreduction even in patients with chemoresistant disease. Prognostic models need to be developed which will identify at diagnosis patients with impaired prognosis who will be candidates for trials which include high-dose therapy early in the course of the disease.

New agents such as thalidomide, revlimid, bortezomib, oblimersen, etc. are under investigation in patients with advanced WM. There is preliminary evidence of activity but their exact place in the management of WM remains to be determined. Advances in the understanding of the biology of the disease will help us to develop more rational treatments and targeted therapies for WM.

F6.04

AMYLOIDOSIS

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Systemic AL amyloidosis is a disorder of protein folding in which certain monoclonal immunoglobulin light chains are transformed into and deposited as AL amyloid fibrils. These amyloid deposits progressively disrupt the normal structure and function of tissues throughout the body, potentially leading to multiple organ failure. Without treatment, AL amyloidosis is often fatal within 2 years. The monoclonal plasma cell disorders that underlie AL amyloidosis are usually subtle and not proliferating, but some patients do have concurrent multiple myeloma or other B-cell malignancies. Conversely, up to 10% of patients with multiple myeloma have some AL amyloid deposits that can be identified histologically, but which are insignificant clinically; this can only be differentiated after comprehensive evaluation. Frequent manifestations of AL amyloidosis include renal dysfunction, cardiomyopathy, neuropathy and hepatomegaly. The diagnosis of amyloid requires histological confirmation, followed by immunohistochemical staining to characterize the fibril protein, although the latter is non-confirmatory in up to 50% of cases with AL type. However immunohistochemistry can usually exclude AA amyloidosis, and DNA analysis may be required to exclude hereditary forms of amyloid which are now known to account for 5-10% of cases of apparently sporadic non-AA amyloidosis. The mere presence of a monoclonal gammopathy in a patient with amyloidosis may be incidental and gravely misleading. AL amyloidosis should be monitored and treatment guided by frequent quantitation of serum free light chains, and, where available, serial SAP scintigraphy to determine the extent and distribution of the amyloid deposits, along with clinical and laboratory measurements of associated organ dysfunction. Chemotherapy regimens in AL amyloidosis are derived from those used in multiple myeloma, but there have been no comparative trials in AL. An important objective in amyloid is to suppress production of amyloidogenic light chains rapidly, which infusional and high dose regimens achieve with broadly similar effect. However, PBSCT has TRM of 15-40% in AL amyloid and it should be restricted to selected patients, or used second or third-line. TRM associated with PBSCT for AL is lower when performed in centers with experience of its challenges in this particular disease. In patients who are sufficiently fit, VAD is a rational initial therapy, whereas monthly i.v. melphalan 25 mg/m^2 ± Dex is an alternative when VAD is contra-indicated, or has proved ineffective. PBSC collection should be considered before proceeding with IDM. Other treatment options include oral MP, dexamethasone, thalidomide based regimens, solid organ transplantation and palliative care. Regression of amyloid and clinical improvement following chemotherapy in AL amyloidosis is always delayed for many months following adequate suppression of the underlying clonal disease, and treat-

ment strategies in individual patients are presently best guided by their early effect on quantitative measurements of circulating free immunoglobulin light chains. Novel therapies directed specifically towards amyloid deposits and their precursor proteins are under development, and some of these are already being tested in patients.

F6.05

CRYOGLOBULINEMIA, POEMS AND GAMMOPATHY-ASSOCIATED NEUROPATHY

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Neuropathies due to monoclonal gammopathies are not common. The differential diagnosis when seeing a patient with a monoclonal protein and a neuropathy includes MGUS-associated neuropathy, amyloidosis, cryoglobulinemia, and POEMS (osteosclerotic myeloma). Among 66 patients with cryoglobulinemia seen at the Mayo Clinic between March 1994 and November 27, 2000, 18% had peripheral neuropathy as a major clinical manifestation. Among 46 patients who received any treatment 27 or 59% achieved no response or had disease progression requiring a change in therapy. Monoclonal gammopathy-associated neuropathy is most commonly associated with IgM monoclonal proteins that are found to have activity against the myelin-associated glycoprotein. Therapies over the years have been many, reflecting the poor outcome associated with each and have included total plasma exchange, interferon, immunoglobulin infusion, as well as symptomatic therapy with amitriptyline and gabapentin. Recently rituximab has been attempted to manage this syndrome, with mixed reports of benefit. The POEMS syndrome is a specific disorder that is associated with monoclonal proteins of the lambda type. A kappa monoclonal protein makes the diagnosis of POEMS suspect. These patients are characteristically much younger than patients presenting with multiple myeloma, median age 51 years (the median age of cryoglobulinemia patients 53 years). Peripheral neuropathy is present in all patients by definition. For those patients who have a solitary sclerotic lesion, radiation to the site is frequently effective. For patients with multifocal lesions not amenable to radiation therapy, melphalan-based chemotherapy or combination chemotherapy with alkylating agents have been reported to result in benefit in 44% of patients. Recently we have undertaken peripheral blood stem cell transplant as a management tool and have transplanted and reported on 16 of these patients, with a median age of 51 years. There was one transplant-related death and an unexpectedly high peri-transplant complication rate, usually cardiopulmonary. Of 14 evaluable patients, all had neurologic improvement or stabilization. Other features of the syndrome have improved substantially as well. These neurologic syndromes are disabling, produce significant morbidity, but are rarely fatal. The median survival of our non-transplanted POEMS cohort is 165 months and of 66 patients with cryoglobulinemia the median survival has not been reached at a seven-year median follow-up.

PLENARY SESSION 7
HOW DO WE USE GENOMICS TO TAILOR THERAPY?

PL7.01

THE TRANSCRIPTOME OF MULTIPLE MYELOMA DEFINES DISEASE SUBGROUPS WITH DISTINCT GENETIC AND CLINICAL FEATURES AND ALSO ALLOWS IDENTIFICATION OF GENES HIGHLY CORRELATED WITH AN AGGRESSIVE CLINICAL COURSE

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Multiple myeloma is likely to be a broad descriptor of several distinct clinical entities arising from unique transformation events. In order to classify myeloma and identify molecular pathways that define these subclasses, we used a combination of unsupervised hierarchical clustering, SAM, and PAM analysis of the expression patterns of ~20,000 genes from CD138-enriched plasma cells from 351 newly diagnosed myeloma patients treated with Total Therapy 2 (TT2). Using training and validation groups the analysis revealed that myeloma could be separated into 7 groups with uniform and unique patterns of gene expression, clinical characteristics and cytogenetic abnormalities. Group I (n=69) was defined by uninformative karyotypes (80%) and a normal polyclonal plasma cell signature suggesting these marrows consists of a mixture of normal and malignant plasma cells a possible indication of a more macro-focal disease subtype. Group II, the largest group (n=85) also contained a high incidence of uninformative karyotypes (55%) and when abnormal were predominantly hyperdiploid (90%). Group III (n=59) also contained a high incidence of uninformative karyotypes (74%) and was also defined by the presence of CCND1 spikes (n=56) a characteristic of the t(11;14). Interestingly, 2 of 3 cases lacking CCND1 had a CCND3 spike characteristic of the t(6;14) signifying that cyclin deregulation is a critical feature of this form disease subtype. Group IV (n=32) was defined by overexpression of proliferation-associated genes and contained an even distribution of hyperdiploid and hypodiploid karyotypes. MAF (n=10) and MAFB spikes (n=9) characterized by the t(14;16) or t(14;20) respectively, clustered together and defined Group V (n=19). Group VI (n=36), not defined by any of the above parameters, was characterized by elevated expression of CST6. MMSET/FGFR3 spikes (n=49) characteristic of the t(4;14) defined Group VII (n=49). Ten of the 49 cases lacked FGFR3 spikes. Specific clinical characteristics were significantly associated with each group. MM cases with an IgA isotype predominated in Groups V, VI, and VII (p=0.009). β2M levels were significantly elevated in Group IV (mean 7.66 mg/L) and Group V (mean 9.09 mg/L) (p=0.003). Creatinine (≥2.0 mg/dL) (p=0.005) and LDH (UI/L) (p=0.003) were significantly elevated in the Group IV. Cytogenetic abnormalities predominated in Groups IV and VII (p=0.0002). When abnormal karyotypes were present, Groups II and IV tended to be hyperdiploid (p=0.001) and Groups IV and VII hyperdiploid (p=0.002). Group VI had a significant reduction in the presence of >3 MRI lesions (p=0.001). Final-

ly, chromosome 13 deletion in 80% of clonotypic plasma cells as detected by interphase FISH was significantly elevated in Groups IV, V, and VII (p<0.001). Multivariate stepwise descriminant analysis was used to identify 15 genes whose expression could define the 7 subgroups with a high degree of correlation between the subgrouop designations indicated by the 350 or 15 gene models. At the time of analysis there was a median follow-up of live patients of 30 months with at least 18 months of follow-up on 227 cases. There were 54 disease-specific events and 25 disease-specific deaths. Kaplan-Meier analysis revealed significant differences in event-free (p<0.0001) and overall (p=0.0012) survival between the 7 subgroups with Groups IV, V, and VII exhibiting relatively poor event-free and overall survival. Data presented here suggest that MM is a broad descriptor of multiple discrete molecular and clinical entities with unique mechanisms of transformation. This classification schema could provide a novel framework for the application of current therapies and may facilitate development of subgroup-specific treatment strategies.

The variability in survival among patients with myeloma can range from months to >10 years. Patients at highest risk are best identified by the presence or absence of an abnormal karyotype. We have shown a significant relationship to survival based on global gene expression-defined subgroups. To better define high-risk disease we performed Kaplan-Meier survival analysis using gene expression quartiles for 33,000 probe sets on 351 newly diagnosed myeloma cases treated with TT2. Using the same survival data above the analyses revealed that elevated expression of 93 genes and reduced expression of 18 genes (2.5% false discovery rate) were associated with reduced survival (p<0.0001). Genes from chromosome 1q (19.5%) and 1p (46%) were significantly over-represented in over-and under-expressed genes, respectively. Indeed G-banded karyotype analysis of 668 cases treated with TT2 revealed that cytogenetic abnormalities containing abnormalities in chromosome 1 were more highly correlated with shorter survival than those with cytogenetic abnormalities lacking chromosome 1 and those lacking cytogenetic abnormalities (p<0.0001). We have previously shown that 1q21 represents an amplicon in myeloma (Sawyer *et al.,* 2004) and overexpression of CKS1B, a crucial regulator of cell cycle progression, mapping at 1q21, was significantly linked to poor survival (p=0.000013). Microarray was highly correlated with QPCR in 49 cases tested (r = 0.78). Longitudinal analysis of CKS1B expression at baseline and relapse in 27 cases revealed increased CKS1B expression at relapse (p=0.0009). Furthermore, CKS1B expression was higher in those patients who experienced a rapid death following relapse versus those who could be salvaged (p=0.009). CKS1B gene expression by microarray was correlated with CKS1B DNA copy number by comparative genomic hybridization in 58 newly diagnosed cases (r=0.58, correlation and p<0.005, permutation test). Virtually all myeloma cell lines over-express CKS1B and FISH analysis of CKS1B revealed CKS1B gene amplification (3X to 8X) in 15 of 17 cases tested. Kaplan Meier analysis of 96 primary myelomas also revealed a strong link between increased CKS1B ploidy and short survival (p<0.0001).

In fission yeast CKS1 directly promote the transcription of CDC20 a regulatory component of the anaphase promoting complex/cyclosome (APC/C) that controls exit form mitosis by the ubiquitinylation of mitotic cyclins (Morris *et al.,* 2003). CDC20 and CKS1B expression was highly correlated (r=0.78) in primary myeloma. Array CGH analysis revealed that CDC20 gene expression is not linked to increases in DNA copy number, suggesting that CDC20 expression may be regulated by CKS1B in myeloma. CKS1B has been shown to directly regulate DNA synthesis by controlling the ubiquitinylation and subsequent proteasomal degradation of p27 (Spruck *et al.,* 2001, Ganoth *et al.,* 2001). Western blot analysis of cytosolic and nuclear protein from plasma cells from 25 newly diagnosed myeloma cases and 7 myeloma cell lines showed a strong correlation between CKS1B mRNA and protein levels and an inverse correlation between CKS1B and p27Kip1 levels. In conclusion these data suggest that multiple myeloma is made up of at least seven distinct molecular entities and that amplification 1q, likely targeting the CKS1B gene in each subtypes reduces p27 and imparts a highly aggressive phenotype in multiple myeloma.

PL7.02

TRANSLOCATION (4;14), FIBROBLAST GROWTH FACTOR RECEPTOR 3 AND MYELOMA

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Dysregulation of fibroblast growth factor receptor 3 (FGFR3) and MMSET by the t(4;14)(p16;q32) translocation occurs in 15% of multiple myeloma (MM) patients and confers a growth and survival advantage to malignant plasma cells. FGFR3 functions as an oncogene in murine models and confers a growth advantage to B cells which proliferate more rapidly in the face of interleukin 6 (IL6) and may even become independent of IL6 in the presence of activating mutations. In that light, activating mutations of FGFR3 which are relatively common in MM cell lines are uncommon in newly diagnosed MM (<5%) and are thought to be associated with disease progression. FGFR3 and t(4;14) detection methodologies including immunohistochemistry, flow cytometry, RT-PCR, FISH and expression profiling are currently being compared and will be presented. Surprisingly, FGFR3 expression is lost in 30% of patients and MMSET expression retained suggesting that MMSET may be the true causal target of t(4;14) in MM. Nevertheless, MMSET is only weakly oncogenic in transgenic murine model systems. The relative roles of FGRFR3 and MMSET or their complementarity thus requires more study.

The clinical impact of t(4;14) translocation has now been demonstrated in 3 large studies each reporting a marked reduction in overall survival. In our own series a t(4;14) was detected in 14/108 (13%) patients by FISH. These patients had a shorter event-free (median 9.5 versus 25.8 months) and overall survival (median 18 months vs 46.3 months; p=0.0053) than patients without the t(4;14). This reflects rapid relapse rather than primary drug resistance with initial response to induction therapy of greater than 80%; nevertheless all 14 patients with a t(4;14) who received alkylating agent salvage failed to respond. As in other studies an association was found between the presence of the t(4;14) and IgA immunoglobulin isotype. Our results indicate that the detection of the t(4;14) by cIg-FISH is associated with a very poor prognosis in MM patients and such patients do not benefit from receiving intensive chemotherapy and autotransplant.

As FGFR3 may play a significant role in MM oncogenesis and because the t(4;14) confers a poor prognosis FGFR3 expression represents a molecular target. Consequently we have assessed the therapeutic potential of several FGFR-selective receptor tyrosine kinase (RTK) inhibitors. These studies have shown that inhibition of FGFR3 in t(4;14) +ve MM cell

lines induces cytotoxic responses demonstrating that these cells remain dependent on FGFR3 signaling despite the complexity of genetic alterations in these end stage patients. One small molecule we have examined is CHIR-258, a kinase inhibitor that targets Class III-V RTK and inhibits FGFR3 with an IC50 of 5 nM in an *in vitro* kinase assay. We first employed the IL-6 dependent cell line, B9 that has been engineered to express wild-type FGFR3 or active mutants of FGFR3 (Y373C, K650E, G384D and 807C), to screen CHIR-258 for activity against FGFR3. CHIR-258 differentially inhibited FGF-mediated growth of B9 expressing wild-type and mutant receptors found in MM, with an IC50 of 25 nM and 80 nM respectively as determined by MTT proliferation assay. Growth of these cells could be rescued by IL-6 demonstrating selectivity of CHIR-258 for FGFR3.

We then confirmed the activity of CHIR-258 against FGFR3 expressing myeloma cells. CHIR-258 inhibited the viability of FGFR3 expressing KMS11 (Y373C), KMS18 (G384D) and OPM-2 (K650E) cell lines with an IC50 of 90 nM, (KMS11 and OPM-2) and 550 nM (KMS18). Importantly, inhibition with CHIR-258 was still observed in the presence of IL-6 or IGFI, potent growth factors for MM cells. U266 cells, which lack FGFR3 expression, displayed minimal growth inhibition demonstrating that at effective concentrations, CHIR-258 exhibits minimal nonspecific cytotoxicity on MM cells. Further characterization of this finding demonstrated that inhibition of cell growth corresponded to G0/G1 cell cycle arrest and dose-dependent inhibition of downstream ERK phosphorylation. In responsive cell lines, CHIR-258 induced apoptosis via caspase 3. *In vitro* combination analysis of CHIR258 and dexamethasone applied simultaneously to KMS11 cells indicated a synergistic interaction. *In vivo* studies demonstrated that CHIR-258 induced tumor regression and inhibited growth of FGFR3 tumors in a plasmacytoma xenograft mouse model. Finally, CHIR-258 produced cytotoxic responses in 4/5 primary myeloma samples derived from patients harboring a t(4;14) translocation. These data indicate that the small molecule inhibitor, CHIR-258 potently inhibits FGFR3 and has activity against human MM cells setting the stage for a phase I clinical trial of this compound in t(4;14) MM. These trials will be activated in the first quarter of 2005.

PL7.03

THE PHARMACOGENOMICS OF MYELOMA

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The immune system has evolved to cope with the continued threat of disease encountered in everyday life which has selected for variation in immune response. Previous infections and inherited genetic variations may affect the risk of developing tumors of the immune system. Another type of variation has been selected for by the need to deal with toxic xenobiotics encountered in the environment. Such inherited variation can affect the way that the body deals with chemotherapy agents either diminishing effectiveness or increasing the risk of side effects. The study of these variations and their clinical impact has been called pharmacogenomics. These aspects have been known for many years but it is the improvements in high throughput genotyping and the completion of the sequencing of the genome which have made them potentially clinically useful.

The most common form of inherited genetic variation takes the form of single neucleotide polymorphism (SNP). There are many millions of these variants and they can have a variety of effects. Changes in non-coding DNA exert no effects but can act as markers for changes that do. Changes in coding sequence, which change protein structure, are less frequent and there may be as few as 50,000 variants. However, perhaps the most relevant variants occur in promoter regions and subtly change the patterns of the expression of the gene they encode rather than altering protein function.

The challenge in a relatively rare disease like myeloma is to generate an adequate patient sample which gives adequate power to test defined hypotheses. BOAC is a project aimed at doing this and can be split into a number of distinct sub-projects:

1. Family studies looking at predisposition genes.
2. Case control studies aimed at defining etiological mechanisms.
3. Outcome studies looking at factors modifying survival and the risk of developing side effects.

The first approach requires sibling pairs with myeloma or pedigrees and can identify high risk myeloma predisposition genes. The second approach aims to find low risk genes which affect many people in the population and can be used to test specific questions like how inherited immune variation affects the risk of myeloma. It can also be used to generate hypotheses about potential chemical/environmental exposures. Using the 3rd approach we will ask a number of questions. Does inherited variation affect the risk of side effects of, or outcome from, alkylating agents? What governs the risk of neuropathy after thalidomide or Velcade exposure? Similarly what governs responses to these agents can be addressed. I will discuss the latest data on the structure of the genome and how this is detected together with its relevance to myeloma.

PL7.04

ASSOCIATION OF GENETIC POLYMORPHISMS IN MYELOMA: UPDATE ON BANK *ON A CURE*

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Although multiple myeloma is clinically defined as the accumulation of clonal, malignant plasma cells in the bone marrow, the disease shows significant heterogeneity with regard to morphology, progression and therapeutic response. This heterogeneity likely is due, in part, to differences in genetic abnormalities, including various combinations of mutations in oncogenes and tumor suppressor genes in the malignant clone. However, it is also very likely that germline genetic polymorphisms contribute significantly to an individual's disease course and response. Our hypothesis is that functional genetic polymorphisms are associated with tumor growth control, drug metabolism/detoxification, and DNA repair mechanisms that will influence risk, chemotoxicity and the course of the disease. Bank On A Cure™ (BOAC) is a collaborative project initiated with the International Myeloma Foundation (IMF) to develop a DNA bank on 10,000+ myeloma patient samples, through international cooperation. Cooperative clinical trials groups (eg. ECOG, SWOG), institutions and individual patients have provided either tissue (blood, marrow from stored banks), or buccal cells (BOAC mouthwash kits), and targeted demographic and clinical information. Our goals in this study are to utilize this unique resource to assess targeted SNP associations with disease progression, therapeutic responses (including

toxicities), and etiology (in a cooperative agreement with the NCI).

As we initiated genotyping studies directed at linking genetic variation with clinical outcomes, we chose to take a very directed approach to the choice of genetic polymorphisms. The current SNP list has now been expanded to a total of 90 gene targets each with a specific rationale for inclusion. This list was derived from targeted searches of genetic variations in genes associated with 1) published gene expression profiles (a polymorphsim could have the same impact as high or low expression); 2) recent profiles in a twin study (affected/unaffected); 3) markers of bone disease and bone microenvironment; 4) genes involved in common pathways affecting myeloma growth and signaling; and 5) genes associated with toxicity.

To date, we have nearly 2,800 DNA samples in banks in the U.S. and the U.K. Over 20,000 targeted genotypes have been completed. Data analysis is ongoing, with some interesting associations being identified. Because IL-6, IL-1β, IL-10, TNF-α and LT-α are important regulators of myeloma cell growth, we examined the association of single nucleotide polymorphisms that affect production or function in these cytokines with progression-free survival (PFS), overall survival (OS), objective response and infection grade, of patients in 3 arms of an ECOG phase III trial E9486. We found significant associations of polymorphisms with outcome, specific to different treatment regimens. There is a significant association between progression free survival and TNF-α genotype (p=0.009) as well as IL-10 genotype in treatment arm that included high dose cyclophosphamide (p=0.017). There is an indication of shorter PFS in patients with TNF-α homozygous A genotype or LT-α homozygous G genotype in treatment arm using high dose interferon α. Overall survival is also associated with TNF-α genotype (p=0.027) and IL-10 genotype in cyclophosphamide treatment arm (p=0.033). Multivariate analysis using the Cox proportional hazard model indicated an adverse OS in patients with the IL-10 homozygous G genotype in the promoter region compared to the common AA genotype after adjustment for other known prognostic indicators (adjusted hazard ratio of 1.5, p=0.007). These genes are also associated with objective response and infection grade, but the association often depends on treatment arms. Our data are consistent with the hypothesis that patients homozygous for the high producer allele in myeloma growth-promoting genes have adverse clinical outcomes.

Individuals deficient in the repair of DNA damage are not only at high risk for developing cancer, but also could show DNA repair dependent responses associated with DNA damaging therapeutic agents. We also examined the association of functional genetic polymorphisms in the DNA repair genes, XRCC1 (SNP positions 280, 399) and ERCC2 (SNP positions 312, 751), with toxicities and clinical outcomes in E9486. DNA from 359 patients was genotyped and examined for association with response, toxicities, blood counts, bone pathology, and survival parameters. Notably, in the interferon arm of the trial significant associations were observed in progression free survival and polymorphisms of XRCC399 (AA median survival 51 months versus AG/GG median survival of 33 months; p=0.008), ERCC751 (AA/AC median survival 33 months versus CC median survival of 48 months; p=0.05), and ERCC312 (AA median survival 49 months versus AG/GG median survival of 34 months; p=0.02). Hazard ratios ranged from 1.7 to 2.05 for survival differences associated with DNA repair genes in this arm. Interestingly, T cell counts are known to be affected by interferon; and DNA repair polymorphisms in this arm were also associated with CD8+ T cell counts. While this represents a single arm of one clinical trial, it is intriguing to consider the impact of genetic polymorphisms on DNA repair in the light of chemotherapeutic agents that may affect cells of the immune system. Previous studies from the ECOG Myeloma Committee of the E9486 trial show highly significant associations of survival and immune status in myeloma patients. Our results suggest genetic polymorphisms in DNA repair genes may influence clinical outcome in certain therapeutic regimens.

We have begun analysis of association of genetic variations on clinical outcome in the intergroup trial S9321. This trial tested a single high dose regimen with autologous stem cell support against a conventional dose regimen, with further randomization of responders to maintenance with interferon or not, in newly diagnosed patients with multiple myeloma. ECOG, CALGB and SWOG enrolled 899 patients with newly diagnosed MM to receive VAD induction x 4 cycles followed by randomization to PBSC-supported high dose therapy (HDT) versus standard dose therapy (SDT) of VBMCP, using CTX 4.5 g/m^2 + G-CSF for PBSC mobilization in all patients. Responders to VBMCP or HDT were randomized to IFN or no maintenance. Preliminary findings (n=600-650) demonstrate functional genetic variants of IL-10, DNA repair genes ERCC2 and XRCC2, and IL-1RA are showing trends in association with outcomes. While these preliminary results are now only suggestive of trends in genetic polymorphisms associated with clinical outcome, completion of the full SNP panel on the entire sample base should provide a extensive association study, and analysis of potential differences in therapy arms of the trial.

PL7.05

MICROARRAY EXPRESSION PROFILING INDICATES UPREGULATION OF THE RIBOSOMAL MACHINERY IN DEL(13)-NEGATIVE CLONES

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In multiple myeloma (MM), deletion of chromosome (chr.) 13q and hypodiploidy are adverse prognostic factors. Prognostic evaluation is frequently based on interphase-fluorescence *in situ* hybridization (FISH) with a single probe for chr. 13q14. CD138-positive bone marrow cells from 97 patients with newly diagnosed MM (58 training group (TG) and 39 validation group VG)) were enriched by magnetic-activated cell-sorting (median purity, 95%). Sorted cells were analyzed by interphase-FISH with probes for chr. 13q14, and additionally 9q34, 11q23, 19q13, t(11;14), and t(4;14). Expression profiling was performed with Affymetrix U133A+B (TG) and HGU133-2.0plus (VG) microarrays. Nearest shrunken centroids classification (NSC) was applied to discriminate clones with FISH-detected del(13q14) vs. those without, using VSN-normalized gene expression values. Chromosomal localization of predictor genes was determined using the MapIt program, and functional relationship was established by Gene Ontology (GO) annotation and creation of GO-slims.

A deletion of chr. 13q14 was found in 27/58 patients (47%) of the training and in 21/39 (54%) of the validation group. Frequencies of trisomies were lower (9q: 48 vs. 74%;

11q: 41 vs. 74%; 19q: 44 vs. 84%) and of IgH translocations higher (48 vs. 16%) in patients with del(13q14). NSC resulted in a predictor for del(13q14) of 378 probe-sets with a cross-validated classification error rate of 22%. The VG is under investigation. Of the predictor genes, 18% were localized on chr. 13 (distributed evenly from 13q12 to 13q33), followed by chr. 19, 11 and 3 (10/6/6%). In the 50 probe-sets with the highest scores, the most frequent localizations of the represented genes were chr. 19, 9, and 13 (12/8/6 of 50). In 8/8 incorrectly classified patients with del(13q14), at least 2 of 3 trisomies (9q, 11q, 19q) were present, hinting at hyperdiploidy. Only 1/5 incorrectly classified patients without del(13q14) harbored 3 trisomies. Biological functions (GO level 3) of predictor genes were related to protein and DNA metabolism (43%), cell growth/maintenance (27%), and cell communication (17%). The most frequent GO term for cellular component was the ribosome (34%). Sixty-nine of 80 human ribosomal protein (RP) genes were represented in the predictor, and made up 33 of the top-50 probe-sets. RP genes were overexpressed in patients without del(13q14) compared to plasma cells from 7 normal donors. Expression levels of RPL12, RPLP2 and RPL13A (on chr. 9, 11 and 19) correlated with the respective chr. copy numbers.

We conclude that FISH-detected del(13q14) is associated with non-hyperdiploidy rather than defining an independent subentity of MM. Overexpression of RP-genes in malignancies has been linked to cell growth, disease progression and drug resistance. A possible pathogenetic role of the upregulation of virtually all ribosomal protein genes observed in del(13q)-negative/hyperdiploid MM clones has to be evaluated further.

PLENARY SESSION 8
AUTOLOGOUS TRANSPLANTATION: REPORTS FROM STUDIES

PL8.01

INTENSIVE VERSUS DOUBLE INTENSIVE THERAPY IN UNTREATED MULTIPLE MYELOMA: UPDATE ANALYSIS OF THE RANDOMIZED PHASE III HOVON 24 STUDY

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The benefit of high-dose therapy with hematopoietic stem cell rescue in the treatment of multiple myeloma has been demonstrated in phase II/III studies. One randomized trial demonstrated a superior long-term clinical outcome of double as compared to single high-dose therapy. In 1995 HOVON started a prospective randomized multicenter trial to compare the efficacy of intensified treatment followed by myelo-ablative therapy and peripheral stem cell transplantation with intensified treatment alone in newly diagnosed patients. We now report the results of a second analysis in 441 eligible patients with stage II (22%) and stage III (78%) disease. The median age was 55 years (range 31-65 years). Remission induction treatment consisted of 3-4 courses of VAD by rapid infusion. Sixty-three patients up to 55 years who had an HLA identical sibling were candidates for an allogeneic transplantation and will be presented separately. After VAD, patients without a sibling were randomized to receive melphalan 140 mg/m^2 divided in 2 doses of 70 mg/m^2 (IDM) without stem cell rescue (arm A) or the same regimen followed by myelo-ablative treatment with cyclophosphamide (120 mg/kg) and TBI with peripheral stem cell transplantation (arm B). Peripheral stem cells were mobilized by cyclophosphamide (4 g/m^2) and G-CSF after VAD. Interferon-α-2a was given as maintenance therapy in both arms.

Of 441 registered patients, 303 were eligible for randomization. Patient characteristics with regard to sex, age, stage of disease, Ig isotype, and β2-M were not significantly different between the two arms. The median follow-up from randomization was 56 months. Overall, 81% of patients received both cycles of IDM (79% in arm A and 83% in arm B) and 79% of patients actually received myeloablative therapy followed by autologous peripheral stem cell transplantation in arm B. The median duration of interferon-α-2a maintenance treatment was 12 months (arm A) vs 7 months (arm B). The CR rate was significantly better in arm B (28% vs 13%, p=0.002), while the overall response rate (PR + CR) was not different (90% vs 86%, p=0.23). The median event-free survival (EFS) from randomization was 22 months (arm B) vs 20 months (arm A) (logrank p=0.016). Median progression-free survival (PFS) was significantly better in patients treated with double intensification (24 vs 23 months, logrank p=0.036). Time to progression (TTP) was significantly worse in arm A (median 25 vs 33 months, logrank p=0.001). The difference for EFS, PFS and TTP between the 2 treatment arms only became evident after at least 4 years of follow-up. Overall survival (OS) was not different between both treatments (median 55 months in arm A vs 50 months in arm B, logrank p=0.38). Multivariate

analysis showed that treatment arm A, higher age, hemoglobin 6.21 mmol/L, stage 3 and elevated serum LDH were significant adverse prognostic factors for EFS. Cytogenetic analysis in 151 registered patients was abnormal in 37% (45% del 13/13q-, 51% abnormal 1p/q, 33% del 6q, 89% complex abnormalities). Cox regression analysis showed that 1p/q was an independent unfavorable prognostic factor for OS, EFS, PFS and TTP (p<0.001), calculated from the start of VAD. Del 13/13q- was highly correlated with 1p/q abnormalities. By combining β2M > 3 mg/L with del13/13q- and 1p/q, prognostic groups could be defined with a significant impact on OS (p<0.000002), EFS (p<0.0002), PFS (p<0.00006) and TTP (p<0.0000002).

In conclusion, in this trial second intensification by myeloablative treatment with cyclophosphamide/TBI when added to intensified chemotherapy alone resulted in a superior EFS, PFS and TTP, but not OS. An update of the follow-up will be made in February 2005 and will be presented.

**C.M. Segeren, P.Sonneveld, B. van der Holt et al, Blood, 2003 101:2144-51*

PL8.02

SINGLE VS. DOUBLE HIGH-DOSE THERAPY IN MULTIPLE MYELOMA: SECOND ANALYSIS OF THE GMMG-HD2 TRIAL

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High-dose therapy (HDT) followed by autologous blood stem cell transplantation (SCT) has resulted in superior response rates as well as longer event-free (EFS) and overall survival (OS) in patients with multiple myeloma (MM). However, almost all patients relapse and succumb to their disease. To improve the clinical outcome, sequential HDT followed by SCT was proposed for newly diagnosed MM.

The GMMG-HD2 trial was designed as a prospective randomized phase III study to assess single vs. double HDT and autologous SCT. The first randomization was performed for induction treatment: 3 to 6 cycles of either standard VAD or VID (vincristine, idarubicin, and dexamethasone, administered orally). Peripheral blood stem cells were harvested during G-CSF-enhanced leukocyte recovery after high-dose chemotherapy with cyclophosphamide (4 g/m^2). According to the second randomization, patients were treated either with a single or with two sequential cycles of HDT (melphalan, 200 mg/m^2), followed by autologous peripheral blood SCT (PBSCT) of at least 2.5 x10^6 CD34-positive cells per kg body weight. Interferon-α (9-14.5. U per week) was administered as maintenance treatment for patients in both randomization arms. A total of 268 MM patients from 46 centers were included; 264 patients are evaluable for the comparison of VAD vs. VID. Four deaths occurred during neutrocytopenia in the VID arm. Therefore, the dose of idarubicin was reduced from 10 to 8 mg/m^2. Response rates (CR+PR rate: 65 vs. 67%) and non-hematologic toxicity were similar after VAD and VID, while haematological toxicity of VID was significantly higher. For the comparison of single vs. double HDT, 261 patients were evaluable. Patients treated with two sequential cycles of HDT and PBSCT had a significantly longer EFS (p=0.03; median 23 months vs. not reached for single vs. double HDT and PBSCT). A β-2-microglobulin (B2M) serum level of greater than 3.0 mg/L was associated with an inferior EFS, while serum albumin levels below 35 mg/l were not. Results of fluorescence *in situ* hybridization (FISH) for the chromosome region 13q14 were available for 49 patients. In 22 of these, deletion of 13q14 was detected. EFS was not significantly different in patients with FISH detected deletion of 13q14 vs. those without. In summary, sequential HDT with melphalan 200 mg/m^2 and autologous PBSCT results in superior EFS compared to single HDT. VAD and VID induction are comparable in anti-myeloma efficacy, while VID has a higher hematologic toxicity. A β2M level > 3.0 mg/L indicated a shortened EFS, while albumin <35 mg/L and FISH detected deletion of 13q14 could not be associated with significantly shortened survival; however, the number of patients assessed by FISH was small. The final analysis of the trial will be presented at the Sydney meeting.

PL8.03

HIGH-DOSE THERAPY/STEM CELL SUPPORT IN MULTIPLE MYELOMA: UPDATE OF THE SPANISH STUDIES (PETHEMA/GEM)

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High-dose therapy (HDT) followed by autologous stem cell support is commonly used in younger patients with multiple myeloma (MM). There are two randomized trials reporting the superiority of HDT in terms of CR rate, EFS and OS when compared with conventional chemotherapy. Furthermore, in one case control and in two population-based control studies HDT was superior to conventional chemotherapy. However, in another prospective randomized trial, HDT was not superior to conventional chemotherapy. In addition, the US Intergroup has recently reported an advantage in EFS (median, 21 vs. 25 months, p=0.05) but comparable CR rate (16% vs 17%) and overall survival (median, 53 vs 58 months). The Spanish PETHEMA group compared the efficacy of HDT/intensification versus continuation of conventional chemotherapy in patients responding to the initial chemotherapy. Thus, from May 1994 to October 1999, 216 patients (122M, 94F), median age 56 years, stage II or III myeloma and ECOGscore < 3 were registered. The initial chemotherapy consisted of 4 courses of alternating VBMCP/VBAD and responding patients were randomized to receive either 8 additional courses of VBMCP/VBAD or intensification with MEL-140 and 12 Gy of fractionated TBI or MEL-200 followed by stem cell support. Maintenance treatment consisted of alpha-interferon and dexamethasone in both arms. Onehundred and eighty-five patients responded to the initial VBMCP/VBAD chemotherapy. Twenty-one of these responding patients were not randomized due to different reasons. One hundred and sixty-four patients were randomized: 83 to continued chemotherapy and 81 to HDT intensification. The degree of response to the initial chemotherapy as well as the main prognostic features were similar in both groups. After a median follow-up of 66 months from the initiation of treatment and analyzed on an intention-to-treat basis, the CR rate (negative electrophoresis) was significantly higher in the HDT arm (30% vs. 11%, p=0.002). However, PFS was not significantly different between HDT intensification and continuation of conventional dose chemotherapy (median, 43 vs 34 months, p=NS) and the OS was similar in both groups (61 months for HDT, 66 months for chemotherapy, p=NS). In conclusion, this trial shows that HDT intensification significantly increases the CR rate with no significant impact on PFS and OS in myeloma patients responding to the initial chemotherapy.

In our current trial (PETHEMA/GEM 2000) patients with MM younger than 70 years are treated with alternating VBMCP/VBAD chemotherapy. Responding patients are intensified with HDT/SCS (busulphan-12/MEL-140 or MEL-200).

Patients achieving CR (negative immunofixation) or near-CR (negative electrophoresis, positive immunofixation) after the first transplant are given maintenance treatment with interferon/prednisone while patients with a remaining visible M-spike (partial or minimal response) are planned to receive a second HDT/SCS with CVB (cyclophosphamide, etoposide, BCNU) or a dose-reduced intensity allogeneic transplant conditioned with fludarabine/melphalan, depending on the donor availability. Patients with primary refractory disease are planned to receive a tandem transplant the second high-dose procedure being either autologous or dose-reduced intensity allogeneic procedure depending on the donor availability. A number of abstracts with the preliminary results from this trial have been submitted to this Workshop: 1) the impact of CR defined by the EBMT criteria (negative immunofixation) on PFS and OS as compared with other categories of response; 2) the prognostic impact of age (<60 vs ≥60 years); 3) high incidence of hepatic venooclussive disease with busulphan-12-/melphalan-140 as compared with melphalan-200; 4) the feasibility and efficacy of a second high-dose procedure (second "auto" or "mini-allo") in patients who had not achieved CR or near-CR with a first transplant; and 5) the results of HDT, including tandem transplant, in patients with primary refractory myeloma. A brief summary on each of the above topics will be also shown in this general presentation.

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PL8.04

UPDATE ON HIGH-DOSE THERAPY - ITALIAN STUDIES

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Since 1996 we have initiated different studies aimed at exploring the role of high-dose therapy with autologous stem cell transplantation as primary treatment for symptomatic multiple myeloma (MM). The "Bologna 96" trial addressed the issue of one versus double autologous transplantation (Tx-1 vs. Tx-2) for patients ≤ 60 years of age. By study design, autologous transplantation was preceded by 4 monthly courses of VAD and subsequent collection of peripheral blood stem cells (PBSC) using high-dose cyclophosphamide (HD-CTX). Tx-1 was given to support melphalan, 200 mg/m^2 (MEL-200); Tx-2 was given to support a first course of MEL-200 followed, within 3 to 6 months, by melphalan 120 mg/m^2 + busulfan 12 mg/kg. An analysis was performed using an intent-to-treat approach on 228 patients who were randomly assigned to Tx-1 (n=115 patients, median follow-up: 53 months) or Tx-2 (n=113 patients, median follow-up: 57 months). In comparison with Tx-1, Tx-2 prolonged the median event-free survival (EFS) of 13 months (p=0.002) and the median time to progression (TTP) of 16 months (P=0.00001). The median duration of overall survival (OS) was 59 months in the Tx-1 arm and 73 months in the Tx-2 arm (P=0.3). Among patients randomized to Tx-1, attainment of complete remission (CR) or near CR (nCR) was an essential prerequisite for extended OS (P=0.004), EFS (p=0.00001) and TTP (p=0.00001). At the opposite, the benefits of double autologous transplantation were the greatest among patients who failed at least nCR. In particular, analysis of response at 3 months after the first transplantation revealed that patients who were in ≤ partial remission (PR) and underwent the second autologous transplantation had a significantly longer duration of OS (p=0.004), EFS (p=0.00001) and TTP (p=0.00001) than patients who had the same response status but received Tx-1. The benefits of Tx-2 were evident not only among patients who converted from ≤ PR after one transplantation to at least nCR after the second transplantation, but also among patients who failed at least nCR after completing the entire treatment program. Among these latter patients, the 6-year projected probability of survival was 67%, while it was 35% among patients in final ≤ PR after Tx-1 (P=0.004). It is concluded that, in comparison with a single transplantation, double autologous transplantation as part of first-line therapy for MM significantly prolonged EFS and TTP among patients with ≤ 60 years of age. The superiority of double over single autologous transplantation in terms of extended OS, EFS and TTP was particularly relevant among patients who failed the important objective of attaining CR or nCR. Mature data derived from the final analysis of the study must be awaited before definite conclusions can be given concerning the impact of double autologous transplantation on the ultimate outcome of patients with MM.

In January 2002, we started the phase II "Bologna 2002" study aimed at evaluating the efficacy and toxicity of combined thalidomide-dexamethasone as primary therapy for MM patients who were candidates to receive double autologous transplantation. By study design, thalidomide and dexamethasone were administered for 4 months and were

followed by collection of autologous PBSC to support two sequential courses of MEL-200. The starting dose of thalidomide was 100 mg/d, with a subsequent increase to 200 mg/d after 14 days; the monthly dose of dexamethasone was 40 mg/d on days 1 to 4, with courses repeated on days 9 to 12 and 17 to 20 on the first and third months of therapy. The first 100 patients who entered the study (of whom, 86% were in advanced clinical stage and 48% carried chromosome 13 abnormalities) were evaluated for response, toxicity and collection of PBSC. For comparison of their outcome, an equal number of pair mates were selected among patients who received primary VAD therapy as part of the "Bologna 96" clinical trial. Matching criteria were age (within 2 years), clinical stage and serum β2-microglobulin (within 1 mg/L). Response to therapy was evaluated using an intent-to-treat approach and stringently defined criteria proposed by the EBMT group. In comparison with VAD, thalidomide-dexamethasone resulted in a significantly higher ≥ PR rate (52% versus 76%, respectively; p=0.0004) and effected more profound reduction in tumor cell mass, as reflected by significantly lower levels of residual IgG (p=0.002) and IgA (p=0.01) serum M components. Side effects registered during therapy with thalidomide-dexamethasone or VAD were different. The major toxicity of VAD was hematologic, particularly granulocytopenia (grade 3-4, 12% of patients). In contrast, non-fatal deep vein thrombosis was the most troublesome complication of primary thalidomide-dexamethasone therapy (15% of patients). In both treatment groups, 91% of patients proceeded to PBSC mobilization with HD-CTX (7g/m^2). Considering 4 x 10^6 CD 34+/kg as the minimum number of stem cells required to safely perform double autologous transplantation, adequate cell yields were obtained in 83% of patients with prior exposure to thalidomide-dexamethasone and in 88% of patients treated with VAD (p=0.3).The median yields of CD34+ cells were 7.85 x 10^6/kg in the thalidomide-dexamethasone group and 10.5 x 10^6/kg in the control group (p=0.2). In conclusion, results of the present study extend and confirm prior observations by our group and others showing that thalidomide-dexamethasone is an effective and relatively well tolerated induction regimen for previously untreated patients with MM. In comparison with VAD, combined thalidomide-dexamethasone significantly augmented both the rate and the magnitude of response, without increasing the toxicity or interfering with subsequent collection of PBSC. Based on these data, thalidomide-dexamethasone may be considered an oral, and easy to administer, alternative to VAD as front-line therapy for MM patients who are candidates to subsequent autologous transplantation.

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PL8.05

HIGH DOSE THERAPY SUPPORTED WITH AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA: LONG TERM FOLLOW-UP OF THE PROSPECTIVE STUDIES OF THE MAG GROUP

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Between 1986 and 2000, we conducted 4 multicenter prospective trials aimed at solving different issues related to the place of high dose therapy (HDT) and autologous blood stem cell (ABSC) transplantation in the treatment of multiple myeloma (MM):

1) To assess the feasibility of the procedure, the first study was a phase II study in which 63 young patients (median age 44 yrs) with newly diagnosed or relapsing stage III or stage II MM were included (Blood 1993;82:2005-9).

2) To assess the optimal timing of HDT and ABSC transplantation, a randomized prospective study was initiated in 1990 in which patients up to 56 years of age with successful ABSC collection (185 out of 202 initially enrolled patients) were randomly assigned to receive HDT and ABSC transplantation (early HDT group) or a conventional-dose chemotherapy (CCT) regimen (late HDT group). In the late HDT group, HDT and transplantation were performed as rescue treatment, in case of primary resistance to CCT or at relapse in responders (Blood 1998;92:3131-6).

3) To examine the issue of patients' age, a third study, initiated in 1992, randomly compared HDT and ABSC transplantation with a conventional-dose chemotherapy regimen in 190 patients aged between 55 and 66 years with symptomatic newly diagnosed MM. In this study, HDT protocol consisted of melphalan (MLP) 200 mg/m^2 (or MLP 140 mg/m^2 + busulfan 16 mg/kg) whereas HDT included a 12 Gray total body irradiation (TBI) in the 2 previous studies.

4) To evaluate the potential interest of combining improvement of tumor mass reduction through a multiple HDT regimen and the reinfusion of tumor decontaminated graft using a CD34 selection technique, a two by two designed randomized trial was initiated in 1996. In this fourth study, 230 young patients aged under 56 with newly diagnosed symptomatic MM were randomly assigned upfront to receive either a single HDT or two sequential HDT. In addition, all patients were independently randomized to be transplanted with unselected ABSC (unselected arm) or CD34-enriched ABSC (CD34 arm).

The median follow-up of all patients who were enrolled in the 4 studies currenthy exceeds 10 years. Up-dated data of each study, particularly of the one versus two HDT part of the last one, will be presented at the meeting. In addition, we will present an analysis of the rate and characteristics of long-term survivors.

PL8.06

UPDATE ON HIGH DOSE THERAPY – MRC STUDIES

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In the Medical Research Council (MRC) Myeloma VII Trial[1] 407 previously untreated patients less than 65 years of age were randomized to receive either the conventional-dose ABCM (doxorubicin, carmustine, cyclophosphamide, melphalan), the Standard regimen, to maximal response or infusional induction chemotherapy with C-VAMP (cyclophosphamide, vincristine, doxorubicin, methylprednisolone) followed by high-dose therapy (HDT) with autologous stem cell transplant (ASCT), the Intensive regimen. The planned maintenance therapy in both arms of the trial was interferon α-2a. The two groups of patients were well balanced with regard to age, performance status, serum calcium and creatinine, hemoglobin and ß2 microglobulin strata. In the Intensive-therapy group 150 of 201 patients received HDT and ASCT. The great majority had melphalan 200mg/m^2 and peripheral blood stem cell reinfusion; 8 received total body irradiation plus melphalan at 140mg/m^2. In the Standard-therapy arm 196 of 200 patients received the protocol

treatment but at least 36 patients (18%) went on to have HDT plus autograft and 4 (2%) an allograft as part of off-protocol therapy. Analysis was on an intention-to-treat basis.

Among the 401 evaluable patients, the rates of complete response (requiring immunofixation negativity) were higher in the Intensive-therapy group than in the Standard-therapy group (44% v 8%, $p<0.001$). In an updated follow-up analysis (July 2004) 258 of 401 patients had died (115 in the Intensive group compared with 143 in the Standard group) with a median follow-up of survivors of 68 months. There was an improved survival in the Intensive group compared with the Standard group, median 56.3 months versus 42.2 months, a difference of 14.1 months ($p=0.004$ log-rank test $p=0.013$ Wilcoxon test). This confirmed and reinforced the conclusion of benefit reported previously[1]. There was an improved progression-free survival in the Intensive group, median 31.2 (95% CI 27.1 to 37.5) compared with 19.5 (95% CI 16.2 to 21.6) in the Standard group ($p=<0.0001$ Log-rank/Wilcoxon tests) with 52 and 11 patients respectively remaining progression-free at the time of this further analysis (based on 395 evaluable patients). A significant interaction between treatment effect and the pre-treatment level of serum ß2 m strata as defined in previous MRC studies was seen ($p=0.003$ in the Cox model). Stratified log-rank analysis showed that within each stratum the Intensive-therapy group had a longer median survival than the Standard-therapy group. This difference was greatest in the high serum ß2m (>8 mg/L) stratum (41.5 months; 95% CI 31.3 to 56.2 as compared with 13.1 months; 95% CI 9.2 to 23.9). There was also a trend toward improving survival in the Intensive-therapy group as the extent of maximum response increased from minimal to partial to complete. The bolt-on studies in cohorts of patients from the trial further emphasized the relevance and importance of depth of response in relation to outcome[2,3]. Previously the odds ratios and 99% confidence intervals for Myeloma VII as well as for the only two strictly comparable studies, carried out by the French IFM and MAG Groups, were calculated. The estimated combined treatment effect was consistent with a significant survival benefit with treatment incorporating HDT as compared with conventional-dose therapy (odds ratio, 0.70; 95% CI, 0.53 to 0.93; $p=0.01$)[1]. Overall the results of the MRC and other trials and extended single center experience indicated that HDT was effective and could now be regarded as a standard component of first-line treatment for patients with multiple myeloma.

In the current MRC Myeloma IX Trial, HDM 200mg/m^2 has been incorporated as the standard component of treatment for all patients fit enough for such an approach with no fixed age limit. Patients with symptomatic myeloma (as defined by the International Myeloma Working Group[4]) enter one of two treatment pathways (based on performance status, clinical judgement and patient preference) and are randomized for type of bisphosphonate (clodronate v zoledronic acid) and induction therapy. In the Intensive pathway infusional CVAD is being compared with the oral CTD regimen (cyclophosphamide, thalidomide, dexamethasone) as induction therapy. Following HDT there is a maintenance randomization between low dose thalidomide (50-100mg daily) and no thalidomide. All younger patients and their siblings are tissue typed and patients with potential HLA-matched donors are offered a reduced intensity conditioning (RIC) allograft to follow the standard HDT autograft (allograft patients are not eligible for the maintenance randomization). In the Non-intensive pathway MP (melphalan, prednisolone) is being compared with an attenuated version of CTD (CTDa) and the maintenance question is the same as in the Intensive pathway. Linked studies, requiring biological sampling at key time-points, include evaluation of the clinical relevance of genetic/cytogenetic changes, monitoring of residual tumor by cellular phenotype and evaluation of serum free light chain (flc) measurement as a prognostic factor and in the monitoring disease activity. The trial was initiated in May 2003 and as at 31 October 2004 (17 months of recruitment) 530 patients had been entered of whom 309 were in the Intensive pathway.

Patients with myeloma have an increased risk of venous thrombotic events (VTEs) and that risk may be increased by treatment with thalidomide. Patients receiving infusional chemotherapy are at increased risk of thrombosis and infection in relation to central venous lines. As of August 2004 (15 months from initiation of Myeloma IX) there had been 35 VTE in the 430 patients entered. In the Intensive pathway there were 13 deep vein thromboses, 9 pulmonary embolic episodes and 5 line-related thromboses corresponding to an incidence of 10.6% VTE in patients receiving CVAD and 9.0% in patients receiving CTD. Although currently full anticoagulation is advised only where particular risk factors are identified, this aspect of toxicity is the subject of continuing close monitoring and dialogue with trial participants.

Myeloma IX has been designed as a framework trial with the possibility of modification/addition during its course. It is clear that the newer agents being introduced in the treatment of myeloma will have an impact on treatment strategies. The introduction of alternative induction chemotherapy for patients who respond inadequately initially is being considered and pilot studies are progressing. For the moment, HDT is regarded as the central plank around which peri-HDT strategies to optimize response and enhance outcomes can be built.

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FOCUS SESSION 8
BONE & TUMOUR MICROENVIRONMENT

F8.01

NEW THERAPEUTIC APPROACHES IN MYELOMA BONE DISEASE

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One of the major clinical features of multiple myeloma is the development of osteolytic bone disease, characterized by intractable bone pain, pathological fractures and hypercalcemia. The location of myeloma cells within the bone marrow microenvironment enables their interaction with a range of cell types, including osteoclasts, osteoblasts and bone marrow stromal cells. It is this interaction that promotes both tumor growth and the development of myeloma bone disease. The reciprocal relationship between myeloma cells and osteoclasts raises the possibility that inhibition of bone resorption may also result in an indirect reduction in tumor growth. Over recent years, significant advances have been made in the therapeutic approaches to the treatment of myeloma bone disease. These novel therapeutic approaches have arisen as a direct result of increases in our understanding of the mechanisms involved in the development of myeloma bone disease and the mechanisms of action of existing anti-resorptive agents such as bisphosphonates.

Bisphosphonates such as clodronate, pamidronate and zoledronate are widely used in the treatment of osteolytic bone disease associated with myeloma. In addition to their beneficial effects in myeloma bone disease, we and others have demonstrated that some bisphosphonates, including zoledronate, can have anti-tumor effects both *in vitro* and *in vivo* in multiple myeloma.[1,2] However the question as to whether bisphosphonates have a direct anti-tumor effect in vivo still remains unanswered. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway resulting in an inhibition of protein prenylation, a mechanism that has been shown to be responsible for both their anti-resorptive and anti-tumor effects.[3,4] The knowledge of the molecular targets of bisphosphonates has enabled the development of bisphosphonates and bisphosphonate analogs that differ in their relative anti-resorptive, anti-tumor and bone affinity potencies. In addition to their potential for the treatment of diseases such as multiple myeloma, these novel compounds are important tools to further elucidate the precise cellular and molecular mechanisms involved in the anti-resorptive and anti-tumor effects of bisphosphonates. The mevalonate pathway is a potential therapeutic target for the treatment of myeloma bone disease. There are a number of compounds in addition to bisphosphonates, which inhibit this pathway, and therefore may be beneficial in the treatment of myeloma bone disease.

Over recent years, our understanding of the mechanisms involved in the development of myeloma bone disease has increased dramatically. The receptor activator of NF-kB ligand (RANKL)/osteoprotegerin (OPG) system was first identified as playing a critical role in normal osteoclast formation and consequent bone resorption. RANKL is expressed by bone marrow stromal cells and osteoblasts, and interacts with its receptor, RANK found on the surface of osteoclast precursor cells to stimulate osteoclast formation. OPG is a soluble decoy receptor for RANKL, which is secreted by osteoblasts and bone marrow stromal cells and binds to RANKL, thus preventing osteoclast formation. The RANKL/OPG system is dysregulated in multiple myeloma, and we and others have shown that targeting this system can prevent the development of myeloma bone disease *in vivo*.[5] Recently, OPG has also been suggested to function as a soluble decoy receptor for TNF-related apoptosis-inducing ligand (TRAIL), a ligand that can induce myeloma cell apoptosis in vitro and has been suggested to play a role in the anti-tumor activity of the immune system *in vivo*. We have demonstrated that OPG released from osteoblast-like cells can protect against TRAIL-induced apoptosis of human myeloma cells *in vitro*.[6] These observations raise the possibility that endogenous OPG may have additional functions as a survival factor for myeloma cells by protecting against the apoptotic effect of TRAIL in the bone marrow microenvironment.

Multiple myeloma is currently an incurable disease, and the associated osteolytic bone disease is a considerable cause of morbidity for these patients. The precise cellular and molecular mechanisms involved in the development of myeloma bone disease remain unclear. Increasing our understanding of the complex relationship between myeloma cells and cells of the bone marrow microenvironment will lead to the development of novel therapeutic strategies for the treatment of myeloma bone disease and tumor growth.

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F8.02

MACROPHAGE INFLAMMATORY PROTEIN -1α: OSTEOLYTIC AND TUMOUR-PROMOTING EFFECTS IN MYELOMA BONE DISEASE

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Multiple myeloma (MM), the second commonest adult hematologic malignancy, is frequently associated with severe bone destruction due to increased osteoclast (Ocl) activity induced by secreted factor(s) produced by the tumor cells. MM cells produce macrophage inflammatory protein-1 (MIP)-1α/CCL3, a C-C chemokine, and MIP-1α levels have been shown to be elevated in bone marrow plasma of MM patients compared with other hematologic malignancies and normal controls. Data from recent studies in our laboratory and others have implicated MIP-1α in MM-associated osteolysis.[1] Although there are several reports of MIP-1α inducing osteoclastogenesis *in vitro*, it is unclear whether MIP-1α enhances osteoclast formation and activity *in vivo*. In addition, it remains controversial whether MIP-1α requires other

downstream factors such as receptor activator of nuclear factor-kB (RANK) ligand (RANKL) for its effects on bone. To investigate this, we utilized an *in vivo* model of osteoclastogenesis and bone resorption in which cytokines are injected over the calvariae of mice and the bones examined for evidence of increased Ocl and bone resorptive activity. Recombinant MIP-1α evoked a striking increase in the number of intensely stained tartrate-resistant acid phosphatase-positive (TRAP+) Ocl in normal mice, an effect dependent on RANK/RANKL signaling because MIP-1α had no discernable effect in RANK-/- mice. To determine the effects of MIP-1· on bone *in vivo*, Chinese hamster ovarian (CHO) cells genetically engineered to secrete human MIP-1α (which does not bind to murine MIP-1α receptors) were inoculated into athymic mice. Mice bearing intramuscular CHO/MIP-1α tumors developed lytic lesions at distant skeletal sites, which occurred earlier and were larger than those in mice with CHO/empty vector (EV) tumors. When experimental metastases were induced via direct intra-cardiac inoculation, mice bearing CHO/MIP-1α tumors, but not CHO/EV tumors developed hypercalcemia and had significantly more osteolytic lesions. Intramedullary CHO/MIP-1α tumours were also associated with significantly more TRAP+ Ocl. Together, these results are consistent with the notion that MIP-1α plays an important role in the pathogenesis of tumour-induced osteolysis and strongly suggest that MIP-1α is sufficient to induce MM-like destructive lesions in bone *in vivo*. We therefore hypothesized that neutralizing MIP-1α bioactivity in vivo should limit the development and/or progression of osteolytic lesions in a model of human MM that utilizes murine 5TGM1 myeloma cells that express MIP-1α mRNA and secrete bioactive MIP-1α. To establish whether the effects of the chemokine are direct, to enhance osteolysis, or indirect and mediated through a reduction in tumor burden, or both, neutralizing anti-murine MIP-1α antibodies were administered to murine 5TGM1 (Radl) myeloma-bearing mice. In this model of myeloma bone disease, 5TGM1 cells injected through the tail veins in syngeneic immunocompetent C57BL/KaLwRij mice or immunocompromized bg-nu-xid mice home to, and expand within, the medullary cavities and spleen resulting in radiographically-detectable lytic lesions in bones, greatly elevated titers of the monoclonal paraprotein (IgG2bk) in serum and splenomegaly. Systemic (intraperitoneal) administration of anti-MIP-1α antibodies was efficacious in ameliorating myeloma disease progression in this model. Mice inoculated with 5TGM1 cells were randomized into groups and treated with the monoclonal rat anti-mouse MIP-1α antibodies or an isotype rat monoclonal IgG2a with a third bearing-bearing group receiving PBS using the same dosing schedule. Treatment with the anti-MIP-1α antibody resulted in a significant reduction in serum IgG2bk titer in 70% of the 5TGM1 bearing-bearing mice, below the median for the isotype IgGk and PBS-treated control groups pooled. Serum paraprotein levels also directly correlated with splenic wet weights. The mean surface areas of the osteolytic lesions visible on radiographs, as assessed by blinded computerized image analysis, were significantly reduced in mice that received the anti-MIP-1α antibodies compared to control IgG- and PBS-treated mice. Histomorphometric analyses of long bones and vertebrae revealed that this blockade of MIP-1α function resulted not only in a reduction in tumor volume, but also a reduction in the number and intensity of staining of TRAP+ Ocl lining bone surfaces. In summary, administration of neutralizing anti-MIP-1α antibodies to 5TGM1 myeloma-bearing mice reduced overall tumor load assessed by serum monoclonal paraprotein titers, prevented splenomegaly, limited development of osteolytic lesions, and concomitantly reduced tumor growth in bone. Altogether, these data strongly implicate MIP-1α in the pathogenesis of MM-associated bone destruction and possibly myeloma itself. Because, in the 5TGM1 model, blockade of osteoclastic resorption in other situations does not decrease tumor burden[2] we conclude that MIP-1α exerts a dual effect in myeloma, on osteoclasts, and tumor cells. Furthermore, our results suggest that small molecule, orally-available antagonists of the cognate receptors that mediate effects of MIP-1α (CCR-1 and CCR-5), which are now in clinical trials for other conditions, may hold promise as beneficial adjuncts to current standard anti-resorptive therapeutic approaches in MM.

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F8.03

RANKL AND MACROPHAGE INFLAMMATORY PROTEIN-1 ALPHA IN MULTIPLE MYELOMA: CLINICAL IMPLICATIONS

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New insights into the pathophysiology of osteoclastogenesis in multiple myeloma (MM) have emerged recently with the characterization of three new molecules that belong to the tumor necrosis factor-superfamily, namely receptor activator of nuclear factor-kB (RANK), its ligand RANKL and osteoprotegerin (OPG), which is the decoy receptor of RANKL. The importance of RANKL and OPG as regulators of osteoclastogenesis has become evident from experiments with transgenic mice. Mice that lack either RANKL or RANK or that over-express OPG develop osteopetrosis because of decreased osteoclast activity. Conversely, OPG knockout mice have numerous osteoclasts and develop osteoporosis and multiple fractures, since OPG cannot inhibit RANKL activity. Myeloma cells have the ability to up-regulate the expression of RANKL and down-regulate the expression of OPG at both m-RNA and protein level in pre-osteoblastic or stromal cells co-cultures. Therefore, RANKL expression has been found to be increased in bone marrow biopsies from patients with multiple myeloma (MM), while RANKL is over-produced by stromal cells, osteoblasts and activated T-cells in areas infiltrated by myeloma cells. An interesting and controversial question is whether RANKL is expressed by human myeloma cells. Some researchers have found that myeloma cells did not express RANKL and did not produce sRANKL. Furthermore, microarray technology studies showed that RANKL gene expression has not been detected in myeloma cells of MM patients. However, other groups have detected RANKL expression of myeloma cells. Despite this controversy, the available data suggest that RANKL/OPG system is involved in the activation of osteoclasts by myeloma cells indirectly through the bone marrow environment. OPG expression is reduced in bone marrow specimens from myeloma patients.

The adhesive interactions of myeloma cells with bone marrow stromal cells inhibit OPG production both at the m-RNA and protein level. Furthermore, myeloma cells decrease OPG availability by internalizing it through CD138 (syndecan-1) and degrading it within their lysosomal compartment. Thus, in MM, the regulation of OPG reduces the availability of OPG in the marrow microenvironment, leading to reduced inhibition of RANKL and increased osteoclast activation. Indeed, when serum OPG levels were evaluated in MM patients, they were found to be decreased, while serum levels of soluble RANKL (sRANKL) were increased; thus, the ratio of sRANKL/OPG has been found to be increased in both newly diagnosed patients and in patients with refractory/relapsed disease. OPG levels were associated with the degree of radiographic skeletal involvement and low OPG levels correlated with impaired performance status. Levels of sRANKL were also related with the extent of osteolysis. The ratio of sRANKL/OPG correlated with the extent of bone disease and markers of bone resorption, such as the N-telopeptide of collagen type I (NTX) in the urine and tartrate-resistant acid phosphatase isoform-5b (TRACP-5b) in the serum, confirming the importance of RANKL/OPG pathway in the pathogenesis of MM bone disease in humans. Furthermore, sRANKL/OPG ratio correlated with serum levels of interleukin-6 (IL-6) in myeloma patients. It has recently been reported that RANKL treatment significantly induced an increase of IL-6 and IL-11 secretion by both bone marrow stromal cells and endothelial cells *in vitro*. IL-6 is a well characterized growth factor for myeloma cells, and this result suggests that RANKL play a significant role in myeloma cell biology. In addition, sRANKL/OPG ratio correlated with β2-microglobulin, stage of myeloma and overall survival in myeloma patients at diagnosis. These results are in keeping with the observation that intravenous administration of either RANK-Fc, a fusion protein of the murine RANK with the human IgG constant region, or recombinant OPG markedly reduced not only bone resorption and skeletal destruction, but also tumor burden in myeloma animal models. These data confirm the crucial role of RANK/RANKL/OPG pathway in both the development of myeloma bone disease and myeloma cell growth. The administration of anti-myeloma treatment, such as high dose therapy with autologous stem cell support (ASCT) in patients after initial chemotherapy or the combination of thalidomide and dexamethasone in myeloma patients with refractory/relapsed disease resulted in the reduction of sRANKL, sRANKL/OPG ratio and bone resorption markers in the serum of treated patients. In addition, ASCT increased OPG levels at five months after transplantation, and OPG values remained elevated at 12 months post-ASCT. However, no correlation was found between these alterations and response to treatment, disease-free survival or overall survival to-date. Serum levels of sRANKL have also correlated with levels of macrophage inflammatory protein-1 alpha (MIP-1α), which is a member of the CC chemokine family and primarily associated with cell adhesion and migration. MIP-1α is chemotactic for monocytes and monocyte-like cells, including osteoclast precursors. It is produced by myeloma cells and directly stimulates osteoclast formation and differentiation in a dose dependent way. Moreover, the addition of a neutralizing antibody against MIP-1α to human marrow cultures treated with freshly isolated marrow plasma from patients with MM blocks MIP-1α induced osteoclast formation. MIP-1α m-RNA has been detected in myeloma cells, while MIP-1α protein levels were elevated in the bone marrow plasma of MM patients and correlated with disease stage and activity. MIP-1α was also elevated in the blood of myeloma patients with severe bone disease, but not in MGUS patients with increased bone resorption. Furthermore, patients with multiple bone lesions exhibited higher MIP-1α secretion from MM cells along with elevated markers of bone resorption compared with those with minimal bone lesions. MIP-1α levels also correlated positively with bone resorption markers, such as urinary deoxypyridinoline, urinary NTX and serum TRACP-5b, providing further evidence for a causal role of MIP-1α in the development of lytic bone lesions in MM. MIP-1α has been found to stimulate proliferation, migration and survival of plasma cells both *in vitro* and *in vivo* studies. Mice, which were inoculated with myeloma cells and treated with a monoclonal rat anti-mouse MIP-1α antibody, showed a reduction of both paraprotein and lytic lesions. In addition, MIP-1α enhanced adhesive interactions between myeloma and marrow stromal cells, increasing the expression of RANKL and IL-6, which further increased bone destruction and tumour burden. These observations are in accordance with the recent finding that myeloma patients with high MIP-1α serum levels had poor prognosis. The positive correlation between MIP-1α and β2-microglobulin that has been observed in MM patients at diagnosis further supports the notion that MIP-1α is not only a chemokine with osteoclast activity function but is also implicated in myeloma growth and survival. These data have provided evidence for the role of RANK/RANKL/OPG and MIP-1α pathways in myeloma biology. Therefore, these molecules may serve as targets for developing novel anti-myeloma therapies. Recently, there was an attempt to disrupt the RANK/RANKL/OPG interaction in 28 myeloma patients who were randomized to receive a single dose of either recombinant OPG or pamidronate. OPG caused a rapid, and sustained dose-dependent decrease in NTX, comparable to that observed with pamidronate, without having severe side-effects. Another recent study in 49 post-menopausal women with osteoporosis, confirmed the safety and bone anti-resorptive effect of a single subcutaneous dose of a human monoclonal antibody to RANKL. These results warrant further clinical trials targeting the RANK/RANKL/OPG pathway as well as MIP-1α in MM patients.

F8.04

TOWARD THE ELUCIDATION OF THE ROLE OF ALTERED WNT SIGNALING MYELOMA AND DEVELOPMENT OF THERAPEUTIC INTERVENTIONS BASED ON THESE FINDINGS

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We recently demonstrated that bone destruction in multiple myeloma (MM) is directly related to elevated expression and secretion, by myeloma plasma cells, of the soluble Wnt signaling inhibitor DKK1 (Tian *et al.,* 2003). Based on additional unpublished data we suspect that DKK1 *cultivates* the bone marrow microenvironment in such a way that it becomes a rich *soil* for myeloma cell growth. The mechanisms by which this occurs is unclear, but we suspect that this is strongly related to shifts in the biology of marrow stromal cells or mesenchymal stem cells (MSC). The DKK1 molecule is thought to disable the ability of these cells to differentiate into mature osteoblasts (Tian *et al.,* 2003; Rawadi *et al.,* 2003). In addition to its effects on MSC it is also pos-

sible that DKK1 has direct and indirect effects on hematopoietic stem cell biology as. Wnt signaling has been shown directly influence the self renewal capacity of hematopoietic stem cells (Murdoch *et al.,* 2003, Reya *et al.,* 2003) and osteoblasts, which require Wnt signals for differentiation, form the so-called bone marrow niche that maintains hematopoietic stem cell viability (Zhang *et al.,* 2003; Calvi *et al.,* 2003). Taken together these data suggests that DKK1 may contribute to anemia and immunosuppression that is frequently seen in patients with myeloma. A recent study has demonstrated that proteasome inhibitors activate osteoblast differentiation and bone formation *in vitro* and in vivo via increased production of BMP-2 (Garrett *et al.,* 2003). Our group has observed that response to bortezomib therapy is strongly correlated with the increase in serum markers of osteoblast differentiation and bone formation (Zangari *et al.,* 2003). Gene expression profiling of bone biopsies and purified plasma cells from four patients 48 hours post-bortezomib and thalidomide revealed significantly elevated expression of BMP-2, ALP, BGLAP, RUNX2, OSF-2/POSTN, NLX5, and GHR, which suggests a rapid activation of osteoblast differentiation. This suggests that MSC are not absent form the marrow but unable to differentiate (likely due to the effects of DKK1). The elevation of BMP-2 by bortezomib is not only of potential importance in osteoblast activation but has also been shown to have direct anti-myeloma effects (Kawamura, *et al.,* 2000).

It is currently not clear whether immature osteoblasts promote myeloma growth or mature osteoblasts kill myeloma. But it does appear that reactivation of osteoblast in myeloma has anti-myeloma effects and may even have bone restorative effects as well. The recent report on the role of DKK1 and bortezomib in the 5TGM mouse model of myeloma (Oyajobi *et al.,* 2004) found that DKK1 was not expressed in myeloma cells, but was, as reported by Gregory et al., 2003, highly expressed in MSC cell lines. High levels of DKK1 in myelomatous bone suggest that the myeloma cells induce production of DKK1 in MSC. They also showed that DKK1 expression was inhibited at nanomolar concentrations of bortezomib in 14M1, 2T3, MG-63 cell lines in a dose and time-dependent manner. Furthermore, nanomolar concentrations of bortezomib stimulated new bone formation in neonatal mouse calvarias; inhibited IL-1 stimulated bone resorption; and induced 5TGM cells' apoptosis. Taken together the data suggest that the Wnt-inhibitors produced by myeloma plasma cells block osteoblast differentiation and uncouple bone turnover but more importantly also appear to directly influence myeloma cell growth. Bortezomib therapy appears to function in part through the inhibition of DKK1 and the reactivation of osteoblasts. If this hypothesis can be proven, this will represent a major shift in the paradigm of cancer chemotherapy that has traditionally focused on attacking the tumor cell directly.

If true, a major emphasis in both clinical and basic research in myeloma should be dedicated to the role of osteoblast or lack thereof, in the natural history of the disease. It is possible that other therapies that reduce DKK1 effects in the marrow such as lithium, a potent inhibitor of the GSK3, kinase or small molecule inhibitors of GSK3, which have the potential to activate Wnt singling downstream of the receptor-ligand complex on the cell surface or other factors that promote Wnt signaling and osteoblast differentiation, e.g. BMP-2, anti-DKK1, PTH, and autologous mesenchymal stem cell transplantation, may be combined to exploit this new direction in myeloma biology and therapy. Along these lines of investigation we have recently used polyclonal anti-DKK1 antibody therapy to treat SCID mice that have been transplanted with primary myeloma. Preliminary data suggest that this treatment results in the activation of osteoblasts, inactivation of osteoclasts, and reduction in tumor burden. An update on the progress of these experiments will be reported. We and others have also shown that there is a statistically significant non-random distribution of elevated DKK1 expression in hyperdiploid and t(11; 14) myelomas (Robianni *et al.,* 2004). As these forms of myeloma tend to have better prognosis than other molecularly defined forms of disease, e.g. FGFR3/MMSET-positive myelomas, it is possible that secretion of Wnt inhibitors by myeloma cells may also suppress Wnt signaling on myeloma cells. This theory is bolstered by the recent discovery that myeloma plasma cells, unlike other B-lineage cells, can signal through Wnt (Qiang *et al.,* 2003, Derksen *et al.,* 2004).

To test whether primary myeloma plasma cells signal through Wnt, we treated freshly isolated CD138-enriched plasma cells from six newly diagnosed myeloma patients with Wnt 3A-conditioned media. Evidence of Wnt signaling was tested by Western blot analysis for β-catenin stabilization. β-catenin was stabilized in all cases. Next, we investigated whether DKK1 could inhibit the Wnt-canonical pathway by adding recombinant human DKK1 protein to two myeloma samples; Wnt-3a mediated β-catenin stabilization was inhibited significantly by DKK1. We then performed Western blots on protein extracts from plasma cells from 69 newly diagnosed cases to determine the relative levels of β-catenin across all samples. These data showed highly divergent levels of stable β-catenin. We then correlated the level of β-catenin with global gene expression profiles on an aliquot of the cells used in the Western blot analysis. A high degree of correlation was observed between levels of β-catenin stabilization and expression of neural adhesion molecules N-cadherin (CDH2) and NCAM1. There was no correlation between β-catenin levels and DKK1 expression on our molecularly defined myeloma subtypes. However there is a strong correlation between the expression of both CDH2 and NCAM1 with DKK1 expression on our microarray analysis of 351 newly diagnosed myelomas. Given that Wnt signaling has been implicated in carcinogenesis and that β-catenin stabilization results in activation of gene transcription, we then treated freshly isolated plasma cells from five newly diagnosed cases with Wnt 3A-conditioned media for 6 hours and then performed gene expression profiling on treated and untreated cells from each case using the U133Plus2.0 microarray. Although there is clear stabilization of β-catenin, which can be blocked by DKK1, we have not been able to identify consistent gene expression changes in primary MM. This study was done on a limited number of cases. The gene expression profiling revealed that myeloma is made up of at least seven subgroups, with DKK1 expression predominating in specific subgroups, it is possible that study of a larger cohort will reveal subgroup-specific changes linked to Wnt3A treatment. We will continue these analyses and will report the updated analysis on Wnt signaling and also the relationship of bortezomib therapy and osteoblast development.

F8.05

MACROPHAGE INFLAMMATORY PROTEIN-1 MAY CAUSE RECIPROCAL REGULATION OF OSTEOCLAST AND DENDRITIC CELL DIFFERENTIATION FROM MONOCYTES IN MYELOMA

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Multiple myeloma (MM), a malignancy of plasma cells, generates a devastating bone destruction by osteoclasts (OC). We and others reported that macrophage inflammatory protein (MIP)-1alpha and beta are among major OC activating factors secreted by MM cells. In contrast to OC, dendritic cells (DC) decrease in number and their function is defective in MM, leading to tumor escape and susceptibility to infection. Because OC and myeloid DC are derived from the same monocytic precursor cells, we hypothesized that differentiation into the two cell lineages is reciprocally regulated and that MM cells modulate the lineage determination process. In this study, we examined roles for MIP-1 as well as MM cells in OC and DC induction from human peripheral blood monocytes. OC were formed from monocytes spontaneously or by exogenous M-CSF and soluble RANKL. Interestingly, addition of anti-MIP-1alpha and beta antibodies in combination inhibited OC formation with a concomitant increase of RANK-negative macrophages. Culture supernatants of thus formed OC revealed MIP-1alpha and beta immunoreactivity, suggesting an autocrine action of MIP-1 in the OC induction. We next investigated a role for MM cell-derived MIP-1 in OC induction from monocytes. When the MIP-1- and RANKL-expressing MM cell line TSPC-1 was co-cultured with monocytes on dentine slices, formation of TRAP-positive multinucleated cells as well as pits were enhanced. Again, anti-MIP-1alpha and beta in combination abrogated these enhancement, suggesting a critical role for MM cell-derived MIP-1 in enhancement of monocyte-derived OC formation and function. In sharp contrast to the OC induction, non-adhesive co-cultures with MIP-1-producing MM cell lines, RPMI8226 and U266, as well as primary MM cells inhibited the induction of $CD83^{+}$, $CD80,^{++}$ HLA class II^{++} mature DC from monocytes in the presence of GM-CSF plus IL-4, followed by TNF-alpha, whereas those with normal lymphocytes did not. Addition of anti-MIP-1alpha and beta in combination restored the differentiation of CD1a+ immature DC by GM-CSF plus IL-4 and further induction of CD83+ mature DC by TNF-alpha suppressed in the co-cultures with RPMI8226 cells. These results suggest that MM cell-derived MIP-1 may play a critical role in the commitment of OC lineage as well as OC maturation and activation and inhibition of DC maturation. Thus, MM cells may affect the reciprocal regulation of differentiation into OC and DC lineages, thereby enhancing bone resorption and concomitantly inhibiting antigen-presenting capacity of DC.

PLENARY SESSION 9
ALLOGENEIC TRANSPLANTATION

PL9.01

STATE OF THE ART OF ALLOGENEIC TRANSPLANTATION IN MULTIPLE MYELOMA

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Allogeneic transplantation for multiple myeloma, performed since the early 1980s, may have the potential to cure a fraction of the patients. However, using conventional high dose myelablative conditioning it is hampered by a significant transplant-related mortality (TRM) of 25-40%. Recent use of reduced intensity non-myeloablative conditioning seems to diminish the treatment related mortality (TRM) to below 20%, but the effect on relapse rate is uncertain.

Conventional myeloablative conditioning treatment usually consists of total body irradiation in a dose of about 10 Gy with lung shielding, fractionated or non-fractionated, and combined with high-dose cyclophosphamide. High-dose melphalan and TBI has also been widely used in myeloma. However, despite high-dose treatment, the relapse rate is significant, indicating that myeloma precursor cells persist in most patients. Due to a transplant related mortality of 25-40% it is not feasible to increase the dosage. In contrast, published and ongoing trials have focused on the idea of using reduced intensity conditioning to exploit the graft-versus-myeloma (GVM) effect. Should be enough to eradicate most of the myeloma cells, sparing the normal cells and thus diminishing transplant-related mortality. It has been shown that engraftment can be obtained and that the GVM effect can be enhanced by adding donor lymphocytes later on to prevent or to treat threatening relapse.

Due to longer follow up, more data are available for myeloablative transplants than for non-myeloablative ones. A comparison of myeloablative transplants performed by the Myeloma subcommittee of the EBMT during the two time periods 1983-1993 and 1994-1998 showed a significant improvement in survival during the latter time period. The improvement was due to decreased transplant-related mortality. This in turn seemed to be due to many factors, e.g. earlier transplantation and more effective treatment of bacterial, fungal and viral infections. No change in relapse rate was seen. The later use of peripheral blood stem cells (PBSC) instead of bone marrow was not the reason for the improvement. On the contrary, a later update indicates that there may be a small, but borderline significant disadvantage of using PBSC, which may be related to a higher risk of chronic GVHD.

Favorable prognostic factors for myeloablative transplantation are low age, low beta2-microglobulin, stage I at diagnosis, responsiveness to previous treatment and only one treatment regimen before transplantation. Recently we have shown that although the female to female combination has the best outcome, the relapse rate in males with a female donor is significantly lower than in males with a male donor, compensating for the higher transplant-related mortality in this combination.

Procedural factors play a role in outcome, but documen-

tation is poor. The most frequently used conditioning regimen for myeloablation is cyclophosphamide 60 mg/kg x 2 plus 10 Gy total body irradiation with lung shielding to 9 Gy. There is no evidence from registry data that improved results are obtained by various other regimens that have been reported to the EBMT registry.

Prophylaxis of GVHD by methotrexate plus cyclosporine is standard and other regimens have not proven to be superior in terms of improving survival, although various T-cell depletion methods have diminished GVHD.

A survey among EBMT centers indicates that reduced intensity non-myeloablative regimens result in lower transplant-related mortality than with myeloablative regimens, particularly in good prognosis patients. However, recent registry analysis indicates a higher relapse rate. This may be partly due to the use in early studies of T-cell depletion without subsequent DLI. Relapse risk might be counteracted by prospective DLI post-transplant.

The use of non-myeloablative allogeneic BMT as a strategy to reduce relapse after autologous transplantation is currently being explored. EBMT is presently running a study comparing tandem autologous-non-myeloablative allogenic transplantation to autologous transplantation alone based on the availability of an HLA matched sibling donor. Scheduled donor lymphocyte transfusions are given post-transplantation depending on response or recurrence. The initial safety analyses indicate 11% early transplant-related mortality following the non-myelablative transplantation.

In summary, non-myeloablative allogeneic transplantation reduces transplant related mortality compared to myeloablative transplantation, but the effect on relapse rate and overall survival is as yet unclear. It should still preferentially be performed in controlled trials. Myeloablative transplantation may be an option for selected patients with stage IIIA disease, preferentially for younger women who are diagnosed when they have stage I disease and who have an HLA matched female sibling donor. Patients who are responsive to previous conventional treatment and have received only one treatment regimen before the transplant are the best candidates. However some patients who have not responded and are poor candidates for conventional treatment or autologous transplantation may be offered an allogeneic transplant if their general condition is otherwise good.

PL9.02

CURRENT STATUS AND PERSPECTIVES OF DOSE-REDUCED CONDITIONING FOLLOWED BY RELATED AND UNRELATED STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Allogeneic stem cell transplantation after dose-reduced conditioning exerts a powerful graft-versus-myeloma effect and is associated with a relative low treatment-related mortality in comparison to standard conditioning. Several strategies to incorporate the dose-reduced allo-approach within the treatment of multiple myeloma have been performed such as a tandem auto-allo transplantation or as salvage therapy after failure to a prior autograft. The most commonly used regimens are either a very low-intensity approach with 2 Gy-total body irradiation with or without fludarabine or melphalan at an intermediate dose of 100 – 140 mg/m^2 in combination with fludarabine. Other regimens such as cyclophosphamide/fludarabine or busulfan/fludarabine or treosulfan/fludarabine have been also investigated. Risk factors for unfavorable outcome reported in smaller series of patients are no chemosensitivity at time of allografting, relapse to a prior autograft or no experience of chronic GvHD. Chromosomal abnormalities in multiple myeloma, especially deletion of chromosome 13 have become an important prognostic factor even after dose-reduced allogeneic stem cell transplantation. In a recent multicenter international analysis involving 120 patients after melphalan/fludarabine conditioning, relapse after prior high-dose chemotherapy was the most significant factor for treatment-related mortality (HR: 2.8), for relapse (HR: 4.14), event-free survival (HR: 3.11), and for overall survival (HR: 2.69).

Therefore, a more successful strategy is using dose-reduced allogeneic transplantation earlier in the course of disease in chemosensitive patients. The most promising strategy is to reduce tumor burden with high-dose melphalan and autografting followed after a 2-3 month interval by a second, dose-reduced allogeneic transplantation to induce the graft-versus-myeloma effect.

So far, we and several groups have published their preliminary experience with that approach using either melphalan (100-140 mg/m^2)/fludarabine or 2 Gytotalbody-irradiation plus/minus fludarabine as conditioning regimen prior to allogeneic transplantation. The preliminary results of all studies were similar: the treatment-related mortality at day 100 ranged from 0-6%, and the mortality at one year ranged from 0-17%. It is notable that no difference could be seen in the treatment-related mortality between the matched-sibling- and the unrelated donors. A complete donor chimerism was seen in nearly all patients and was probably supported by the immunosuppressive effect of the preceding autologous transplantation.

The rate of acute GvHD grade II – IV ranged from 32-44%. Severe grade III – IV GvHD was seen in 6-18% of the patients. Chronic GvHD ranged between 28-64%, and extensive chronic GvHD was seen in 8-46% of the patients. The overall response rates for all studies ranged from 68 – 83% including a high rate of complete remissions of 52 – 83%.

The overall survival at two or three years ranged from 62-78%, and the progression-free survival ranged from 54-56%. Despite these encouraging preliminary results, for all studies a longer follow-up is necessary to assess the influence of the high rate of complete remissions on survival and to determine whether this treatment approach is a curative approach in patients with myeloma. Several international ongoing studies compare this two-step auto-allo-approach with tandem autograft within randomized studies, such as the EBMT, the Bone Marrow Transplant Clinical Trial Network (BMT-CTN) and several national myeloma study groups.

Currently preclinical and clinical research is focused on two major issues: 1) reducing the treatment-related morbidity and mortality of allografting and 2) enhancing the remission status after transplantation by adoptive immunotherapy to prolong disease-free survival and hopefully cure the patient.

Major efforts are made to reduce TRM by reducing acute and chronic GvHD after transplantation by using poly- and monoclonal antibodies for *in vivo* T-cell depletion. Anti-thy-

mocyte globulin (ATG) and alemtuzumab (campath 1-H) have been shown to reduce the incidence of acute and chronic GvHD, but since it has been shown that chronic GvHD is a strong factor for preventing relapse, some concern has been raised about losing the graft versus myeloma effect by using *in vivo* T-cell depletion. Recently, we could show that in myeloma patients alemtuzumab resulted in less GvHD after unrelated stem cell transplantation in comparison to ATG, but also in a lower rate of complete remissions and a higher risk of relapse. To enhance remission status and prevent relapse adoptive immunotherapy with donor lymphocytes can be used. The response rate after DLI ranges between 30 and 50%, but only few achieved complete remission. Despite the efficacy of DLI there is a substantial risk of acute and chronic graft-versus-host disease (GvHD). The largest study reported 57% acute GvHD and 47% chronic GvHD. The risk of severe GvHD can be avoided by using a dose-escalating DLI approach. In order to enhance the remission rate of DLI we combined thalidomide with DLI in a phase II study achieving a remission of more than 60% with no severe grade of GvHD. Since achieving molecular remission after allogeneic transplantation is associated with long-term freedom of disease and probably cure, molecular remission should be the target for any post-transplant strategies. The ultimative goal for adoptive immunotherapy would be the separation of the GvHD from the graft-versus-myeloma effect, which would result in a more specific tumor targeting with less toxicity. Several targets, such as hematopoiesis restricted minor histocompatibility antigens or idiotype proteins have been used for T-cell specific DLI or as donor vaccine and will be further explored to treat minimal residual disease after allografting in order to induce molecular remission and long-term freedom of disease.

PL9.03

HIGH DOSE OR REDUCED INTENSITY CONDITIONING FOR MULTIPLE MYELOMA?

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Allogeneic stem cell transplantation is of proven benefit for a variety of hematologic malignancies. The ability to eradicate disease is based on the cytotoxic effects of the high dose preparative regimen and the allogeneic activity of donor lymphoid cells. There is currently intense debate about the relative contributions of the preparative regimens vs. the allogeneic effect of donor lymphocytes (GVL) on the ability to cure leukemias. This is especially true in multiple myeloma (MM), a disease where historically, the transplant-related mortality has ranged between 30-50% even in relatively young patients. This has led to exploration of reduced-intensity regimens designed more for immunosuppression than cytoreduction, with the hypothesis that donor lymphoid engraftment would hopefully lead to disease eradication through a graft-versus-leukemia (GVL) effect.

A variety of lower intensity conditioning regimens are being investigated for their safety and potential efficacy prior to allogeneic transplant for patients up to age 70. These regimens generally combine fludarabine with intermediate doses of cyclophosphamide, melphalan or single-dose total body irradiation. Some regimens consist of intermediate does of melphalan alone. Lower mortality rates of between 5-15% have been reported with CR rates of 20-40%. Unfortunately, several studies indicate that patients with MM who have significant residual disease rarely achieve durable complete responses after non-ablative allografting. In addition, efforts to make the reduced intensity regimens even safer by reducing GVHD through the use of antibodies, have had the paradoxical effect of reducing GVL. These observations would indicate that the GVL effect in MM is relatively modest and in the setting of significant residual disease may be inadequate to result in durable, long-term remissions.

The observation that primarily patients with minimal disease have the best outcomes has led to another strategy, utilizing a tandem transplant approach where the non-ablative allograft follows a standard autologous transplant using high dose melphalan alone. Most of these studies have reported transplant-related mortality rates of 15-20%; an apparent reduction over high dose regimens. Complete response rates of 25-70% have been reported, with 1-year survivals of 60-80%. Even with a prior autologous transplant to achieve a state of minimal disease, however, studies with sufficient follow-up have indicated a continuing risk of relapse at least within the first 3 years after allogeneic transplant. This risk is currently estimated to be 50-60% even with a tandem auto/reduced, intensity allograft approach. Currently it is unknown which of these non-ablative regimens is the safest and most efficacious. Furthermore, the data on tandem auto/allo transplants are too preliminary to determine what the long-term survival and disease-free survival for such patients will be and whether this approach will result in a higher proportion of durable remissions than high-dose, ablative regimens. Some insight may be gained by examining the experience in chronic myeloid leukemia (CML). The GVL effect in CML is well established and at least as potent, if not more so than MM. Durable CR to donor lymphocyte infusions (DLI) used to treat relapse after allogeneic transplant are obtained in 60-75% of patients with CML compared to 22-28% in MM. Non-ablative transplants have been performed for patients with CML in an effort to reduced transplant-related toxicity. In a recent survey from the EBMT, the rates of relapse at 2 years were 35%. With an ablative transplant regimen, Slattery and colleagues (1997) have demonstrated a relationship between steady-state busulfan (BU) concentration and relapse in patients receiving HLA-compatible transplants for CML in chronic phase. In that study there were seven relapses among the 22 patients with BU steady-state concentrations below the median value of 917 ng/mL and none among the 23 patients at or above the median (p=0.0003). Since 1995, the FHCRC has utilized dose-adjusted BU, targeting a steady state concentration of at least 900 ng/mL. A total of 144 patients with CML in chronic phase have received a transplant using marrow (BM, n=110) or mobilized peripheral blood stem cells (PBSC, n=34). Four patients died of transplant complications before day 100; the estimated 1 and 3 year non-relapse mortalities were 10% and 14%. The estimates of relapses at 1 and 3 years were 3% and 5%; only 1 patient has died of relapse. Thus in a disease with a relatively high sensitivity to the GVL effects of allogeneic cells, the rates of relapse after ablative regimens are highly dependent on dose intensity as measured by steady state BU levels. This would argue that adequate cytoreduction in CML, prior to allografting is important to ensure long-term remissions. It is very likely that in MM, a disease with the same or lower sensitivity to GVL, the same principle applies. Thus future efforts at improving outcomes after allogeneic transplant for patients with multiple myeloma should focus on strategies to make high dose, ablative regimens more tolerable and safer. Although non-ablative allogenic transplants for patients with MM can be used to extend this therapy to older patients, this strategy is unlikely to be curative for very many patients due to the lack of cytoreduction and the relatively weak GVL effects.

PL9.04

STRATEGIES TO IMPROVE THE GRAFT-VERSUS-MYELOMA EFFECT OF ALLOGENEIC STEM CELL TRANSPLANTATION AND DONOR LYMPHOCYTE INFUSIONS IN MULTIPLE MYELOMA

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Donor lymphocyte infusions. The existence of graft versus myeloma is best illustrated by the effect of donor lymphocyte infusion (DLI) given to patients with relapsed disease after allogeneic stem cell transplantation (Allo-SCT). Response rates to DLI between 30-50% have been reported. We recently updated the outcome of DLI in 54 patients for relapse after partially T-cell depleted Allo-SCT.[1] Twenty-eight (52%) patients responded, 19 (35%) with a partial response and 9 patients (17%) with a complete response. Progression free and overall survival was a median of 19 and 23 months respectively. Six patients are in sustained CR more than 2 years after DLI. In a subgroup analysis, deletion of chromosome 13, as determined by double color FISH in diagnostic bone marrow samples, had no impact on outcome, indicating that these patients are candidates for early allogeneic transplantation followed by DLI in case of no response.

Targets involved in graft-versus-myeloma. Acute and chronic GVHD after DLI occurring in 57% and 47% of patients respectively were the strongest predictors for response.[1] This strongly suggests that like in leukemia the targets for the cytotoxic donor cells are minor histocompatibility (mHa) antigens expressed on both recipient normal and myeloma plasma cells. Nonantigen specific mechanisms in association with GVHD, such as cytokines or tumor specific antigens however may be involved as well in GVM. GVM may occur without GVHD and recently it was shown that in patients achieving CR to DLI this was associated with high antibody responses to highly expressed myeloma –associated antigens.[2]

We have isolated myeloma reactive T-cell clones from patients following myeloablatieve and non-myeloablative Allo-SCT. The clones were isolated from the blood post transplant using irradiated peripheral blood mononuclear cells and EBV transformed B cells from the patients obtained prior transplantation. One CD4+ clone lysed both autologous EBV cells and autologous myeloma cells indicating a common polymorphic peptide functioning as target for graft derived cytotoxic T cells.[3] Another CD4+ clone recognized the (non autologous) MM cell line UM9, autologous myeloma cells, monocytes and PHA-induced T-cell blasts but not CD40L stimulated autologous B cells and stromal cells. Additional studies showed that the CD4+ clone was activated in a HLA-DP*0401 restricted fashion and that the antigen was expressed by 27% HLA-DP*0401 positive EBV transformed B cell lines (P.Holloway *et al*: in press). The high prevalence of this HLA class II-restricted antigen, together with its apparent hematopoietic restriction makes it an antigen of interest for cellular immunotherapy.

Enhancement of the graft versus myeloma effect.

Although 30 -50% patients treated with DLI benefit from a positive GvM effect and probably an equal percentage of patients treated with nonmyeloablative allogeneic stem cell transplantation, the number of (sustained) molecular remissions is low especially in patients with high risk features. The mechanism of immune escape/resistance to Allo-reactivity including DLI are greatly unknown and may include down regulation of target antigens and/or accessory molecules on myeloma cells and effector cells, presence of inhibitory molecules and cytokines and/or ineffective antigen presentation. Recently the role of antigen presenting cell (APC) genotype in DLI was studied in mice showing that timing is critical for obtaining GVH and GVT reactivity. Sykes *et al.* showed that DLI in mixed hematopoietic chimeras results in better leukemia-free survival compared to DLI in full donor chimeras.[4] They hypothesize that recipient APC present in mixed chimeras at the time of DLI may exert more optimal presentation of recipient antigens leading to superior activation of infused donor T cells, whereas absence of recipient DC in full donor chimeras may result in non-responsiveness. In patients, induction of allo immune responses after DLI not only depends on the number of infused donor T cells but also on the interval between SCT and DLI. Furthermore, mixed chimeras induced with nonmyeloablative conditioning have attained striking remissions of refractory lymphoid and solid malignancies both with and without DLI.[5] These data indicate that the APC genotype plays an important role in transplantation immunity, and may be a critical factor for induction of potent GVT/GVM reactivity. These studies stimulated us to analyze chimerism patterns of cellular subsets in patients following non myeloablative allogeneic stem cell transplantation. Although mixed chimerism for many months to years was the usual pattern in the T cell compartment, dendritic cells became of donor origin almost completely within a few months after transplantation. Our observations and the murine studies indicate that administration of autologous APC post NMA and DLI might enhance the GVM effect. Clinical studies with autologous APC as pre-emptive therapy after NMA and with DLI combined with autologous APC for treatment of relapse are in preparation.

Other potential interesting options are the application of immune modulating agents like thalidomide (analogs) and bortezomib to increase a specific anti-tumour reaction following Allo-SCT and/or DLI. Among the many actions of thalidomide immunological properties include potentiation of NK cell activity, stimulation of CD8+ cells and Il-2 secretion. In a study with 26 relapsed patients response (PR + CR) to low dose thalidomide combined with DLI was 50% including 22% CR suggesting a synergistic anti-myeloma effect.[6] In a phase I study in refractory myeloma patients the thalidomide analog CC 4047 (actimid) induced increased CD45RO expression on CD4+ and CD8+ cells, with a concomitant decrease in CD45RA+ cells and significantly increased serum interleukin (IL)-2 receptor and IL-12 levels. In a leukemia mouse model bortezomib inhibited graft-versus-host disease with retention of the graft versus leukaemia effect. The combination of allogeneic BMT and Bortezomib significantly improved the survival.

These preliminary studies will stimulate the further exploration of strategies aimed at improving molecular response rate of Allo-SCT and DLI. Molecular remissions are the only guarantee for long term myeloma free survival and probably cure.

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PL9.05

FEASIBILITY AND EFFICACY OF A PLANNED SECOND TRANSPLANT ("AUTO" OR "MINI-ALLO") INTENSIFICATION IN PATIENTS WITH MULTIPLE MYELOMA NOT ACHIEVING COMPLETE REMISSION (CR) OR NEAR-CR WITH A FIRST AUTOLOGOUS TRANSPLANT: RESULTS FROM A SPANISH PETHEMA/GEM STUDY

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Background. It has been shown in a non-randomized setting that continuing treatment intensification, including tandem transplant, results in an increased CR rate. A randomized trial showed that tandem transplant resulted in a significantly longer EFS and OS in patients failing to achieve CR or near-CR with a single transplant. However, other studies failed to show a significant benefit from a second transplant.

Objective. To investigate the feasibility and efficacy in terms of response up-grading and survival from a second transplant intensification in patients with chemosensitive disease who failed to achieve CR or near-CR with a first transplant in a large multicenter trial.

Patients and Methods. Patients diagnosed with multiple myeloma (MM) from Oct 1999 to Dec 2003 younger than 70 years received 6 courses of VBMCP/VBAD chemotherapy and responding patients were intensified with busulphan/melphalan or MEL-200 followed by stem cell support. Patients with a persistent M-spike on electrophoresis (i.e., not having achieved CR or near-CR) were planned to undergo a second transplant (either a second auto with CVB - cyclophosphamide, etoposide and BCNU - intensification or a dose-reduced intensity *allo* with fludarabine/MEL-140 conditioning, depending on sibling donor availability).

Results. The complete data on 141 patients candidates for a second transplant have been validated by two of the authors (LR, JB). It is of note that 79 (56%) did not receive the planned second HDT procedure because of the following reasons: patient refusal -24 pts-, poor PS -14 pts-, lack of CD34 -10 pts-, physician decision -10 pts-, progressive disease before the second transplant -13 pts-, others -8 pts-. Patients who did not proceed with the second transplant were significantly older (58 vs. 54 yrs, p= 0.007) and had higher serum beta2-microglobulin levels (5.4 vs. 3.5, p= 0.039). Forty-eight patients received a second autologous transplant while 13 underwent a *mini-allo*. Thirty-five percent of the patients given a second autologous transplant achieved an up-graded response (CR or near-CR: 8%, PR: 12% and MR: 15%) while 65% showed *no change*, progressive disease or early death. A response up-grade was observed in 45% of patients undergoing a *mini-allo* procedure (CR: 31%, PR: 7%, MR: 7%). The CR rate was significantly higher with the allogeneic procedure (31 vs. 4%, p= 0.02). However, the TRM was higher with the *miniallo* procedure (4% vs. 22%, p=NS). The survival from the second high-dose procedure was not significantly different between the two transplant modalities (2nd auto vs "mini-allo"). The results of this study will be updated for the Workshop presentation

Conclusions. 1) In about one-half of the patients in whom a tandem transplant is planned the second high-dose procedure is not performed; 2) a dose-reduced intensity allogeneic transplant; after an autologous procedure results in a significantly higher CR rate than a tandem autologous transplant, 3) with the current follow-up we found no significant differences in survival between the two modalities of second transplant intensification.

FOCUS SESSION 9
HOMING MECHANISMS AND SIGNAL TRANSDUCTION

F9.01

BONE MARROW HOMING OF MULTIPLE MYELOMA CELLS

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A major feature of multiple myeloma (MM) cells is their restricted localization in the bone marrow (BM). As it is believed that MM originates from an antigen-selected B cell, disease spreading is likely to be mediated by a circulating cell that can (re-) enter the BM microenvironment. Using the *in vivo* 5T2MM model, we could demonstrate that homing of MM cells from the blood circulation to the BM is not a random process but occurs selectively.[1] In analogy to the homing mechanism of normal leucocytes,[2] it can be assumed that MM homing is also a multi-step process, involving particular adhesion molecules, chemotactic signals and proteases. Our group could identify the functional expression of different types of molecules indicating that MM cells home to BM according to this model. Both human MM cells as well as murine 5TMM cells were found to express different adhesion molecules, including VLA-4, CD38 and CD44, that allow binding to BM endothelium, the first step in the homing cascade.[3] Moreover, we demonstrated that 5T33MM cells adhere preferentially to BM endothelial cells when compared to other types of endothelial cells and that this adhesion is at least partially mediated by CD44 variant 10.[4] BM endothelium also expresses different molecules that cause chemotaxis of MM cells. We found that both human MM cells and murine 5T2MM cells migrate to laminin-1, a major component of the basement membrane of BM endothelium. This migratory effect was found to be mediated by the 67kd laminin receptor.[5] Other BM-EC produced chemotactic molecules to which MM cells are responsive include MCP-1 and IGF-1.[6,7] Interestingly, the expression of some of the receptors for these chemotactic signals, like 67kd laminin receptor and IGF-1 receptor is upregulated on MM cells after contact with BM-EC.[5,8]

The BM homing of MM cells is also stimulated by BM fibroblast-derived chemokines. We could demonstrate that these cells produce MCP-1, -2 and –3, that all induce MM cell migration through the CCR2 receptor.[9] We found that MM cells express additional chemokine receptors i.e. CCR1 and CXCR-4, that have also BM-derived ligands, i.e. MIP-1α and SDF-1, respectively. Evaluating the expression of these different chemokine receptors on a large panel of BM samples from MM patients, we found recently that the $CD138^+$ cell chemokine receptor expression phenotype offers a prognostic value; complete loss of this expression on MM cells seems to be associated with unfavorable disease status (in preparation). A major step in the migration of MM cells is the passage through the basement membrane underneath the BM endothelium, which necessitates that the tumor cells can degrade the extracellular matrix. We demonstrated that both human and murine MM cells express the metalloproteinase-9 (MMP-9).[10,11] This MMP degrades collagen-IV, an important component of the BM endothelium basement membrane. Its production is also upregulated when MM cells are exposed to BM-EC and in human MM cells this involves HGF.[11]

In conclusion, our work of the last decade revealed that the homing of MM cells to BM is a complex process that involves multiple molecular pathways. Targeting one or more of these pathways might affect disease evolution and can provide (a) new therapeutical tool(s). Specific *in vivo* experiments in the 5TMM model lead already to the promising observation that antagonists/inhibitors against adhesion molecules (CD44v10)[4] or receptors for chemotactic molecules (67kD)[5] can indeed impair the BM homing of MM cells.

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F9.02

ROLE OF INSULIN-LIKE GROWTH FACTOR AND ITS RECEPTOR IN THE 5TMM EXPERIMENTAL MOUSE MODEL

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Insulin-like growth factor-1 (IGF-1) is an endocrine factor mostly involved in metabolic control that also exerts anti-apoptotic functions in many cancer cells.[1,2] Although IGF-1 is mainly produced by hepatocytes in response to growth hormone stimulation, it is now well recognized that besides the liver, IGF-1 is also produced by different cells in other organs like the bone marrow (BM) (e.g. chondroblasts, fibroblasts and osteoclasts), where autocrine or paracrine mechanisms of actions are important. It has previously been demonstrated that IGF-1 is an important survival and proliferation factor for myeloma (MM) cells *in vitro*.[3,4] The IGF-1 receptor (IGF-1R) /CD221, a tyrosine kinase receptor with unique characteristics that differentiate it sharply from the insulin receptor, is predominant in mitogenesis, transformation and protection from apoptosis. Its expression has been demonstrated on human MM cells [3,5] and was correlated with poor prognosis of the patients.[6]

In the present work we investigated the expression and role of the IGF-1/IGF-1R pathway in the 5TMM experimental mouse model. This model, originally developed by J. Radl [7], is a syngeneic fully immunocompetent murine model that resembles the MM disease closely.[8,9] Its major clinical characteristics are the BM homing of the MM cells, the presence of a monoclonal serum M component, the induction of angio-

genesis and the development of osteolytic lesions (in the 5T2MM model). The expression of the IGF-1R α/β on both 5T2 and 5T33MM cells was demonstrated by FACS analysis.[10,11] Its expression was higher on CD45 positive MM cells and was correlated with the invasive behavior of the cells, both *in vitro* and *in vivo*.[12] Binding of IGF-1 on its tyrosine kinase receptor resulted in the phosphorylation of the downstream signaling molecules ERK1/2 and Akt. IGF-1 induced F-actin assembly [13] resulted in the migration of the MM cells [10] in a PI3K dependent way [14]. Induction of DNA synthesis occurred by a PI3K/Akt-MEK/ERK pathway. We could also demonstrate that IGF-1 induced VEGF secretion, one of the prominent angiogenic factors in MM, and this by the MEK-ERK pathway.

To investigate the molecular sequelae stimulated in 5TMM cells after exposure to IGF-1 (6h), microarray analyses were performed (Affymetrix), revealing distinct transcriptional profiles. The major groups of genes were associated with higher metabolic functions, higher synthesis of DNA and proteins and altered microtubuli. The expression of the IGF-1R by the MM cells was BM microenvironmentally dependent. We demonstrated an induced expression of the IGF-1R by the 5TMM cells, isolated from the BM 18h after injection of the cells into naïve mice. Moreover when these MM cells were harvested from the BM of diseased mice, their migratory capacity towards BM stromal factors was significantly increased compared to the cells before injection.[11] Direct contact with BM endothelial cells was involved in this upregulation.

To investigate the *in vivo* role of the IGF-1/IGF-1R system, different strategies can be followed. Here, 5T33MM injected mice were treated with a highly selective IGF-1 receptor tyrosine kinase inhibitor, picropodophyllin (PPP), of the cyclolignan family.[15] The tumor burden of the BM of the treated animals was 77% lower than that of the vehicles (with a 90% decrease of serum paraprotein). This was associated with a 66% reduction of BM microvessel density (as assessed after CD31 staining). In a separate experiment, Kaplan Meier analysis demonstrated a significant increase in survival of PPP treated animals when compared to vehicles (28 vs. 18 days). *In vitro* analysis demonstrated direct effects of PPP on IGF-1 induced proliferation, VEGF secretion and migration of the MM cells.[16]

Together these data demonstrate the relevance of the IGF-1/IGF-1 receptor pathway in multiple processes in the pathobiology of MM and show the potential of IGF-1 receptor tyrosine kinase inhibitors in therapeutical strategies targeting MM.

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F9.03

AKT IN MYELOMA: THERAPEUTIC IMPLICATIONS

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The AKT kinase is a downstream target of phosphatidylinositol 3'kinase (PI3-K). PI3-K is activated via binding of its p85 regulatory subunit to growth factor receptors or adapters. Ras can also stimulate PI3-K by directly binding to the p110 kinase subunit. PI3-K then phosphorylates phosphatidylinositols (PI) which function as second messengers activating AKT. Phosphorylated PI bind to the pleckstrin homology domain of AKT, resulting in membrane localization of the kinase where it can be phosphorylated on serine/threonine residues for full activation. By virtue of its ability to dephosphorylate PIs, the tumor suppressor PTEN phosphatase inhibits signaling through AKT.

In situ immunohistochemistry, with a phospho-specific antibody, demonstrated frequent activation of AKT in patients multiple myeloma (MM) cells. AKT activation was not present in MGUS plasma cells nor in non-malignant hematopoietic cells. This latter finding indicates a therapeutic window may exist in patients. *In vitro* work with MM cell lines and limited numbers of primary specimens suggests that oncogenic ras mutations and marrow IL-6/IGF-1 stimulation could account for the observed AKT activation. Other potential mechanisms of kinase activation, still to be tested, include stimulation via MM cell binding to stromal cells or extracellular matrix and loss-of-function alterations in PTEN.Downstream signaling from AKT diverges into viability-promoting pathways or a proliferation-promoting pathway and both of these are present in MM cells. The viability-promoting influences are due to AKT-dependent alteration of several different effectors, including forkhead transcription factors, BAD, NF-kB, ASK-1 and GSK. Indeed, AKT has been shown to protect against MM cell apoptosis induced by dexamethasone and PS-341 and, thus, targeting the PI3-K/AKT pathway in combination with such apoptosis-inducing agents makes a lot of sense.

In our work, we have chosen to target the proliferation-promoting pathway. Proliferation is stimulated by AKT-dependent signals flowing through the tuberous sclerosis complex of proteins to the mammalian target of rapamycin (mTOR). Once activated, mTOR induces phosphorylation and activation of p70S6kinase (p70) and the 4E-BP1 translational repressor. Phosphorylated p70 is critical for ribosome biogenesis. 4E-BP1 phosphorylation releases the eIF-4E translation initiation factor, allowing it to participate in an initiation complex where the mRNA cap binds the 40s ribosomal subunit, followed by scanning of the mRNA for an initiation codon and initiation of translation. The so-called cap-dependent translation of cell cycle proteins occurs in this manner, allowing G1-S transit.

We have used the mTOR inhibitors rapamycin (RAPA) and CCI-779 (CCI) as therapeutic agents in MM. Therapeutically achievable concentrations in MM cells inhibited p70 and 4E-BP1 phosphorylation, prevented release of unbound eIF-4E, inhibited D-cyclin and myc expression and induced G1 arrest. Of great interest is the fact that sensitivity to G1 arrest correlated with AKT activity of MM cells. Myeloma cells expressing heightened AKT activation, either due to oncogenic ras mutation, loss-of-function PTEN mutation, or gene transfer, demonstrated hypersensitivity to RAPA/CCI in terms of *in0 vitro* G1 arrest, down-regulation of D-cyclin expression and *in vivo* anti-tumor effects in a xenograft model. As cells retain a cap-independent salvage pathway of translation when mTOR is paralyzed, which is due to internal structures in the 5'UTR of mRNAs (internal ribosome entry sites or IRESes) that can independently recruit the ribosomal 40s unit, we have hypothesized that AKT activity can inversely regulate such IRES function, preventing this salvage pathway of translation.

The finding that high AKT activity sensitizes MM cells to mTOR inhibitors is clinically relevant. First, the high degree of AKT activation in patient MM cells would predict for frequent efficacy of mTOR inhibitors. Second, as AKT activity protects against most anti-tumor treatments, the heightened sensitivity to mTOR inhibitors makes these drugs particularly useful for these potentially resistant MM clones. Third, mutated ras-containing myeloma, which is associated with an aggressive phenotype, and which exhibits heightened AKT activation, may be especially sensitive. The design of future phase II trials of mTOR inhibitors in myeloma should, thus, attempt to assay pre-treatment AKT and ras status as possible markers of response.

F9.04

IN MYELOMA CELLS, BIM PRO-APOPTOTIC FUNCTION IS NEUTRALIZED THROUGH ENDOGENOUS MCL-1/BIM COMPLEX FORMATION ON MITOCHONDRIA RATHER THAN BY SEQUESTRATION TO THE DYNEIN

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Multiple myeloma is a fatal malignancy of B-cell origin characterized by the accumulation of plasma cells within the bone marrow. IL-6 is a major myeloma cell survival factor that induces the activation of both Ras/MAP kinase and JAK/STAT pathways, the latter being involved in cell survival. Thus, IL-6 starvation or IL-6 signaling blockade by AG490 (a JAK 2 inhibitor) triggers apoptosis. In myeloma cells, others and us have shown that Mcl-1 is the major anti-apoptotic protein, since Mcl-1 antisenses induce apoptosis while Bcl-2 or Bcl-xL antisenses have no effect. In the present study, we examined the pro-apoptotic BH3-only molecule Bim which is implicated in apoptosis induced by growth factor deprivation. The three major Bim isoforms (EL, L and S) are expressed in viable human myeloma cell lines and are essentially associated with mitochondria. The Bim isoforms are negatively regulated by IL-6. Blockade of IL-6 signaling induces the up-regulation of Bim isoforms concomitant to the down-regulation of Mcl-1. Of major interest, in viable myeloma cells, Bim is strongly associated with Mcl-1, with Bcl-2 and to a lesser extent with the dynein light chain. Endogenous Bim/Mcl-1 and Bim/Bcl-2 complexes are essentially present on the mitochondria. Bim is always complexed in healthy cells; indeed when removing Mcl-1, Bcl-2 and dynein by immunoprecipitation there is no free Bim left. Bim/Mcl-1 endogenous interaction is disrupted upon apoptosis induction, either by IL-6 starvation or AG 490 treatment. Of note, Bim/Bcl-2 complex is not affected under the same conditions. In parallel to disruption of Bim/Mcl-1 interaction and Bim release, Bax activation was observed. Therefore, in myeloma cells Bim function is neutralized through endogenous complex formation with Mcl-1 on mitochondria rather than by sequestration to the dynein. Under apoptosis induction, Mcl-1 drastic down-regulation leads to Bim release, which in turn exerts its pro-apoptotic function through Bax activation. We provide evidence that disruption of the Mcl-1/Bim interaction is an interesting therapeutic approach which could be achieved by BH3 peptidomimetics.

F9.05

INHIBITION OF MULTIPLE MYELOMA CELL PROLIFERATION AND INCREASE OF APOPTOSIS THROUGH REGULATION OF THE NF-KB AND JNK PATHWAYS BY SILENCING TRAF6 C-DOMAIN MRNA

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Several members of the tumor necrosis factor receptor-associated factor (TRAF) family, including TRAF2, TRAF5, and TRAF6 have been implicated in regulating signal transduction from various TRAF family members. However, the unique biological function of TRAF6 is largely determined by its TRAF-C domain, which does not interact with peptide motifs that are recognized by TRAF1, -2, -3 or -5.

Based on TRAF6, CD40, and RANKL sequences and crystal structures, we targeted the TRAF6 C-domain residues from 420 to 440 because the TRAF6 interaction domain with CD40 or RANKL resides in residues 333 to 508. We found that silencing TRAF6 mRNA with the C-terminal domain siRNA significantly inhibited MM cell proliferation maximally at 72 hours whereas effects on inducing MM cell apoptosis were most prominent at 48 hours. The decrease in cell proliferation and increase in cell apoptosis occurred in a concentration (of siRNA)-dependent fashion. Furthermore, NF-kB mRNA expression and protein levels were also reduced using a TRAF6 C-domain siRNA. We also examined the effect of TRAF6 siRNA on the JNK pathway since this signaling pathway is also associated with cell cycle effects in myeloma. We measured JUN kinase kinase (JNKK), which activates the MAP kinase homologues SAPK and JNK in response to IL-1 receptor stimulation. The results showed that the phosphorylation of JNKK is clearly reduced after knockdown of TRAF6 gene expression by siRNA. Furthermore, we examined c-Jun, a component of the transcription factor complex AP-1, which binds and activates transcription at TRE/AP-1 elements. The transcription activity of c-Jun is regulated by SAPK/JNK binding to c-Jun and phosphorylation of c-Jun at Ser63/73. We found that total endogenous c-Jun is reduced after silencing TRAF6 mRNA in the RPMI8226 MM cell line. In contrast, our data demonstrated that the TRAF6 C-domain siRNA does not affect TRAF5 or TRAF2 gene expression. In addition, introduction of siRNA derived from the Zn-finger sequence into RPMI8226 MM cells failed to inhibit TRAF6 production. These studies suggest that the TRAF6 C-domain may be an excellent target to block myeloma cell signaling important for the survival and proliferation of MM cells.

PLENARY SESSION 10
CHAIRMAN'S SYMPOSIUM

PL10.01

HUMAN APURINIC/APYRIMIDINIC ENDONUCLEASES LEAD TO GENETIC INSTABILITY IN MULTIPLE MYELOMA

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Multiple myeloma (MM) is associated with significant genomic instability. As endonucleases play an important role in mediating homologous recombination, which is elevated in myeloma, chaos with spontaneous decay, oxidation, and DNA replication errors frequently give rise to abasic sites, 3-mismatched nucleotides, and non-conventional 3-ends in the genome. These DNA modifications can lead to mutations that require immediate repair. Apurinic/apyrimidinic (AP) endonucleases (Ape1 and Ape2) are the key proteins involved in the repair of DNA modifications described above. Ape1 is the major abasic endonuclease implicated in base excision repair and 3-end processing with endonuclease activity and is responsible for repair of alkylation and oxidative DNA damage in cells. The protein also functions as a redox factor and maintains transcription factors in an active reduced state and regulate their DNA binding activity such as p53, Jun, Fos, NF-kappaB, HIF-1, and PAX. In this study we have evaluated role of AP endonuclease in multiple myeloma (MM). Gene expression profile analysis showed > 2 fold elevation of Ape1 or Ape2 or both in 5 of 6 MM cell lines and 12 of 15 patient samples. Immunocytochemistry confirmed upregulation of Ape1 protein in MM cell lines. A plasmid degradation assay confirmed significantly elevated endonuclease activity in MM cells compared to normal plasma cells. To identify the predominating endonuclease activity, the degradation assay was carried out in the presence of specific endonuclease inihibitors. Aurintricarboxylic acid (ATA; Ca^{++}/Mg^{++} dependent endonuclease inhbitor), harmane (the specific inhibitor of apurinic/apyrimidinic endonuclease with associated glycosylase activity), and methoxy amine (MA; specific inhibitor of apurinic/apyrimidinic endonucleases) effectively inhibited endonuclease activity in MM cells, confirming the predominant role of apurinic/apyrimidinic endonucleases (Ape1 and Ape2) in mediating increased endonuclease activity in MM. Next we investigated the role of elevated APE endonuclease activity in DNA recombination and subsequent genomic rearrangements. Using a plasmid-based assay we have previously demonstrated significantly elevated homologous recombination (HR) in MM. HR activity was suppressed (85+/- 2%) by exposure of MM cells to methoxyamine (MA; inhibitor of AP endonucleases). Next, we evaluated whether inhibition of HR by MA can affect the frequency of acquisition of new genetic changes in myeloma cells using single nucleotide polymorphism (SNP) arrays (Affymetrix) as an indicator of genomic instability. In three independent experiments, MA reduced the acquisition of new LOH loci by an average of 71%. These data suggest that dysregulated APE endonucleases significantly contribute to genomic instability, acquisition of new mutations in MM cells and progression of disease and provide the rationale for targeting endonuclease activity to prevent disease progression including development of drug resistance.

PL10.02

BONE MARROW MICROVESSEL ENDOTHELIAL CELLS IN MULTIPLE MYELOMA HARBOR MYELOMA-ASSOCIATED CHROMOSOMAL TRANSLOCATIONS

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Introduction. Studies in malignant disease have indicated that tumor growth is dependent on the formation of new blood vessels. In multiple myeloma (MM), previous investigations have documented that microvessel density (MVD) is increased in the bone marrow of patients with active MM, and that markedly elevated MVD is of prognostic significance. Moreover, MM endothelial cells, which exhibit a distinct angiogenic phenotype, interact with MM cells via a variety of chemokines and their receptors. We have recently observed that microvessel endothelial cells of patients with B-cell lymphomas carry lymphoma specific aberrations (NEJM 351:250-9, 2004). It was therefore the aim of the present study to determine whether MM-associated chromosomal translocations are present in bone marrow microvessel endothelial cells.

Materials and Methods. Using a combined immunohistochemical and fluorescence *in situ* hybridization assay (FICTION) on bone marrow biopsies, we investigated the endothelial cells of 5 patients with MM for cytogenetic aberrations known to be present in the myelomatous plasma cells (4 cases with a t(11;14)(q13;q32), one case with a t(4;14)(p16.3;q32)). Results were also compared with those obtained in other hematologic malignancies (6 anaplastic large cell lymphomas, 1 angioimmunoblastic T-cell lymphoma, 4 chronic myeloid leukemias, 3 acute myeloid leukemias and 1 acute lymphoblastic leukemia).

Results. We observed that microvascular endothelial cells of all investigated cases harbored the disease-specific chromosomal aberrations. The MM specific translocations were found in a median of 42% of endothelial cells (range, 21% to 71%). This percentage of genetically aberrant endothelial cells was lower compared to chronic myeloid leukemia (median 63%), but higher than in anaplastic large cell lymphoma (median 15%).

Conclusion. Our findings suggest a genetic relationship between MM cells and their endothelium, which reflects a novel aspect of tumor angiogenesis in MM.

PL10.03

THE COEXPRESSION OF CD11^{a} AND CD45^{bright} IS THE HALLMARK OF PROLIFERATING MYELOMA CELLS

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To identify new potential therapeutic targets in multiple myeloma (MM), we have defined the phenotype of the subset of proliferative myeloma cells (n=66) in comparison with that of normal PC (n=25). Proliferation was evaluated by *ex vivo* incorporation of BrdU (labeling index, LI). Surface PC phenotype was performed in a four-color assay with CD38, CD45, CD138 and the mAb indicated. For intracellular BdrU staining, cells were first labeled with CD38, CD45 and CD138, fixed and permeabilized before BrdU staining. At least 1000 normal PC and 10000 myeloma cells were analyzed. We show that all bone marrow PC, either malignant or normal, always included a subset of proliferative PC

(BrdU+) that was always located within the CD45++ fraction. Indeed, CD45++ myeloma cells (median 12%) had a labeling index (LI) 7.5-fold higher that of CD45+/- myeloma cells (7.1% versus 0.94%). Actually, in all cases of MM, CD45++ myeloma cells were always the most proliferative myeloma cells. As observed for myeloma cells, LI of normal PC was heterogeneous i.e., higher in the CD45++ population of PC: CD45++ PC (median 65%) had a LI 5.7-fold higher of that of CD45+/- PC. Compared to myeloma cells, LI of PC were higher in both subsets, of 20.5% and 3.6% for CD45++ and CD45+/-, respectively. Non-malignant PC from blood or tonsil were homogeneously CD45++ and did proliferate (LI> 10% and up to 45% for reactive PC). In all PC (normal, reactive, malignant), we found an inverse correlation between CD45 and Bcl-2, confirming a known inverse correlation between proliferation and Bcl-2 expression. Our data suggest that a minor cycling Bcl2 lowCD45++ population of myeloma cells differentiate into a no more cycling major Bcl2 high CD45+/- population of myeloma cells that accumulates. We further characterized the CD45++ myeloma cells phenotype: CD11a and to a less extent HLA-DR were expressed only by CD45++ myeloma cells in contrast to CD40 and CXCR4 that were expressed by all myeloma cells. Moreover, all CD45++ myeloma cells coexpressed CD11a. Thus, the-to-be-killed population of myeloma cells could be targeted through CD45 or CD11a.

PL10.04

BCL-XL, MYC, AND MUTANT RAS TRANSGENIC MOUSE MODELS OF PLASMA CELL MALIGNANCIES

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While there have been several mouse models of plasma cell malignancies, until recently, few reports describe spontaneous development of plasma cell tumors in the bone marrow. In an effort to develop such a model, we have made several transgenic mouse lines that incorporate targeted expression of myc, bcl-xL, and mutant ras. Our transgenic lines include: 1) bcl-xL driven by the kappa Ig 3' enhancer (3'KE-bcl-xL); 2) myc driven by the IgH Emu enhancer (Emu-myc); 3) myc driven by a C° intron enhancer (TV3); 4) mutant ras driven by 3'KE (3'KE-ras); and an additional experimental approach using retroviral abl-myc infection. Through cross breeding of the 3'KE-bcl-xL line with myc lines, we have been able to show genetic cooperativity and have generated multiple mouse lines that show 100% incidence of plasma cell tumors. Double transgenic myc/bcl-xL mice produce plasma cell tumors that infiltrate the bone marrow and produce monoclonal Ig, and recapitulate many of the features of human multiple myeloma. V-Abl-myc infected bcl-xL transgenic mice also show evidence of plasma cell tumors that infiltrate the bone, compared to v-abl-myc infected littermate controls that developed only extramedullary plasma cell tumors. The effect of bcl-xL on v-abl-myc-induced tumors was notably more evident in older mice compared to younger mice (9 week median survival versus 17 week median survival). The 3'KE-ras transgenic mice do not develop malignancy, but aged mice develop immunoglobulinemia and accumulations of plasma cells in extramedullary sites. When crossed to Emu-myc mice, bi-transgenic animals develop fatal B-cell tumors (median survival 10 weeks). Additional cross-breedings with ras transgenic mice are in progress. In some cases, we have developed *in vitro* plasma cell culture lines from double transgenic mice, that can grow as tumors by adoptive transfer in recipient mice. More recently we have developed a lenti-viral system to target plasma cell tumor lines with GFP, that will provide marking for tracking sites of tumor development. This system will also allow us to transduce additional genetic deregulation into tumor lines. In addition, the transgenic model system allows us to develop multiple tumor lines that will likely demonstrate genetic heterogeneity, as seen in human myeloma patients. Gene expression profiles are being conducted on each line. This model system provides an opportunity to develop panels of plasma cell tumors that can be examined for differences in localization, progression, gene expression, and therapeutic responses. Progress in the development of this model will be presented.

PL10.05

FINAL RESULTS OF THE IFM9904 PROTOCOL: DOUBLE TRANSPLANT ± ANTI-INTERLEUKIN-6 MONOCLONAL ANTIBODY IN HIGH-RISK *DE NOVO* MULTIPLE MYELOMA PATIENTS LESS THAN 65 YEARS OLD

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IFM Group

The median survival of patients with high-risk *de novo* multiple myeloma (MM) (β2 microglobulin >3 and chromosome 13 deletion by FISH analysis), is approximately 2 years when treated with high-dose therapy (Facon *et al,* Blood 2001; 97:1566). For this subgroup of patients, in 1999, the IFM group initiated two trials, the IFM9903 and 9904 protocols. In both protocols, the induction regimen consisted of VAD (4 courses) followed by melphalan 200 mg/m^2 (HDM200) plus autologous peripheral blood stem cell transplantation (APBSCT). When a HLA-sibling donor was available, APBSCT was followed by miniallogeneic PBSCT: IFM9903 protocol. When no family donor was available, patients were randomized to receive a second APBSCT prepared by a higher dose of melphalan, HDM220 ± anti-IL6 monoclonal antibody (BE-8, 250 mg total dose, Diaclone Besançon, France): IFM9904 protocol. The aim of this study was to evaluate prospectively the impact of the addition of anti-IL6 moAb to a higher dose of melphalan (Moreau *et al,* Br J Haematol 2000;109:661).

From 12/1999 to 08/2004, 225 patients less than 65 years of age with *de novo* MM with both β2 microglobulin>3 and chromosome 13 deletion by FISH analysis at diagnosis without an HLA-identical sibling donor were included in the IFM9904 protocol. Twenty-eight patients included after 03/2004 are too early to evaluate. Thus 197 patients are included in this survival analysis. Thirty of the 197 (15%) were not randomized to receive or not anti-IL6 moAb + HDM220 because of disease progression (9), early death (3) or severe infection (10) during VAD induction therapy, refusal (2), protocol violation (4) or toxic death during the first APBSCT (2). One hundred and sixty-seven (85%) were randomized, 84 in arm A = HDM220 without anti-IL6 moAb, 83 in arm B = HDM220 + antiIL6 moAb.

At the reference date of September 1st 2004, the median event-free survival (EFS) and overall survival (OS) of the whole group of 197 patients from the time of diagnosis are 30 and 39 months, respectively. Patients who proceeded to randomization (arm A + B, n = 167) had a significantly better OS and EFS as compared with patients (n = 30) who were not randomized, median OS 45 months vs 11 (p<0.001) and median EFS 31 months vs 10 (p<0.001). The addition of anti-IL6 in the conditioning regimen before the second APBSCT did not

improve either EFS or OS: median EFS 34 months in arm A (without anti-iL6 moAb) vs 30 in arm B (p=0.84), and median OS 38 months in arm A vs 46 in arm B (p=0.25). These survival rates (> to 50% at 3 years) are superior to what has been previously described in this high-risk subgroup of patients, indicating that a double transplant strategy (HDM200 / HDM 220) may prolong survival. Nevertheless, the addition of anti-IL6 moAb to HDM220 during the second conditioning regimen did not influence the outcome of patients. The final results of the 9904 protocol for the whole cohort of 228 patients will be presented during the 10th International Myeloma Workshop.

PL10.06

FIRST INTERIM ANALYSIS OF THE JOINT HOVON-50/GMMG-HD3 RANDOMIZED STUDY ON THE EFFECT OF THALIDOMIDE COMBINED WITH ADRIAMYCIN, DEXAMETHASONE AND HIGH DOSE MELPHALAN IN PATIENTS WITH MULTIPLE MYELOMA

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In November 2001 a randomized phase 3 trial was initiated in patients with multiple myeloma stage II/III under 66 years. The primary objective of the study was to determine whether thalidomide as part of induction therapy before, and as maintenance after intensive treatment improves response rate, event-free and overall survival. In the standard arm induction therapy consists of 3 cycles of VAD (vincristine, doxorubicin, dexamethasone) given as rapid infusion, followed by stem cell collection after CAD (cyclophosphamide, doxorubicin, dexamethasone) + G-CSF, intensification with 1 or 2 cycles of high dose melphalan (HDM), 200 mg/m^2 + PBSCT and maintenance treatment with interferon-α 3x10^6 U, thrice weekly until progression or relapse. In the experimental arm patients receive thalidomide 200 mg/day (which can be escalated to 400 mg/day) combined with doxorubicin, dexamethason (TAD) as induction and Thalidomide 50 mg/daily as maintenance until progression or relapse. Patients randomized to TAD receive low dose low molecular weight heparin (LMWH, nadroparine 2850 IU (5700 IU > 90 kg) during induction therapy as prophylaxis against venous thrombo embolism (VTE). In the HOVON part of the study patients all patients with an HLA-identical family donor and in the GMMG part only high risk patients (Δ 13 and high β-2M) are candidates for inclusion in a satellite study with non myeloablative allogeneic stem cell transplantation (200cGy conditioning only) between 2 and 6 months after HDM. As of October 15, 2004, 995 patients were included and the study will be closed in (early) 2005. Forty-eight (HOVON) patients were included in the auto/allo study of which 1 patient died from steroid refractory-graft-versus host disease and 2 patients from progression (one patient before NMA and 1 patient 5 weeks after NMA) Thirty cases of VTE in 412 evaluable patients were reported (7%), 11 (6%; 95% CI 3-10%) in arm A and 19 (9%; 95% CI 6-14%) in arm B (p=0.15). During VAD or TAD only, 8 (4%) and 17 (8%) VTE cases were reported (p=0.08). When compared with historical controls this LMWH prophylaxis reduces the incidence of VTE in *de novo* MM patients who receive treatment with Thalidomide in combination with dexamethasone and/or anthracyclines. An interim analysis including at least 400 patients will be performed in March 2005. Both arms will be compared for response, event-free survival, overall survival and toxicity. The results of this interim analysis will be presented during the workshop.

PL10.07

MELPHALAN-PREDNISONE (MP), MP-THALIDOMIDE AND HIGH-DOSE THERAPY USING MELPHALAN 100 MG/M^2 FOR NEWLY DIAGNOSED MYELOMA PATIENTS AGES 65-75 YEARS: INTERIM ANALYSIS OF THE IFM 99-06 TRIAL ON 350 PATIENTS

T Facon, JY Mary, C Hulin, L Benboubker, M Attal, JL Harousseau, B Pegourie, M Renaud, G Guillerm, L Voillat, C Chaleteix, J Troncy, M Monconduit, P Casassus, H Maisonneuve, V Dorvaux, J Jaubert, M Dib, C Doyen, on behalf of the Intergroupe Francophone du Myelome (IFM)

The results of the IFM 95-01 trial comparing MP to dexamethasone-based regimens were consistent with the fact that the standard MP remained the reference treatment for patients (pts) older than 65 years with multiple myeloma (MM) (Blood 2003;102,147a). In May 2000, the IFM designed a new trial, IFM 99-06, for pts aged 65-75 years, comparing standard MP (12 courses at 6 weeks intervals) to MP-thlidomide (THAL) (same MP, THAL at the maximum tolerated dose but ≤400 mg/day, stopped at the end of MP) and a MEL100-based treatment (intermediate-dose MEL procedure developped by the Italian MM study group); VADx2, cyclophosphamide 3 g/m^2 for stem cell collection, 2 courses of MEL100. IFM99-06 is planned to randomize 500 pts, according to a 3 (MP) – 2 (MP-THAL) – 2 (MEL100) allocation, and schedules two main comparisons; MP vs MP-THAL and MP vs MEL100. The primary end point is overall survival. In the absence of clear toxicity data for MP-THAL and MEL100 in elderly MM pts, in the setting of a large randomized study, two interim analyses were planned, after the enrollment of 200 (40%) and 350 (70%) pts (followed at least 4 months), to be reviewed by an independent expert review committee (R.Schots / B.Van Camp, Brussels, Belgium; M. Boccadoro, Torino, Italy; S.Chevret, Paris-St-Louis, France). The first interim analysis was performed during the first months of 2003 (reference date for analysis January 2, 2003; 200 pts enrolled between May, 2000 and June, 2002). The experts considered that (i) the adverse events were as expected with the treatments used (ii) noclear-cut advantage or disadvantage of either MP-THAL or MEL100 over MP was observed according to the statistical rule of the first interim analyses (iii) the trial should continue as planned. Following a request of the french regulatory agency AFSSAPS, we were asked to look rapidly at some thalidomide toxicities and at the feasability of MEL100. The incidence of deep venous thrombosis (DVT) was 6%, 9% and 3.5% for MP, MP-THAL and MEL100, respectively (no toxic death due to DVT). Peripheral neuropathy was observed in 25% of MP-THAL pts (14/57 pts, at a median time of 12 months). MEL100 was feasible; approximately 80% of the pts received the cyclophosphamide and 65% received the 2 MEL100 treatments. No toxic death was noted among 74 courses of MEL100 (39 pts). The second interim analysis, performed on 350 pts (382 pts enrolled on August 1, 2004) is in progress during July/August 2004 (reference date June 1, 2004). Results of the second interim analysis will be presented at the meeting.

PLENARY SESSION 11: IMMUNE BIOLOGY

PL11.01

TUMOR IMMUNE INTERACTIONS IN MYELOMA

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Clonal expansion of a transformed cell is essential, but not sufficient for the development of clinical cancer. This is particularly puzzling in the case of monoclonal gammopathies, because many of the cytogenetic and genomic changes initially described in myeloma have now also been detected in pre-malignant monoclonal gammopathy of undetermined significance (MGUS). The immune system has been postulated to play a role in surveillance of tumors in mice. The role of immune effectors in the control of transformed cells in patients with gammopathies remains to be fully defined. We have studied tumor reactive immune effectors in the blood and tumor bed of patients with MGUS and myeloma. We have recently shown that freshly isolated T cells from blood or tumor bed of patients with progressive myeloma lack tumor reactive rapid effector function. Targeting tumor antigens to Fcγ receptors of dendritic cells (DC) leads to enhancement of cross presentation and generation of tumor reactive effector T cells (Dhodapkar *et al,* J Exp Med 2002;195:122). Using this approach, T cells from the tumor bed of even patients with progressive tumors can be activated to yield tumor reactive killer T cells (Dhodapkar *et al,* PNAS 2002;99:13009). Anti-tumor reactivity in these cultures was specific for autologous tumor, and mostly not directed against Ig derived determinants. Natural killer T cells are distinct lymphocytes that recognize glycolipid antigens in the context of the CD1 family of antigen presenting molecules. We find that clinical progression in myeloma is associated with a loss of ligand reactive rapid effector function in NKT cells, which can be restored *ex vivo* using dendritic cells (Dhodapkar *et al*., J Exp Med 2003;197:1667). Direct analysis of tumor-specific effector T-cell function in MGUS now indicates the presence of an active anti-tumor effector T-cell response (Dhodapkar *et al.*, J Exp Med 2003;198:1753. This response is enriched in the marrow and is specific for antigens expressed by tumor cells in each patient. Preliminary studies also suggest the common occurrence of humoral responses against a panel of defined tumor antigens in these patients. Together, these data provide direct evidence for active and tumor specific immune recognition in the bed of a human non-viral *preneoplastic state*, and strongly suggest that the development of *clinical myeloma* is regulated at least in part at the level of the host immune response consisting of both innate and adaptive immune effectors. Premalignant lesions may therefore be attractive targets for vaccine based approaches against cancer.

PL11.02

T CELLS IN MYELOMA: LESSONS FROM A MURINE MODEL

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Background. Multiple myeloma (MM) cells produce myeloma protein with V-regions that express unique tumor-specific antigenic determinants called idiotopes (Id). Id+ myeloma protein may, upon immunization, induce both Id-specific antibodies and Id-specific T cells. Id-specific antibodies are probably of little use in the treatment of MM since they should be blocked by copious amounts of myeloma protein present in extracellular fluids. Moreover, MM cells express little surface immunoglobulin (Ig), and thus provide few targets for Id-specific antibodies. As for Id-specific T cells, CD8+ T cells might play a role in immunotherapy of MM but their relevance has been little studied. Here, we will focus on the role of Id-specific CD4+ T cells in eradication of MM cells.

A murine model for Id-specific CD4+ T cells in MM. The mouse MOPC315 plasmacytoma produces M315 (IgAλ2) myeloma protein. It has been shown that M315 protein is endocytosed and processed by antigen-presenting cells (APC), and that a fragment of the λ2 Ig light chain corresponding to amino acids 91-101 is presented on MHC class II molecules on APC to cloned Id-specific CD4+ T cells *in vitro*.[1-4] This particular $\lambda 2^{315}$ Id-peptide differs from the λ2 germ line amino acid sequence in positions 94, 95 and 96, and thus represents an Id-peptide that depends on somatic mutations commonly present in Ig V-genes in MM cells.[1, 2, 5] Since MOPC315 is MHC class II negative itself, it cannot directly present V-region Id-peptides to CD4+ T cells. However, when APC are added to a mixture of MOPC315 and Id-specific CD4+ T cells *in vitro*, growth of MOPC315 cells is inhibited.[6] A parallel situation was found in tumors: M315 secreted by MOPC315 cells is endocytosed by tumor-infiltrating APC that in turn present Id-peptide to Id-specific CD4+ T cells.[7, 8] Once activated, such Id-specific CD4+ protect against challenges with MOPC315 cells in a T-cell receptor transgenic mouse model.[9, 10] Despite these encouraging results, if high loads of MOPC315 cells are injected, resistance of TCR-transgenic mice is partly overcome. In this instance, once myeloma protein concentration in serum exceeds 50 μg/mL, T cell tolerance develops by deletion of Id-specific T cells in the thymus [11], in the periphery, and even in the tumor [4]. These results indicate that Id-specific CD4+ cells can successfully fight MM cells, however, if they fail to do so, MM cells expand and T cell tolerance ensues.

Novel developments in the MOPC315 model which are currently being addressed:

1. Is secretion of myeloma protein required for stimulation of Id-specific CD4+ T cells and protection against a tumor challenge? Based on the model described above, one might predict that secretion of myeloma protein is required for elicitation of tumor protective CD4+ responses. However, cell-associated Ig could be cross-presented by APC engulfing dead myeloma cells, as has been described for MHC class I-restricted tumor-specific antigen. To test this issue, we are employing three MOPC315 variants. One variant, MOPC315.26, secretes only a free λ2 light chain. Another variant, MOPC315.37, does not secrete any myeloma protein but retains free λ2 chain intracellularly. A third variant, MOPC315.36, does not produce any myeloma protein at all. By use of these three sublines we have been able to address whether secretion of Id+ myeloma protein is required for Id-priming of APC, activation of Id-specific CD4+ T cells, and rejection by Id-specific TCR-transgenic mice.

2. Rejection of MM cells by Id-specific CD4+ T cells studied in a collagen matrix. It has been difficult to study how Id-specific CD4+ T cells actually reject MOPC315 myeloma cells. To approach this question, we have embedded

MOPC315 cells in a collagen matrix placed in a s.c. site in TCR-transgenic mice. This approach has allowed us to study early events of rejection. Data will be presented which demonstrate that MM cells are rejected as a consequence of a complex interaction of tumor cells, macrophages and Id-specific CD4+ T cells.

3. Do Id-specific CD4+ T cells protect against disease in a bone marrow mouse model for multiple myeloma? The MOPC315 cell line is a mineral oil-induced plasmacytoma (MOPC) and is usually injected i.*p*. or s.c. where it forms local tumors. It has been questioned whether such local plasmacytomas represent valid models for human MM disease. To address this issue, we repeatedly injected MOPC315 cells i.v. and isolated tumor cells from flushed femurs between each passage. After 9 cycles, a MOPC315 variant has been obtained that has been tagged with luciferase. By use of *in vivo* imaging in intact animals, we are now testing whether this variant homes to the bone marrow. If so, we will test whether Id-specific CD4+ T cells protect against growth of MM cells in bone, a predilection site for MM disease. These studies should indicate whether Id-specific CD4+ T cells might be useful in the treatment of human MM bone marrow disease.

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PL11.03

DENDRITIC CELL BIOLOGY IN MULTIPLE MYELOMA

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Dendritic cells (DC) include specialized subsets of cells, which link the innate and cognate immune systems, thereby, initiating and directing immune responses. Human blood DC (BDC) include the CD11c+ myeloid subsets (CD16+, CD16- CD1c+ and CD16- BDCA3+) and the plasmacytoid CD123hi BDCA-2+ subset (Blood 2002; 100:4512). A new clinically useful BDC counting method shows that BDC are altered in a variety of clinical states and that BDCA-2+ DC are reduced in multiple myeloma (J Immunol Methods 2004; 284:73). The function of DC in multiple myeloma patients is abnormal (Blood 2001;98:2992) but this can be restored (Brit J Haematol 2004; 125:743). Studies on human BDC have established that they have different phenotypic and functional characteristics to in vitro cytokine generated monocyte derived DC (Mo-DC). Those data and the advantage of *patient manufacturing* as opposed to *in vitro manufacturing*, combined with their different migration capacities make BDC a theoretically attractive alternative for DC immunotherapies. We have developed a single step monoclonal antibody selection method for obtaining human DC for clinical immunotherapy procedures (J Immunol Methods 2003; 274:47). Preclinical *in vitro* studies indicate that BDC prepared from multiple myeloma patients are functionally effective and generate cytotoxic T cell responses. A phase I clinical trial has been given ethical approval but the challenge for multiple myeloma may remain with the definition of target tumor associated antigens.

PL11.04

PHENOTYPIC AND FUNCTIONAL ALTERATIONS OF GAMMA/DELTA T CELLS IN MYELOMA

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The role of innate immunity in natural tumor immunity and tumor immunotherapy has recently been revisited. Innate effector cells (NK cells, NKT cells, macrophages, and γδT lymphocytes) may act as the earliest defenders against tumor initiation and amplify immune responses mediated by adaptive immunity in a setting of minimal residual disease after high-dose chemotherapy. Among innate effector cells, circulating Vγ9/Vδ2 (γδ) T cells are naturally cytotoxic against a variety of hemopoietic tumor cell lines, including myeloma cell lines. Interestingly, γδ T cells can be activated by aminobisphosphonates (NBPs) which are commonly used in MM to treat or prevent osteoclast activation and bone disease. We have phenotypically and functionally characterized peripheral γδ T cells of normal donors and MM patients. On average, total counts of γδ T cells were similar in normal donors and MM at diagnosis. However, only 40% of MM patients at diagnosis showed *in vitro* γδT cell proliferation to zoledronic acid (Zol) as compared to 100% of normal donors. Total counts of peripheral γδ T cells were significantly lower in MM with defective Zol-induced proliferation (ZolPRO-) compared with normal donors and MM without defective Zol-induced proliferation (ZolPRO+). Immunophenotyping showed that memory γδ T-cells (CD45RA-CD27+), which have strong proliferative capacity, were minimally represented in ZolPRO- MM patients. By contrast, effector γδ T cells (CD45RA-CD27-) and terminally differentiated effector γδ T cells (CD45RA+CD27-), which have strong effector functions but low proliferative capacity, were predominantly represented in ZolPRO-MM patients. To explore the effector funtions of ZolPRO+and ZolPRO-MM patients, Zol-stimulated γδ T were tested for the expression of CD107a and CD107b antigens (as specific markers of cytotoxic activation), CD69 antigen (as marker of cell activation), IFN-γ production (both at the intracellular and extracellular levels), and cytotoxic activity against myeloma cell lines and primary myeloma cells. No differences were observed in the effector functions of γδ T cells of ZolPRO+ and ZolPRO- MM patients. Zol-induced antimyeloma activity was dependent on cell-to-cell contact and direct γδ TCR engagement, as shown by Transwell and blocking experiments with anti-γδ TCR or anti-αβ TCR mAbs. It has recently been proposed that γδ T cells can be activated by phosphoantigens-like metabolites derived from the mevalonate pathway of stressed or transformed cells.

We have confirmed that the Zol-induced activation of γδ T-cell effector functions *in vitro* is dependent on the mevalonate pathway of myeloma cells. It is currently under investigation whether the phenotypic and functional differences between ZolPRO+ and ZolPRO- MM patients are related to differences in the signaling of myeloma cells to Á‰ T cells via the mevalonate pathway and whether these differences may influence the immunocompetence and clinical outcome of MM patients.

PL11.05

THE ROLE OF DENDRITIC CELL GENOTYPE IN THE INDUCTION OF T-CELL RESPONSES AGAINST HEMATOPOIETIC MALIGNANT CELLS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Allogeneic stem cell transplantation (SCT) with or without donor lymphocyte infusion (DLI) can be curative in a variety of hematologic malignancies. DLI is very effective in chronic myeloid leukemia (CML), but multiple myeloma (MM) patients respond much less and more often relapse. The genotype of dendritic cells (DC) may play an important role in the induction of an efficient anti-tumor alloresponse. We evaluated chimerism of myeloid DC (MDC) and plasmacytoid DC (PDC) in relapsed patients prior to DLI. We found that both DC-subsets in relapsed CML patients (n=14) were predominantly of recipient origin, whereas DC-subsets in relapsed MM patients (n=7) were of donor origin. Murine transplant models show that the origin of the DC is crucial in elucidating graft versus tumor response, donor APC do not efficiently cross-present recipient antigens to donor CD8+ T cells. To test the hypothesis that recipient DC can boost anti-tumor alloresponses in MM patients after SCT, we wrote a DC vaccination protocol. Prior to RIC-SCT we cryopreserve monocytes from the patient to generate DC (MODC) for vaccination after allo-SCT during the phase of stable minimal residual or progressive disease. We observed that despite high-dose chemotherapy (HDM) MODC of MM patients can be efficiently matured, and have similar functional activity as MODC of healthy donors. We investigated whether recipient DC were efficient in expanding donor alloreactive T cells. Donor T cells obtained from a patient after SCT expanded 3.5 fold after stimulation with recipient MODC. These T cells produced higher levels of IFN-γ upon re-stimulation with recipient MODC than on re-stimulation with MODC of the stem cell donor or an MHC-mismatched donor. From this T-cell culture, a number of CD8+ T-cell clones were obtained that recognized an HLA-A2-restricted alloantigen on recipient MODC and EBV-LCL. These data suggest that recipient MODC are potent in inducing alloimmune responses *in vitro*. We now are testing *in vitro* the efficacy and specificity of the generated T cells.

In conclusion, chimerism data after allogeneic transplantation as well as animal studies suggest that patient DC are superior in generating anti-tumor alloresponses. We showed that cryopreserved monocytes collected after autologous transplantation and before reduced intensity allogeneic transplantation can be used to generate functional recipient DC. A clinical protocol is started, using MDC after reduced intensity allotransplant in patients with stable minimal residual or slowly progressive disease.

FOCUS SESSION 11: EXPERIMENTAL AGENTS

F11.01

A PHASE I, MULTI-DOSE, DOSE ESCALATION STUDY OF SGN-40 (ANTI-HUCD40 MONOCLONAL ANTIBODY) IN PATIENTS WITH REFRACTORY OR RECURRENT MULTIPLE MYELOMA

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CD40 is a type I transmembrane protein that upon binding to CD40 ligand regulates important biological effects in the immune system. CD40 is also highly expressed on hematologic tumors, which has raised interest in the potential for its use as a tumor target for antibody-based cancer therapy. SGN-40 is a humanized monoclonal antibody that selectively binds to human CD40 and induces apoptosis and growth inhibition of a wide variety of B-cell derived cancer cell lines *in vitro*. Our preclinical work has confirmed the *in vitro* cytotoxicity of SGN-40 against human multiple myeloma (MM) cells via several mechanisms. These include induction of cytotoxic ligands of TNF superfamily; suppression of IL-6-induced proliferative and anti-apoptotic effects, as well as antibody-dependent cell-mediated cytotoxicity (Tai, et al, Cancer Research 2004; 64, 2846-52). Since ~90% of MM cells express CD40, targeting CD40 using SGN-40 is a potential novel treatment strategy. Based on these preclinical data, a phase I study is being conducted to define the toxicity profile, characterize the pharmacokinetics (PK), and evaluate antitumor effects of SGN-40 in patients with refractory or recurrent MM. Four weekly doses ranging from 0.5 to 16 mg/kg are planned to be administered to groups of at least three patients per cohort. Patients will be followed for up to 6 weeks after their last dose. Currently, a total of thirteen patients have been treated with SGN-40 at dose levels of 0.5, 1.0, and 2 mg/kg. One patient treated at the 2 mg/kg level experienced a decrease in ANC to a grade 3 level over the four week treatment period. Three additional patients were treated at the 2 mg/kg level with no effect on ANC noted. Decrease in CD19 positive B cells were noted for patients treated at all dose levels. Changes in serum and urine M protein levels were measured to estimate potential anti-tumor effects of SGN-40. Of the nine patients evaluable for changes in M protein, two patients have had stable serum M protein, over the 10 week study period. Clinical evaluation with dose escalation of this agent will continue.

F11.02

ANTAGONIST ANTI-CD40 ANTIBODY CHIR-12.12 CAUSES TUMOR REGRESSION AND PROLONGS SURVIVAL IN MULTIPLE MYELOMA XENOGRAFT MODELS

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CD40 is expressed on most B-cell malignancies including multiple myeloma and represents an attractive target for antibody therapy. We have generated a novel, highly potent, fully human antagonistic anti-CD40 monoclonal antibody, CHIR-12.12, using XenoMouse mice (Abgenix, Inc). The antibody can mediate anti-tumor activity potentially by at least two mechanisms: CHIR-12.12 can block CD40-ligand mediated survival signals and it can lyse tumor cells by antibody-dependent cellular cytotoxicity (ADCC). We have previously reported that CHIR-12.12 mediates stronger killing of CD40- and CD20-expressing lymphoma cells than rituximab by ADCC *in vitro* and significantly inhibits the growth of both rituximab-responsive and rituximab-resistant human lymphoma xenografts *in vivo*. In this study, we examined *in vitro* and *in vivo* efficacy of CHIR-12.12 against human multiple myeloma. The human MM cell line IM-9, which expresses both CD40 and CD20, the target antigen for CHIR-12.12 and rituximab respectively was used for the study. CHIR-12.12 induced lysis of target tumor cells by ADCC in a dose dependent manner reaching maximum cell lysis at 0.1 ug/mL concentration. The maximum specific lysis of IM-9 cells by CHIR-12.12 was greater than the lysis induced by rituximab (64% vs 45%, n=3, $p<0.01$). In addition, the EC50 of CHIR-12.12 was on average 5.9 picomolar, which was 10-fold lower than the EC50 of rituximab. Greater ADCC by CHIR-12.12 was not due to higher density of CD40 molecules on the target tumor cells compared to CD20 molecules. IM-9 cells expressed 35590 8858 CD40 molecules compared to 93783 2247 CD20 molecules. The in vivo CHIR-12.12 efficacy was then evaluated in IM-9 xenograft model. In an un-staged conditional survival model, where treatment began one day after intravenous inoculation of IM-9 tumor cells, CHIR-12.12 significantly prolonged the survival of tumor-bearing mice in a dose-dependent manner with 60% survival in the 0.1 mg/kg CHIR-12.12 treated group and 80% survival in the 1 and 10 mg/kg groups respectively on day 56 (Log Rank Test: $p<0.01$ and $p<0.001$, respectively). All animals in the control IgG1 and bortezomib treated groups were suppressed between day 18 and day 26 due to severe disease related to tumor development (i.e., hind limb paralysis and significant body weight loss). In a staged subcutaneous model, where treatment began once the tumor volume was 150-200mm^3, CHIR-12.12 administered weekly at 0.1, 1 and 10 mg/kg significantly inhibited tumor growth with a tumor volume reduction of 17% ($p>0.05$), 34% ($P<0.01$) and 44% ($p<0.001$) respectively. Bortezomib, when tested at 0.5 mg/kg twice a week did not inhibit tumor growth. At the maximally tolerated dose (MTD) of 1 mg/kg twice a week, bortezomib inhibited tumor growth by 30% ($p<0.01$). Taken together, these data demonstrate that the anti-CD40 mAb CHIR-12.12 has potent activity against human multiple myeloma *in vitro* and xenograft models *in vivo*.

F11.03

CHIR-258, A NOVEL MULTI-TARGETED TYROSINE KINASE INHIBITOR, FOR THE TREATMENT OF t(4;14) MULTIPLE MYELOMA

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The t(4;14) translocation that occurs uniquely in a subset (15%) of multiple myeloma (MM) patients results in the ectopic expression of the receptor tyrosine kinase, fibroblast growth factor receptor3 (FGFR3). Wild-type FGFR3 induces proliferative signals in myeloma cells and appears to be weakly transforming in a hematopoeitic mouse model. The subsequent acquisition of FGFR3 activating mutations in some MM is associated with disease progression and is strongly transforming in several experimental models. The clinical impact of t(4;14) translocations has been demonstrated in several retrospective studies each reporting a marked reduction in overall survival. We have previously shown that inhibition of activated FGFR3 causes morphologic differentiation followed by apoptosis of FGFR3 expressing MM cell lines, validating activated FGFR3 as a therapeutic target in t(4;14) MM and encouraging the clinical development of FGFR3 inhibitors for the treatment of these poor-prognosis patients.

CHIR-258 is a small molecule kinase inhibitor that targets Class III-V RTK and inhibits FGFR3 with an IC50 of 5 nM in an *in vitro* kinase assay. Potent anti-tumor and anti-angiogenic activity has been demonstrated *in vitro* and *in vivo*. We employed the IL-6 dependent cell line, B9 that has been engineered to express wild-type FGFR3 or active mutants of FGFR3 (Y373C, K650E, G384D and 807C), to screen CHIR-258 for activity against FGFR3. CHIR-258 differentially inhibited FGF-mediated growth of B9 expressing wild-type and mutant receptors found in MM, with an IC50 of 25 nM and 80 nM respectively as determined by MTT proliferation assay. Growth of these cells could be rescued by IL-6 demonstrating selectivity of CHIR-258 for FGFR3.

We then confirmed the activity of CHIR-258 against FGFR3 expressing myeloma cells. CHIR-258 inhibited the viability of FGFR3 expressing KMS11 (Y373C), KMS18 (G384D) and OPM-2 (K650E) cell lines with an IC50 of 100 nM, 250 nM and 80 nM, respectively. Importantly, inhibition with CHIR-258 was still observed in the presence of IL-6, a potent growth factors for MM cells. U266 cells, which lack FGFR3 expression, displayed minimal growth inhibition demonstrating that at effective concentrations, CHIR-258 exhibits minimal nonspecific cytotoxicity on MM cells. Further characterization of this finding demonstrated that inhibition of cell growth corresponded to G0/G1 cell cycle arrest and dose-dependent inhibition of downstream ERK phosphorylation. In responsive cell lines, CHIR-258 induced apoptosis via caspase 3. *In vitro* combination analysis of CHIR-258 and dexamethasone applied simultaneously to KMS11 cells indicated a synergistic interaction. In vivo studies demonstrated that CHIR-258 induced tumor regression and inhibited growth of FGFR3 tumors in a plasmacytoma xenograft mouse model. Finally, CHIR-258 produced cytotoxic responses in 4/5 primary myeloma samples derived from patients harboring a t(4;14) translocation. These data indicate that the small molecule inhibitor, CHIR-258 potently inhibits FGFR3 and has activity against human MM cells. These results support the further study of CHIR-258 in t(4:14) myeloma.

F11.04

TARGETING THE INSULIN-LIKE GROWTH FACTOR-I RECEPTOR (IGF-IR) IN MULTIPLE MYELOMA CELLS USING SELECTIVE IGF-IR TYROSINE KINASE INHIBITORS

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In multiple myeloma (MM) emerging evidence provided by us and others suggest that the IGF-IR as an important mediator of tumor cell survival and thus resistance to cytotoxic therapy. Since IGF-IR is not an absolute requirement for maintenance of normal cell homeostasis, interfering with IGF-IR signaling at the receptor tyrosine kinase (RTK) level represents an attractive strategy to improve anti-cancer treatment. However, most IGF-I RTK inhibitors have the disadvantage of not to fully discriminating between the IGF-I RTK and the insulin RTK, i.e. are diabetogenic. Recently, members of the cyclolignan family have been shown to selectively inhibit the phosphorylation of the IGF-IR β-chain without downregulating the RTK activity of the insulin R. The effects of two of these compounds, picropodophyllin (PPP) and deoxypodophyllotoxin (DPPT), were studied *in vitro* using a panel of nine MM cell lines and freshly purified tumor cells from MM patients and also *in vivo* using the 5TMM mouse MM model. Both IGF-I RTK inhibitors effectively inhibited growth in all MM cell lines providing increased apoptosis and G2/M-arrest. Notably, the two drug-resistant subclones of the MM cell line RPMI 8226, Dox40 and LR5, were also highly sensitive to the IGF-I RTK inhibitors. In addition, PPP and DPPT showed inhibitory effects on primary MM cells cultured in vitro on bone marrow stromal cell feeder layers. Inhibition of the IGF-I RTK with PPP was previously shown to be ATP non-competitive suggesting interference with the IGF-IR at the substrate level. To identify tyrosine phosphorylation site(s) on the IGF-IR β-chain potentially regulated by the IGF-I RTK inhibitors, IGF-IR extracted from RPMI 8226 cells was analyzed by tryptic phosphopeptide mapping following 32P-orthophosphate-labeling and treatment with PPP or DPPT. The cleaved phosphopeptides were separated by thin layer electrophoresis/chromatography and analysed by a modified radio-Edman degradation. However, the results show that the IGF-I RTK inhibitors do not downregulate any specific tyrosine phosphorylation site of the IGF-IR in MM cells. Analysis of the same cell line using *in vitro* kinase assay suggests that PPP/DPPT-induced inhibition of the IGF-IR is conducted via a general downregulation of the tyrosine kinase activity of the receptor. Extraction of tyrosine phosphorylated proteins from the RPMI 8226 cell line followed by SDS-PAGE electrophoresis/silver staining and mass spectrometric analysis revealed a number of signaling molecules potentially affected by treatment with the IGF-I RTK inhibitors. Both PPP and DPPT seemed to e.g. impair tyrosine phosphorylation of CDK1/cdc2, a cell cycle associated protein also known as a potent regulator of apoptosis. Taken together, we show that treatment of MM cells with selective IGF-I RTK inhibitors decreases survival/proliferation and also affects the function of crucial intracellular signaling proteins, thus emphasizing the pivotal role for IGF-IR signaling in MM.

F11.05

IN VITRO ACTIVITY OF A NOVEL SMALL MOLECULE CYCLIN DEPENDENT KINASE INHIBITOR, CYC202 (SELICICLIB OR R-ROSCOVITINE), IN MULTIPLE MYELOMA

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A perturbation of the cell cycle is central to tumorigenesis, resulting in unrestricted cell proliferation. Cyclin dependent kinase (CDK) inhibitors have the potential to induce cell cycle arrest, followed by apoptosis, in cancer cells. CYC202 (seliciclib or R-roscovitine; Cyclacel, Dundee, UK) is a potent inhibitor of CDK currently undergoing phase II clinical testing. *In vitro* data have shown this tri-substituted purine to have greatest potency against CDK2/cyclin E, although it also inhibits CDK2/cyclin A, CDK7/cyclin H, and CDK9/cyclin T. It has been tested against a broad range of tumor cell lines, ultimately inducing cell cycle changes and apoptosis. This orally bio-available compound has also induced regression of human tumor xenografts in nude mice, prompting its clinical testing. Phase I studies have demonstrated favorable pharmacokinetics and toxicity profiles, and phase II trials are currently ongoing in combination with gemcitabine /cisplatin for non-small cell lung cancer and as a monotherapy in B-cell malignancies. We have previously shown that MM cell lines demonstrate relatively low levels of p21WAF1, suggesting that CDKs are constitutively activated, thereby promoting uncontrolled cell cycle regulation, growth, and proliferation. Conversely, inhibiting CDK activity may therefore trigger MM cell growth inhibition. Here we studied the *in vitro* activity of CYC202 in MM cells. Our data demonstrates that CYC202 has potent cytotoxic effects against MM cells that are both sensitive (MM1.S, RPMI 8226, U266, H929) and resistant (MM1.R, Dox-40, LR5, MR 20) to conventional chemotherapy. MM cell line cytotoxicity, as evidenced by MTT assays, is noted at 24 hours, with an IC_{50} ranging from 25-50 μmol. In contrast, this dose was not toxic to normal peripheral blood mononuclear cells. Cell cycle analysis demonstrated an increase (35-50%) in MM cells in sub-G1 phase at 24 hrs induced by CYC202 (25 Ìmol), suggesting that CYC202 triggers apoptosis. Caspase-8 and poly ADP-ribose polymerase (PARP) cleavage, evidenced by western blot analysis further confirmed apoptosis of MM cells. Importantly, CYC202 triggered a rapid down-regulation of MCL-1, a known anti-apoptotic protein in MM. Treatment of MM cells with CYC202 also resulted in decreased phosphorylation of retinoblastoma protein. Protein expression of certain CDK, specifically CDC2, CDK4, and CDK6, was also down-regulated after treatment. Ongoing studies are delineating the specific signaling cascades affected by CYC202 treatment. Future studies will be aimed at identifying combinations of CYC202 with both novel and conventional agents to enhance cytotoxicity, abrogate drug resistance and improve patient outcomes.

F11.06

INHIBITION OF HUMAN PLASMACYTOMA CELL GROWTH BY A NOVEL JAK KINASE INHIBITOR

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Novel strategies in cancer therapy aim at inhibiting distinct signal transduction pathways that are aberrantly activated in malignant cells. Protein tyrosine kinases of the JAK family are associated with a number of cytokine and cytokine-like hormone receptors and regulate important cellular functions such as proliferation, survival, and differentiation. Constitutive or enhanced JAK activation has been implicated in neoplastic transformation and abnormal cell proliferation in various hematologic malignancies. In multiple myeloma (MM), JAK kinases play a critical role because of their association with cytokine receptors of the IL-6/gp130 family. A novel small-molecule inhibitor was developed that shows a 100 to 1,000-fold selectivity for JAK1, JAK2, JAK3, and TYK2 relative to other kinases including Abl, Aurora, c-Raf, FGFR3, GSK3β, IGF-1R, Lck, PDGFRα, PKBβ, and Zap-70. Growth of MM cell lines and primary patient cells was inhibited by this compound in a dose-dependent manner. The IL-6 dependent cell line INA-6 and derived sublines were sensitive to the drug, with IC_{50}s of less than 1 μM, in [3H]-thymidine uptake and a colorimetric, tetrazolium compound (MTS) based assay (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI). Importantly, INA-6 and patient tumor cell growth was also inhibited in the presence of bone marrow stromal cells, which by themselves remained largely unaffected. Growth suppression of INA-6 correlated with a significant and dose-dependent increase in the percentage of apoptotic cells, as evaluated by Apo2.7 staining after 48 hours of drug treatment. In addition, the compound blocked IL-6 induced phosphorylation of STAT3, a direct downstream target of JAK kinases and important transcription factor triggering anti-apoptotic pathways. In other myeloma cell lines, the drug overcame the protective effect of gp130 cytokines on dexamethasone induced apoptosis. In MM1.S cells, it completely blocked IL-6 induced phosphorylation of SHP-2 and AKT, both known to mediate the protective effects of IL-6. In contrast, AKT phosphorylation induced by IGF-1 remained unchanged, demonstrating selectivity of the compound. These studies show that disruption of JAK kinase activity and downstream signaling pathways inhibits myeloma cell growth and survival as well as circumvents drug resistance, thereby providing the conceptual basis for the use of JAK kinase inhibitors as a novel therapeutic approach in MM.

F11.07

THE COMBINATION OF TIPIFARNIB AND BORTEZOMIB RESULTS IN SYNERGISTIC MYELOMA CELL DEATH VIA DOWNREGULATION OF PHOSPHO-AKT AND INDUCTION OF APOPTOSIS

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Introduction. Despite advances in myeloma treatment and an improved understanding of myeloma biology, myeloma remains an incurable disease. Targeted agents represent a unique mechanism by which biologically based therapies can be applied and tested. Our group has previously reported that the combination of the farnesyltransferase inhibitor lonafarnib and the proteasome inhibitor bortezomib result in synergistic myeloma cell death when using myeloma cell lines or primary tumor cells from patients. We report on our preliminary experience using the combination of tipifarnib and bortezomib as a model for future clinical trials combining tipifarnib and bortezomib for treatment of relapsed and refractory myeloma.

Methods. MM.1S and MM.1R cell lines were tested with different concentrations of each agent. Cell death was assayed using the MTT assay as well as flow cytometry for annexin V and PI. Western blots and immunoblots were performed to investigate the effects of therapy on AKT, Bcl-2, Bcl-XL, Mcl-1, mTOR, Stat/JAK, caspase 3, 8, and 9.

Results. As previously reported by Alsina *et al,* MM.1S were more resistant to the effects of tipifarnib than MM.1R: a 48 hour MTT assay using MM.1S demonstrates modest effects for single agent bortezomib at a dose of 8nm or tipifarnib at a dose of 5uM. The combination of bortezomib and tipifarnib at subtherapeutic doses resulted in enhanced myeloma cell death, with an associated reduction in *p*-AKT expression as early as 24 hours after incubation. When the MM.1R cell line was used, there was more activity for either single agent even at low doses, and the combination effect was more pronounced. MM.1R cell death, as measured using MTT assay and flow cytometry after 48hrs, was approximately 90% when FTI (5um) was combined with 8nm PS-341. In this case FTI alone was only 37% and PS-341 (8nm) was only 40%. We have previously shown that the combination of FTI and bortezomib mediates myeloma cell death via downregulation of *p*-AKT. This was tested in MM.1S cells, with clear demonstration that *p*-AKT is unaffected by either single agent, but markedly reduced when bortezomib and tipifarnib are combined for 48 hours. Similarly, in MM.1R cells 8nm bortezomib alone had only modest effects on *p*-AKT levels, while the combination of 8nm bortezomib and tipifarnib (5um), markedly reduced and nearly eliminated any *p*-AKT expression at 48 hours.

Conclusions. The combination of tipifarnib with bortezomib shows similar degrees of synergy as we have previously reported with lonafarnib and bortezomib. This has been confirmed with MTT assays, and flow cytometry, with a similar pattern of effect on *p*-AKT expression. The differential effect of the combination on MM.1R and MM.1S suggests that mechanisms of dexamethasone resistance may be central to the effect seen with our combination. Further studies are planned evaluating the impact of sequence of administration (as we have shown with lonafarnib) and the effects of tipifarnib and bortezomib on other downstream targets of AKT are planned as part of the preclinical testing prior to initiation of a clinical trial in humans.

PLENARY SESSION 12: IMMUNOTHERAPY

PL12.01

ACTIVE SPECIFIC IMMUNOTHERAPY IN MULTIPLE MYELOMA – AN OVERVIEW

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Tumor cells express a variety of tumor antigens which can be exploited in a therapeutic approach. The majority of these antigens are low-avidity self-antigens to which the host is tolerant. Despite these facts, such antigens have been utilized in a variety of malignancies for active specific immunotherapy. Immune responses have been elicited and clinical effects observed with practically no side effects.

Antigens which should preferentially be selected for vaccination therapy might be those which may spontaneously break tolerance. In multiple myeloma, natural cellular immunity against the idiotype has been noted, which T cells might lyse autologous myeloma cells. A natural cellular immunity has also been shown against MUC-1 and telomerase. MUC-1 and telomerase specific T cells capable of lysing myeloma cells could be generated from patients with myeloma. T cells lysing the autologous target cells were mainly type I MHC class I restricted, but MHC class II restricted T cells were also present. The advantage of utilizing the idiotype is the strict tumor specificity. MUC-1 and telomerase are also good candidates for targeting as they are expressed in practically all patients by the majority of the tumor cells. MUC-1 might be an immuno-dominant antigen. Dendritic cells (DC) pulsed with myeloma cell RNA induced *in vitro* a MUC-1 CTL response. Other antigens as NY-ESO-1, MAGE-3, sperm-17 may also be considered but these antigens are only expressed in a minority of myeloma patients and might therefore not be useful in a universal vaccination approach.

Vaccination therapy might not in all probability be effective in advanced disease. Tumor cells secrete T cell suppressive factors. A lot of studies have shown a variety of T cells defects in patients with advanced disease while at low stage T cell functions are mainly preserved. A high serum M component concentration may induce a progressive deletion of idiotype specific CD4 T cells making idiotype immunization ineffective as both CD4 and CD8 specific T cells seem necessary for myeloma cell rejection. Vaccination should therefore be exploited early during the course of the disease or after cyto-reductive therapy. However, patients might have impaired T-cell functions after high dose chemotherapy. Measures should probably be taken to restore T cell functions during such conditions.

So far, in all vaccination trials in myeloma the autologous idiotype has been used as the vaccine. The idiotype has been given either as the protein alone together with various adjuvant cytokines, GM-CSF, IL-12, IL-2 or loaded onto autologous DC. It is very difficult to summarize the results of these disperate studies including between 200-300 patients at different stages of the disease and applying various immunization schedules. Moreover, in most DC trials an optimal vaccination approach, based on the present knowledge, has not been applied. The following conclusions might be drawn: 1) idiotype specific T cells (proliferative response, gγFN response, CTL response) might be induced; 2) both CD4 and CD8 idiotype specific T cells are detected mainly of type I; CD4 T cells seems to prevail when protein is used; 3) the frequency of idiotype specific T cells is low; 4) the frequency of patients mounting an idiotype specific T cell response varies considerably between studies; the highest frequency is seen in patients vaccinated at an early stage (depending on schedule close to 100%) or post-transplant provided sufficient time has elapsed from the intensive chemotherapy (20-50% of the patients); 5) clinical efficacy is modest, varying between 10 and 20% at the best; most of the responses are minor responses; 6) the procedure is safe.

A reasonable conclusion based on present data is that the idiotype is not an optimal tumor antigen for targeting in a vaccination approach. There are other antigens which might be more promising. Whole tumor cell preparations may be an attractive alternative. Whole tumor cells have been used as vaccines in a lot of randomised phase III trials in colorectal carcinoma, renal cell carcinoma and melanoma. Vaccination induced significantly improved overall and disease free survival. The MUC-1 protein has been used as a vaccine for non-small lung carcinoma patients stage III B in remission or stable phase after chemotherapy. Vaccination alone compared to observation induced an improved progression free and overall survival. These vaccine preparations need urgently to be tested in multiple myeloma. There might also be other non-idiotype candidates.

Vaccination against tumor cells is an attractive treatment alternative in various malignancies including myeloma. Vaccination should be applied early during the course of the disease or in remission with the aim to prevent disease progression or relapse. Novel vaccine preparations have to be tested in myeloma. Joint efforts are necessary to be able to enrol sufficient numbers of patients in well designed vaccination trials within a restricted time frame. More than 10 years have elapsed since we first started to immunize myeloma patients with the idiotype. The clinical progress has been slow, although we have learnt a lot about idiotype immunization.

PL12.02

TUMOR LYSATE DENDRITIC CELL VACCINATION IN MULTIPLE MYELOMA

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Multiple myeloma (MM) is still a fatal disease. Despite advances in high-dose chemotherapy and autologous stem-cell support, relapses of the underlying disease remain the primary cause of treatment failure. Novel therapeutic approaches that have a mode of action different from and non-cross-resistant with cytotoxic chemotherapy are required to eradicate tumor cells that have become multidrug-resistant. To this end, immunotherapy aimed at inducing or enhancing myeloma-specific immunity in tumor-bearing patients may be desirable. Indeed, in the post-allograft relapse setting of MM (in which patients are chemotherapy refractory), long-lasting disease remission has been achieved after infusion of donor lymphocytes, suggesting that chemotherapy and T cell-mediated cytolysis kill myeloma cells by different modes of action that are non-cross-resistant.

Idiotype (Id) proteins are tumor-specific antigens and active immunization against Id determinants on malignant B cells has produced resistance to tumor growth in transplantable murine B-cell lymphoma and plasmacytoma. Early clinical trials of Id-pulsed dendritic cell (DC) vaccination in MM by many groups including ours reported disappointing results. Less than 50% of patients mounted an Id-specific immune

response, and clinical responses have rarely been observed. To improve the efficacy of DC vaccination in MM, we investigated the used of Id-pulsed mature DC administered subcutaneously. Five patients with stable partial remission following high-dose chemotherapy were vaccinated with DC vaccines starting at least 4 months post-transplantation. After 3 DC vaccinations, Id-specific T-cell responses were elicited in 4 patients (4 by ELISPOT assay and 2 by proliferation assay), and anti-Id B-cell responses were elicited in all 5 patients. The cytokine-secretion profile of activated T cells demonstrated a type-1 response. A 50% reduction in serum Id protein was observed in one immunologically responding patient that persisted for more than one year, and stable disease resulted in the other 3 patients. The remaining patient without an immune response to the vaccination relapsed. These results are promising but also call for additional improvements to optimize DC-based immunotherapy in this disease. One of the strategies is to search for and use other myeloma antigens for immunotherapy in patients.

To explore the possibility of using myeloma cells, which may contain a multitude of tumor antigens that can stimulate an increased repertoire of anti-tumor T cells, as the source of tumor antigens for immunotherapy, myeloma-specific cytotoxic T lymphocytes (CTL) were generated from patients by culturing T cells with autologous DC pulsed with myeloma freeze-and-thaw cell lysate. These CTL lines proliferated in response to autologous primary myeloma cells and DC pulsed with autologous, but not allogeneic, tumor lysate and secreted predominantly IFN-γ and TNF-α. The CTLs had strong cytotoxic activity against autologous tumor lysate-pulsed DC and primary myeloma cells. Interestingly, some of the CTL killed, to a lesser degree, autologous Id-pulsed DC and allogeneic myeloma cells, suggesting that Id was sometimes a part of tumor antigens present in the tumor lysate and that there were shared tumor antigens between patients. No killing of autologous peripheral blood mononuclear cells, purified B cells, or Epstein-Barr virus-transformed B-cell lines was observed. These data demonstrate that CTL induced by tumor lysate-pulsed DC specifically kill autologous tumor cells, but not normal blood cells.

To evaluate the antimyeloma effects of the specific CTL *in vivo*, a myeloma SCID-hu host was established by inoculation of myeloma cells in the implanted human bone in the mice. After myeloma was established, defined by the appearance in mouse serum of human Ig or light chain secreted by the tumor cells, a myeloma-specific CTL line was injected into the tumor sites. The treatment consisted of three weekly injections of $10x10^6$ T cells. Adoptive transfer of the specific T cells, but not a control CTL line, strongly suppressed tumor growth *in vivo*. Eight weeks after tumor inoculation, the control mice and the mice that had received control CTL had large tumors (5-10 g) in and around the implanted human bones, while specific CTL-treated mice had no visible tumor mass or circulating human Ig. Two weeks after the final injection, circulating human T cells were still detected, indicating that human T cells survived in the host. Taken together, these findings indicate that myeloma-specific CTL can control or eradicate tumor cells both *in vitro* and *in vivo*, and provide a rationale for vaccination with tumor lysate-pulsed DC in myeloma patients.

Currently, we are conducting a phase-II study to evaluate the efficacy of intranodal DC vaccination in myeloma patients. Two groups of patients are targeted: group A consists of patients with indolent or smoldering MM who receive Id-pulsed, CD40L-mature DC vaccines; group B consists of patients with advanced MM who receive tumor lysate-pulsed, CD40L-mature DC vaccines. KLH is added to the vaccine, and a low dose of IL-2 is administered to patients following each vaccination. To induce an optimal tumor reduction without compromising immunotherapy, patients with advanced disease are vaccinated up-front to generate tumor-specific T cells, and blood T cells, which may contain the specific T cells, will be collected. After high-dose chemotherapy and tandem autologous transplantations, collected T cells will be reinfused and 4 additional DC vaccines will be given to boost the specific immunity. To date, 15 patients have been enrolled (10 in group A and 5 in group B). In this presentation, I will mainly discuss the results obtained from the 5 patients who received tumor lysate-pulsed, CD40L-mature DC vaccines.

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PL12.03

TUMOR ANTIGEN IMMUNIZATION OF HUMAN ALLOTRANSPLANT DONORS IN MYELOMA

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The unique antigenic determinants (idiotype [Id]) of the immunoglobulin secreted by myeloma cells can serve as a tumor-specific antigen for active immunotherapy. We are developing a therapeutic strategy to induce tumor-specific T-cell immunity in bone marrow transplant (BMT) donors and to transfer this immunity to recipients to enhance the specific antitumor effect of the allograft. Five HLA-matched sibling donors were vaccinated with Id proteins isolated from the plasma of myeloma patients prior to bone marrow harvest. The recipients were administered booster Id immunizations following transplantation. Vaccination induced specific cellular and/or humoral immune responses against Id and the vaccine carrier molecule, keyhole limpet-hemocyanin (KLH), in all five donors. Two patients died within 30 days of BMT due to transplant-related complications. Id-specific and KLH-specific T cell responses were detected in all three remaining patients post-, but not pre-BMT. In at least one patient, the Id-specific T cell immunity persisted beyond 18 months after the completion of vaccinations. All three surviving patients converted from partial to complete

responses following BMT. Two of the three patients remain in complete remission 7 years and 8 years after BMT, and the third died of renal failure after 5.5 years while in complete remission from myeloma. Our results suggest that myeloma Id vaccination induces specific T cell immunity in healthy donors that is transferable by BMT, is associated with prolonged disease-free survival of recipients, and may represent a general strategy to enhance tumor-specific immunity in other malignancies for which defined tumor-specific antigens exist..

PL12.04

DNA VACCINATION AGAINST MYELOMA POST AUTOLOGOUS OR ALLOGENEIC TRANSPLANTATION

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Therapeutic vaccination against myeloma presents a challenge. Not only do myeloma cells occupy the immune system and suppress normal immunity, but there is toleragenic pressure on specific T cells which might have recognized and responded to myeloma antigens. It is clearly necessary to bring patients into remission before contemplating vaccination, and, in that setting, provided immune capacity can be restored, there is a possibility of instituting a continuing immune attack on emergent tumor. Autologous transplantation meets this requirement in part, and provides a window of opportunity for vaccination. More information on the immune capacity of these patients is required and we have investigated recovery of the ability to respond to conventional vaccines. Allogeneic transplantation has obvious problems, but vaccination of donors prior to DLI, or even prior to transplant, is an attractive option, possibly combined with subsequent vaccination of the patient. Clearly a safe and effective vaccine has to be the goal for all these settings.

DNA plasmid vaccines have the advantage of containing a natural stimulator of innate immunity in the CpG repeats of the bacterial backbone. Sequences encoding the antigen of choice can be placed in the plasmid under the control of a strong promoter. Following injection, antigen is expressed in host cells and gains access to the antigen processing pathways leading to immunity. In our design, immunoenhancing sequences from tetanus toxin are fused to the tumor antigen sequence to activate high levels of T-cell help. The CD4+ Th cells are derived from the non-tolerized large anti-microbial repertoire and they promote induction of anti-tumor immunity against weak antigens, even in a toleragenic setting. The power of DNA technology allows us to modify antigen and immunoenhancing sequences to direct immune outcome towards antibody, CD4+ T cells or CD8+ T cells as effectors.

The classical tumor antigen for myeloma is secreted idiotypic Ig. Although specific, it is difficult to know how to attack the neoplastic plasma cells via this antigen. Binding of idiotypic peptides to MHC Class I will be extremely variable and unpredictable, making clinical utility difficult. Indirect attack by CD4+ T cells is evident from models and is intriguing. We have shown that our DNA fusion vaccines can induce anti-idiotypic CD4+ T cells able to protect against myeloma, but whether this pathway will be sufficient to suppress myeloma cell growth in patients is the question. We are cautiously exploring these vaccines in patients following autologous transplantation.

Cytotoxic T cells (CTL) are powerful agents to attack target cells and should be effective against myeloma cells. We have now developed modified DNA fusion gene vaccines aimed to induce epitope-specific CTL, and these can kill cancer cells in vivo. For myeloma, we have focused on cancer-testis antigens and have induced protective immunity against the classical mouse P1A cancer-testis antigen in pre-clinical models. To move to human application, we have tested the MAGE-A2 and MAGE-A3 antigens, known to be expressed especially in the later stages of myeloma. Using mice transgenic for human HLA-A2, we were able to induce CTL against both these antigens. A similar design can induce CTL against the minor histocompatibility antigens HA-1 and HA-2, which are relevant targets for graft-versus myeloma effects following allogeneic transplantation. Our DNA fusion gene vaccine design can also be used to vaccinate against viral epitopes, and we have investigated the immmunodominant epitope from the pp65 matrix protein of cytomegalovirus as a target epitope. In HLA-A2-expressing mice, we can induce high levels of epitope-specific CTL able to kill human cells infected with a virus expressing pp65. These results have allowed us to test the vaccine in donors of allogeneic transplants for patients at risk of CMV reactivation.

In our trials, and in trials of DNA vaccination against infectious diseases, there appear to be no significant safety issues.

Translating promising data from pre-clinical models to patients takes time. Although we are seeing immune responses in patients, we need to optimize performance for these larger animals. One problem relates to the dose and volume of DNA vaccine injected, each critical for optimal antigen expression *in vivo*. To overcome this, we are using physical methods such as electroporation. This technique increases antigen expression dramatically and leads to high levels of antibody or effector T cells in large animals. Priming with naked DNA followed by boosting with electroporation is particularly effective. Electroporation will be incorporated into one arm of a new trial against of our fusion vaccine design aimed against prostate cancer. Prime/boost with non-viral vectors is desirable for treatment of cancer since it might be necessary to continue to boost immunity to maintain pressure on tumor cells. Epitope-specific CTL can be focused on tumor cells, and double attack using a second vaccine should prevent escape. The need for new vaccines against infectious diseases, and the testing of fusion gene vaccines against a range of tumors will provide data which can support the quest for effective vaccines against myeloma.

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PL12.05

DENDRITIC CELL BASED VACCINATION AND BEYOND

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The potential susceptibility of multiple myeloma (MM) to immune based therapy has been demonstrated in the allogeneic transplantation through graft versus myeloma effect. A major focus of investigation, therefore, has been to use of immune-based therapies in a minimal disease setting to decrease the risk of relapse and potentially achieve curative outcomes. Although current vaccine strategies have achieved anti-myeloma immune responses, meaningful clinical responses have not been observed. In an effort to improve upon these responses, we have developed dendritic cell (DC) -MM cell fusion vaccination. Preclinical results in both a murine model and with human cells confirm feasibility of presenting a wide array of myeloma-related antigens through this approach and the development of CTLs able to lyse primary myeloma cells. We have initiated a clinical study evaluating DC-MM fusion cell vaccination. Preliminary results confirm feasibility as well as development of immune response following vaccination in 5 patients. These studies are also providing an opportunity to identify novel myeloma-associated antigens by screening the myeloma cDNA expression library using the SEREX technique for eventual development of an antigen cocktail. We have identified series for antigens using this technique and have begun to validate individual antigens. Additionally, to improve immune response and to achieve clinically meaningful responses we have begun to study dysregulated immune function in myeloma. We have observed dysregulation of CD4+CD25+ T regulatory cells (Treg). The initiation of immune response is controlled by CD4+CD25+ Treg cells which can modulate anti-tumor immune responses. This property of Treg cells to modulate immune response, specifically the anti-tumor immune responses, may function as a barrier to cancer immunotherapy. In order to overcome such suppression, signaling through Toll-like receptors (TLR) can induce adjuvant effect by increasing local production of chemokines and proinflammatory cytokines, and by enhancing antigen-presentation by APC. We have evaluated the role of Treg cells and the effects of TLRs in myeloma and observed significant increases in CD4+CD25+ cells in MM patient samples compared to in normal donors. However, Treg cells in MM are dysfunctional. LPS which functions through Toll-like receptor 4 (TLR4), is able to overcome suppression of T-cell proliferation by Treg cells in normal controls, however, it has significantly limited influence in MGUS and MM. Understanding the molecular basis of Treg cell dysfunction is underway with a view to eventually target these mechanisms to improve immuno-therapeutic strategies in myeloma. Additionally, novel immunomodulatory agents are being evaluated to provide T-cell co-stimulation to improve immune responses post vaccination. In conclusion, these stepwise improvements in immunotherapeutic strategies directed at myeloma are likely to lead to immune, and more importantly, clinical responses that will eventually help achieve a cure in multiple myeloma.

FOCUS SESSION 12
DRUG RESISTANCE: MECHANISMS AND THERAPEUTIC STRATEGIES

F12.01

DRUG RESISTANCE IN MULTIPLE MYELOMA

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In multiple myeloma (MM), acquired or *de novo* multidrug resistance (MDR) prohibits the ultimate cure of the disease. Acquired MDR (aMDR) is generally conferred by a selection of cells that are capable of overcoming the injury of cytotoxic treatment. Examples of the mechanisms that are involved in classical aMDR are i) reduced intracellular drug accumulation by increased expression of ABC transporter proteins such as P-glycoprotein (Pgp), breast cancer resistance protein (BCRP) or the multidrug resistance associated protein (MRP1-7); ii) increased drug detoxification by glutathione-S-transferases (GST) that catalyze the conjugation of glutathione (GSH) to many chemotherapeutics; iii) alterations in drug targets such as topoisomerases IIα/II, or altered expression or point mutations in the hormone binding domain of the glucocorticoid complex. To some extent, expression of anti-apoptotic proteins like Bcl-2 or Mcl-1 has been shown to modify the drug response in myeloma cell lines and these may represent targets for drug therapy and to overcome drug resistance. Pharmacological approaches to circumvent classical aMDR, for example by blocking Pgp-mediated drug efflux have not been succesful so far. Recently, increasing evidence has been obtained about the important role of *de novo* MDR, which is instituted by IL-6 and IGF-1-mediated paracrine growth stimulation of MM cells in the bone marrow micro-environment. Three intracellular signalling pathways have been identified that mediate IL-6 and possibly IGF-1-induced cell stimulation, i.e., the PI3-kinase/AKT pathway, the JAK/STAT pathway and the Ras/MEK/ERK pathway. Activation of these pathways through signaling in the BM micro-environment may induce cell proliferation and inhibit apoptosis, and in this way protect the MM cell from drug-induced cytotoxicity. Recently developed new anti-myeloma drugs may disrupt the interaction of MM cells with the BM micro-environment by inhibiting angiogenesis (thalidomide, linalidomide) or downregulating adhesion molecules through NF-κβ, inhibition (bortezomib). Arsenic trioxide (As_2O_3) has anti-tumor activity and induces apoptosis in association with downregulation of bcl-2. In addition, multiple other effects have been observed, including inhibition of NF-κβ and VEGF. The *in vitro* effects in MM include inhibition of STAT3 activation and JAK-STAT signaling. As_2O_3 decreases MM cell adhesion to the bone marrow micro-environment and sensitizes chemoresistant cells to chemotherapy. While these new agents have demonstrated significant activity in relapsed/refractory MM, their intracellular targets and the potential mechanisms of drug resistance are largely unknown. However, they clearly interfere with *de novo* MDR through interaction with the intracellular signaling pathways and/or with DNA methylation/acetylation. We have recently started a new technique to analyse the complex effects of bortezomib on intracellular signaling activation status by using a peptide phosphorylation array on which the major intracellular signaling kinases are spotted. In this program the downstream effectors of these pathways and how they are expressed fol-

lowing therapeutic intervention, are investigated.The results show that the effects of bortezomib are complex and illustrate the potential of the MM cell to overcome targeted therapy and the need for effective drug combinations.

F12.02

ANTI-APOPTOTIC SIGNALING IN MULTIPLE MYELOMA CELLS: THERAPEUTIC IMPLICATIONS

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Apoptosis is a mechanism of controlled cell death critically important in many biological processes. Most anti-tumor agents trigger apoptotic signaling, and defects in this process lead to the development of drug-resistance. In multiple myeloma (MM), loss of apoptotic response to chemotherapy may result from the following: 1) genetic abnormalities; 2) overexpression of anti-apoptotic proteins and/or multidrug resistance (MDR)-related genes; 3) MM cell binding to bone marrow (BM) extracellular matrix proteins, and BM stromal cells (BMSC); and 4) cytokines in the BM milieu, including interleukin-6 (IL-6) and insulin-like growth factor-I (IGF-I). Recent studies are now unveiling the mutagenic role of the BM microenvironment in the progression of MM. Adhesion of MM to BMSC triggers secretion of various cytokines from BMSC, which promote MM cell growth and block cytotoxicity of chemotherapy by inducing anti-apoptotic signaling. For example, high serum levels of IL-6 and IGF-I observed in MM patients contribute to chemoresistance and treatment failure; Dexamethasone (Dex)-induced apoptosis in MM cells is abrogated *in vitro* by IL-6 or IGF-I. Cytokines themselves induce multiple growth signaling cascades: Ras-Raf-MAPK, JAK-STAT, and PI3K/Akt signaling pathways. Besides triggering MM cell growth, the interaction of MM and BMSC also upregulates anti-apoptotic proteins, such as Bcl2 family members and inhibitor of apoptosis proteins (IAP), which confer drug-resistance in MM. Genomics and proteomics-based studies show that anti-MM agents trigger activation of cellular defense machinery. For example, treatment of MM cells with Dex not only induces cell-death signaling, but also concomitantly triggers transient transcription of genes encoding growth factor receptors (IL-6R, IGF-IR), thereby enhancing anti-apoptotic signaling. Similarly, bortezomib-induced apoptosis correlates with induction of anti-apoptotic proteins, such as heat shock proteins (Hsp90, Hsp70, and Hsp27). Activation of these proteins may reduce the cytotoxicity of drugs and/or confer drug-resistance. These findings provide the rationale for combining biochemical inhibitors of growth/survival signaling pathways and/or anti-sense against anti-apoptotic signaling molecules (Mc11, Bc12, SHP2, Hsps, IAPs) with conventional anti-MM agents, to enhance MM cell-death.

Our studies have also delineated anti-MM agent-induced stress signaling pathways, which allows for identification of molecular targets to amplify apoptosis. For example, anti-MM drugs induce apoptosis via release of mitochondrial proteins cytochrome-c or Smac, suggesting that active peptides against these molecules may increase the apoptotic threshold of drugs. Already our preliminary data suggest that Smac mimetic induces synergistic anti-MM activity when combined with other drugs. The finding that Dex-induced apoptosis occurs without cyto-c release or activation of JNK provides the rationale for amplifying apoptotic signaling by combining Dex with agents that trigger cyto-c release and activate JNK. Proteomic analysis of MM cells in the BM before and after treatment with bortezomib showed impairment of DNA repair enzymes, thereby providing rationale for clinical protocols combining bortezomib and DNA damaging agents. Gene profiling and proteomic studies have identified novel drugs that specifically target molecules on the MM cell surface (IL-6R, IGF-1R, VEGFR), cytoplasm (Hsp90, Hsp27, IKK/NF-kB), mitochondria, ER (Bcl2) and nucleus (Telomestatin, HDAC inhibitors). Delineation of cell death and growth cascades provides the rationale to inhibit growth and amplify apoptosis, in order to enhance anti-MM activity of available therapies, prevent drug resistance, and improve patient outcome in MM.

F12.03

DRUG RESISTANCE IN MYELOMA

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F12.04

MECHANISMS OF DRUG RESISTANCE TOWARDS THALIDOMIDE AND ITS IMMUNOMODULATORY DERIVATIVES IN MULTIPLE MYELOMA CELLS: ROLE OF C/EBPβ

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Despite the fact that many novel therapies have been introduced in the treatment of multiple myeloma (MM), drug resistance develops and all patients eventually relapse. Therefore, it is critical to identify factors, which mediate drug resistance in MM. C/EBPβ belongs to the C/EBP family of transcription factors which share substantial sequence similarity in the C-terminal basic leucine zipper region and play an important role in the regulation of cell proliferation and differentiation. Increased levels of C/EBPβ have been found in different types of tumors, and mice deficient in C/EBPβ show impaired generation of B lymphocytes, suggesting that C/EBPβ plays a critical role in B lymphopoiesis. The promoter of IL-6, the most important growth and survival factor for MM cells, is activated by C/EBPβ, and thus C/EBPβ was originally named NF-IL6 (nuclear factor involved in IL-6 expression) (Akira *et al.* 1990). In addition, it has been shown that MIP-1α, which is also a major growth and survival factor for MM, increased expression of C/EBPβ (Matsumoto *et al.* 1998). These data suggest that C/EBPβ might play an important role in the pathogenesis of MM.

In proliferation assays we analyzed 27 malignant hematologic cell lines for their sensitivity to the immunomodulatory derivates (IMiD) CC-4047, including multiple myeloma, Hodgkin, Burkitt's lymphoma, pre-B-ALL and T-cell leukemia cell lines, Our analyses revealed that 13 cell lines were sensitive to CC-4047 with an inhibition of proliferation of at least 60%. To examine the mechanisms that mediate resistance or sensitivity to CC-4047, we investigated the transcriptional profile of CC-4047 treated MM.1S MM cells by oligonucleotide microarray analysis. This analysis revealed that treatment with CC-4047 resulted in a signifi-

cant regulation of several genes, including FHF-2, C/EBPβ, c-myb, GFI-1 and HUMSPARC. MM.1S cells, which were sensitive to CC-4047, showed a strong down-regulation of C/EBPβ mRNA in response to CC-4047. In western blotting, C/EBPβ protein including all isoforms (LIP, LAP) completely disappeared with treatment by CC-4047 in MM.1S cells. In contrast, treatment with thalidomide had only minor effects on C/EBPb expression. Interestingly, pretreatment of MM1.S cells with IL-1, increased C/EBPβ expression, but this induction could also be completely abrogated by CC-4047 treatment. Further experiments showed, that all cell lines sensitive to CC-4047 consistently demonstrated a down-regulation of all C/EBPβ isoforms.

Our studies suggest that C/EBPβ might be an important transcription factor controlling drug resistance towards thalidomide and its derivatives in MM cells. Further studies are warranted to understand the molecular basis underlying the resistance towards IMiDs in order to define new strategies for breaking drug resistance in MM cells.

F12.05

MCL-1 IS TRIGGERED BY BORTEZOMID AND ITS LACK CONFERS RESISTANCE TO BORTEZOMIB AND MELPHALAN BUT NOT DOXORUBICIN

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Bortezomib is a proteasome inhibitor recently approved by the FDA for treatment of relapsed refractory MM. To date, however, mechanisms of MM cell sensitivity versus resistance to Bortezomib are undefined. Mcl-1 is an anti-apoptotic protein which protects MM cells against spontaneous and dexamethason-induced apoptosis. Here we examine the role of Mcl-1 bortezomib-induced apoptosis. We studied modulation of Mcl-1 expression triggered by bortezomib in MM1S cells and two murine embryonic fibroblast cell lines (MEF): Mcl-1 deleted (Mcl-$1^{\Delta/null}$) and MEF Mcl-$1^{Control}$. We similarly evaluated conventional therapies (doxorubicin and melphalan) for MM. Treatment with doxorubicin, melphalan and bortezomib for 48h inhibits MM1S cell growth, measured by MTT assay, in a dose dependent-manner with IC_{50} of 50 nM, 9 μM and 5 nM, respectively. At 6h and 24h bortezomib, but not melphalan (up to 40 μM) or doxorubicin (up to 200nM), induces appearance of cleaved-form (28kd) of Mcl-1 associated with caspases-8, -9, and -3, as well as PARP cleavage. Down-regulation and cleavage of Mcl-1 is observed after 24 h at bortezomib concentrations as low as 2.5 nM. The pan-caspase inhibitor Z-VAD-FMK inhibits induction of Mcl-1 cleavage, suggesting that it is caspase-dependent. We therefore hypothesized that Mcl-1 may be an early target in bortezomib-induced-apoptosis. To address this issue, we compared the percentage of apoptotic Mcl-$1^{\Delta/null}$ vs Mcl-$1^{control}$ MEF, defined as the percentage of cells in sub-G1 phase by flow cytometry (PI staining). Both cell lines were sensitive to doxorubicin: 50% vs 45% apoptosis at 50 nM and 85% vs 80% apoptosis at 100 nM in Mcl-1control and Mcl-$1^{\Delta/null}$ MEFs, respectively. In contrast, melphalan (10 μM) failed to induce apoptosis in Mcl-$1^{\Delta/null}$ MEFs: 60% vs 10% apoptotic cells in Mcl-$1^{control}$ and Mcl-$1^{\Delta/null}$ MEFs, respectively. Similar results of sensitivity between the two cell lines were confirmed by using MTT and ^{3}H-thymidine incorporation. Interestingly, in melphalan-treated-cells PARP is cleaved, but no morphological apoptosis is observed. Furthermore, cell cycle analysis in melphalan treated-cells demonstrates accumulation of Mcl-$1^{\Delta/null}$ MEFs in G2/M phase. Bortezomib (up to 20 nM) similarly failed to induce apoptosis in Mcl-$1^{\Delta/null}$ MEFs: 80% vs 17% apoptotic cells in Mcl-$1^{control}$ and Mcl-$1^{\Delta/null}$ MEFs, respectively. Lack of induction of sub-G1 Mcl-$1^{\Delta/null}$ cells by bortezomib correlated with absence of both PARP cleavage and morphological signs of apoptosis. Interestingly and as in MM1S cells, only Bortezomib induces Mcl-1 cleavage in Mcl-$1^{control}$ MEF. These results suggest that bortezomib and melphalan, but not doxorubicin, trigger distinct Mcl-1-dependent induced-apoptosis pathways: bortezomib triggers Mcl-1 cleavage prior to PARP cleavage; and melphalan triggers PARP cleavage, with downstream Mcl-1 required for progression from G2/M to sub-G1 phase. In conclusion, our study demonstrates that Mcl-1 is not only an anti-apoptotic protein but also an important mediator of bortezomib and melphalan-, but not doxorubicin, induced apoptosis.

POSTER SESSION 1: CYTOGENETIC AND MOLECULAR ANALYSIS; MOUSE MODELS

P0.101

BENEFIT OF THALIDOMIDE/DEXAMETHASONE OVER DEXAMETHASONE SEEN IN ALL SUBGROUPS OF MULTIPLE MYELOMA: ANALYSIS OF THE DATA FROM THE E1A00 CLINICAL TRIAL

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Background. A cytogenetic and molecular classification of multiple myeloma (MM) has recently been developed, termed the TC classification (Blood 2004; 104:607-618). The goal of this study was to determine if subsets of MM defined by the TC classification exhibited a differential response to Thal/Dex therapy.

Methods. Samples for the study were derived from a phase III randomized trial of thalidomide plus dexamethasone (Thal/Dex) versus dexamethasone alone (Dex) in newly diagnosed MM coordinated by ECOG. Samples were collected following diagnosis of MM, prior to any therapy. Overall 207 pts were enrolled in the trial: 103 randomized to Thal/Dex and 104 to Dex. Of the 207 pts, 74 had baseline bone marrow aspirate samples for research studies. Plasma cells were isolated by CD138 bead selection using MACS separation columns as described by the manufacturer. (Miltenyi Biotec) Cells were analyzed for purity by Wright-Giemsa stain. Total RNA was isolated from patient samples using the RNeasy® Kit as described by the manufacturer (Qiagen, Inc.). RNA purity and integrity was confirmed using an Agilent Bioanalyzer. Synthesis of cDNA and biotin-labeled cRNA as well as fragmentation and hybridization to the Affymetrix Hu133A chip were done according to manufacturer recommendations (Affymetrix, Inc.,CA). Microarray gene expression analysis was successfully performed in 46 pts in whom adequate RNA was available. Response to therapy was assessed using standard ECOG criteria, and the overall results of the study have been presented earlier (Rajkumar *et al.,* Blood 2004;104).

Results. Of the 46 pts studied, 19 were classified as having a response and 16 as no response; 10 pts unevaluable and 1 unknown for response were excluded from the analysis. The gene expression values for MMSET, FGFR3, c-maf, mafB, CCND1, CCND2, CCND3 were determined, and the TC classification assigned to each patient as described previously (Blood 2004; 104:607-618). Given the low number of samples available for analysis, the pts were categorized into two groups (group 1: 6p21, 11q13, D1 and D1+D2, and group 2: 4p16, 16q23, and D2) based on TC class. The groupings are based on the presence of many shared biological and clinical features (prognosis, bone disease, chr 13 deletion, ploidy). In group 1 (n=17), response rates were 43% with Dex alone versus 70% with Thal/Dex, p=0.35; response rates were 33% versus 58%, respectively if unevaluable pts were not excluded, p=0.39. Corresponding values for Group 2 (n=18) were 33% versus 67%, p=0.35, respectively; response rates were 25% versus 60%, respectively if unevaluable pts were not excluded, p=0.19. In the 4p16 subgroup of MM which is associated with an adverse prognosis, none of the 3 pts responded to Dex, while 3 of 4 pts (75%) responded to Thal/Dex.

Conclusions. These preliminary results suggest Thal/Dex has increased activity over Dex in both TC classification groups; differences are not statistically significant, probably because of the small sample sizes. Although sample size is small (7) and results preliminary, the difference in response rates between Thal/Dex and Dex in the 4p16 adverse prognosis subgroup merits further study.

P0.102

A NEW MOLECULAR CLASSIFICATION OF MULTIPLE MYELOMA USING GENE EXPRESSION PROFILING AND FLUORESCENCE *IN SITU* HYBRIDIZATION PREDICTS FOR EVENT-FREE SURVIVAL

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The aim was to establish a new molecular classification of multiple myeloma (MM) based on changes in global gene expression attributable to cytogenetic aberrations detected by interphase FISH (iFISH) in order to (i) predict event-free survival(EFS) and (ii) investigate differentially expressed genes as a basis for a group specific and risk-adapted therapy.

Patients and Methods. Overall, 105 newly diagnosed MM-patients (65 trial (TG)/40 independent validation group (VG)) and 7 normal donors (ND) were included. Bone marrow aspirates were CD138-purified by activated magnetic cell sorting. RNA was *in vitro* transcribed and hybridized to Affymetrix HG U133 A+B GeneChip (TG) and HG U133 2.0 plus array (VG). CCND1 and CCND2 expression was verified by real time RT-PCR. iFISH was performed on purified MM-cells using probes for chromosomes 11q23, 11q13, 13q14, 17p13 and the IgH-translocations t(4;14) and t(11;14). Expression data were normalised (Bioconductor package gcrma) and nearest shrunken centroids (NSC) applied to calculate and cross validate a predictor on 40 patients of the TG with a comprehensive iFISH panel available combined with CCND overexpression. Differentially expressed genes were identified using empirical Bayes statistics for pairwise comparison.

Results. Overexpression of a D-type cyclin (D1 or D2) was found in 61/65 patients with MM compared to ND. CCND3 overexpression only appeared concomitantly with CCND2 overexpression. Four groups could be distinguished: (1.1) CCND1 (11q13) overexpression and trisomy 11q13, (1.2) CCND1 overexpression and translocations involving 11q13 i.e. t(11;14), (2.1) CCND2 overexpression without 11q13+, t(11;14), t(4;14), (2.2) CCND2 overexpression with t(4;14) and FGFR3 upregulation. A predictor of 6 to 566 genes correctly classifies all 40 patients of the TG (estimated cross validated error rate 0%). An independent VG of 40 patients was used. Genes with highest scores in NSC are: (1.1) CCND1, ribosomal proteins (e.g. RPL 28, 29), GPX1, CCRL2, (1.2) CCND1, TGIF, RAET1E and NCAM (non-overexpression), (2.1) CCND2, (2.2) FGFR3, WHSC1, CCND2, IRTA2, SELL, and MAGED4. Distribution of clinical parameters (i.e. beta2M, Durie Salmon stages, ISS) was not significantly different between the groups. The distribution of del(13)(q14q14) was (1.1) 31.5%, (1.2) 37.5%, (2.1) 37.5% and (2.2) 100%. (p<0.01). I.e. HGF, DKK1, VCAM, CD163 are differentially expressed between all 4 groups and ND (adjusted p<0.001). The groups defined by the predictor show significantly different EFS after autologous stem cell transplantation according to the GMMG-HD3 protocol

(median: (1.1) 18 / (1.2) not reached (no event)/(2.1) 22 / (2.2) 6 months; log-rank-test: p=0.004).

Conclusions. CCND1 or CCND2 overexpression is nearly ubiquitous in MM and attributable to defined cytogenetic aberrations. Gene expression and iFISH allow a molecular classification of MM which can be predicted by gene expression profiling (GEP). Classes show a distinctive GEP-profile as well as different EFS interpretable as risk stratification and indicator of therapeutic targets.

P0.103

CYTOGENETIC ABNORMALITIES IN PATIENTS WITH MULTIPLE MYELOMA STUDIED BY FLUORESCENT *IN SITU* HYBRIDIZATION: COMPARISON OF SIMULTANEOUS IMMUNOFLUORESCENT LABELING OF MALIGNANT PLASMA CELLS AND IMMUNOMAGNETICALLY SELECTED PLASMA CELLS

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Chromosomal aberrations such as 13q14 deletions or translocations involving 14q32 are described to be common cytogenetic findings in multiple myeloma (MM). Especially, deletions of 13q14 has been associated with an adverse outcome and it has been proposed as one of the most important prognostic factors for MM patients. Because metaphase cytogenetic studies in MM are hampered by a low proliferative activity of myeloma cells *in vitro*, interphase fluorescent *in situ* hybridization (FISH) using specific DNA probes is the technique most widely used for the determination of genomic aberrations in this disease. In the present study we have performed fluorescence *in situ* hybridization experiments with probes directed to the 13q14 and 14q32 chromosomal regions in 46 patients with MM. For identification of malignant plasma cells in bone marrow samples, we have used a cytoplasmic immunoglobulin (cIg) labeling methodology (Ahmann *et al.,* 1998). This method allowed us to identify simultaneously monotypic plasma cells by monoclonal antibody fluorescence (anti-κ or anti-λ) and detect chromosomal abnormalities by FISH (cIg-FISH). FISH studies revealed that monoallelic deletions of 13q14 or monosomy 13 were present in 24 of 46 (52 %) patients, 14q32 abnormalities were observed in 11 of 22 (50 %) patients with MM. Conventional cytogenetic analyses were performed in 39 cases and revealed numerical and structural changes in 7 (18%). Our results confirmed that by combining immunofluorescent labeling of myeloma cells and FISH, cytogenetic abnormalities can be proved also in patients with a low percentage of neoplastic plasma cells. In 19 patients, we compared the results of 13q14 deletion obtained by the cIg-FISH method with FISH analyses performed on immunomagnetically selected plasma cells (MACS). Both methods provided similar results. We conclude that cIg-FISH and MACS procedures represent reliable methods that can increase the incidence of chromosomal aberrations required for prognostic evaluations of patients with MM, but the cIg-FISH technique seems to be cheaper and less time consuming.

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P0.104

CHROMOSOME ABNORMALITIES DETECTED BY FLUORESCENCE *IN SITU* HYBRIDIZATION ONLY HAVE PROGNOSTIC EFFECT IN YOUNGER MYELOMA PATIENTS

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Fluorescence *in situ* hybridization (FISH) was performed on 228 cases of myeloma (MM) including 33 smoldering/asymptomatic MM (SMM). The patients ranged in age from 27 to 93 with 95(42%) being more than 70 years of age and 63 (28%) more than 75. Overall 163 patients were studied at diagnosis with the rest from 3 to 130 months after diagnosis. Only 105/161 newly diagnosed patients could be staged according to the international prognostic index (IPI) owing to the failure to test β2 microglobulin in many of the referring district general hospitals. Purified plasma cells were tested for deletion/monosomy 13 (Δ13) (96/228 deleted 42%), IgH rearrangement (101/228 rearranged 44%) followed by specific testing for t(11;14)(q13;q32) (36/228 positive 16%), t(4;14)(p16;q32) (22/228 positive 10%) and t(14;16)(q23;q32) (7/228 positive 3%), p53 deletion (8/228 deleted 3%), and numerical abnormalities of the centromeres of chromosomes 3, 6, 7, 9, 10, 11, & 17. Combined results from all probes were used to estimate ploidy by comparison with hypothetical results obtained for these probes from our own and published full karyotypes. This suggested that 49/228 (21%) were hypodiploid or near tetraploid. Shorter survival was seen for patients with Δ13 (22 mo vs not reached, p=0.004), any IgH rearrangement (24 mo vs nr, p=0.035), t(14;16) (6 mo vs nr, p=0.002) and p53 deletion (3 mo vs nr, p=0.041). However, all of these effects were exaggerated in patients aged 70 or under (p=0.003, p=0.005, p=0.015 and p<0.001 respectively) and abolished in those over 70 (p=0.53, p=0.34, p0.06 and p=0.56 respectively). The p values for survival with hypodiploidy decreased from 0.17 at age ≤70 to 0.02 at age ≤65 and showed a strong linear trend with respect to age (p=0.007). The absence of significance of chromosome abnormalities in older patients was due to the poor survival of the non-affected patients, but other factors, eg paraprotein type other than IgG, were still significant in predicting a poorer prognosis in these patients. It is not clear whether the change in significance of chromosome abnormalities with age is simply due to the natural history of the disease, or whether the effect is due to the more aggressive therapy likely to be given to younger patients. The patients classifiable by IPI were studied to see if Δ13 results added any prognostic information; for those studied at diagnosis there was a trend to worse survival based on the additional effect of Δ13 but this did not quite reach significance (p=0.06).

P0.105

BIOLOGICAL AND PROGNOSTIC IMPLICATIONS OF IGH, RB AND P53 ABNORMALITIES IN MULTIPLE MYELOMA: A STUDY OF THE MYELOMA SPANISH GROUP (GEM-2000)

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Aim. To evaluate the prognostic and biologic significance of IGH translocations (Tx), and RB and P53 deletions (Del) assessed by FISH in a large cohort of patients with multiple myeloma (MM) homogeneously treated.

Patients and Methods. A total of 313 patients enrolled in the GEM 2000 Spanish protocol were included in the study at the time of diagnosis. The median age was 63 years (range, 41-70 years). Bone marrow plasma cell infiltration was above 8% in all patients. Interphase FISH studies for the detection of IGH rearrangements was carried out by means of LSI IGH, dual color, break apart rearrangement probe (Vysis). Patients with IGH Tx were explored for 11q13 partner (LSI IGH/CCND1, dual fusion translocation probe), for 4p16 (LSI IGH/FGFR3) and for 16q23 (BAC clones 356D21, 484H2, 10205 and 10206, kindly provided by R. Fonseca). The presence of Del of 13q and 17p were evaluated with a specific probe for RB -LSI 13 (RB1)- and for P53 -LSI P53 (17p13.1)- respectively. Clinical and survival analysis was carried out in the 175 patients with extensive follow-up information. The median overall survival (OS) for the whole group was 50.1 months and the median follow-up for survivors was 22.5 months.

Results. FISH analysis detected chromosomal abnormalities in 191 (61%) of 313 patients. Tx of IGH were observed in 121 out of 313 cases (39%) with the following distribution between partners: t(11;14) in 40/313 (13%), t(4;14) in 41/313 (13%), t(14;16) in 12/313 (4%) and unknown chromosomal partner in 28/313 (9%). Del of RB gene was found in 137 out of the 306 cases analyzed (45%), while P53 in 34 of 313 patients (11%). A significant association between t(4;14) and Del of RB was observed (78%, p=0.02). No correlation between IGH Tx involving 11q13, 16q23 or unknown partners and RB Del was found. P53 Dels were not significantly associated with the other abnormalities. A strong association was noted between IGH Tx (excluding Tx involving unknown partners) as well as RB Del and non-hyperdiploid MM as detected by DNA content evaluated by flow cytometry (87%, p<0.001 and 77%, p<0.001 respectively).As mentioned above all patients were uniformly treated according to GEM 2000 protocol (6 courses of VMCP/VBAD followed by high dose therapy). In the univariate analysis, patients with t(4;14) and patients with Del of RB showed a significantly worse median OS when compared with patients without these abnormalities (21.2 and 30.7m respectively vs 50.1m, p<0.01). RB Del conferred shorter survival in those patients with t(14;16) or IGH Tx with an unknown partner (median OS: 26.3m vs not reached, p=0.03). The presence of RB Del was associated with a higher frequency of failure to respond after transplantation (relative risk=6.9). Interestingly, t(4;14) was significantly more frequent in younger patients. In the multivariate analysis, three variables retained their independent prognostic influence: RB Del, % of S-phase PC and creatinine.

Conclusions. The presence of t(4;14) and Del of RB are associated with poor prognosis in MM patients treated with high-dose therapy.

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P0.106

THE CHROMOSOMAL PATTERN 14q-TRANSLOCATION PLUS 13q-DELETION IS CHARACTERISTIC FOR MULTIPLE MYELOMA AFTER A PRECEDING MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Introduction. Multiple myeloma (MM) may be preceded by a monoclonal gammopathy of undetermined significance (MGUS), but it is at present unclear whether or not MM post-MGUS is biologically and clinically different from *de-novo* MM.

Materials and Methods. We have performed a molecular cytogenetic analysis of 32 cases of MM post-MGUS (median time between recognition of MGUS and transition to MM, 7.6 years; range, 2.6 years to 19.5 years) and compared the findings with those of 256 patients with *de-novo* MM, in whom no previous history of MGUS had been documented. FISH studies of clonal plasma cells (cytoplasmic Ig positive) were performed with probes for IgH translocations [t(14q32)], t(11;14)(q13;q32), t(4;14)(p16;q32), and deletion of 13q14 [del(13q14)].

Results. FISH results are summarized in Table 1:

Abnormality	*MM post-MGUS*	*MM de-novo*	*p*
Any t(14q32)	24/32 (75%)	114/256 (44.5%)	.003
t(11;14)(q13;q32)	9/32 (28.1%)	32/256 (12.5%)	.05
t(4;14)(p16;q32)	2/32 (6.3%)	27/256 (10.6%)	.37
del(13q14)	19/32 (59.4%)	102/256 (39.8%)	.17
del(13q14) plus t(14q32)	18/19 (94.7%)	57/102 (55.8%)	.001
del(13q14) plus t(11;14)	6/19 (31.6%)	9/102 (8.8%)	.02

Serial studies of MGUS plasma cells and MM post-MGUS plasma cells from 12 of these patients have thus far indicated that all chromosomal abnormalities observed at MM post-MGUS were already present in the MGUS plasma cells; most notably, there was one patient with t(4;14) plus del(13q) who had both abnormalities at the time of MGUS 94 months prior to transition to MM.

Conclusions. Collectively, our data suggest that MM post-MGUS is characterized by a distinct chromosomal pattern, in particular a high frequency of t(14q32) plus del(13q14), frequent occurrence of a t(11;14), but low frequency of a t(4;14). We are currently studying the t(14q32) plus del(13q) chromosomal pattern in MGUS to investigate its potential value as a risk factor for transition from MGUS to MM.

P0.107

IMMUNOCYTOCHEMICAL EXPRESSION PROFILE OF MARROW PLASMA CELL CYCLIN A, D1, D2, D3 AND CYCLIN DEPENDENT KINASE INHIBITOR, P16 AND P21 HAS A RESPONSE PREDICTIVE VALUE IN UNTREATED MYELOMA PATIENTS

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Recently Bergsagel *et al.* have published a hierarchical model of myeloma evolution based on cytogenetics and Cyclin D expression profiles. This model includes five subsets of patients that carry IgH translocations involving Cyclin D1-3 that are known to have both good or poor prognostic features. However the sequential role of Cyclin A and p21 has not been determined yet. We had reported the flow cytometric expression profiles of cyclin and cyclin dependent kinase inhibitors (CDKI) previously (Blood 98: 4230,2001) and had not been able to see the cyclin-CDKI relationship observed in normal subjects, in unsorted marrow samples of myeloma patients. Encouraged by these findings we aimed: (1) to analyze the cyclin and CDKI in marrow plasma cells by immunocytochemistry; (2) to evaluate the proliferative status of plasma cells by Ki67 expression, mitotic index; and (3) to compare the results with FISH findings, clinical parameters and outcome.

Patients. Thirty-four myeloma patients diagnosed in 2002-2004, aged 54(33-77), M/F: 21/13 whose bone marrow biopsy specimens could be retrieved were included in the analysis. All patients were treated with VAD or MP as first line treatment.

Methods. Immunocytochemical staining was performed using Zymed ABC Px Kit (manually), Ventana Benchmark (automated) and monoclonal antibodies: Cyclins A, D1, D3, p16 and p21 (LabVision), Cyclin D2 (Santa Cruz) and Ki67 (LabVision). Immunocytochemistry evaluation was performed by an independent pathologist (IK) who was uninformed about the clinical parameters. 13q(13q14.3) and t(11,14)(IgH/CCDN1 XT) probes (Vysis) was used for FISH staining on sections or CD38/138 sorted plasma cells.
Results. The percentage of patients expressing each cyclin or CDKI was as follows: Cyclin A: 6/34, Cyclin D1:8/34, Cyclin D2:8/33,Cyclin D3:3/34, p16: 7/34 p21:6/32. The presence of at least one Cyclin D was:18/34. Since cyclins are proliferative elements, we categorized the presence of both cyclins and lack of the relevant CDKI as a maximum proliferative group (P-max,n=14) and vice versa a P-min (n=6). The other combinations were grouped as others and included 14 patients. Age, β2MG, CRP, Ki67, 13q abnormalities or t(11,14) distribution was not different between the groups. However, overall response(>50% Ig reduction) rate was higher in the P-min compared to P-max patients(p=0.024).

		Age	*Beta2M*	*CRP*	*Ki67(<25%)*	*response rate*
P-min	n: 6	64(54-77)	4.5(3.2-7.6)	11(3.3-46)	3/4	5/6
P-max	n:14	54(49-70)	3.3 (0.8-16.9)	10(1-158)	6/9	4/14
P-others	n:14	56(33-70)	2.2 (0.4-16)	3.3(1-18)	2/9	6/14

Conclusions. Our preliminary results show the feasibility of an immunocytochemical detection of cyclins and CDKI in marrow plasma cells. In this small group of untreated myeloma patients absence of these cyclins and presence of p16/p21 is associated with better response and had a predictive value greater than β2MG, CRP or Ki67.

P0.108

CLINICAL IMPLICATIONS OF t(11;14)(q13;Q32), t(4;14)(p16.3;q32) AND 17p13 DELETIONS IN MYELOMA PATIENTS TREATED WITH HIGH DOSE THERAPY

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Introduction: FISH is able to recognize chromosomal deletions and translocations with a greater sensitivity than conventional cytogenetics. Specific abnormalities have been associated with prognosis. Initial observations suggest a poor outcome for patients with 17p13.1, chromosome 13 abnormalities (13) and t(4;14)(p16.3;q32). In contrast a good outcome has been shown in some series for patients with t(11;14)(q13;q32). We analyzed the value of FISH in patients receiving high dose therapy.

Patients and Methods: We studied by cIg-FISH 226 patients undergoing high dose therapy at the Mayo Clinic between 1/1990 and 9/2001. All patients had a pretransplant cIg-FISH done on cytospin slides from marrow aspirates for t(11;14)(q13;q32), t(4;14)(p16.3;q32), and -17p13.1(p53). Information was available regarding 13 for all patients (+ in 52%).

*Results:*The prevalence of the abnormalities were: t(11;14)(q13;q32) 17% (n=197), t(4;14)(p16.3;q32) 13% (n=153), and -17p13.1 11% (n=168). The overall survival (OS) was significantly shortened in patients with t(4;14)(p16.3;q32) (18.2 vs. 43.3 mo, p=0.001) (Figure) and patients with 17p13.1 (14.7 vs. 38.6 mo, p=0.01). OS was not different for patients with the t(11;14)(q13;q32) (36.2 vs. 34.8 mo, p=ns). Likewise time to progression (TTP) was shortened in patients with t(4;14)(p16.3;q32) (8.5 vs 17.7 mo, p=0.001) and 17p13.1 (8.3 vs. 16.2 mo, p=0.005). TTP was also not affected significantly by the t(11;14)(q13;q32) (20.7 vs. 14.9 mo, p=NS). To dissect the specific contribution of t(4;14)(p16.3;q32) we did a subset analysis of patients who also had 13, since 85% of patients with t(4;14)(p16.3;q32) are expected to have 13. Of 84 studied for both abnormalities 22 had both 13 and t(4;14)(p16.3;q32). The OS was significantly shorter in patients with both abnormalities versus those with 13 alone (26.6 vs. 18.2 months, p=0.001). When a multivariable analysis of the impact of 13 and t(4;14)(p16.3;q32) were placed into a Cox model, the hazard function for t(4;14)(p16.3;q32) was greater than 13 (2.6 versus 1.6). Chromosome 13 abnormalities had only borderline significance in this model (p=0.06).

Conclusions: We have been unable to corroborate the improved outcome after transplant for patients with t(11;14)(q13;q32). As has been reported in patients with conventional and high dose therapy 17p13(p53) and t(4;14)(p16.3;q32) have clinical importance for estimation of OS and TTP. In this patient group the t(4;14)(p16.3;q32) carried a greater adverse impact than did 13, and identifies a subset of patients whose time to progression is 8.5 months. These patients likely do not benefit from autologous transplant and are candidates for novel therapeutic approaches.

P0.109

FAVORABLE SURVIVAL OF MULTIPLE MYELOMA PATIENTS WITH t(11;14)(q13;q32) PLUS NORMAL CHROMOSOME 13q

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Introduction. Previous studies have shown that specific chromosomal abnormalities are of major prognostic significance in patients with multiple myeloma (MM). It has been recently suggested that a t(11;14)(q13;q32) is an indicator of favorable outcome in MM. In this investigation, we analyzed 163 patients with newly diagnosed MM (53% treated by high-dose therapy) to address the question whether or not the simultaneous occurrence of a t(11;14) and a deletion 13q [del(13q)], an established negative prognostic factor in MM, has any impact on prognosis.

Materials and Methods. DNA-specific probes for IgH (14q32) and cyclin-D1 (11q13) as well as rb-1 (13q14) were used for interphase FISH analysis of clonal plasma cells (cytoplasmic Ig positive).

Results. A t(11;14) by FISH was shown in 27 of the 163 MM patients (16.6%); the abnormality was present in the majority (median, 89%) of clonal plasma cells. Immunohistochemical analysis of CYCLIN-D1 expression was carried out in 72 patients, of whom 11 had a t(11;14) by FISH; all 11 patients had evidence for CYCLIN-D1 protein expression. Presence of a t(11;14) did not show a significant correlation with standard MM features; there was also no association with CD20 expression by MM cells. Presence of any 14q-translocation (52% of patients) was associated with similar overall survival times (OS) compared to patients lacking a t(14q), whereas patients with a t(11;14) experienced prolonged OS (median, 70+ months vs. 59.8 months among patients without t(11;14); p=0.071). This survival advantage was even greater among the 16 patients with t(11;14) who were also normal for 13q (p=0.02); however, occurrence of a del(13q) concomitantly with a t(11;14) was indicative for shortened progression-free survival (17.7 months vs. 31.6 months; p=0.17) and OS (p=0.07). A survival benefit of MM patients with a t(11;14) was particularly evident for the population receiving standard-dose chemotherapy (median OS not yet reached; p=0.02). By multivariate Cox regression analysis, low serum b2M at diagnosis (p=0.001), absence of a del(13q) (p=0.004), high-dose therapy (p=0.034), and presence of t(11;14)/no del(13q) (p=0.069) emerged as independent favorable parameters for OS. Thus, according to the cytogenetic pattern, three prognostic groups of patients could be discriminated (p<0.001): patients with good [t(11;14), no del(13q)], intermediate [no t(11;14), no del(13q)], and poor prognosis [no t(11;14), del(13q)].

Conclusions. We conclude that MM with t(11;14) represents a heterogeneous entity, and only the cytogenetic pattern t(11;14)/no del(13q) characterizes the most favorable prognostic group of MM, with sensitive disease to multiple lines of anti-MM therapy.

P0.110

SUPERIOR OVERALL SURVIVAL IN PLASMA CELL MYELOMA WITH CYCLIN D1 PROTEIN EXPRESSION IDENTIFIED BY IMMUNOHISTOCHEMISTRY

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The expression of cyclin D1 is dysregulated in approximately half of cases of plasma cell myeloma through a variety of mechanisms, including the translocation t(11;14)(q13;q32), aneusomy, or other abnormalities. Recent studies using quantitative mRNA analysis have suggested that increased cyclin D1 mRNA expression is associated with a favorable prognosis. Previous attempts to examine the significance of cyclin D1 protein expression by immunohistochemistry have been hampered by the use of antibodies with weak staining and high background. In this study, we employed a newly available, commercial antibody that gives superior staining in B5 fixed tissues. We performed immunohistochemistry for cyclin D1 on bone marrow core biopsies from a series of 44 newly diagnosed plasma cell myeloma patients who were uniformly treated on a phase II study of rituxan, melphalan and prednisone. Twenty-two patients (50%) were positive for cyclin D1, defined as any plasma cells with positive nuclear staining. Cyclin D1 positive and negative cases displayed no significant differences in the initial levels of β2m (3.6+/-0.5 mg/L vs. 3.5+/-0.5 mg/L, p=0.860), number of bone marrow plasma cells (63+/-5.5% vs. 47+/-6.2%, p=0.063), or proportion of cases classified as SWOG stage 3-4 (2 of 22 (9%) vs. 5 of 22 (23%), p=0.412). The cyclin D1 positive cases displayed a superior overall survival with an estimated 3-year survival of 95% for cyclin D1 positive cases versus 56% for cyclin D1 negative cases (p=0.032). The cyclin D1 positive cases also displayed a trend towards superior progression-free survival (median progression free survival of 15.7 months for Cyclin D1 positive versus 12.8 months for cyclin D1 negative, p=0.13). This study demonstrates that cyclin D1 immunohistochemistry, which could be readily performed in most routine pathology laboratories, is capable of identifying a subset of plasma cell myeloma with a favorable survival. Additional studies are ongoing to determine if these results can be generalized to other forms of therapy as well. If confirmed, routine cyclin D1 immunohistochemistry at the time of diagnosis may offer important prognostic information that could identify lower risk patients for whom less intensive therapies might be appropriate.

P0.111

SUBTELOMERIC CHROMOSOME REARRANGEMENTS IN THE PROGRESSION OF MULTIPLE MYELOMA: IDENTIFICATION OF A NEW RECURRING CRYPTIC TRANSLOCATION der(19)t(1;19)(p36.33;p13.3)

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Multiple myeloma (MM) is characterized at the cytogenetic level by chromosome aneuploidy and complex structural aberrations involving both balanced and unbalanced translocations, deletions, duplications, and chromosome instability. Subtelomeric regions are gene rich and could be involved in cryptic translocations, such as the t(4;14)

(p16.3;q32), which are not resolved by chromosome banding methods. In an attempt to identify other cryptic chromosome aberrations occurring in the progression of MM we have analyzed 25 patients and three cell lines showing complex karyotypes with a panel of subtelomeric fluorescence *in situ* hybridization (FISH) probes. The panel consisted of 41 telomere specific FISH probes (excluding the p arms of 13, 14, 15, 21, 22), and 7 centromeric and/or locus specific probes which serve as markers for chromosome identification (Vysis, Downers Grove, IL, USA). The subtelomeric probes are estimated to be within 300 kb from the end of each chromosome. The chromosome analysis was performed on metaphase preparations from patients and cell lines which had previously been characterized by both G-banding and spectral karyotyping (SKY). At least one cryptic telomere rearrangement was found in all patients and cell lines with the average being 2.2 rearrangements (range 1-6). This analysis indicated that the most frequently rearranged telomeres were, in decreasing order of frequency, 14q(17), 4p(12), and 8q(11). The recurring translocation t(4;14)(p16;q32) was identified in nine patients (three were complex three-way translocations) and one cell line. Two new recurring aberrations of chromosome 19 were identified, including an isochromosome 19p which was found in two patients and one cell line. Iso19p has not been previously reported in MM and is cryptic to SKY and G-band analysis. The second new aberration was a cryptic der(19)t(1;19)(p36.33;p13.3) translocation which was identified in four patients. This translocation was found only on chromosome 19s with previous translocations to the long arm, suggesting this aberration occurs as a late secondary event in the progression of the disease. Interestingly, in three of the four cases the 1p subtelomeric region translocated to a der(19)t(1;19)(q12;q13) resulting in a der(19)t(1;19)(p36.33;p13.3) t(1;19)(q12;q13), while in the fourth case a der(19)t(1;19)(p36.33;p13.3) t(13;19)(q10;p10) was identified. During the progression of MM large whole-arm 1q translocations (jumping 1q) can occur on the distal long arm of 19 and can show gene duplications in 1q12~21. These 1q aberrations are associated with aggressive disease. Three of the four patients with the der(19)t(1;19)(p36.33;p13.3) translocation also showed the t(4;14)(p16;q32), and all four showed a loss or deletion of chromosome 13, suggesting that this new subtelomeric translocation may also be associated with these other poor prognostic markers. The types of subtelomeric aberrations reported here indicate a higher level of cryptic chromosome instability in the progression of the MM clone than has previously been recognized, which could in turn cause changes in expression of telomere-proximal genes altered by these events.

P0.112

FLUORESCENT *IN SITU* HYBRIDIZATION BASED TRISOMY INDEX IDENTIFIES HYPERDIPLOID MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Multiple myeloma is characterized by complex genetic changes and aneuploidy. Recent studies have shown that 2 major genetic subtypes of multiple myeloma exists as defined by the composition of their chromosome number. Hyperdiploid MM (48 to 74 chromosomes, median 53 chromosomes) is characterized by trisomies, especially of chromosomes 3,7,9,11,15, and 19, while the non-hyperdiploid (less than 48 chromosomes or more than 74 chromosomes) myelomas are associated with primary translocations like t(11;14), t(4;14) and t(14;16). The oncogenic event leading to the develoment of hyperdiploid myeloma is/are currently unknown. Study of ploidy in myeloma is hampered by limitations of current methods. Conventional karyotype only provides useful information in the minority of patients while DNA content estimation by flow cytometry suffers from lack of standardization, poor correlation with cytogenetics and conflicting prognostic usefulness. These problems are compounded in monoclonal gammopathy of undetermined significance (MGUS) where the number of clonal plasma cells is small. Fluorescent *in situ* hybridization (FISH) would overcome many of these shortcomings and have shown that most myelomas and a significant proportion of MGUS have aneuploidy in the clonal plasma cells. In this study, we derived a trisomy index based on the use of centromeric FISH probes for a number of chromosomes that could identify hyperdiploid myeloma with a high degree of sensitivity and specificity. The index is derived from pooled cytogenetic data from 2 large cohorts of myeloma patients with abnormal karyotype. Using the criteria of 2 or more trisomies out of either 2 of combinations of 5 commonly trisomic chromosomes (7,9,11,15,18 or 3,9,11,15,19), hyperdiploid myeloma can be detected with a sensitivity of 80-88% and specificity of 94-96% depending on which combination is used. We then validated this index by using FISH probes for the 2 different combinations of chromosomes in 2 independent cohorts of patients who had known ploidy status either by karyotyping or DNA content measurement by flow cytometry. Again, hyperdiploid MM was identified with high sensitivity and specificity. Using this index we found that out of the 28 SMM/MGUS (11 SMM and 17 MGUS) patients who had a normal karyotype, 15 had hyperdiploid SMM/MGUS. This percentage (54%) is remarkably similar to the percentage of hyperdiploid MM reported in the literature. We have therefore derived a robust yet highly applicable index based on FISH that accurately identifies hyperdiploid myeloma. Furthermore we now report that hyperdiploid SMM/MGUS exists at a percentage not significantly different from that reported for symptomatic myeloma, suggesting that hyperdiploid MM may originate early during disease evolution.

P0.113

ANALYSIS OF GENE EXPRESSION IN MONOCLONAL GAMMOPATHIES POLYMERASE CHAIN REACTION BY REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTION WITH TAQMAN PROBES

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Array studies have identified a high number of deregulated genes in MM plasma cells (PC) from patients with monoclonal gammopathies (MG). We studied 20 selected genes in patients with MG to analyze the relationship of gene expression with several clinical and biological characteristics of the disease.

Materials and Methods: Seventy-four patients were included in the analysis: 2 patients with plasma cell leukemia (PCL), 6 with progressive multiple myeloma (MM), 54 with untreated symptomatic MM, six with smoldering MM (SMM) and 6 with monoclonal gammopathies of undetermined significance (MGUS). Gene expression was analyzed in purified PC obtained from bone marrow aspirates. Purifications were made through magnetic separation by using CD138 antigen expression (purity was always >90%). Gene expression was assessed by quantitative real time RT-PCR according to the *EAC Program* Protocol (Leukemia 2003, 17:2318). RT-PCR was carried out using TaqMan Probes with reagents obtained from Applied Biosystems according to the Assay-on-demand protocol. The following genes were analyzed: CCD1, FGFR3, C-MAF, RB1, P53, P16/P14, P15, PTEN, MGMT, MLH1, SOCS-1, ZHX2, RAN, CHCL1, RHAMM, MMSET, DNMT1, BIK, DAD-1 and XBP1. The ABL gene expression was used as a house-keeping gene to correct RNA quality and target-gene expression.

Results: According to patient diagnosis within the proposed model for MG evolution (MGUS -> SMM -> symptomatic MM -> progressive MM -> LCP) six genes showed statistically significant differences in their RNA expression: BIK, DNMT1, MLH1, P15 & P53, which were progressively over-expressed in the final stages, and ZHX2, that showed an opposite expression picture. These differences were also maintained when symptomatic MM were divided into the three Durie & Salmon categories. By contrast, when we compared the gene expression with the recently proposed International Prognostic Index, only DNMT1 and ZHX2 kept its direct and inverse association with the advanced stages, respectively. According to the proliferative activity, DAD and RAN were over-expressed in those cases with high numbers of S-phase PC; by contrast, PTEN was infra-expressed in cases with less than 1.8% S-phase PC. Immunophenotyping and gene expression had interesting associations; for instance, CD56+ MM displayed a low cyclin D1 expression and a high c-maf and p53 expression. As expected, cyclin D1 and FGFR3/MMSET expression was related to the presence of the IgH translocations, since they showed a higher expression in cases with the t(11;14) and t(14;16), respectively. Interestingly, cases with Rb deletion did not show a lower Rb mRNA expression, only showing a higher expression of RHAMM. Response is still pending to be evaluated and, despite it being too soon to complete the survival analysis, p15 and p16 over-expression were associated with a better survival.

Conclusions: Gene expression analysis by RT-PCR in plasma cells from MM patients correlates with singular clinical and biological disease characteristics.

P0.114

IN VIVO GENE EXPRESSION PROFILES IN MULTIPLE MYELOMA BEFORE AND AFTER INDUCTION CHEMOTHERAPY AND IN HEALTHY DONORS, USING AN EASY AND EFFICIENT GENE EXPRESSION METHOD: MICRO FLUIDIC CARDS

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Background. In multiple myeloma (MM) the result of the interactions between MM cells and the bone marrow microenvironment is induction of osteoclast formation and activation with bone resorption outweighing bone formation.There is increasing evidence that progression of osteolytic bone disease (OBD) is dependent on disturbances in the relationship between receptor activator of nuclear factor-κB (RANK), its ligand – RANKL and the antagonist osteoprotegerin (OPG). Other cytokines, such as tumor necrosis factor (TNF)-α, TNFβ, matrix metalloproteases (MMP), macrophage inflammatory protein (MIP)-1α, MIP-1β and members of the interleukin system also play a role, either by RANKL-dependent or -independent pathways.

Aim. To study gene expression profiles of 20 genes, suspected to by involved in osteolytic bone disease and disease progression in multiple myeloma, by comparison of gene expression profiles before and after induction chemotherapy, and between myeloma patients and healthy donors.

Methods. Bone marrow biopsies from 10 newly diagnosed multiple myeloma patients before and after VAD induction chemotherapy, and biopsies from 8 healthy volunteers where taken un-fixated and bedside frozen in liquid nitrogen. The biopsies were homogenized followed by mRNA purification and creation of cDNA. Gene expression profiles of 20 genes and 2 housekeeping genes were made using a new real-time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) technique, Micro Fluidic Card (MFC) (Applied Biosystems, Foster City, USA). MFC is a customized 384-well microtiter plate with PQ-PCR primers and probes lyophilized in each 2 μL reaction well.

Results. Compared with healthy donors, our data suggest an up-regulation of RANKL, hepatocyte growth factor (HGF), (MIP)-1α, MMP13, MMP14, syndecan-1 and frizzled-related protein (FRZB). We observed no overall deregulated expression of MIP-1β, MMP-1, MMP-2, MMP-7, MMP-8, MMP-9, DKK-1, OPG, RANK, VEGF, osteopontin, IL-1α, IL6, IL7 and IL13. After induction chemotherapy we found a reduction in the gene expression levels of syndecan-1, osteopontin, MMP-2, MMP-13, MMP-14, IL6, HGF and FRZB. The sensitivity of MFC was found to by 1-2 CT reactions lower compared with standard RQ-PCR technique using 25 a μL reaction volume.

Conclusions. The use of Micro Fluidic Card is an efficient and easy way to examine gene expression of multiple genes although less sensitive than standard techniques. Our study is too small to make clear conclusions, but we found a gene expression pattern with increased expression of some genes suggested to be involved in OBD and disease progression in multiple myeloma.

P0.115

GENE EXPRESSION PROFILING OF THALIDOMIDE IN MULTIPLE MYELOMA: A TIME COURSE STUDY

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Microarray based gene expression profiling of drug treated tumor cells is a powerful way to determine the action of anti tumor agents. Gene expression profiling of thalidomide (s-thal) treated multiple myeloma (MM) cells showed that groups of genes with specific function in the pathophysiology of MM had consistent increases and decreases. We have previously shown that the genes for NF-κB and Bcl2 are downgraded by s-thal. The U266 mm cell line was cultured with s-thal 0-1000uM (Cellgene Corporation USA) and RNA extracted after 2,4 and 24 hours exposure to the IC_{50} (% viability) of s-thal and gene expression profiling established by microarray methodologies. The table below shows the

mean fold changes in expression of genes associated with the caspase cascade and a gene CLARP associated with the production of an apoptotic protein.

	APF 1	*CASP9*	*CASP8*	*CASP7*	*CASP3*	*CLARP(FLIP)*
1 hour	1.59	1.06	1.38	-1.17	-1.04	1.05
4 hours	1.1	1.72	-1.2	-1.06	1.66	-1.36
24 hours	-1.2	1.82	-2.3	1.58	1.27	-2.43

Progressive upregulation of caspases 9, 7 and 3 occurs as the cascade progresses with the exception of caspase 8 as in these cells s-thal appears to affect only the intrinsic pathway. The anti apoptotic gene CLARP was also downregulated which is consistent with the previously demonstrated anti apoptotic effect of s- thal These changes are being further investigated at the protein level.

P0.116

CHARACTERIZATION OF MULTIPLE MYELOMA CELL LINES BY COMPARATIVE GENOMIC HYBRIDIZATION (CGH), ARRAY-BASED CGH AND REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION

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Multiple myeloma cell lines (MMCL) represent a unique and homogeneous model for myeloma research that has contributed greathy to gain insight on the disease biology and mechanism of action and efficacy of new drugs. Accordingly, a thorough characterization of the genetic and antigenic properties of MMCL is desirable. In the present work we have characterized ten cell lines derived from plasma cells obtained from MM patients with different techniques.

Ten MM cell lines were analyzed: MM_1S, MM_1R, MM_{144}, U_{266}, $U_{266}LR7$, $U_{266}DOX$, MGG, $RPMI_{8226}$, NFR, and OPM_2. All had been previously characterized by flow cytometry, conventional cytogenetics and fluorescence *in situ* hybridization. Now we have analyzed them by conventional CGH, CGH-Array and RT-PCR. CGH-array was performed by using 330 BAC clones containing human genomic DNA (1 Mb BAC set from the Wellcome Trust Sanger); 110 BACs were localized on chr. 13 while the remaining contained well-known oncogenes and tumor-suppressor genes. RQ-PCR was carried out in an ABI Prism 7700 sequence detector with the EAC protocol and reagents obtained with Assay-on-Demand© protocol for the following genes: CCND1, FGFR3, C-MAF, RB1, P53, P16/P14, P15, PTEN, MGMT, MLH1, SOCS-1, ZHX2, RAN, CHC1L, RHAMM, DAD1, BIK, XBP-1, DNMT-1 and MMSET.

OPM_2 was the only cell line showing DNA hyperdiploidy by FCM, but CGH and CGH-array detected an overall increase in the number of copies in all cell lines MM. The mean of changes per case by CGH was 21 (range 16-25). In all cell lines, CGH-arrays, showed a common deletion region on 13qcenter-13q22 (size 6 Mb). This region was limited by the probes bA26A3 -ba129M14 and includes the RB gene. Despite this, RT-PCR demonstrated that RB transcripts were present in all cell lines, although in a lower number than normal controls (around 10 times lower). A similar finding was observed for the CHCL gene, present on 13q14. In addition, CGH-array globally revealed gains on 1q25, 3q26.2, 8p12, 17p13 and 20q11 and losses on 4q34.3 and 16p13. CCDN1 was moderately elevated in most cell lines, although >1000 times in U_{266} cell lines, which harbor the t(11;14). FGFR3 and MMSET were only significantly over-expressed in the OPM_2 cell line, which was the only one with the t(4;14), Interestingly, this cell line also had a DNA loss at 4p16 that however did not result in FGFR3 infra-expression. RHAMM was homogeneously over-expressed in all cell lines, especially in $U_{266}LR7$, which harbors a 5q33 gain where the gene is located. The expression of the remaining genes analyzed by RT-PCR globally showed a slight heterogeneity, although there were important differences between the OPM_2 and U_{266} cell lines as compared to the remaining cell lines. All cell lines displayed a very high percentage of cells in S-phase (33%±18,4%), but OPM_2 and $RPMI_{8226}$ showed the biggest percentage of proliferating cells. However, they showed the highest P14/P16 expression; a possible explanation for this apparently contradictory result is a failure in this inhibitory mechanism to stop the cell cycle. As far as correlation with drug sensitivity, 3q25, 5q33 & 16p13 gains and 4q23 & 6p21 losses were seen in the MM1R (Dexa-resistant) line while they were absent in the MM_1S line (Dexa-sensitive). In addition, gains on 5q, 12q & 20q as well as losses on 11q & 16p were observed to be associated with melphalan resistance. However, the expression of the 20 selected genes analyzed by RT-PCR did not show important differences between cell lines according to the drug sensitivity, with the exception of p14/16 & p15, that were notably infra-expressed in the MM_1S cell line.

This study illustrates the complexity of genetic abnormalities of MM cell lines which could be used as a reference for investigations on freshly isolated plasma cells.

P0.117

COMPARATIVE ANALYSIS OF CLINICAL PARAMETERS WITH MARROW PLASMA CELL CYCLIN A, D1, D2, D3 AND CYCLIN DEPENDENT KINASE INHIBITOR P16 AND P21 EXPRESSION PROFILES: PRELIMINARY RESULTS IN PATIENTS WIT *DE NOVO* MULTIPLE MYELOMA

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Cyclins are expressed during specific phases of the cell cycle and are controlled by cell cycle dependent kinase inhibitors (CDKI). p16 and p21 control cyclin D and cyclin A, respectively. Recently Bergsagel *et al* have published a hierarchical model of myeloma evolution based on cytogenetics and cyclin D expression profiles. This model includes five subsets of patients that carry IgH translocations involving cyclin D1-3 that are known to have both good or poor prognostic features. These authors define cyclin D and p16 perturbations as early stage events. However the sequential role of Cyclin A and p21 has not been determined yet. We have reported flow cytometric expression profiles of these cyclin and CDKI previously (Blood 2001; 98:4230) and we were not able to detect the parallelism between cyclin-CDKI observed in normal subjects, in unsorted marrow samples of some myeloma patients. Encouraged by these findings we aimed: (1) to analyze the same cyclin and CDKI's in marrow plasma cells by immunocytochemistry; (2) to evaluate the proliferative status of plasma cells by morphological and clinical parameters.

Patients. Thirthy-four myeloma patients diagnosed in our Department between 2002-2004, aged 54(33-77), M/F:21/13 whose bone marrow biopsy specimens could be retrieved were included in the analysis. All patients were treated with

VAD or MP as first line treatment.

Methods. Immunocytochemical staining was performed using Zymed ABC Px Kit (manuel), Ventana Benchmark (automated) and monoclonal antibodies: cyclins A, D1, D3, p16 and p21(LabVision) and cyclin D2 (Santa Cruz). Negative control was present in each experiment. Morphological assessment was performed by an independent pathologist (IK) who was uninformed about the clinical parameters. Results were reported as positive (equal or more than 20% of plasma cells) or negative (less than 20%). The percentage of patients expressing each cyclin or CDKI was as follows: cyclin A: 6/34, cyclin D1: 8/34, cyclinD2: 8/33, cyclin D3: 3/34, p16: 7/34 p21:6/32. The presence of at least one cyclin D was 18/34. Since cyclins are proliferative elements, we categorized the presence of both cyclins and lack of the relevant CDKI as a maximum proliferative group (P-max,n:16) and the vice versa a P-min (n=6). The other combinations were grouped as others and included 12 patients. Some prognostic clinical characteristics and response to initial chemotherapy regimen are summarized:

	Age	*Beta2M*	*CRP*	*Complete response rate*
Pmin n=6	59(48-68) N.S	2.2 (0.4- 6.2) N.S	8(1-46) N.S	5/5 p=0.0001
Pmax n=16	53(49-77)	4.5(0.8-16.9)	5(1-158)	2/15

Although it is too early to draw definite conclusions, our preliminary results show the feasibility of immunocytochemical detection of cyclins and CDKI in marrow plasma cells. In this small group of untreated myeloma patients absence of these cyclins and presence of p16/p21 is associated with better response to initial chemotherapy. This technique may add value to new prognostic scoring systems. We plan to present more data with additional new patients during the Workshop.

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P0.118

ONLY 1% CYCLIN D3 REARRANGEMENT IN OVER 450 PLASMA CELL DYSCRASIAS

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Rearrangement of cyclin D3 (CCND3) on chromosome 6 at band p21, usually by translocation with the immunoglobulin heavy chain locus at 14q32, has been proposed to be a primary rearrangement in multiple myeloma (MM). It is thought that this may lead to a similar type of disease to that associated with a t(11;14)(q13;q32) translocation, which up-regulates cyclin D1. Many studies have been published showing that the frequency of the latter abnormality is 15-20% in MM and similar in MGUS. It is also a frequent finding in primary amyloidosis (AL). Little information is available about the frequency of the t(6;14). One review suggested that its frequency in MM is ~4% but there is no information for MGUS or AL.

We studied 462 cases of plasma cell dyscrasia (MM, n=334, asymptomatic MM, n=28, MGUS, n=40, AL, n=6, currently unclassified, n=54) for whom adequate material was available. The age range of the patients was 27-93, median 66. Samples came from 50 different hospitals across the UK. These were tested for CCND3 rearrangement by fluorescence *in situ* hybridization (FISH) on purified plasma cells using probes spanning the CCND3 locus. Only 5 cases (1%) showed a rearrangement. Four of these had a t(6;14)(p21;q32), three of them unbalanced with loss of the derived chromosome 6, and the fifth was a t(6;22)(p21;q11), confirmed by conventional cytogenetic analysis. Four of these patients had MM, all with different paraprotein types (IgGκ, IgGλ, IgAκ and non-secretory). The fifth had an isolated plasmacytoma with no increase of plasma cells in the marrow although 25% of those purified showed the abnormality. These patients were aged 36-82 and all are still alive 18-40 months after testing.

This very low rate of CCND3 rearrangement needs to be placed in the context of the other FISH-detected abnormalities in these patients. 199/499 (40%) showed deletion 13, 215/504 (43%) showed an IgH rearrangement with 79/502 (16%) having a t(11;14), 49/506 (10%) a t(4;14) and 7/250 (3%) a t(14;16). The low frequency of IgH rearrangement in our series has been shown to be largely due to the high proportion of elderly patients (36%>70, 22%>75) and the inverse relationship between incidence of IgH abnormalities and age (p=0.001). Despite the low overall IgH abnormality rate, our incidence of t(11;14) is similar to that of other series. It is therefore interesting that the frequency of CCND3 rearrangement is so low.

P0.119

RESPONSE TO PRIMARY THERAPY WITH THALIDOMIDE DEXAMETHASONE IN MULTIPLE MYELOMA IS NOT ADVERSELY AFFECTED BY t(4;14) AND del(13)

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Background. Multiple myeloma (MM) is a plasma cell (PC) neoplasm characterized by a profound genomic instability. In spite of this karyotypic complexity, several recurrent genomic abnormalities have been identified. These abnormalities define specific subgroups of MM patients and are supposed to be involved in the pathogenesis of the disease. In this context, it has been shown that one of these subgroups, involving at least one-half of MM patients, carries at diagnosis translocations involving the immunoglobulin heavy-chain (IgH) locus and/or deletion of chromosome 13 (del(13)). Among IgH translocations, t(4;14)(p16;q32) is one of the most commonly reported. Both del(13) and t(4;14) have a poor prognostic relevance and are often associated at diagnosis. Translocation t(4;14) results in the production of a chimeric fusion transcript between MMSET and IgH, and, in about 70% of t(4;14) positive cases, it leads to the activation of the fibroblast growth factor receptor 3 (FGFR3).

Aim. We investigated the frequency of t(4;14) and del(13) in a series of 52 previously untreated patients with symptomatic MM who received first-line remission induction therapy with thalidomide and dexamethasone in preparation for subsequent autologous transplantation. The relationship between these chromosomal abnormalities and response to treatment was also analyzed.

Methods. For this purpose we isolated the CD138+plasma cell fraction from the bone marrow taken at diagnosis from MM patients. We analyzed: 1) the presence of t(4;14) by RT-PCR of the hybrid transcript MMSET/IgH; 2) the over-

expression of FGFR3 by Real-time RT-PCR; and 3) the presence of del(13) by FISH analysis. The relationship between these two chromosomal abnormalities and response to thalidomide-dexamethasone was also investigated.

Results. Translocation t(4;14) was detected in 15/52 patients (29%). Among these patients, 10/15 (67%) displayed both MMSET/IgH fusion gene and FGFR3 over-expression, thus supporting the discrepancy between MMSET/IgH positivity and FGFR3 over-expression. Del(13) was detected in 19/47 patients who could be evaluated (40%). Patients with translocation t(4;14) were more likely to carry also del(13) than t(4;14) negative patients (64% vs. 31%, respectively; p=0.05). Patients with translocation t(4;14) and/or del(13) had the same probability of responding to thalidomide-dexamethasone remission induction therapy than patients who lacked these unfavorable karyotypic abnormalities.

Conclusions. The frequency of t(4;14) in our series of newly diagnosed MM patients was 29%, a value higher than that found in other series reported so far. At the opposite, the presence of del(13) was consistent with data from the literature. Translocation t(4; 14) and/or del(13) had no adverse influence on response to primary therapy with thalidomide-dexamethasone which can thus be considered as a valid treatment option in preparation for autologous transplantation in preparation for autologous transplantation even for patients with adverse chromosomal abnormalities.

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P0.120

t(4;14) POSITIVE MULTIPLE MYELOMA IS CHEMOSENSITIVE TO DEXAMETHASONE AND/OR THALIDOMIDE BUT NOT ALKYLATING AGENTS: RAPID RELAPSE AND NOT PRIMARY DRUG RESISTANCE EXPLAINS POOR OUTCOMES

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The t(4;14) simultaneously dysregulates expression of fibroblast growth factor receptor 3 (FGFR-3) and MMSET and has been detected in 15% of MM patients. Despite being an early event in the genesis of clonal gammopathy the t(4;14) is somewhat surprisingly reported as a poor prognostic factor in patients treated with both conventional chemotherapy and those undergoing autologous stem cell transplantation (ASCT). Presumably either primary drug resistance or, alternatively, rapid relapse following treatment must explain this outcome. To address this clinical issue we analyzed 130 patients who were treated with induction chemotherapy followed by melphalan 200 mg/m^2 at our institution between 1998-2000. Using FISH analysis we identified 18 of these patients with a t(4;14). These patients had a predominance of the IgA isotype (50.0%) compared with t(4;14) negative patients (17.7%) (p=0.0019). Otherwise baseline characteristics including sex, age, β-2 microglobulin, CRP, calcium, creatinine, Hb, albumin or percentage of bone marrow plasma cells were indistinguishable from the general MM population. Sixteen t(4;14) patients received induction chemotherapy with 4-5 cycles of vincristine, adriamycin and dexamethasone(VAD) with two receiving pulsed dexamethasone alone. Fourteen of the eighteen (78%) responded with > 50% paraprotein decrease. However, 4 of these 14 patients demonstrated early progression of disease during later cycles of VAD or during stem cell collection and required further salvage prior to ASCT. Use of high dose melphalan and ASCT resulted in a further 55% of patients achieving a >50% paraprotein decrease with a mean paraprotein reduction of 49% (range 0-99%). Nevertheless the median progression free survival (PFS) post ASCT was only 9.9 months, significantly shorter than for t(4;14) negative patients (p=0.0001). Given the surprisingly short PFS following high dose alkylating agents we next examined all salvage regimens employed. Ten patients at some point received a conventional dose alkylating agent as salvage (cyclophosphamide or melphalan). Response was dismal with stable disease in 6 patients and progressive disease in 4 patients. In contrast 14 patients at some point received salvage thalidomide or high dose dexamethasone either as a single agent or in combination. Five patients demonstrated a partial response (>50-90% reduction), 4 patients a minor response and 5 patients had stable disease for an overall response rate of 64%. Two patients treated with bortezomib demonstrated partial responses. We conclude from this preliminary analysis that t(4;14)+ve MM is poorly responsive to alkylating agents, including high dose melphalan and thus that use of these agents (including ASCT) is generally of no long term value in this patient population. Given the high response rates of 81% at induction and 64% at salvage, induction and maintenance regimens containing high dose dexamethasone and/or thalidomide are favored. Nevertheless, since response to these agents is often short lived novel therapeutic regimens are required. In this regard the early clinical study of targeted FGFR-3 tyrosine kinase inhibitors which have shown pre-clinical promise is encouraged.

P0.121

ECTOPIC FIBROBLAST GROWTH FACTOR RECEPTOR 3 EXPRESSION MEDIATED BY ISOTYPE SWITCH RECOMBINATION

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Fibroblast growth factor receptor 3 (FGFR3) is dysregulated in approximately 15% of cases of multiple myeloma (MM) occurring by chromosomal translocation in the IgH switch region which results in the juxtaposition of FGFR3 next to the regulatory elements of the IgH locus, causing its ectopic expression in plasma cells. The acquisition of FGFR3 activating mutations in some tumors indicates a role for FGFR3 in tumor progression. In order to study its role in MM progression we developed a strain of transgenic mice in which the expression of the activated form of FGFR3 is mediated by isotype switch recombination by replacing the IgH gamma1 constant region with an FGFR3-IRES-EGFP cassette in a BAC that covers the entire murine IgH locus. We predict that in the transgenic mice B cells that undergo switch recombination to γ1 on the productive allele will also undergo switch recombination to γ1 at the transgenic locus and express FGFR3 and EGFP. To evaluate FGFR3 expression *in vitro*, we collected splenocytes from our transgenic mice and induced their proliferation and differentiation to plasma cells (mainly IgG1) using LPS and IL4. After four days of stimulation, the average number of EGFP positive cells went from 0.2% to 80%. We then analyzed FGFR3 expression by RT PCR, Northern and Western blot and detected LPS/IL4 inducible FGFR3 expression in plasma cells mediated mainly by the Igamma1 promoter and in a subset of them by the Vh promoter. We then tested whether forced FGFR3 expression in B cells would affect their proliferation rate and abil-

ity to differentiate *in vitro* by MTT assay, cell cycle analysis and PI staining on LPS/IL4 stimulated splenocytes at different time points. With this system, we did not observe any FGFR3 mediated alteration of splenocytes growth parameters when compared to wild type controls. Similarly, *in vivo* no monoclonal gammopathy has been observed by serum protein electrophoresis and all the transgenic mice remaine tumor free at 1.5 years. Although we have not been able to demonstrate a tumorigenic role for FGFR3 in switched plasmacells we were able to obtain regulated FGFR3 expression in them. It is possible that FGFR3 expression alone is not sufficient to induce plasma cell transformation and that FGFR3 has a dispensable role in tumor initiation, but that it could still play a role in tumor progression. Consistently, FGFR3 expression is lost in about 25% of multiple myeloma wih a t(4;14). To address this hypothesis we are currently crossing these mice with MMSET (the other gene dysregulated in the t(4;14)) transgenic mice to investigate a possible collaborative role between these two genes in the development of MM.

P0.122

FIBROPLAST GROWTH FACTOR RECEPTOR 3 EXPRESSION IN MAINTENANCE THALIDOMIDE TREATMENT FOLLOWING HIGH DOSE THERAPY IN MYELOMA

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Myeloma is characterized by chromosomal translocations into the immunoglobulin heavy chain genes in 55 – 70% of cases. Translocation t(4;14) is found in 15% and confers a poor prognosis in both standard and high dose therapy. FGFR3, one of the candidate genes localized to der(14), is a tyrosine kinase receptor not normally expressed in plasma cells. It is upregulated in approximately 2/3 of t(4;14)+ tumors. While FGFR3 overexpression increases cellular proliferation *in vitro*, the poor prognostic effect of t(4;14) appears to be independent of FGFR3 expression. As thalidomide is an inhibitor of bFGF, one of the ligands of FGFR3, we investigated the effect of high dose therapy with maintenance thalidomide and steroid in a phase III randomized controlled trial (ALLG MM6) on FGFR3 expression, for a possible impact of FGFR3 expression on response and disease behavior. Marrow was obtained before high dose therapy and after 12 months of maintenance treatment, either with thalidomide and prednisone or prednisone alone. After flow-purification of CD38hi CD138+ plasma cells, the presence of t(4;14) was assessed by RT-PCR of the IgH-MMSET hybrid transcript, while FGFR3 expression was quantitated by Taqman real-time RT-PCR, using beta-actin as internal control. Of 57 patients examined for IgH-MMSET fused mRNA, 9 (16%) were positive for t(4;14). Five of the 9 tumors had increased FGFR3 expression, consistent with previous findings. However, 28 of 73 samples (38%) demonstrated upregulated FGFR3 expression, indicating that FGFR3 overexpression may not be confined to t(4;14)+ myeloma. Our previous investigations have indicated no evidence of activating mutations, and trans-acting or epigenetic mechanisms may be involved. Of 11 patients in whom analyses before and after treatment have been completed, 3 carried t(4;14) and 8 had initial upregulation of FGFR3. Six of the 8 patients, of whom 4 were on thalidomide, demonstrated a reduction in FGFR3 expression following therapy. In contrast, one t(4;14)+ tumor demonstrated a marked increase in FGFR3 expression following thalidomide treatment, possibly indicating a compensatory upregulation of the receptor due to FGF inhibition, or progressive disease. We await data on response and disease behavior. In summary, the study of a possible relationship between FGFR3 expression and the effect of thalidomide may provide important information on the significance of FGFR3 in the poor prognostic impact of t(4;14) in myeloma.

P0.123

DYSREGULATION OF MMSET IS TUMORIGENIC IN TRANSGENIC MICE

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Approximately 15% of multiple myeloma (MM) are characterized by a t(4;14) translocation that causes the simultaneous dysregulation of MMSET on der(4) and fibroblast growth factor receptor 3 gene (FGFR3) on der(14). We reported several lines of evidence indicating a role for FGFR3 in myeloma tumorigenesis. First, activated FGFR3 is an oncogene capable of transforming fibroblasts. Second, FGFR3 activating mutations are acquired by MM cells during tumor progression. Third, targeted inhibition of FGFR3 leads to terminal differentiation and apoptosis in two t(4;14) MM cell lines. However, expression of FGFR3, but never of MMSET, is lost in about 25% of t(4;14) MM. Therefore, the overexpression of MMSET in all MM tumors with a t(4;14) translocation, and its homology to MLL, the oncogene on 11q23 translocated in acute leukemia suggest a critical role for MMSET in MM.

To determine whether MMSET is an oncogene *in vivo*, we have generated transgenic mice in which MMSET expression in driven in lymphocytes by the lck proximal promoter juxtaposed to the Emu enhancer. Using the same expression vector we and others have obtained specific, high levels of transgene expression in B and T cells from spleen, bone marrow and thymus. Four transgenic lines were generated and although we detected MMSET expression in T cells in each of them, unexpectedly no expression in B cells was seen. Nevertheless B lymphoid tumors expressing MMSET developed at 23 months of age in each line (18/51 mice). Only 1/19 wild type matching control mice developed splenomegaly. Predominantly the tumors arose in the mesenteric lymph nodes, and splenomegaly was not a prominent feature. By Southern blot, monoclonal rearrangements of IgH, IgL and TCR were detected within the same tumor population. Histological examination showed heterogeneity among different tumor speciments. However, the lymphomas were predominantly of the diffuse large B cell type, with prominent histiocytes. In addition, frequent lymphoproliferative disease was seen even in mice without tumors. In conclusion, this is the first report that MMSET is an oncogene capable of transforming lymphoid cells in an animal model. We are currently crossing these mice with FGFR3 transgenic mice to assess cooperation between these two oncogenes in tumorigenesis. Obviously a more restricted expression of MMSET in germinal center cells is required to investigate the role of MMSET in MM. Therefore, as we have done for c-myc, we are generating new transgenic mice in which MMSET expression will be activated sporadically in germinal center B cells by somatic hypermutation.

P0.124

DELETION 13 BY FLUORESCENT IN SITU HYBRIDIZATION PROVIDES PROGNOSTIC INFORMATION ON OVERALL SURVIVAL AND TIME TO PROGRESSION INDEPENDENT OF SERUM BETA 2 MICROGLOBULIN AND BONE MARROW PLASMA CELL LABELING INDEX IN MYELOMA PATIENTS UNDERGOING STEM CELL TRANSPLANTATION

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Introduction. The bone marrow plasma cell labeling index (PCLI) and the beta 2 microglobulin (β2M) when combined provide a powerful staging system highly predictive of outcomes in myeloma patients. This staging system lacks information on cytogenetics and it has been demonstrated that deletion 13 (13) by conventional cytogenetics is associated with poor outcomes. In conventionally treated patients 13 by FISH is an independent prognostic variable. We analyzed the importance of 13 by FISH in a cohort of patients receiving high dose therapy and assessed its value relative to known prognostic parameters, PCLI and β2M.

Patients and Methods. We studied 238 patients undergoing high dose therapy between January 1990 and September 2001. The minimum follow-up of patients was 34 months. The median age of the cohort was 56 years. Interphase fluorescence *in situ* hybridization with Probes LSI13/ D13S319 was performed with simultaneous immunofluorescent detection of bone marrow plasma cells by the presence of cytoplasmic immunoglobulin. PCLI was done by a slide-based immunofluorescence method with antibodies to bromodeoxyuridine.

Results. Of the 238 patients studied 215 had a successful FISH analysis and 212 of these had a PCLI. Deletion 13 was absent in 104 (48%) and present in 111 patients. The overall survival and time to progression for patients lacking and having deletion 13 was 47.8 vs 26.4 (OS) and 19.0 vs 11.7 (TTP) months respectively. A Cox model was constructed with the PCLI, the presence or absence of deletion 13 and serum β2M. Although all three variables were independently predictive of survival and time to progression, β2M was the weakest of these three variables. The hazard function for survival for PCLI, deletion 13 and β2M was 1.4, 1.6 and 1.1 respectively. A staging system was constructed assigning 1 point for the presence of deletion 13 or a PCLI 1%. Patients fell into 3 categories of 0 points (N=69), 1 point (N=104) and 2 points (N=39). The overall survival for the three groups were 56.5, 36.6 and 13.7 months, respectively. The time to progression for the three groups were 22.3, 15.4 and 8 months (p<0.001). When analysis was limited to those patients transplanted within 12 months of diagnosis the results remained significant (p<0.003) with median survivals of stage 0, 1 and 2 of 72.5, 59.1 and 15.4 months, respectively. TTP for stage 0, 1 2 in this same cohort of patients transplanted < 12 mos after diagnosis is 30.6, 21.5 8.2 months, respectively.

Conclusions. Deletion 13 by FISH is an independent predictor of survival and time to progression providing information independent of β2M and the PCLI. Combining the PCLI with FISH del 13 identifies patients who following transplant have a median time to progression of 8 months and a median OS of 13.7 months. This group may not benefit from autologous transplant and are candidates for novel therapeutic approaches.

P0.125

MOLECULAR CONSEQUENCES OF MAFB UP REGULATION IN PLASMA CELLS AS CAUSED BY THE RECURRENT t(14;20)(q32;q12) IN MULTIPLE MYELOMA

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Chromosomal translocations of the immunoglobulin heavy chain gene region at 14q32 are regularly involved in B lymphoid malignancies. They initiate transformation either by deregulation of existing (proto) oncogenes or creation of new hybrid genes with transforming properties. Previously, we reported a novel recurrent translocation, t(14;20)(q32;q12). After cloning the regions containing the breakpoints in the der(14) and der(20) chromosomes from the parental cell line, we analyzed ectopic mRNA expression of genes in the breakpoint regions of both derivative chromosomes. Ectopic gene expression was observed for the transcription factor MAFB in der(14). The breakpoint scatter in four other cell lines with a t(14;20) - all expressing MAFB - is comprised within a region of 0.8 Mb. Provisional data indicate that this t(14;20) is associated with an adverse prognosis.[1] A possible aberrant regulation of cellular proliferation and/or apoptosis (underlying the oncogenic properties of the plasma cells) is currently being addressed in studies using inducible siRNA (short interference RNA) to knock down MAFB in cell lines with t(14;20). Conversely, inducible MAFB up-regulation in cell lines lacking the t(14;20) is studied to test for the downstream genes in that respect.

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P0.126

CLINICAL USEFULNESS OF mRNA EXPRESSION ANALYSIS OF THE 14q+ CHROMOSOME-ASSOCIATED PROTOONCOGENES AND RAS GENES IN MULTIPLE MYELOMA

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Purpose. Chromosomal translocations involving immunoglobulin heavy chain gene locus (14q+) is responsible for the development of multiple myeloma (MM). The transcriptional activation of 14q+-associated protooncogene mRNA (CCND1, FGFR3, c-MAF, MAFB, MUM1, and c-MYC) and N/K-Ras mutations are frequently found in myeloma cells. To investigate their clinical significance and to classify MM based on the genetic alterations, we established a RQ/RT-PCR system to quantify their mRNA expression and detected Ras mutations in MM cell lines and samples.

Methods. In patients' specimens, 1ug of total RNA extracted from purified plasma cells using CD138 bead selection was reverse transcribed for cDNA, diluted and used for RQ-PCR. mRNA expression of the abovementioned six genes was quantified by RQ/RT-PCR with the aid of Light Cycler. We studied gene status in 19 MM cell lines, 45 MM, 3

MGUS and 3 reactive plasmacytosis patients. In order to detect N/K-Ras mutations at codons 12,13 and 61 at the mRNA level, the same cDNA was used for RT-PCR followed by direct sequencing analysis. IgH-MMSET chimeric mRNA was also amplified from the same cDNA.

Results. In 19 MM cell lines, mRNA expression of the CCND1, FGFR3, c-MAF and MAFB genes was extremely high when 14q+ chromosomes involving these gene loci existed. In 45 MM patients, twelve (26.6%), nine (20%), four (8.9%) and two (4.4%) showed ectopic expression of the CCND1, FGFR3, c-MAF and MAFB genes. Interestingly, the expression of CCND1 and FGFR3 or c-MAF or MAFB was mutually exclusive in the same samples, whereas 3 out of 9 FGFR3/IgH-MMSET-positive samples coexpressed c-MAF or MAFB. Ras mutation was detected in 7 cell lines and in 5 MM patients. Three out of the 7 cell lines possessed K-ras mutations, although all of the 5 MM samples carried N-ras mutations. Overexpression of c-MYC was significantly associated with low hemoglobin concentration, high serum M protein ratio, high total protein concentration and high risk category by Bataille *et al* (high β2-microglobulin or high CRP) ($p<0.05$). As well, the presence of Ras mutation was significantly associated with high β2-microglobulin ($p<0.05$). Overall survival of the MM cases expressing at least one of the CCND1, FGFR3, cMAF, MAFB was shorter when compared to those lacking them (MST: 14.8 month vs not reached) ($p<0.05$ by log-rank test).

Conclusions. Some distinct developmental pathways of MM have been identified from our study, one of which arises from CCND1 deregulation, and the others from FGFR3 deregulation and from c-MAF or MAFB deregulation. In contrast, overexpression of c-MYC correlated with tumor progression markers, suggesting that it is the secondary event. Ras mutation occurred in 10% of the MM cases and was more frequently found in 14q+-negative group. Our simple diagnostic system will provide new insight into understanding genetics-based MM classification and into its application for appropriate molecular targeting therapies.

P0.127

ALLELIC HOMOZYGOSITY OF HYALURONAN SYNTHASE 1 GENE MAY PREDISPOSE TO MULTIPLE MYELOMA BY PROMOTING ABERRANT SPLICING OF HAS1 TRANSCRIPTS

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Hyaluronan synthase 1 (HAS1) maps to chromosome location 19q13.4, synthesizes an extracellular matrix molecule, hyaluronan (HA), and plays a significant role in progression of many types of malignancy. Evidence suggests that HAS1 is a transforming element during tumor development. Furthermore, we have detected up-regulation of HAS1 transcripts in MM and identified aberrant splice variants of this gene, HAS1Va, Vb, and Vc that are expressed at early stages of the disease. The statistical analysis of 58 MM showed that expression of HAS1Vb, which is an intronic splice variant, either alone or in combination with HAS1 and its other variants strongly correlates with reduced survival ($p=0.01$). These variants are expressed as truncated functional proteins in MM. Our results suggest that HAS1 intronic splicing is the first described biomarker for the circulating B-cell compartment of the MM clone. To understand the mechanism underlying the aberrant splicing of HAS1 gene in MM, as a first step we are currently identifying and investigating the mutations and/or SNP that promote abnormal splicing of HAS1, potentially through the activation of a cryptic (donor and acceptor) splice sites of the gene, and/or by the disruption of cis-splicing elements, including exonic/intronic splicing enhancers (ESE, ISE) and suppressors (ESS, ISS), in addition to modulation of splicing branch sites and polypyrimidine tracts of splicing. Using a TaqMan SNP genotyping assay and allelic discrimination we measured the frequency of polymorphism detected on the HAS1 gene upstream of alternative exon 4 in 40 MM patients and 70 healthy donors. Our results suggest that in healthy individuals, the frequency of each of the two alleles was 50%, half heterozygous and half homozygous for HAS1. In contrast, 83% of analyzed MM patients are homozygous for HAS1 ($p<0.0001$). This increased homozygosity appears to be germline derived, confirmed by analysis of T cells, as a source of non-malignant cells, from 9 MM patients. Thus, homozygosity of HAS1 may be a disposing factor of MM. The expression analysis of HAS1 and variants in the same group of individuals demonstrated that intronic HAS1splice variants were detectable only in homozygous patients for the HAS1 gene. Thus, HAS1 homozygosity may regulate aberrant splicing events, such as exon skipping and/or activation of cryptic splice sites that give rise HAS1 splice variants, since this particular polymorphism is located on an ESE, as identified using *in silico* methods. Our preliminary analysis shows that a HAS1 SNP located upstream of alternative exon 4 disrupts ESE, accommodating alternative exon 4 skipping. However, this analysis also suggests that other mutations and/or aberrations are required in any given cell to achieve the HAS1 intronic splicing we have identified in MM patients. We speculate that HAS1 SNPs which overlap with ESE may account for the variable penetrance of specific proximal or distal mutations elsewhere in the gene, thus affecting the severity of the clinical phenotype of MM.

P0.128

MULTIPLE MYELOMA IS INDUCED BY SOMATIC ONCOGENE ACTIVATION IN THE GERMINAL CENTER

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Multiple myeloma (MM) is a disease of immunoglobulin genes hypermutated, isotype switched, long-lived plasma cells that home to the bone marrow. Recreating this disease in a transgenic mouse has represented a challenge since it has been impossible so far to express an oncogene of interest uniquely in plasma cells. In fact, there are not known plasma cell restricted promoters, and the classic immunoglobulin (Ig) regulatory elements (Emu and 3'E) are also active at early stages of B-cell development and lead to lymphomagenesis when utilized in transgenic animal models.

We have identified chromosome translocations into the Ig locus as a primary event in the pathogenesis of MM. They result from errors during the switch recombination or somatic hypermutation processes and cause the dysregulation of several oncogenes. In the attempt to regulate the expression of such oncogenes in murine plasma cells, we designed a construct in which an inactive HA-myc transgene, placed under the control of the kappa chain regulatory elements, is activated sporadically in a germinal center B cell by somatic hypermutation. To monitor for plasma cell dyscrasia, we periodically analyzed the serum of our C57Bl6 transgenic mice by SPEP. At 30 weeks of age we could detect monoclonal spikes in 25% of transgenic mice, compared to none in the wild type controls. At 40w, the incidence increases to 60% in transgenic mice versus 0% of controls, and keeps

progressing to 84% by 50w (0% of controls) and to 100% by 60-70 weeks. For each informative mouse, we found that the levels of the monoclonal spikes, (IgG1 in 7/8 cases analyzed) slowly increased over time. Unlike other mouse models of plasma cell neoplasia, no lymphosplenomegaly nor ascites was detected and the mice remain asymptomatic. Upon dissection of a 12 month old mouse, the bone marrow, but not the spleen, contained 40% of HA+ fully differentiated CD45-/CD138+ plasma cells. Remarkably, in two young mice, monoclonal spikes appeared 2 weeks after vaccination with NP-CGG. These spikes were sustained and the monoclonal protein was reactive to the NP antigen.

In conclusion, we have developed a novel and unique strategy to precisely target transgene expression to germinal center cells undergoing somatic hypermutation. Also, transgene activation occurs sporadically, as it does in human cancer. We believe that the time at which the oncogene is expressed is critical in determining the ultimate tumor phenotype. We postulate that the only germinal center B-cell that is able to tolerate ectopic c-myc expression is the one receiving a strong survival signal from its high affinity B-cell receptor, destined to home to the bone marrow and differentiate into a long-lived plasma cell.

P0.129

A ROLE FOR THE METASTASIS-ASSOCIATED PHOSPHATASE PRL-3 IN MULTIPLE MYELOMA

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Phosphatases of regenerating liver (PRL phosphatases) constitute a novel class of small (20kDa) prenylated tyrosine phosphatases with possible oncogenic activity. To date, the exact cellular role of the PRL (PRL-1,-2, -3) has not been determined. However, PRL-3 has been implicated in promotion of cell motility, invasion, and metastasis. It is expressed in colon carcinoma cells, but only in metastatic disease. The same expression pattern has been shown in gastric carcinomas. In gene expression profiling studies of two IL-6-dependent myeloma cell lines (OH-2 and IH-1) stimulated with or without IL-6, IL-21 or TNF, we found that the PRL-3 gene was induced in both cell lines and by all of the mitogenic cytokines. We also found that the PRL-3 gene was upregulated in myeloma cells from patients with newly diagnosed myeloma as compared to normal bone marrow plasma cells and MGUS plasma cells. There was a highly significant difference in PRL-3 gene expression across seven subgroups of multiple myeloma identified by unsupervised hierarchical cluster analysis of gene expression profiles from a large cohort of patients. This suggests that the PRL-3 gene may be involved in the molecular pathogenesis of specific myeloma patient subgroups. The PRL-3 gene expression was higher in primary myeloma cells than in myeloma cell lines, possibly reflecting that the gene is induced by mitogenic cytokines in the bone marrow microenvironment. Expression of PRL-3 mRNA was confirmed by quantitative RT-PCR in four IL-6-dependent and four IL-6-independent myeloma cell lines. The IL-6-dependent cell lines ranked 1, 2, 3, and 5 in expression level, another indication of the importance of the microenvironment for expression of this gene. With the exception of Cro-AP5, a primary effusion lymphoma cell line, all non-myeloma hematologic cell lines (n=11) had either undetectable levels of PRL-3 mRNA or lower levels than the IL-6-dependent myeloma cell lines. By immunoblots we demonstrated that PRL-3 protein expression was induced after stimulation with the same cytokines as used in the gene expression profiling experiments. By confocal microscopy we could demonstrate that anti-PRL-3 stained mitotic myeloma cells in a specific pattern. The PRL-3 protein seemed to cycle between cytosol and nucleus in a cell cycle-dependent way and co-localize with alpha-tubulin in the spindle apparatus and centrosome during mitosis. We also studied PRL-3 protein expression with immunohistochemistry. Twenty of the 22 MM biopsies were stained, and only plasma cells/myeloma cells were stained in the bone marrow biopsies. In conclusion, these data suggest that PRL-3 is important in myeloma biology and may represent a new potential therapeutic target.

P0.130

CLASS SWITCH RECOMBINATION IN MULTIPLE MYELOMA: SEQUENCE ANALYSIS OF THE VDJ-S REGION REVEALS LONG-TERM SWITCH JUNCTION STABILITY AND ONGOING MUTATION UPSTREAM OF SWITCH MU

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Immunoglobulin (IgH) variable region (VDJ) domains of B cells associate with new constant region (C) domains by class switch recombination (CSR). This process is mediated through switch regions (S) occurring upstream of each C region, such as S-mu for IgM and S-gamma for IgG. On switching from IgM to IgG, VDJ and C-gamma join by *looping out* the intervening DNA containing C-mu, generating a VDJ–(recombined S-mu/S-gamma)–C-gamma segment. In multiple myeloma (MM), S regions have been shown to mediate t(4;14) translocations in a subset of MM patients. In this study we analyzed bone marrow and blood samples of five MM stage III IgG patients at separate timepoints, usually diagnosis and relapse (1.6 to 4.2 years apart), to determine if the S junction and upstream VDJ-S segment was homogeneous within a time point, and stable over the course of malignancy. Each patient has a unique clonotypic VDJ region contiguous with a unique S junction arising after CSR. This 5-7 Kb VDJ-S region was amplified from bulk bone marrow samples by long distance PCR. In each case a single DNA product was amplified, cloned, and sequenced with primers covering the VDJ-S region. Unique S junctions for patients 1-5 were detected at positions 125, 251, 435, 590, and 750 from the beginning of S-mu, respectively. A single S junction was detected in each patient, which remained constant between time point samples, suggesting S junction stability. The sequence and mutation profile of the S junctions, with more frequent mutations in the upstream S-mu region suggests normal CSR has occurred. Interestingly, mutations were observed in the VDJ-S sequences of later timepoint samples in 4/5 patients. Most notably, 19 new mutations were detected in one patient, including 3 bp and 64 bp deletions 135 and 223 bp downstream of the intronic enhancer, respectively. The significance of these mutations is unclear considering previous studies showing similar kinds of mutations in the VDJ-S of normal B cells. Nevertheless, it seems this process continues in clonotypic cells of some patients throughout malignancy. It is possible that ongoing mutation, especially deletions, may lead to *remodelling* of the

VDJ-S region. In the context of Ig translocations this process may stabilize translocated oncogenes downstream of Ig enhancers, or increase oncogene expression levels conferring a selective advantage on remodelled tumor cells. Presently, we are examining the VDJ-S and downstream translocated DNA regions of t(4;14) patients for changes that may occur over the course of the malignancy.

P0.131

HOW DO CHROMOSOMAL DELETION EVENTS AFFECT THE EXPRESSION OF GENES THAT ARE LEFT BEHIND?

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Previous studies have implied that distinct chromosome deletion events may be associated with several malignant disorders. Using various bioinformatics tools to analyze genes in the vicinity of 13q14, multiple candidate tumour suppressor genes and transcription factors have been identified which are associated with distinct malignancies. These include BRCA2 and BRCA3 (13q12.3 and 13q21 resp: breast cancer; Wagner *et al,* 2004), D13S25 (13q14: chronic lymphocytic leukaemia; Chena *et al.,* 2003) and RB1 (13q14.1; retinoblastoma, osteosarcoma; Kivela *et al.,* 2003). Although these key genes are associated with malignant disorders, little is known about the true functions of these candidate genes. We are interested in the association of contradictory malignant phenotypes with deletion of similar chromosome regions. For example, osteosarcoma (OS) and multiple myeloma (MM) display bone formation and breakdown characteristics respectively, and yet have been mapped to the same general area, 13q14 (Ozaki *et al.,* 2002; Shaughnessy *et al.,* 2000). Apart from the genes already described above, other genes involved in bone homeostasis are also present. These may be differentially expressed due to subtle distinct deletion events, or the same deletion may result in differential expression when cell lineage specific signaling pathways are taken into consideration, resulting in the distinct phenotypes observed. We present data on the generation of a panel of FISH probes specific for chromosome 13 deletions in an attempt to differentiate between OS and MM. We have generated probes for various sequence tagged sites (STS) loci as well as various candidate genes which may contribute to the malignant and bone homeostatic phenotype. Each probe has been validated with the aid of commercial probes by hybridization to donor lymphocyte metaphase spreads and cross hybridization of STS allelic variants. We demonstrate use of the probes in control and patient lymphocytes, and in normal bone marrow mononuclear cells, as well as B-cell, MM and OS human cell lines. We have specifically tested our probes against cell lines with and without 13q14 deletions (including U266 and HS Sultan). We also present data on real-time PCR analysis of gene expression for selected candidate genes (e.g. RANKL, RB1 etc.) and have correlated these expression data with chromosome deletion patterns.

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P0.132

THE MECHANISM OF BMP-INDUCED APOPTOSIS IN MYELOMA CELLS: A ROLE FOR p53

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Only a few naturally occurring cytokines are able to inhibit myeloma cell growth, and among them BMP-4 is a potent inhibitor of growth as well as an inducer of apoptosis in myeloma cells. To study the mechanism behind BMP-4-induced growth inhibition, we performed gene expression profiling by microarrays in two human myeloma cell lines after BMP-4 stimulation for 4 hours. We found that BMP-4 upregulated several known p53 target genes like p21/Cip1, Bax, cyclin G, Gadd45 and dual specificity phosphatases. p53 is activated by several post-translational modifications, including phosphorylation at several serine/threonine residues. We found that BMP-4 treatment lead to phosphorylation of p53, and by conventional Western blotting we demonstrated that BMP-4 phosphorylated p53 at serine 15. Furthermore, by DNA precipitation of p53 applying a p53 binding element from the Gadd45 promoter we demonstrated that phosphorylated Smad 1/5/8 was bound to p53.

Because some myeloma cell lines are resistant to BMP-4-induced growth inhibition, we examined whether this BMP-4 resistance was caused by p53 mutations in the BMP-4-resistant cell lines. We sequenced the p53 gene in eight myeloma cell lines, and found a link between p53 mutations and BMP-4 responsiveness. Introduction of normal p53 via adenoviral constructs in the p53 mutated cell lines restored BMP-4 responsiveness to some degree, and this was further confirmed by studies in a myeloma cell line with a temperature-sensitive p53 mutation. In conclusion, we demonstrate that p53 activation is involved in BMP-4-induced growth inhibition of multiple myeloma cells.

P0.133

IDENTIFICATION OF GENES THAT ARE REGULATED BY MYELOMA CELL GROWTH FACTORS

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Myeloma cells often proliferate in response to interleukin-(IL-)6, IL-10, IL-15, IL-21, tumor necrosis factor (TNF), insulin-like growth factor-1 and other cytokines. In the bone marrow, cytokine-dependent myeloma cells are likely to be redundantly growth-stimulated by several different cytokines. We hypothesized that different growth-regulating cytokines induce expression of a set of common genes, the transcription of which is indispensable for proliferation. Since attacking individual cytokines in targeted treatment might be fruitless due to redundancy, identifying and targeting pivotal proteins induced by all of the mitogens is potentially a more promising approach. We isolated RNA from the cytokine-dependent myeloma cell lines IH-1 and OH-2 after 4-hour stimulation with or without IL-6, IL-21, TNF or IL-6+HGF, and did gene expression profiling with U133-A and U133-B chips from Affymetrix. We listed genes, whose transcription was commonly induced by the cytokines in both cell lines. This list included a high num-

ber of genes related to cell signaling and cell proliferation. Among the genes were STAT3 and MCL1, both known to be important for myeloma cell proliferation and anti-apoptosis. Other genes on the list were JUNB, SGK, BCL3, BCL6, IFI16, IL10 and PTP4A3. Based on published data on gene function, we chose to focus on SGK (serum/glucocorticoid regulated kinase-1), BCL3 (B-cell leukemia/lymphoma 3), and PTP4A3 (protein-tyrosine phosphatase type 4A, 3; PRL-3) as possible targets for future treatment. We have verified the expression of these three genes by RT-PCR and Western blots and confirmed that their expression is indeed induced by IL-6 and other mitogens in four different cytokine-dependent myeloma cell lines. For additional data on SGK and PTP4A3, please refer to separate abstracts at the meeting. BCL3 was first found to be overexpressed in B-cell CLL with t(14,19), and has later been showed to be overexpressed in several cancers. The gene product, Bcl-3, belongs to the IκB family of proteins, and participates in regulation of NFκB-dependent transcription. Its putative role as an oncogene is controversial. We also performed gene expression profiling of RNA from CD 138-enriched bone marrow plasma cells from 351 newly diagnosed myeloma patients and from 24 healthy individuals. Using the 95th percentile of BCL3 expression in normal plasma cells as cut off, 48 myeloma patients (14%) had increased BCL3 gene expression values.

In conclusion, a series of genes were identified that may be important mediators of cytokine-induced growth of myeloma cells. One of these genes was BCL3. The upregulation of BCL3 in myeloma cell lines after mitogenic stimulation and the increase of gene expression in a subset of myeloma patients indicate a possible role for Bcl-3 in multiple myeloma biology.

P0.134

TUMOR-SPECIFIC SOMATIC MUTATIONS TARGETING BCL-6 IN MULTIPLE MYELOMA

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Introduction: Somatic mutations target multiple loci in normal B cells, and generally, but not invariably, localise to the germinal center (GC). Mutations in Ig variable (V) region genes ensure affinity maturation, but the consequences of mutations affecting the 5'- untranslated region (UTR) of the BCL-6 gene are less well understood. BCL-6 is a transcriptional repressor, which functions to regulate germinal center formation, and GC-B-cell survival and maturation. Mutations in BCL-6 occur in ~30% of normal GC B cells and in a variable fraction of lymphoid malignancies. There are indicators however, from malignant B-cells that mutations in specific motifs in the 5'UTR of BCL-6 overtly regulate levels of gene expression, with relevance for tumor origins. The mutational mechanism may also, in part give rise to aberrant chromosomal translocations mapping to this locus. Biallelic and *hot spot* mutations are characteristic features, and interestingly some of these mutations appear common to B-cell tumors, although their significance as yet is unresolved. However, the question of whether polymorphisms could account for some of these imprints has not been studied systematically. In order to probe true mutational events in multiple myeloma (MM), and their relevance for understanding the tumor cell of origin, we analyzed mutations in the 5'UTR of BCL-6 in malignant cells and compared them to germline polymorphisms in matched T cells.

Methods. DNA was isolated after MACS sorted CD138+ bone marrow cells from 4 MM and 1 MGUS patients. A 750 bp fragment of the BCL-6 5'UTR was amplified using a nested PCR strategy. Taq error rate was assessed in a comparable-sized fragment of the beta-globin gene amplified using identical PCR conditions. PCR products were cloned and individual colonies (5 or more for each case) sequenced. To identify polymorphisms of the BCL-6 gene, we amplified the 5'UTR using DNA isolated from sorted, circulating T cells from the same patient.

Results. Two mutations, Δ520 and G397C were present in 4/5 and 2/5 cases, respectively, and have previously been reported in other B-cell tumors. These alterations were unequivocally identified as polymorphisms, as they could be found in clones obtained from myeloma cells and T cells in each case. True mutations were identified in 3/5 cases. To distinguish these from Taq errors, each mutation was required to be present in at least 2 individual myeloma clones and confirmed in an independent PCR reaction. Interestingly, biallelic identical mutations (T115A, G147A) could be identified in one case, with one allele identified by polymorphism, suggesting specific molecular constraints. A known recurrent mutation (C423G) was identified in another case. Biallelic mutations and recurrent mutations are also a feature of tumor-specific BCL-6 mutations in myeloma. Whether recurrent mutations may be specific to MM will require a study of a larger number of cases, using the approach described here. We have preliminary evidence in lymphoma that proteins can bind individual mutational motifs in the 5'UTR of BCL-6 in lymphoma cells (Jardin F *et al.,* unpublished data). Their functional outcome is currently under study.

Conclusions. The level and pattern of somatic mutations in BCL-6 in MM confirm derivation from a GC B-cell, which has undergone a comparable level of targeting at this locus as normal circulating memory B cells harboring BCL-6 mutations. Such mutations in myeloma clearly do not perturbate BCL-6 function to prevent further maturation, as may be occurring in GC-lymphoma.

P0.135

MCL-1 IS OVEREXPRESSED IN MULTIPLE MYELOMA AND ASSOCIATED WITH RELAPSE AND SHORTER SURVIVAL

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Multiple myeloma (MM) is a fatal malignancy of B-cell origin characterized by the accumulation of plasma cells within the bone marrow. The expression of the pro-survival members of the Bcl-2 family has been shown to be a key process in the survival of myeloma cells. More particularly, Mcl-1 expression turned out to be critical for their survival. Indeed, knockdown of Mcl-1 by antisenses induces apoptosis in myeloma cells. Finally, Mcl-1 was found to be the only anti-apoptotic Bcl-2 family member whose level of expression was modified by cytokine treatment of myeloma cells. Indeed, this anti-apoptotic protein is upregulated by inteleukin-6, which plays a critical role in MM. For these reasons, we evaluated the expression of Mcl-1 *in vivo* in normal, reactive and malignant plasma cells (PC) i.e., using a four-color staining and flow cytometry analysis. For this

study, myeloma cell from 51 patients with MM and 21 human myeloma cell lines were evaluated. We show that Mcl-1 is overexpressed in MM in comparison with normal bone marrow PC (fifty-two percent of patients with MM at diagnosis overexpress Mcl-1(p=0.017)). Our results show that the expression of Mcl-1 is associated with disease progression. Indeed, 81% of patients at relapse (p=0.014 for comparison with diagnosis) overexpress Mcl-1. Of note, only myeloma cell lines but not reactive plasmocytoses have abnormal Mcl-1 expression, although both plasmocyte expansion entities share similar high proliferation rates (>20%). Of interest, Bcl-2 as opposed to Mcl-1, does not discriminate malignant from normal PC. This shows that the overexpression of Mcl-1 is clearly related to malignancy rather than to proliferation. It will be important to know whether the overexpression of Mcl-1 is related to an abnormal response to cytokines like interleukin-6 or to mutations of the promoter of the Mcl-1 gene as already described in B chronic lymphocytic leukemia. Finally, the level of Mcl-1 expression is related to disease severity, the highest values being correlated with the shortest event-free survival (p=0.002). In conclusion, Mcl-1 which has been shown to be essential for the survival of human myeloma cells *in vitro* is overexpressed *in vivo* in MM and correlates with disease severity. Mcl-1 represents a major therapeutic target in MM.

P0.136

TUMOR NECROSIS FACTOR-ALPHA 238A GENOTYPE IS ASSOCIATED WITH AN EARLIER ONSET OF MULTIPLE MYELOMA AND BETTER RESPONSE TO THALIDOMIDE

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The individual differences among the polymorphic regions of the cytokine genes involved in the pathogenesis of myeloma have been investigated by various groups: Neben *et al* have found the tumor necrosis factor (TNF) 238. A allele to be associated with a higher serum level of TNF-alpha and with better response to thalidomide. The IL 10-1082 G promoter gene genotype has been found to be associated with high secretory pattern and in increased frequency among myeloma patients compared to normal controls by Zheng *et al.* Van Ness *et al.* have reported the IL-10-G genotype to be associated with shorter survival compared to IL-10-A allele carriers. With an aim to analyze the association between the frequencies of the cytokines known to be important in the pathogenesis of myeloma, TNF-alpha, IL-6, IL-10 and response to thalidomide, we isolated DNA from peripheral blood of 29 patients. Patients with a median age of 59 (42-83), M/F:18 /11 diagnosed and treated in our center between 2002-2004 were analyzed. All patients received thalidomide 200-400 mg/day for a minimum duration of two months. Response was determined according to EBMT criteria. To determine the TNF 238 and 308(G/A), IL-6 174(G/C), IL 10 1082(GCC/ACC/ATA) bp polymorphic allele frequencies, cytokine Genotyping Kit (Pel-Freeze) was used. Evaluation of results was done based on interpretation and definition of phenotypes (low and high secretory patterns) according to the previously published reports. TNF-A homozygous allele could not be observed among all patients. The frequencies of both TNF-α 238A and TNF-α 308A alleles was 21% but not in the same patients. IL-10 1082 GCC/ACC/ATA 16% (high), 58% (intermediate), 25% (low) respectively. IL-6 GG/GC alleles, which have been linked with high secretory pattern, constituted the majority of the patients (29/29). TNF-238A genotype patients compared to the TNF-238G genotype gave a better response to thalidomide (83% vs 39%) (p=0.054).This finding was not valid for the 308 bp location. Chi square analysis revealed a significant association between younger age, TNF-238A allele and better response (p=0.014). IL-10 phenotypes were more complicated with an accumulation in the intermediate level of secretion. The analysis of TNF-alpha genotype within this group did not show any impact of A allele. When we analyzed patients who did not receive thalidomide among a total of 59 patients there was a trend to TNF-alpha A(high) and IL-10 A(low) allele among patients younger than 55 (ASH2004).

Conclusion. Cytokine gene SNP such as TNF-alpha 238A is associated with better response and earlier age of onset in myeloma. However all our patients carried the IL-6 genotype reported to be associated with high secretory pattern. Investigation of other genes in linkage with TNF on the neighboring MHC region may be necessary for understanding the molecular pathogenesis of this association.

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P0.137

GENOMIC AND CYTOGENETIC CHARACTERIZATION OF MULTIPLE MYELOMA CELLS UNCOVERS RECURRENT GENETIC ABERRATIONS AS GAIN AND OVEREXPRESSION OF SELECTIN FAMILY GENES AND TRANSCRIPTION FACTORS SUCH AS TCF4 OR E2F5

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In 50% of the cases of multiple myeloma (MM), the genetic transformation begins with a chromosomal translocation that juxtaposes the IGH gene locus and a functional oncogene. In addition, other genetic aberrations, such as gains and/or losses of genomic regions, also take place in MM that do contribute to the tumor phenotype. Therefore, the detection of genes that are gained and overexpressed in MM would eventually offer novel therapeutic approaches. Our objective was to characterize the cytogenetic and genomic changes occurring in MM cell lines with the purpose of detecting amplified genomic regions and the identification of candidate genes that are simultaneously gained and overexpressed in MM. We analyzed 9 MM-cell lines: RPMI-8226, U-266, OPM-2, SK-MM-2, LP-1, JJN-3, L-636, KARPAS-620, NCI-H929. First, by a multiprobe FISH approach we identified the IgH translocation partners. Second, we screened them by conventional comparative genomic hybridization (c-CGH) analysis to identify deleted or gained regions. Third, 2 microarray analyses were performed: 1) expression-profile studies by hybridization of total mRNA onto the CNIO OncochipTM. 2) CGH-array studies by hybridization of total DNA onto the same platform. This array contains 7237 cDNA clones: 1984 ESTs and 5253 cancer-related genes.

We selected by c-CGH three of the most commonly gained or amplified regions in cell lines and patients: 1q21-q32, 8q21-q23 and 18q11-q22. Then, we found 40, 10 and 7 genes amplified and over-expressed in these regions, respectively. Amplified regions were complex and included several genes of interest. Based on annotated functional data, we found several interesting candidates. In the 1q21 region,

several selectin family members (SELL, SELP and SELE) are gained and overexpressed. These proteins are cell surface components, members of a family of adhesion/homing receptors, which play important roles in leukocyte-endothelial cell interactions. In 8q21: E2F5 gene is a member of the E2F family of transcription factors (related to cell cycle control and action of tumor suppressor proteins), and TPD52 (tumor protein D52) has been found to be expressed in some primary tumors and cell lines (mainly of epithelial origin). Finally, in 18q21, we selected initially MALT1 and TCF4. MALT1 gene has been found to be recurrently rearranged in chromosomal translocation with BIRC3 and IGH locus in mucosa-associated lymphoid tissue lymphomas. This gene may play a role in NF-kappaB activation. TCF4 gene encodes an immunoglobulin transcription factor that is expressed predominantly in pre-B cells, although it is found in other tissues as well. In addition to validating these functional candidates, we are currently exploring all others identified genes with the double purpose of gaining relevant information about the disease pathogenesis and identifying potential molecular therapeutic targets in multiple myeloma.

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P0.138

MGMT PROMOTER HYPERMETHYLATION AND IMMUNOHISTOCHEMICAL ANALYSIS IN PLASMA CELLS DYSCRASIAS

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Background. O6-Methylguanine-DNA methyltransferase (MGMT) is a DNA repair gene that removes mutagenic and cytotoxic adducts protecting against mutagenesis and malignant transformation. Hypermethylation of the CpG island located in the promoter region of MGMT is primarily responsible for the loss of MGMT function in many tumor types since methylated regions of the MGMT promoter are in a *closed* nucleosome structure, which is associated with global alterations in heterochromatin and increased numbers of hypermethylated genes and limits transcription-factor binding. Tumors with MGMT methylation have a new mutator phenotype characterized by the generation of transition point mutations in genes involved in cancer etiology.

Aims. The aim of this study was to investigate the methylation status of the MGMT gene in plasma cell dyscrasias and to correlate it with the protein expression.

Patients and Methods. A total of 45 cases: 29 MM, 13 MGUS, 1 plasma cell leukemia (PCL) and 2 polyclonal plasmacytoses were included. Bone marrow plasma cells were purified using magnetic microbeads labeled with CD138. Briefly DNA was treated with sodium bisulfite, and then amplified using two sets of primers (methylated and unmethylated). The epigenetic silencing of the MGMT gene was determined using methylation-specific PCR (MSPCR). Bone marrow sections corresponding to the DNA samples in 29 of these cases were stained with monoclonal antibody MGMT.

Results: Promoter hypermethylation at MGMT was identified in 7 of 29 MM cases and in 1 of 13 MGUS. We did not observe methylation in PCL or in polyclonal cases.

In immunohistochemical (IH) analysis 7 of 17 MM and 4 of 11 MGUS were negative. Among 7 MM samples in which MGMT protein expression was absent 4 showed promoter hypermethylation and the other three were found unmethylated. In MGUS cases, loss of MGMT protein expression was found in 4 of 11, and only one of these was methylated.

Discussion. In MM patients we found methylation in 23% and we did not find correlation with age, gender, disease stage, type of paraprotein, type of light chain, or calcium levels. Interestingly we observed a trend toward poorer prognosis for patients who had MM with MGMT methylation (p=0.08). Methylation is already detectable in MGUS (7.7%), although less frequently than in MM, suggesting that it could be an early event in the development of MM. Promoter hypermethylation of MGMT is involved in on the silencing of the gene though absence of MGMT expression is not always associated with methylation; therefore other mechanisms (mutations, etc..) could be responsible for the loss of protein expression.

Conclusions. These findings suggest that the silencing of MGMT gene could be a relevant oncogenic event in monoclonal gammopathy evolution, being associated with poorer prognosis.

P0.139

APPLICATION OF THE MYELOMA BANK

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Introduction. Frozen samples archived in the Myeloma Bank (MB) can be used for experimental procedures and monitoring of multiple myeloma and also for diagnostic purposes and for decision-making about targeted treatment. There are 1 749 samples in theMB collected since 2001. The MB contains separated cells and plasma of bone marrow, serum, plasma and mononuclear cells of peripheral blood. Myeloma bank samples are used for research purposes, particularly to study prognostic clinical features of pts. with multiple myeloma (MM)

Patients and Methods. Bone marrow samples from patients with newly diagnosed multiple myeloma, relapsed or with maximal therapeutic response were evaluated. Enriched positive fraction of $CD138^+$ myeloma cells and negative fraction of $CD138^-$ cells were obtained by immunomagnetic separation. The samples were kept in liquid nitrogen.

Results. Monitoring of amplifications, deletion of DNA and nucleoli organizers, assessment of activity of telomerase and telomere length, interphase fluorescence *in situ* hybridization can be performed on separated cells. Separated cells can be used also as tumor antigen for dendritic cells-based vaccines and for preparation of myeloma-specific T lymphocytes. Cytogenetic studies by fluorescence *in situ* hybridization to detect the 13q14 deletion have been performed in 40 patients with myeloma. We have found the 13q14 deletion in 10 of 40 (25%) of cell-non specific samples and in 25 of 40 (62.5%) of enriched myeloma cell suspensions. We examined telomerase activity in 36 pts. with MM. Twelve (33.3%) patients were positive, 7 (19.4%) were negative. In 17 (47.2%) cases the telomerase activity in selected and non selected cells was the same.

Conclusions. Our results indicate that interphase fluorescence *in situ* hybridization on immunomagnetically selected cells increases the detection of the 13q14 deletion in bone marrow samples. Detection of telomerase activity in new by diagnosed pts. cannot be used as a prognostic factor.

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P0.140

THALIDOMIDE DOES NOT AFFECT NUCLEOSOME ACETYLATION IN A MYELOMA CELL LINE

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The U266 multiple myeloma (MM) cell line was exposed to thalidomide (s-thal) (Cellgene Corporation USA) at the IC_{50} (% viability) dose for 24 hours, the RNA extracted and gene expression profiling established using microarray methodologies. A 49-fold upregulation in the H4FL gene involved in DNA binding was found. The transcriptional signature for s-thal was compared to that of suberoylanilide hydroxamic acid, SAHA a powerful deacetylase inhibitor and showed a concordance of transcriptional signature in 78 per cent of 81 genes. However histone deacetylase inhibition by s-thal could not be demonstrated in an isolated liver enzyme HDAC activity assay or in a whole cell system nor could direct acetylation of the four core histones be demonstrated. Despite the perturbation of the changes in the H4FL gene and close transcriptional signature it does not appear that s-thal obtains its anti tumor effect via changes in histone acetylation.

P0.141

A NOVEL SCID-HU *IN VIVO* MODEL OF HUMAN MULTIPLE MYELOMA

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Human multiple myeloma (MM) xenografts in immunodeficient mice have limitations as a model for the human disease since they lack the human bone marrow (huBM) microenvironment. In contrast, murine models harboring a huBM microenvironment with implantation of patient MM cells in the huBM recapitulate the *in vivo* pathophysiology of MM and have significant advantages over conventional murine models for pre-clinical evaluation of investigational drugs. However, there are some limitations in using patient MM cells in such models since i) a limited number of MM cells can be harvested from an individual patient, thus limiting the number of mice that can be injected with cells from the same patient; and ii) only a fraction of engrafted specimens produce measurable paraprotein in a wide range of weeks. To overcome these limitations, we have developed a novel murine model of MM by engrafting INA-6, a cytokine-dependent human MM cell line into SCID mice previously implanted with a human fetal bone chip (SCID-hu mice). INA-6 cells require either exogenous IL-6 or interaction with the bone marrow stromal cells (BMSCs) to proliferate *in vitro*. In this model, we monitored the *in vivo* growth of INA-6 cells stably transfected with a green fluorescent protein (GFP) expression vector (INA-6^{GFP+}). Serum soluble human IL-6 receptor (shuIL-6R) and fluorescence imaging of host animals were sensitive indicators of tumor burden with time dependent increase. Fluorescence imaging was able to detect the myeloma cell growth earlier than measurement of sIL-6R levels. INA-6 MM cells grew in SCID-hu mice, but not in SCID mice injected subcutaneously or intravenously without the human fetal bone. We have further confirmed the feasibility of this model in monitoring the response to therapeutic agents such as dexamethasone or investigational agents by detecting reduction in the intensity of the fluorescent lesions as well as shuIL-6R in SCID-hu mice following anti-MM treatment. This highly reproducible model therefore allows evaluation of investigational drugs targeting MM cells in the huBM milieu.

P0.142

PLASMA CELL TUMORS IN TRANSGENIC MICE

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Accurate mouse models of human plasma cell tumors, such as multiple myeloma (MM), are needed to study the events that are involved in the initiation and progression of these neoplasms, elucidate the genes that confer tumor susceptibility and resistance, and test intervention strategies that might lead to a better outcome. The cellular oncoprotein MYC, the plasma cell differentiation and survival cytokine IL-6, and death suppressors of the BCL2 family are key pathogenetic factors in human MM, suggesting that the transgenic expression of these factors in plasma cells in mice leads to experimental model systems of neoplastic plasma cell development that are of great relevance for humans. We have studied plasma cell tumor formation in the following transgenic mouse strains: (i) BALB/c.H2-Ld-IL-6 congenics that harbor a widely expressed human IL-6 transgene (originally developed by T. Kishimoto, Osaka University, Japan; backcrossed onto BALB/c by M. Potter, NCI, Bethesda, MD); (ii) BALB/c congenics that carry the EμSV-Bcl-2-22 transgene (developed by A. Harris and J. Adams, WEHI, Melbourne; backcrossed onto BALB/c by S. Silva, Karolinska Institute, Stockholm); (iii) C57BL/6 mice that contain a human MYC gene driven by the human Igλ or Igκ enhancers (λ-MYC and κ-MYC mice); (iv) gene-insertion mice that harbor a His6-tagged mouse Myc gene in three different locations of the mouse Ig heavy-chain locus (iMyc mice); and (v) iMyc mice that carry the 3'KE-Bcl-X_L transgene (developed by B. Van Ness, University of Minnesota, MN). All transgenic mouse strains exhibit *spontaneous* development of B cell and plasma cell tumors in extraosseous lymphoid tissues. Combining two transgenes in one mouse strain (e.g., Myc + IL-6 or Myc + Bcl-X_L) results in a predictable acceleration of plasma cell tumors. Bone marrow infiltration with malignant plasma cells, osteolytic lesions and pathological fractures are not uncommon, but occur invariably in conjunction with soft tissue infiltration by tumor cells, particularly in mice with incipient plasma cell leukemia. Genetic background effects are readily observed. These findings demonstrate that genes involved in human myelomagenesis are crucial for plasma cell tumor development in mice. The principal shortcoming of the present mouse models of plasma cell neoplasia is their failure to recapitulate the primary bone marrow manifestation of tumor growth, a hallmark of human MM. Including additional MM progressor genes, such as those involved in Wnt signaling, may overcome this shortcoming. The development of transgenic mice expressing the Wnt pathway inhibitor Dkk1 in plasma cells (collaboration with J. Shaughnessy and B. Barlogie, University of Arkansas, Little Rock, AR) is one step in this direction.

P0.143

INSERTION OF MYC INTO THREE DIFFERENT LOCATIONS OF IMMUNOGLOBIN HEAVY CHAIN LOCUS ESTABLISHES NEW MOUSE MODELS OF HUMAN BURKITTLYMPHOMA t(8;14)(q24;q32) TRANSLOCATIONS AND MOUSE PLASMACYTOMA t(12;15) TRANSLOCATIONS

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MYC-activating chromosomal t(8;14)(q24;q32) translocations are widely believed to be the initiating events in the pathogenesis of the human post-germinal center non-Hodgkinlymphoma, Burkittlymphoma (BL). The corresponding *Myc*-activating t(12;15) translocation in mice causes inflammation-induced peritoneal plasmacytomas (PCT) in strain BALB/c and BALB/c congenics that carry a human BCL2 transgene. The t(12;15) trans-location is also the underlying reason for the *spontaneous* lymph node PCT that arise in BALB/c mice that harbor a widely expressed human IL-6 transgene. To generate a mouse model of human t(8;14) and mouse t(12;15) translocations, we inserted a histidine-tagged mouse *Myc* gene, Myc^{His}, into three different locations of the mouse immunoglobulin heavy-chain locus *Igh* just 5' of the following loci: Eμ, Cμ (thereby deleting Eμ), and Cα. We have designated the newly developed gene-insertion strains $iMyc^{E\mu}$, $iMyc^{C\mu}$ and $iMyc^{C\alpha}$. The $iMyc^{E\mu}$ mice provide a model for the human t(8;14) and mouse t(12;15) translocation most commonly observed in human endemic BL (eBL) and a subset of IL-6 transgenic mouse PCT, respectively. The $iMyc^{C\mu}$ mice mimic the human t(8;14) translocation that is frequently found in sporadic BL (sBL) and immunodeficiency (AIDS)-associated BL (iBL). The corresponding t(12;15) translocation in mice occurs in precursors of inflammation-induced peritoneal PCT. The $iMyc^{C\alpha}$ mice recapitulate the mouse t(12;15) translocation present in the great majority of inflammation-induced BALB/c PCT. Human t(8;14) translocations with homologous fine structure are sometimes found in sBL/iBL. All three *iMyc* gene-insertion strains are prone to develop mature B-cell and plasma cell tumors, including BL-like lymphomas, diffuse large B cell lymphomas, and PCT. Tumor onset, incidence, and spectrum are linked to the integration site of Myc^{His}, which determines the levels of Myc^{His} expression and the proclivity of the B cells and plasma cells to undergo Myc-dependent apoptosis. The iMyc mice afford a promising model system to identify genes that collaborate with *Myc* in neoplastic plasma cell development; e.g., those encoding the plasma cell survival factor IL-6 and the death suppressor $Bcl\text{-}X_L$. Ongoing studies in our laboratory use the iMyc mice to test and design new treatment and prevention strategies for human plasma cell tumors, such as multiple myeloma. One promising compound along this line is the synthetic triterpenoid CDDO-Im (2-cyano-3, 12-dioxooleana-1, 9-dien-28-oic acid imidazolide), which induces apoptosis in iMyc tumors even when these exhibit resistance to apoptosis induction by other drugs due to interruptions in the Myc-$p19^{Arf}$-Mdm2-p53 tumor suppressor pathway or up-regulation of the inhibitor of apoptosis (IAP) gene *Birc5* (survivin).

P0.144

THE SCID-rab MODEL: A NOVEL SYSTEM FOR STUDYING BIOLOGY AND MANIFESTATIONS OF PRIMARY HUMAN MYELOMA, AND FOR DEVELOPMENT OF ANTI-MYELOMA THERAPY

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Scientific and ethical concerns regarding the use of human fetal bones in the SCID-hu model prompted us to develop a novel system that uses rabbit bones implanted subcutaneously in unconditioned SCID mice. The implanted bones were directly injected with unseparated bone marrow cells from 20 myeloma (MM) patients. Successful engraftment of tumor cells from more than 84% of patients led to increased production of patients' M-protein isotypes and typical MM manifestations, including induction of osteolytic bone lesions, increased osteoclast activity ($p<0.0001$) and angiogenesis ($p<0.0001$) of rabbit origin. MM cells grew exclusively in the rabbit bone but were able to metastasize into another rabbit bone implanted at a remote site in the same mouse. Cells from patients with extramedullary disease also grew along the outer surface of the rabbit bones. MM cells from 3 patients injected into SCID-hu and SCID-rab mice resulted in similar growth rate and pattern. We then exploited this model to test the clonogenic ability of CD138-expressing MM plasma cells. CD138-imminomagnetic bead separated MM cells (>95% purity) from 14 of 17 (82%) patients were successfully engrafted and produced typical myeloma in SCID-rab mice. To test the role of neomicrovessels in MM, hosts engrafted with myeloma cells from 5 patients (1402±492 μg/mL pre-treatment levels) were injected subcutaneously, into the surrounding area of the implanted bone, with ultra-pure human platelet TSP-1 (10 μg/day/mouse) or phosphate-buffered saline (PBS) for 3 to 6 weeks. Changes in levels of tumor burden were monitored by weekly measurements of human monotypic immunoglobulins (hIg) using ELISA and histologically confirmed by immunohistochemical staining of rabbit bone sections for human cIg. Microvessels, visualized by factor VIII immunohistochemical staining, were counted in 5 non-overlapping areas. Whereas all PBS-treated mice had increased hIg levels by 583%±193% from 686±182 μg/mL pre-treatment levels ($p<0.03$), TSP-1 treatment resulted in marked reduction of tumor burden in 3 of 5 experiments by 60%±11% and a stable disease in 2 additional experiments ($p<0.01$). Histological examination (H&E, cIg) of myelomatous bones revealed lower tumor dissemination in TSP-1-treated hosts, an effect that was associated with inhibition of angiogenesis by 62%. Microvessel numbers in TSP-1- and control PBS-treated hosts were 14±2/mm^2 and 37±3/mm^2, respectively ($p<0.0005$). TSP-1 had no direct effect on tumor cell survival and growth *in vitro*, suggesting that the anti-myeloma effect of TSP-1 was primarily through its anti-angiogenesis properties. Our study demonstrates the ability of the SCID-rab model marked by nonmyelomatous and nonfetal microenvironment, to support sustained growth of primary MM and highlights its use for studying myeloma biology, and development of novel therapeutic interventions for this disease.

P0.145

EPIGENETIC INACTIVATION OF CIP/KIP CKIS IN THE CELL CYCLE CONTROL PATHWAY IN MULTIPLE MYELOMA

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Dysregulation of cell cycle is important in oncogenesis. We analysed the potential inactivation of the CIP/KIP family of the cyclin E/CDK/RB pathway by gene promoter hypermethylation in multiple myeloma (MM). The methylation-specific polymerase chain reaction (MSP) with primers for methylated (M-MSP) and unmethylated (U-MSP) alleles of the *p21*, *p27* and *p57* genes was used to study 6 cell lines, and 57 primary myeloma marrow samples. Seven cell lines including AF10, U266, WL2, NCI-H929, RPMI8226, LP1 and ARH-77 were analysed. *p21* and p27 were completely unmethylated in all cell lines. *P57* was completely methylated in WL2 but completely unmethylated in others. At diagnosis, *p57* methylation occurred in three (5.3%) patients and there was no *p21* and *p27* methylation in 57 patients with MM. Therefore, epigenetic dysregulation of the CIP/KIP-CDK2-cyclinA/E pathway in MM is infrequent. A review of the literature showed a marked variation in the frequencies of methylation of these genes, which might be attributable to difference in methodologies used to detect gene methylation.

POSTER SESSION 2: MALIGNANT CLONE AND IMMUNOPHENOTYPING

P0.201

PROBING THE CELL-OF-ORIGIN OF MULTIPLE MYELOMA IN AN *IN VIVO* MODEL

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Multiple myeloma and related plasma cell dyscrasias are characterized by monoclonal expansion of plasma cells. However, plasma cells are terminally differentiated cells with a limited replicative lifespan and it is puzzling how they could be the source of aggressive and often fatal neoplasms. We postulate that the myeloma clonogenic progenitor may reside in a more immature compartment with greater self-renewal capacity. To investigate the clonogenic progenitor in myeloma, we are creating a flexible *in vivo* system that will enable the delivery of stochastic, sequential, somatic mutations to precisely defined plasma cell progenitors. Distinct types of avian leukosis virus (ALV) receptors will be expressed in the non-committed germinal centroblast and the committed plasmablast, rendering each cell type differentially susceptible to gene transduction by retroviruses carrying envelope proteins specific for either receptor. This system promises to provide a reliable model for plasma cell dyscrasias and to illuminate the regulation of critical early steps in pathogenesis. Data on the initial analyses of transgenic animals will be presented at the meeting.

P0.202

TUMOR STEM CELLS IN MULTIPLE MYELOMA ARE BIOLOGICALLY DISTINCT FROM THEIR MATURE PLASMA CELL PROGENY

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Previous work from our laboratory has suggested that the cellular organization of multiple myeloma (MM) parallels the hierarchical nature of both normal hematopoiesis and myeloid leukemias in which clonogenic MM stem cells resembling post-germinal B cells give rise to terminally differentiated MM plasma cells (PC). We investigated whether MM stem cells and PC were biologically distinct and studied each cell type using assays that distinguish normal hematopoietic stem cells (HSC) or their susceptibility to various antitumor agents. The human MM cell lines RPMI 8226 and NCI-H929 were initially examined as we previously found that they recapitulate clinical specimens and contain distinct cell populations based on the expression of CD138; $CD138^{+}$ cells resemble typical MM PC, whereas $CD138^{neg}$ cells are phenotypically similar to B cells and have greater clonogenic capacity. Flow cytometric studies demonstrated that the $CD138^{neg}$ cells were smaller and less granular by light scatter than $CD138^{+}$ PC. In addition, $CD138^{neg}$ cells expressed higher levels of the intracellular enzyme aldehyde dehydrogenase that is normally found at high levels in self-renewing HSC. Furthermore, cells expressing the side population phenotype after staining with the DNA binding dye Hoechst 33342 were identified in each cell line and found to be exclusively $CD138^{neg}$. We also compared the effects of clinically utilized agents on $CD138^{+}$ and $CD138^{neg}$ cells and

treated separated RPMI 8226 cells with dexamethasone (dex, 100nM), bortezomib (velcade, 10nM), CC5013 (revlimid, 1 μM), rituximab (10 μg/mL) or alemtuzumab (campath,10 μg/mL). After 72 hours, cells were washed and plated to assess clonogenic capacity. $CD138^+$ PC were significantly inhibited by dex (27±11% recovery compared to untreated control cells), velcade (14±6%) and revlimid (44±27%), whereas rituximab (92±25%) and campath (97±18%) had little activity. In comparison, $CD138^{neg}$ cells were not significantly affected by dex (82±19%), velcade (88±29%), or revlimid (91±14%), but were inhibited by rituximab (63±22%) and campath (47±27%). Similarly, the clonogenic MM growth of $CD138^{neg}$ cells from 4 clinical MM samples was not affected by dex (84±9%), velcade (82±24%), or revlimid (93 ± 11%), but was significantly inhibited by rituximab (19±7%) and campath (15±11%). Clonogenic MM precursors share several biological properties with normal self-renewing HSC, and these parameters may provide additional means to isolate MM stem cells. Furthermore, MM stem cells and PC display different susceptibilities to a variety of clinical antitumor agents, and these results may explain the dramatic, but transient, responses seen with many agents. Therapeutic strategies that result in long-term remissions may require the inhibition of both MM PC to reduce clinical symptoms and MM stem cells that are responsible for relapse.

P0.203

CD27 DELINEATES TWO ENTITIES IN MULTIPLE MYELOMA WITH DIFFERENT PRESENTING FEATURES AND CLINICAL OUTCOME

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CD27 is a marker of memory B cells, which differentiate into plasma cells (PC) through CD27-CD70 interactions in the presence of cytokines (Agematsu *et al.,* Blood, 1998). Preliminary studies have shown that normal PC express CD27 whereas some myeloma cells have lost its expression (van Oers *et al.,* Blood, 1993; Katayama *et al.,* Br J Haematol, 2003; Guikema *et al.,* Br J Haematol, 2003). Recent studies using microarrays have shown that the CD27 gene was the second most significantly under-expressed gene in multiple myeloma (MM) (Zhan *et al.,* Blood, 2002). However, there are few studies available at the protein level to evaluate the clinical impact of the loss of CD27 in patients with MM. For these reasons, we evaluated the expression of CD27 using flow cytometry on normal PC (n= 7), malignant PC of patients with MM either at diagnosis (n=74) or relapse (n=27), and on 20 human myeloma cell lines (HCML). Normal samples all express CD27 on 100% of PC (including tonsil, bone marrow and reactive PC). In MM, the percentage of CD27+ myeloma cells is 50% at diagnosis (vs 100% on normal PC) and 10% in relapse. Actually, CD27 is completely lost in 38% of patients at diagnosis and in 52% of those in relapse. HCML lost CD27 in 18 of 20 cases (90%). Thus CD27 is clearly lost as soon as the malignant transformation occurs and with disease progression. We also studied the prognostic impact of CD27 expression in a cohort of 42 patients (pts) with *de novo* MM prospectively treated according to ongoing IFM protocols; 19/42 (45%) were CD27+ (group A) and 23 (55%) were CD27- (group B). The 2 groups were identical regarding clinical and biochemical characteristics at presentation. Chromosomal analysis revealed that t(11;14) was significantly less frequent and t(4;14) significantly more frequent in group B. Moreover the expression of CD221 (IGF1-R) was significantly increased in group B (p=0.03). Survival analysis showed that CD27 expression influences clinical outcome: overall survival was 92% at 3 years in group A vs 50% in group B (p=0.017). Two other factors were associated with a poorer survival: t(4;14) positivity (p=0.05) and CD221 expression (p=0.007). Multivariate analysis showed that these 3 factors: CD27 negativity, presence of t(4;14) positivity and CD221 expression were closely linked, these features probably representing a specific entity of aggressive MM. This needs to be confirmed on a larger population of patients. In conclusion, CD27 delineates new entities in MM which could be of strong prognostic value.

P0.204

CIRCULATING PLASMA CELLS DETECTED BY FLOW CYTOMETRY AS A PREDICTOR OF SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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The presence of circulating plasma cells (PC) detected by immunofluorescence in patients with multiple myeloma (MM) has been shown to be associated with a worse prognosis (Blood, 1996; 88: 1780-1787). However, PC detection by immunofluorescence, is complex and not readily available in most clinical laboratories. Therefore, we investigated the prognostic significance of circulating PC detected by flow cytometry in patients with newly diagnosed MM.

Methods: Flow cytometry was performed in patients with newly diagnosed MM within the first week of diagnosis prior to the initiation of treatment. Mononuclear cells were isolated by density gradient centrifugation. The number of circulating PC was measured by gating on CD38 positive, CD45 negative mononuclear cells. A total of 50,000 events were analyzed for each patient and the number of PC detected among these events was recorded.

Results: Between May 1998 and January 2003 flow cytometry was performed on 303 patients with newly diagnosed MM (123 females and 180 males). The median age was 65 years (range 29-94) and the median follow-up was 24.5 months (range 1-76). According to the International Staging System (ISS), 69 (23%), 128 (42%) and 78 (26%) patients had stage 1, 2 and 3 disease, respectively. In 28 patients (9%) the ISS could not be determined. In 80 patients (26%) no circulating PC were seen; 163 (53%) patients had 0-5, and 104 (35%) patients had 6-10 circulating PC, the remaining 36 (12%) patients had more then 10 circulating PC. Median overall survival for the whole cohort was 47 months. Patients with 5 or less circulating PC had a median survival of 59 months, while patients with more than 5 circulating PC had a median survival of 40 months (p=0.0014). On multivariate analysis (Cox proportional hazard model), the prognostic value of circulating PC was independent of beta2-microglobulin (p=0.0422), lactate dehydrogenase (p=0.0058) and C-reactive protein (p=0.0246). In the patients with ISS 2 or 3, the median survival was 26 months if there were more then 5 circulating PC, while the median survival was 48 months if the circulating PC number was 5 or less (p=0.0244). There was no difference in survival in ISS 1 patients dependent on the number of circulating PC, however the median survival in this group has not been reached.

Conclusions: The number of circulating PC measured by flow cytometry in patients with newly diagnosed multiple myeloma is an independent predictor of overall survival.

P0.205

INTENSITY OF EXPRESSION OF CD56 (NCAM) ON MYELOMATOUS PLASMA CELLS

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Background: It was shown that the absence of CD56 (neural cell adhesion molecule) on malignant plasma cells (PC) is a hallmark of plasma cell leukemia and of a special subset of multiple myeloma (MM) (Pellat-Deceunynck *et al.,* Leukemia 1998; 12:1977-82). It was also found that expression of CD56 correlates with the presence of lytic bone lesions in MM and distinguishes MM from monoclonal gammopathy of undetermined significance and lymphomas with plasmacytoid differentiation (Ely *et al.,* Am J Pathol 2002; 160: 1293-9). The aim of this study was to evaluate the intensity of CD56 expression on bone marrow (BM) myelomatous PC and to assess clinical correlations.

Materials and Methods: The study group consisted of 80 MM patients (42M 38F, median age 64, range 39-80yr ;12 at stage I, 18-II, 50 -III according to D.S.; 52 had osteolysis; monoclonal protein was IgG in 56 patients, IgA in 16, IgM in 1, Bence Jones in 7) and 12 had plasma cell leukemia (PCL) patients. Controls were 10 healthy subjects. Immunophenotyping was done on freshly collected BM samples using triple staining combination of CD138/CD56/CD38 monoclonal antibodies analyzed by flow cytometry (Cytoron Absolute and FACSCalibur-Becton Dickinson). Plasma cells were identified as cells showing high-density expression of CD38 and CD138 (syndecan-1). Antigen expression intensity was calculated as relative fluorescence intensity (RFI) and for direct quantitative analysis the QuantiBRITE test (Becton Dickinson) was applied. Mean channels of phycoerythrin fluorescence were defined and antibody bounding capacity (ABC) was then calculated using QuantiCALC software.

Results: In 54 patients (67.5%) PCs showed CD56 expression. Out of all CD38++/CD138+ BM cells, the mean proportion of PC with CD56 expression, was 79±23%, median 91%. RFI values ranged from 7.6 to 23.3 in particular patients (15.9±3.6, median 15.6) and the number of CD56 binding sites (ABC) on MM plasma cells ranged from 2255 to 58469 (14199±15038, median 8866). A correlation was found between RFI and ABC values (r=0.76; $p<0.05$). In 26 MM patients considered as CD56 negative, myeloma mean proportion of all BM $CD38^{++}$ cells with CD56 expression was 5.0±4.3%, median 4.0%. A correlation was found between proportion of all BM $CD38^{++}$ cells with CD56 expression and ABC (r=0.60) and RFI (r=0.61) indices ($p<0.05$). Normal PC did not express CD56. In patients with CD56+myeloma a correlation was found between proportion of CD56+cells and percentage of PC in BM smears in morphological analysis (r=0.65). No differences among analyzed cases were seen between occurrence of CD56 expression and presence of osteolysis, stage of disease and monoclonal protein isotype. Response rate to chemotherapy was similar in CD56 positive and CD56 negative plasma cell proliferation; 58 and 55% respectively. Of 12 PCL cases 6 showed CD56 expression on PC in BM and 3 on those in peripheral blood.

Conclusions: In two thirds of MM patients CD56 molecule could be considered as a *tumor associated antigen*. Intensity of CD56 expression on PCs varies among particular CD56 positive MM patients and differences in expression level may be as big as many times. There is a relationship between proportion of BM CD56 positive PCs and density (ABC) and intensity (RFI) of expression of this molecule. PCL cases show heterogeneity in expression of CD56.

P0.206

A CHIMERIC MONOCLONAL ANTIBODY SPECIFIC FOR KAPPA MULTIPLE MYELOMA PLASMA CELLS MEDIATES ANTIBODY-DEPENDENT CELL CYTOTOXICITY OF TUMOR CELLS

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Current front-line therapies for multiple myeloma such as high dose chemotherapy and autologous stem cell transplant have improved progression-free survival. However, patients invariably relapse with refractory disease and salvage therapies are generally ineffective. Antibody-mediated immunotherapy offers an attractive alternative; however, with the exception of idiotype, few antigen targets have been identified that would facilitate specific immunotherapy of MM. We have previously described a murine monoclonal antibody that recognizes a conformation-dependent epitope on free human kappa light chains and a cell surface antigen, KMA, expressed on kappa MM plasma (MM_κ) cells (Goodnow and Raison, J Immunol 1985; 135: 1276-80). Here we show that the murine antibody, mKap, binds specifically to a range of kappa-type multiple myeloma (MM_κ) cell lines and mediates *in vivo* anti-tumor activity in a SCID mouse human myeloma xenograft model.

With the rationale of producing a safe and effective therapeutic for MM_κ, a chimeric human IgG1 version of the murine antibody, cKap, was expressed in CHO cells. mKap and cKap exhibit similar antigen binding specificity to soluble and cell-associated antigen as determined by ELISA and flow cytometric analysis respectively. Kinetic analysis of antigen binding by cKap and mKap using surface plasmon resonance showed that cKap retains the affinity of the murine parent antibody (K_D cKap = 18.2nM; KD mKap = 20.9nM). *In vitro*, cKap mediates significant ADCC of MM_κ cells using human PBMC effectors at E:T ratios as low as 25:1. These results highlight the specificity and functional activity of cKap and indicate the potential of this chimeric antibody as an immunotherapeutic for MM_κ

P0.207

GENETIC ABNORMALITIES AND PATTERNS OF ANTIGENIC EXPRESSION IN MULTIPLE MYELOMA. A STUDY OF THE MYELOMA SPANISH GROUP (GEM-2000)

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Introduction: Myelomatous plasma cells (PC) show a high heterogeneity both in their immunophenotypic characteristics as well as in their cytogenetic features. So far, no extensive studies have been carried out to explore whether such antigenic diversity is associated with specific genetic characteristics. We have investigated the relationship between the immunophenotypic profile at PC and both their DNA ploidy status (evaluated by flow cytometry), and specific genetic features (ascertained by FISH) in a large series of 915

patients with newly diagnosed MM.

Materials and Methods: A total of 915 patients enrolled in the PETHEMA/GEM protocol (six alternating cycles of VBCMP/VBAD followed by autologous stem cell transplantation -ASCT-) were included in the study at diagnosis. Median age at the time of entering the study was 59 years (range, 32-70 years). Immunophenotypic studies by flow cytometry were carried out by using an appropriate panel of quadruple monoclonal antibodies (MoAb) combinations: CD38 /CD56 /CD19 /CD45; CD138 /CD28 /CD33 /CD38 and CD20 /CD117 / CD138/ CD38. A double-staining procedure for nuclear DNA (with propidium iodide, IP) and surface PC antigens (with anti-CD38 and anti-CD138 MoAbs, provided both by Cytognos, SL, Salamanca, Spain) was used to specifically analyze the PC DNA content -PC DNA ploidy status-. Acquisition of information on $2x10^4$ stained cells corresponding to the whole BM cellularity was assessed on a dual-laser FACSCalibur flow cytometer using the CellQUEST software program (BD Biosciences –BDB- San José, CA, USA).

Results: The non-hyperdiploid MM group (n=454, 52%) was associated with a significantly higher frequency of positivity for CD28 and CD20 as well as a higher incidence of CD56- and CD117- cases (p<0.001). Remarkably, 13q deletion and IGH gene rearrangements, which were significantly more common in non-hyperdiploid MM, showed a strong association with CD117- cases. IGH translocation to 11q13 was associated with reactivity for CD20 (p<0.001), down regulation of CD56 (p<0.001) and lack of expression of CD117 (p=0.001). By contrast, IGH translocations to other chromosome partners were almost exclusively found among CD20-and CD117-cases (p<0.001).

Conclusions: Genetic categories in MM exhibit a particular immunophenotypic profiles which in turn are strongly associated with the DNA ploidy status.

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PO.208

GENETIC ABNORMALITIES IN PERIPHERAL BLOOD LYMPHOCYTES OF MULTIPLE MYELOMA PATIENTS AS DETECTED BY FLUORESCENT *IN SITU* HYBRIDIZATION

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Multiple Myeloma (MM) is a terminally differentiated B-lymphocyte malignancy characterized by accumulation of plasma cells in bone marrow. In spite of the variety of treatments available, it remains an incurable disease with an overall survival of 3-4 years. Even though some patients show complete remissions with no detectable plasma cells in the bone marrow, they still relapse. We have identified clonotypic B lymphocytes in peripheral blood of MM patients with malignant characteristics and the ability to xenograft the disease to immunodeficient mice. We analyzed chromosomal abnormalities in peripheral lymphocytes of MM patients to determine whether or not they may be a reservoir of disease showing abnormalities characteristic of MM clonal plasma cells. We also tested the reinfused blood on one patient having an autotransplant. We used an automated scanning system that records the location of each cell, enabling us to analyze cells first through morphology and afterwards through FISH. We recorded both patterns for each cell. We scanned cytospin slides stained with May-Grunwald Giemsa of 36 patients with MM to recognize lymphocytes. We then performed fluorescent *in situ* hibridization (FISH) using two commercial probes, firstly, to detect deletion of chromosome 13 (D13S319 Vysis), and secondly, to detect t(4;14) (p16;q32), abnormalities that correlate with adverse prognosis. Lymphocytes from PBMC of healthy donors had 2.8±1% with ΔCh13, and 0% with t(4;14). For this study we set a cut off value for each abnormality of 10% for ΔCh13 and 2% for t(4;14) to identify lymphocyte positivity. We found that peripheral lymphocytes from 12/36 (33,3%) of MM patients show genetic alterations, either ΔCh13 and/or t(4;14). 9/27 patients' lymphocytes had ΔCh13 (33%) and 3/10 had t(4:14) (30%). For all cases with BM samples available for testing, ΔCh13 or t(4;14) correlate with peripheral findings and were expressed by a sizeable proportion of their plasma cells. For one MM patient with a large compartment of activated lymphocytes in PBMC, 60% had ΔCh13; 63% of plasma cells from this same patient were ΔCh13. For another MM patient sorted sIgM+B cells included a small proportion with t(4;14) translocations. For the reinfused autografts, preliminary data indicates the presence of cells with detectable chromosomal abnormalities This demonstrates that MM includes clonal cells with lymphocyte morphology that harbour chromosomal abnormalities such as deletions or IgH switch region translocations known to be clinically significant in MM, further implicating them as a source of relapse in this disease. Update of these results and a more thorough analysis, will be presented at the workshop.

PO.209

COMPUTER SIMULATION OF MULTIPLE MYELOMA IN THE CONTEXT OF SYSTEMS BIOLOGY

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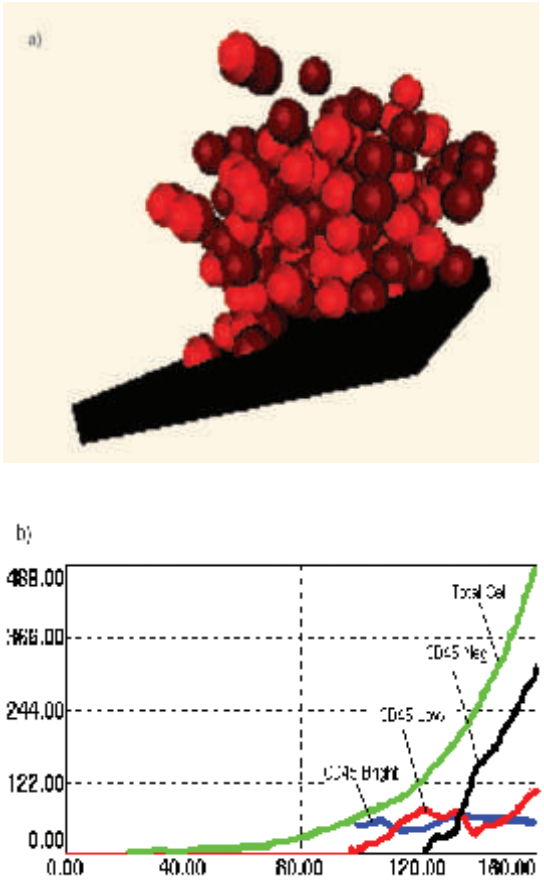
Cancer is complex adaptive system. We assume that multiple myeloma (MM) can be studied in the context of complexity using Systems Biology (SB). SB is a new field in biology aimed at understanding biology at systems level. We developed the *In Virtuo* experimentation for SB using virtual environment. Multiagent systems provide an attractive computer framework for SB and *In Virtuo* approachs. We implement a computer simulation supporting the maturation model of myeloma cells based on the CD45 expression. One has shown that CD45 expression decreases with the maturation of the cells and CD45 annihilation is a critical prognostic for patient survival. CD45 expression is also necessary for IL6 proliferation signals but inhibits IGF-1 proliferation signals. Moreover, CD45 expression is stimulated by IL6 and its activity inhibited by dimerization. The kinetics of CD45 dimerization depends of the molecule isoform. So, we have developed a computer simulation based on a multiagent system which integrates the model of myeloma cell previously described in their micro-environnement. The simulation shows that kinetics of MM tumor presents an exponential shape (Figure 1) which is characteristic of the first stages of tumor growth. Next, we can observe the loss of tumor connectivity, which was previously shown to occur with tumor evolution. We assume that CD45 isoform is an important feature for the proliferation signal choice (data not shown). Holistic study, by *In Virtuo* simulation enables simulation of emergent behavior of MM. The simulation shows the importance of the microenvironement and the CD45 isoform for tumor evolution. Moreover, we assume that our original approach may allow research of new therapeutic targets and therapies.

Figure 1. (a) 3D visualization; (b) Kinetics of total cells (total cell), cells with high expression of CD45 (CD45 Hight), cells with few CD45 (CD45 Low), cells with no CD45 (CD45 neg).



PO.210

FLOW CYTOMETRIC IMMUNOPHENOTYPIC ANALYSIS OF 21 CASES OF PLASMA CELL LEUKEMIA

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The aim of the study was to determine expression of adhesion molecules CD11a (LFA-α), CD18 (LFA-β), CD11b, CD29, CD49d, CD44 (H-CAM), CD54 (ICAM-1), CD56 (N-CAM) and CD117 (c-kit) on peripheral blood (PB) and bone marrow (BM) lymphoid cells in 21 plasma cell leukemia patients (PCL), at diagnosis and in the control group of 10 healthy subjects. Immunophenotyping was performed on freshly collected blood and bone marrow samples by means of flow cytometry. Plasma cells were identified as showing high-density expression of CD38 and CD138 (syndecan-1). Results of analysis are presented both as relative and absolute (omitted in abstract) values of numbers of cells with antigen expression and as relative fluorescence indices (RFI) of studied antigens. Statistical analysis was performed using Wilcoxon's test. All below presented differences are statistically significant. The study revealed in PCL patients a significantly higher relative and absolute number of CD54+ cells (*in brackets: means±SD of PCL pts vs control*) both in BM (63±29% vs 13±5%) and PB (49±25% vs 8±3%) as well as that of CD38+ cells both in BM (84±12% vs 54±11%) and PB (74±11% vs 52±7%). In turn, PCL patients showed a decreased relative number of BM: CD11a+ cells (40±28% vs 73±10%), CD18+ cells (47±25% vs 88±7%), CD11a+ CD18+ cells (42±27% vs 72±10%), CD44+ cells (71±26% vs 93±4%), CD11b+ cells (17±12% vs 35±10%) and PB: CD11a+ cells (58±28% vs 96±3%), CD18+ cells (58±29% vs 99±0,2%), CD11a+CD18+ cells (58±29% vs 96±3%), CD44+ cells (86±15% vs 98±0,9%). In BM of PCL patients compared with the control there were decreased RFI of CD18 (15.0±1,3 vs 16,6±0.7) and CD29 (8.6±1,4 vs 10.4±0.8) and increased RFIs of CD54 (16.9±2.5 vs 13.0±0,5) and CD11a (18.4±1.5 vs 14.7±0.9). In PB of PCL patients RFIs of CD29 (10.4±1.2) was lower than this in control (11.6±0.9) while RFIs of CD38 (16.9±3.0 vs 14.8±1.3), CD54 (16.1±2.8 vs 12.3 ±0.3), CD11a (20.4±1.8 vs 18.3±0.8) were higher. In all examined cases PB and BM leukemic cells with high expression of CD38 and CD138 showed co-expression of CD54 antigens, in 10 cases-also CD56, in 5-CD117, in 7-CD29 and CD49d, in 4(4 in PB, 1 in BM) out 13 – CD11b, in 5 out 9 – CD44. On the other hand, in all cases PB and BM leukemic cells showed lack or weak expression of CD18, CD11a, CD11a+CD18+.

Conclusions: Immunophenotype of leukemic plasma cells characterizes mainly increased expression of CD38, CD54 and CD138 and disturbed expression of CD18, CD11a and CD11b. In the half cases tumor cells show expression of CD56 and CD117.

PO.211

AN UNUSUAL PLASMA CELL NEOPLASM ASSOCIATED WITH A t(11;14)(q13;q32) LYMPHOCYTOSIS AND CYCLIN D1 DEREGULATION

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We describe a patient who presented with lymphocytosis, back pain and mild anemia. On examination there was no lymphadenopathy, hepato-splenomegaly or any other palpable masses which was confirmed by CT scan. PBC showed Hb 12.2, WBC 28.6x10^9/L, lymphocytes 24.5x 10^9/L, neutrophils 3.5 and platelets 140x10^9/L There was no evidence of renal failure. The patient had serum immunoparesis but no paraprotein was detected and a trace of lambda light chain Bence-Jones proteins was detected by immunofixation. MRI scan of vertebra revealed abnormal signaling in keeping with myeloma but with no evidence of lytic bone lesions. The lymphocytosis was unusual and was investigated further. The lymphocytes were small to medium sized with condensed chromatin, regular nuclear outline, no visible nucleolus and scanty irregular cytoplasm.The majority of cells did not have features of plasma cells and only a few (~5%) demonstrated plasmacytoid features. Greater than 80% of cells were positive for CD20 and CD23. The cells were negative for CD5, CD19, CD45, surface Ig and surface CD138 by flow cytometry. However cytoplasmic CD138 could be demonstrated with alkaline phosphatase anti-alkaline phosphatase.Two thirds of the cells expressed cytoplasmic Ig with lambda light chain restriction and were positive for CD38 and FMC7. Bone marrow histology showed a hypercellular marrow, packed with atypical small lymphoid cells entirely effacing hemopoiesis and a sheet of cells with eccentric mildly pleomorphic nuclei, eosinophilic

nucleoli and moderately abundant cytoplasm, positive for CD20, CD23,CD138 and cyclin D1 but negative for CD5. Immunocytochemistry revealed lambda light chain restriction with staining in the cytoplasm. CCND1(11q13)/IGH(14q32) fusions characteristic of t(11;14) with amplification of the region containing the CCND1 locus(11q13 /extra CCND1 signal) and ATM locus (11q23/extra ATM signal) were detected by FISH in > 80% of PB lymphocytes with no evidence of D13S319(13q14), TP53(17p13) and p16(9p13) deletions or translocation t(4;14) involving the FGFR3/MMSET and IGH loci.We are characterizing the site of the breakpoint at the DNA level. The majority of clinical and laboratory findings in this case are consistent with a diagnosis of multiple myeloma. t(11;14) with deregulation of cyclin D1 is present in 15-20% MM/PCL and amplification of 11q23 is seen in 62% of MM. Nevertheless the CD20+, CD23+ "CLL like" cells lacking morphologic plamacytic differentiation and surface CD138 in the peripheral blood is unusual. The presence of CCND1/IGH fusions in the PB cells and cyclin D1 deregulation in the BM plasma cells show that these two populations belong to the same primary clone.

POSTER SESSION 3: STAGING, PROGNOSTIC FACTORS AND EVALUATION OF RESPONSE

P0.301

COMPLETE RESPONSE IN MULTIPLE MYELOMA: DEFINITION OF CLINICAL AND BIOLOGICAL PARAMETERS

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The frequent achievement of a complete response (CR) is a relatively recent feature in the treatment of multiple myeloma. This can now be achieved by either high dose chemotherapy followed by stem cell rescue or by standard combination chemotherapy. Does a CR translate to improved survival and can selected clinical and/or biological features discriminate between patients destined to be long term survivors? To obtain further information we conducted a detailed clinical and biologic set of analyses in CR patients treated on ECOG study E9486. These patients were treated with either VBMCP or VBMCP and interferon-·2 or VBMCP plus high dose cyclophosphamide. Of 653 patients entered onto this study 628 were eligible and CR rates were identified using conventional definitions. The latter included analysis of the serum M protein with immunofixation, detection of the clone in the marrow by immunofluorescence and by allele specific oligonucleotide polymerase chain reaction (ASO-PCR). In addition using two color flow cytometry we determined the quantitative levels of blood immune cells shown by us previously to be associated with favorable clinical outcome. Objective response was found in 420 of 628 (67%), while 85 achieved a CR (13.5%). Median length of time to CR was 13.2 months with a range of 1.1 - 40.8 months. Baseline characteristics were assessed among three groups, CR, PR and non-responders. The Beta 2 microglobulin, PCLI, number of clonal cells in the marrow, creatinine, hemoglobin and C-reactive protein did not differ between CR and PR or non-responders. Differences were detected regarding proportion of IgA M-protein (34% vs. 28% vs. 20%, p=0.02) and CD19+ blood levels (median of 230 vs. 246 vs. 202, p=0.045), however, when comparing CR and PR the difference did not reach statistical significance. We are currently evaluating the level of blood CD4+ T cells in association with survival in this cohort since we have previously found a positive association between myeloma survival and CD4+ levels. The median survival from randomization was 2, 3.9 and 5.2 years for non-responders, PR and CR patients, respectively. In a 2-year landmark analysis CR patients maintained a better survival compared to PR patients (post 2-year median survival of 3.6 vs 2.4 years, p=0.006). Using a Cox proportional hazard model CR status, gender, Beta 2 microglobulin, PCLI, sIL-6R, plasmablastic morphology, percent clonal plasma cells and CD19 were significantly associated with survival. This study confirms that CR can be achieved in multiple myeloma patients and that combinations of clinical and biological parameters may be of help in discriminating which patients will have both a CR and prolonged survival.

P0.302

PROGNOSTIC IMPACT OF COMPLETE REMISSION IN MULTIPLE MYELOMA. PRELIMINARY RESULTS OF A PROSPECTIVE STUDY IN A SERIES OF HOMOGENOUSLY TREATED PATIENTS

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Grupo Español de Mieloma (GEM)

The EBMT Myeloma Subcommittee redefined the response criteria for MM in 1998. In synthesis, a negative immunofixation (IF) was established as a requisite for CR and the criteria for relapse and progression were also established. Several reports, including the study of the Spanish Registry of MM Transplantation, have confirmed the prognostic influence of CR defined by the EBMT criteria, and suggest that patients with a negative IF form a subgroup with better prognosis than cases with a negative electrophoresis but positive IF. Nevertheless, many of these studies are retrospective and not always patients have been uniformly treated. Here we report on the GEM-2000 prospective experience based on a large series of patients uniformly treated.

Patients and Methods. All cases in the GEM-2000 protocol have been treated with 6 cycles of alternating chemotherapy (VBMCP/VBAD) followed by BUMEL or MEL200 as pretransplant conditioning regimen. Only patients evaluated after autologous transplant are included. Complete remission has been defined as CR when the IF was negative; if patients achieve an EEF(-) but IF(+), the response was called Electrophoretic Complete Remission (ECR). Other response categories have also been evaluated: Partial Response (PR); Minimum Response (MR); Stable Disease (SD) and Progression. The univariable statistical analysis of survival was complited with a multivariable model adjusted with other previously-selected risk factors. This preliminary study includes 528 cases (number of cases per response category: CR=181; ECR=73; PR=181; MR=26; SD=23; Progression=12). According to the protocol design, patients with PR or less underwent a second transplant.

Results. At present the median follow up of the 484 living patients is 27 months (8-59 mo, SD±11). Survival (85% at 4 yr, CI 95%: 77-92 mo; median not reached) and Event-Free Survival (EFS) (4 yr: 56%; CI 95%: 67-44 mo, median 49 mo) in the CR groups was better than in any of the other responding groups (p ranging between 0.00009 and 0.03). However, the other response categories (ECR vs. PR vs. MR vs. SD) do not discriminate different prognostic subgroups (Survival at 4.5 yr=77%, 64%, 80% and 60% mo, unreached medians, p=0.8; EFS, medians: 35, 37, 35 and 28 mo, p=0.3, respectively). As expected, patients in progression displayed the worse prognosis with respect to any of the other groups (Survival and EEF: p<0.00000). The multivariable analysis confirms the statistical significance of CR vs. the other responses (Survival: OD for CR 3.7, p=0.001, model p<0.00000. EEF: OD for CR 2.2, p=0.001, model p=0.006).

Conclusions. These preliminary data corroborate our previous retrospective study and support that obtaining a CR defined by IF is a necessary step towards in order to achieve prolonged Survival and EFS. In our hands the ECR category, frequently designated with the equivocal term of *Near Complete Remission*, does not have a significant influence on disease outcome, although longer follow-up is still needed. Other terms such as immunophenotypical or molecular CR needs to be prospectively evaluated.

P0.303

PRE-TRANSPLANTATION LEVEL OF MINIMAL RESIDUAL DISEASE DETECTED BY QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION IS A PROGNOSTIC PARAMETER IN PATIENTS WITH MULTIPLE MYELOMA

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Background. High-dose chemotherapy with autologous stem cell transplantation has improved outcome and survival of patients with multiple myeloma. However, the majority of patients suffer from relapse. Using real-time quantitative (RQ) PCR we have shown before (Haematologica 89,2004) that the amount of residual tumor cells in the bone marrow of patients before transplantation is of prognostic relevance. In this study we evaluated in a larger group of patients with multiple myeloma whether a pre-transplantation level of clonotypic cells in the bone marrow is predictive for time-to-progression (TTP) and overall survival (OS). Further, we compared results with known prognostic factors.

Patients and Methods. Bone marrow samples of 19 patients with stage II/III multiple myeloma were obtained after induction therapy but before transplantation. Immunoglobulin heavy chain (IgH) RQ-PCR using patient-specific Taqman probes was performed to quantify pre-transplantation tumor levels. The proportion of clonotypic cells was assessed as IgH/2 beta-actin ratio in percent. Medical records of patients were reviewed for prognostic factors and outcome.

Results. The median level of residual tumor cells in bone marrow of all patients at the time before transplantation was 0.3%. At 23 month median follow-up after transplantation the median TTP and OS in our study were 14 and 36 month, respectively. The threshold level of 0.03% clonotypic cells identified two prognostic groups (p<0.0001, log rank). Twelve patients in the bad prognostic group had an early relapse with a median TTP of 9 months (range: 3-17). All patients in the good prognostic group had ongoing remissions after a median follow-up of 24 month (range: 13-44 month). Univariate analysis was performed including other prognostic factors at the time before transplantation such as cytogenetic abnormalities, beta2-microglobulin, hemoglobulin, platelet count, LDH, CRP, serum albumin and age. Besides the pre-transplant level of minimal residual disease, CRP level was predictive for TTP. In multivariate analysis using a step-wise Cox regression model grouping by pre-transplantation tumor level was the only prognostic factor for TTP (p=0.05). Moreover, low pre-transplantation tumor levels also showed a trend for a better OS, but in multivariat analysis only normal cytogenetics were predictive for a superior outcome (p=0.03).

Conclusions. Quantitative molecular assessment of pre-transplantation tumor level in the bone marrow is an independent prognostic parameter for the progression-free survival of patients with multiple myeloma and thus helps to guide therapeutic interventions.

P0.304

HIGH FREQUENCY OF MOLECULAR REMISSIONS IN MULTIPLE MYELOMA PATIENTS ACHIEVING A HEMATOLOGIC COMPLETE REMISSION ON TOTAL THERAPY II

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Total therapy II (TT-2) represents a very intensive treatment approach for newly diagnosed myeloma patients consisting of hematopoietic growth factor-dependent induction therapy followed by melphalan 200 mg/m^2-based tandem autotransplant, consolidation chemotherapy and dexamethasone/interferon alpha maintenance. A stringently defined hematologic complete remission (CR) was obtained in 48% of the first 446 patients enrolled on TT-2. CDR3-PCR to evaluate minimal residual disease (MRD) in myeloma has revealed molecular complete remission (m-CR) in 50% of 40 reported allotransplant cases compared to approximately 15% among 75 reported autotransplants myeloma. We evaluated the frequency of m-CR in 20 TT-2 patients with a qualitative PCR for CDR3. CDR3 probes were generated from RNA of CD138 purified plasma cells. Light-density (<1.077 g/cm^3) bone marrow cells served as the source of DNA post-therapy to assess MRD. At the time of CDR3-PCR analysis, 13 patients were in hematologic CR, 1 in near CR (immunofixation positivity as the only evidence of disease), 4 in partial remission (PR) (bone marrow < 5% plasma cells, decrease in serum M protein ≥75% and in urine M protein ≥90%) and two had less than a PR. Four patients were tested for MRD prior to the first transplant (2 < PR, 1 PR, 1 CR), two prior to the second transplant (2 PR) and 14 at a median of 21 months (range 6 months to 2 years) after the second transplant (12 CR, 1 near CR, 1 PR). Six of the 13 CR patients had abnormal metaphase cytogenetics at diagnosis, including 5 with deletion of chromosome 13. A m-CR was observed in 10/13 (77%) hematologic CR patients, including 4/6 with abnormal cytogenetics. p53 exon 6 amplification was seen in all patients who were CDR3-PCR negative indicating good quality DNA. Not unexpectedly, all seven patients failing to achieve a stringently defined hematologic CR had persistent disease as assessed by CDR3-PCR. Eight of the 10 peripheral blood stem cell collections of m-CR patients were analyzed for the presence of MRD and 4 were positive, indicating that a m-CR can be achieved despite infusion of myeloma-containing grafts. Our results suggest that high intensity therapy as applied in TT-2 can indeed achieve a more marked tumor cytoreduction as reflected by a higher m-CR rate than previously reported with autotransplantation and that infusion of clean grafts is not an absolute prerequisite to obtain m-CR, if post-transplant consolidation and maintenance therapy is applied.

P0.305

EVALUATION OF METHODS FOR THE DETECTION OF MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA USING A CELL LINE MODEL

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Immunoglobulin heavy chain (IgH) rearrangement provides a clone specific marker for the molecular detection of tumor cells and monitoring minimal residual disease in multiple myeloma. The unique sequences of the CDR3 region can be used to design allele specific oligonucleotides (ASO) and a individualized detection system for each patient. We have compared several PCR-based methods in a model system using the monoclonal IgM lambda immunoglobulin producing human cell line E11. For tests with consensus primers, 7 different primer pairs were used (VH1-6 with JH, and FR3 with Cx). PCR products were detected by agarose gel electrophoresis and high-resolution fragment analysis. For high-resolution fragment analysis, PCR products were generated with fluorescent primers and separated using a Long Read Tower automated sequencer and Genescan computer program (Bayer, Visible Genetics). In methods using the clone-specific ASO-CDR3 primers, sequencing of the IgH gene and identification of the specific CDR3 region sequence was performed first. ASO-PCR with the CDR3-JH primer set generated approximately 90 bp PCR products that were detected by agarose gel electrophoresis, fragment analysis and quantitative real time PCR. The detection limit of each technique was determined by serial dilutions of the tumor cell line with normal peripheral blood mononuclear cells and serial tumor cell cDNA dilutions with human placental DNA. Methods with consensus primers had a detection limit of 10^{-3} for tumor cells both with agarose gel electrophoresis and Genescan detection, while the sensitivity of ASO-PCR was 10^{-4} with both methods. Real time ASO-PCR was able to detect E11 cells with a detection limit of 10^{-5}. The specificity of ASO primers was tested on mononuclear cells from healthy controls and myeloma patients. The lack of PCR products in these controls indicated the specific annealing of the primers in the E11 cell system only. We determined that the most sensitive method was using ASO primers and real time PCR. This optimized method is currently being applied on serial bone marrow, peripheral blood and stem cell collection samples from multiple myeloma patients. The predictive value of the assay will be determined in a longitudinal clinical study.

P0.306

PROTEIN EXPRESSION PROFILING TO DEVELOP A MINIMAL RESIDUAL DISEASE FLOW ASSAY FOR THE MRC MYELOMA IX TRIAL

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Immunofixation and free light chain quantitation are sensitive approaches for assessing response to therapy in myeloma. They are however indirect measurements which cannot quantify residual disease levels accurately. Studies utilizing ASO-PCR and/or flow cytometry have demonstrated that detection of disease at the 0.01% level after high-dose therapy for myeloma is associated with rapid disease relapse. However, ASO-PCR is expensive, not applicable to all patients and not ideally suited for monitoring disease in real time. Flow cytometry is sufficiently sensitive and rapid, but is not suitable for patients more than three months after therapy. This is because normal long-lived plasma cells, which are phenotypically similar to myeloma plasma cells, may regenerate after this time point and reduce the sensitivity of the minimal residual disease (MRD) assessment. The aim of this study is to develop an MRD flow assay suitable for monitoring residual myeloma at all time points, utilizing the high-level analytical software developed for mRNA microarray analysis. Samples from myeloma patients (n=43), normal controls (n=26) and MGUS (n=14) were assessed with a panel of 66 test antibodies. Protein expression was calculated from the geometric mean fluorescence intensity. Plasma cells were identified using CD38, CD45 and CD138 gating with four-color flow cytometry. The dChip analysis program was used to identify discriminating antibodies, with hierarchical cluster analysis to identify complementary combinations. An iterative process was used: increasing numbers of patients were assessed with smaller more targeted antibody panels until highly specific combi-

nations were identified. In particular, we have focused on the identification of proteins that are differentially expressed between neoplastic plasma cells and normal long-lived plasma cells. In conjunction with CD38/CD138 gating antibodies, the antibody combinations CD27/CD39, CD81/MPC-1, CD31/CD63, and CD40/ CD117 are highly specific for identification of neoplastic plasma cells. Most of these antigens are expressed at approximately three-fold lower levels by neoplastic plasma cells than their normal long-lived counterparts, with the exception of CD117 which is aberrantly overexpressed. Analysis of cytoplasmic IgG/IgA and kappa/lambda expression coupled with conventional CD19/CD56 assessment may be used to guide the requirement for extended MRD analysis. This MRD flow assay is routinely applicable for the detection of neoplastic plasma cells when they represent as few as 0.01% of total leukocytes. This MRD flow assay is as sensitive as, and more specific than previously reported assays and is applicable to samples at any stage of the disease without knowledge of the presentation phenotype. This assay is currently being applied prospectively for analysis of residual disease in the MRC Myeloma IX trial, which has recruited over 480 patients to date.

PO.307

COMPARISON OF THE INTERNATIONAL PROGNOSTIC INDEX AND DURIE-SALMON STAGING SYSTEM TO DETECT DIFFERENCES IN THE LEVELS OF BIOCHEMICAL MARKERS OF BONE REMODELING AND SERUM CYTOKINES IN MULTIPLE MYELOMA

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Recently, a new International Prognostic Index (IPI) for multiple myeloma (MM) has been proposed. This is based on serum levels of B2M and albumin, but in contrast to the classical Durie & Salmon classification (D-S), the IPI does not use information on bone disease. Biochemical markers of bone remodeling (BMBR) levels and some serum cytokines have shown correlation with the tumor burden. We have investigated the levels of these parameters according to different IPI stages as well as in D-S classification.

Materials and Methods. 103 patients MM untreated patients (median age-67; ratio M/F- 59/48) were included in the study. We have measured four bone resorption markers (Pyrt, Dpyrt, Beta-crosslaps and Trap5b), two bone formation markers (bone alkaline phosphatase and osteocalcin), and several serum cytokines (IL-6, SIL-6R, TNF-α, IL-1β, HGF, VEGF, OPG, sRANKL, MIP-1α, IGF-I and syndecan-1). All them were measured in serum by ELISA, except Pyrt and Dpyrt, which were measured in urine by HPLC. Statistical methods: chi-square; nonparametric test (Kruskal-Wallis, U Mann-Whitney).

Results. Only four parameters showed significant different levels according to the IPI stage (Table 1).

		Pyrt	Dpyrt	Bcrosslap#	IL-6#	TNF-α*	HGF#
IPI	I	79.7	14.6	0.33	2.9	3.4	1196
	II	108.3	17.9	0.7	4.6	5.1	1301
	III	116	25	1.34	6.4	6.9	2192
D-S	I	74	12.2	0.63	3.1	4.8	1391
	II	107.1	17	0.65	4.2	5.1	1760
	III	122.3	26.9	1.38	4.9	6.2	1616

**p<0.05, #p<0.01, &p<0.001*

The levels of IL-6, HGF, TNFα and B-crosslaps progressively increased from stage I to stage III, with statistical significant differences. The serum levels of IGF1, MIP, sRANKL, VEGF and sIL6R as well as those of the remaining cytokines described in M&M were not significantly different along several IPI stage. Upon comparing the behavior of these markers in the D-S & IPI classification, as expected, Pyrt & Dpyrt were different according to D-S staging, but not upon using the IPI system. By contrast, the differences in levels for cytokines such as TNF-α and HGF observed at the IPI did not appeared with the D-S classification.

Conclusions. The levels of cytokines but not those of BMBR (except B-crosslaps) correlate with the different stages of the IPI system.

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PO.308

CONFIRMATION OF THE PROGNOSTIC VALUE OF THE INTERNATIONAL STAGING SYSTEM FOR MULTIPLE MYELOMA PROPOSED BY THE INTERNATIONAL MYELOMA WORKING GROUP AND SOUTHWEST ONCOLOGY GROUP STAGING SYSTEM WITH SIMILAR RESULTS USING β_2MICROGLOBULIN ALONE

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A simple, reliable staging system in myeloma is important for consistent comparison of data from clinical trials from different institutions and for identification of poor risk groups for which novel or aggressive treatment is justified. In an attempt to simplify and universalize myeloma staging, an International Staging System (ISS) has been proposed by the International Myeloma Working Group (IMWG). Three stages based on β_2-microglobulin (B_2M) and albumin have been proposed; Stage 1 (S1): B_2M <3.5 mg/L and albumin ≥3.5 g/dL; Stage 2 (S2): B2M 3.5-5.5 mg/L or B2M <3.5 mg/L and albumin <3.5 g/dL; Stage 3 (S3): B_2M ≥5.5 mg/L. To assess the prognostic value of the new International Staging System, we performed a retrospective analysis of 898 consecutive patients (pts) with previously untreated, symptomatic multiple myeloma. The median (med) overall survival was 53 months (mo.) (n=325) for Stage 1, 42 mo. (n=269) for Stage 2 and 26 mo. (n=304) for Stage 3, p<0.01. The proposed ISS criteria successfully identified 3 stages of patients in this cohort with median overall survival similar to the corresponding stage of patients from IMWG. The prognostic value of the SWOG staging system (Stage 1, B2M < 2.5 mg/L; Stage 2, B2M 2.5-<5.5 mg/L; Stage 3, B2M > 5.5 mg/L and albumin > 3.0 g/dL; Stage 4, B2M <5.5 mg/L and albumin < 3.0 g/dL) was also evaluated in the same 898 patients at MDACC. The overall median survival was 64 mo. (n=169), 45.7 mo. (n=425), 28.1 mo. (n=254), and 16.2 mo. (n=50) for SWOG Stages 1, 2, 3, and 4, respectively (p<0.01). Thus, although more complex than the ISS, the SWOG system was also confirmed. Using B2M alone with identical cutoffs for analysis resulted in identical survival in both the ISS Staging System (S1: med OS 53 mo., S2: med OS 42 mo., S3: med OS 26 mo., p< 0.01) and the SWOG Staging System (S1: med OS 63.9 mo., S2: med OS 45.9 mo., S3: med OS 26.4 mo., p<.01). The prognostic value of the ISS for pts with creatinine (Cr) ≥2.0 mg/dL was poor in our analyses using either B_2M and albumin (med OS 29 mo., 33 mo. and 27 mo. for S1, S2 and S3, respectively, p.45) or B2M alone (med OS 29 mo., 33 mo., 27 mo. for S1, S2 and S3, respectively, p.45). When serum creatinine was normal, both systems successfully identified distinct stages of patients with different sur-

vivals. However, when the serum creatinine was >2 mg/dL, both the ISS and SWOG staging systems lost their prognostic power. We were however, able to identify adjusted incremental ranges of B_2M alone in patients with creatinine 2-4 g/dL that were predictive of survival (S1: B_2M<6 mg/L, 54 mo. OS; S2: B2M 6-15, 28 mo. med OS; S3: B_2M >15, 15 mo. med OS p.08), suggesting that adjusted prognostic systems based on B2M and creatinine level should be evaluated and considered in future analyses. Based on validation of the ISS proposed by the IMWG, and staging criteria proposed by SWOG, it seems reasonable to recommend international acceptance of one of these systems. However, validation of a system using B_2M alone, with incremental adjustments based on creatinine in the future seems warranted.

P0.309

THE SEARCH FOR A NEW AND IMPROVED STAGING SYSTEM FOR NON-INTENSIVE CHEMOTHERAPY: THE UK MEDICAL RESEARCH COUNCIL'S EXPERIENCE

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Background. Patients with multiple myeloma vary greatly at presentation and, consequently, there are currently many reported prognostic factors. These factors measure general features, tumour burden, haemopoeitic function, skeletal and renal disease. Attempts to combine prognostic factors and create a useful prognostic index to measure overall survival have resulted in several prognostic indices, including the International Prognostic index (IPI) and the MRC 6-factor prognostic index (MRC6PI). Any new prognostic indices need to be evaluated against existing systems and validated on independent datasets.

Methods. Within the MRC 5th and 6th myeloma trials, 999 patients were randomised to receive ABCM combination chemotherapy. Previous log-rank and Cox regression analyses of 39 prognostic factors derived a MRC 6-factor prognostic index (MRC6PI) comprising Sβ2m, age, performance status, blood urea, platelets and corrected serum calcium for this 999 ABCM patient *test set*. Three independent *validation* data sets of 599 ABCM based chemotherapy patients from the 8th MRC trial, 845 melphalan patients from the 4th and 5th MRC trials and 271 non-randomised ABCM patients from the MRC 6th trial were used to evaluate MRC6PI as well as compare against Sβ2m, Durie-Salmon index, MRC Cuzick index and IPI. Logistic regression was used to determine predictive ability of MRC6PI for short (6 months), medium (2.5 years) and long-term (7.5 years) survival. The first three factors of MRC6PI, i.e. Sβ2m, age and performance status, were combined to develop a simple 3-factor model (MRC3PI).

Results. MRC6PI was the best discriminator between good, intermediate and poor risk groups for overall survival when compared to Sβ2m, Durie-Salmon index, MRC Cuzick index and the IPI, for both the test set and the three independent validation sets. Although all the staging systems were highly significantly associated with overall survival (log-rank p<0.001), MRC6PI was the most significant and Durie-Salmon the least; Durie-Salmon and IPI tended to classify a smaller percentage of patients into the good risk group. The predictive ability of MRC6PI for short, medium and long-term survival ranged from 62-74% overall correct. MRC3PI simple score was also shown to be a good predictor of overall survival with a similar predictive ability as MRC6PI.

Conclusions. MRC6PI was shown to be the best discriminator of good, intermediate and poor risk groups for overall survival when compared to the other reported staging systems. Logistic regression indicated that MRC6PI had a good predictive ability. We recommend that Sβ2m, age, performance status, blood urea, platelets and corrected serum calcium, i.e. the six components of this MRC6PI, are recorded in all future clinical trials. The simple MRC 3-factor score could be considered as a simple alternative in the clinic.

P0.310

POORER SURVIVAL WITH AGE IN MYELOMA IS CLOSELY LINKED TO HIGHER INTERNATIONAL STAGING SYSTEM STAGE AT PRESENTATION BUT NOT CORRELATED WITH OTHER KNOWN INDEPENDENT RISK FACTORS

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Up to now no systematic analysis on the impact of different age categories on survival in patients with multiple myeloma has been reported. Information on possible correlations of host and tumor related prognostic factors with different age categories are lacking. We studied these parameters in a large cohort of patients with multiple myeloma (n=10.750) submitted by participating institutions and groups in the international staging system (ISS) project. Prognostic factors were recorded and age was calculated at start of initial chemotherapy. Patients were grouped into 6 age cohorts (<40, 40-<50, 50-<60, 60-<70, 70-<80 and ≥80 years). p values were calculated with the Jonckheere-Terpstra test and Spearman's correlation coefficient was used where appropriate. The sequential median survivals constantly decreased by decade from 61 months to 60, 53, 40, 32 and 24 months in the 6 patient cohorts from age < 40 years to age >80 years examined, respectively, with a median value of 44 months (p<0.0001). The distribution of prognostic factors by age revealed a highly significant correlation between high serum β_2 microglobulin (≥3.5 mg/dL) and age, ranging from 45% in patients in the youngest to 75% of patients in the oldest age cohort (r=0.17 (0.15-0.19), p<0.0001). A similar correlation was seen between low serum albumin (<3.5 g/dL) and age: the proportion of patients with low serum albumin levels increased from 32% in patients at age < 40 years to 54% in patients > 80 years (r=-0.11(-0.13,-0.09), p<0.0001). Consequently, as $S\beta_2M$ and serum albumin constitute the prognostic parameters of the ISS, a close correlation between ISS stage and age was found (p<0.0001). The proportion of patients with ISS stage I ($S\beta_2M$<3.5 mg/dL and serum albumin ≥3.5 mg/dL) was 40% in patients aged <40 years and only 12% in those aged ≥80 years. In contrast, 44% of patients of the oldest and 31% of the youngest age cohort presented with ISS stage III. In addition, a similar, albeit lesser trend was noted for decreasing hemoglobin with age (r=-0.08(-0.10,-0.07, p<0.0001) and increasing serum creatinine with age (r=0.08(0.06, 0.10), p<0.0001). The parameters reflecting the biology of the myeloma clone did not vary between different ages cohorts. Bone marrow plasma cell infiltration (BMPC) ≥33%, CRP levels >=0.8 (mg/dL) and normal LDH was seen in similar frequencies in the different age categories. Similarly, no age dependent variation in cytogenetically defined prognostic variables was seen. The proportion of patients with Del 13 and of those with t(11; 14),t(4; 14) did not differ between the different age categories (these data were obtained in a limited number of patients only (616, 544 and 418 patients, respectively). In conclusion, age was identified as important prognostic factor in the six different age cohorts examined. Poorer survival

with higher age is closely linked to higher ISS stage. In addition, creatinine and low haemoglobin correlate, albeit to a lesser degree, with increasing age, but not other known risk factors such as those reflecting adverse biologic features of the tumor clone (LDH, BMPC, CRP, del 13, t(11;14) t(4;14).

P0.311

SIGNIFICANCE OF NEW INTERNATIONAL STAGING SYSTEM FOR SURVIVAL AFTER AUTOLOGOUS TRANSPLANTATION: A SINGLE CENTER EXPERIENCE IN 133 MULTIPLE MYELOMA PATIENTS

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Background. Reliable and simple staging of MM is important for accurate prognostic evaluation and for the comparison of data from different clinical trials. Attempts to improve the widely accepted Durie-Salmon (DS) staging system have led to the development of numerous new prognostic systems, that have not been universally accepted. Recently new International Staging System (ISS) was presented by Greipp *et al.,*[1] It has shown promise in patients treated by conventional as well as high-dose chemotherapy and is based on a simple combination of serum β_2-microglobulin (β2M) and albumin (alb) values (stage 1 = β2M < 3.5 mg/L and alb ≥3.5 g/dL; stage 2 = β2M < 3.5 mg/L and alb <3.5 g/dL, or B2M ≥3.5 mg/L to <5.5 mg/L; stage 3 = β2M ≥5.5 mg/L).

Methods and Results. We have retrospectively analysed 133 patients with MM undergoing autologous transplant (ASCT) in our center. All patients had the same therapy and were transplanted to one year after diagnosis. The aim of our analysis was to evaluate both ISS and DS systems in our set of patients. Clinical stages according to DS were as follows: stage I in 16 patients (12%), stage II in 17 cases (13%) and stage III in 100 cases (75%). Among the 100 patients with DS III, there were 94 patients with multiple osteolytic lesions. The size and number of bone lytic lesions varied considerably in DS III subgroup. Clinical stages according to ISS were the following: stage 1 in 48 patients (38%), stage 2 in 56 cases (45%), and stage 3 in 21 cases (17%). Initial values of β2M and albumin were not available for 8 patients. Patients with clinical stage III according to Durie-Salmon had stage 1 according to ISS in 28% of cases, ISS stage 2 in 51% of cases and ISS stage 3 in 21% of cases. Median OS of patients with DS stage I was 67.6 months, with DS stage II 71.0 months, and with DS stage III 71.3 months. Differences in survival among patients with clinical stages according to DS system were not statisticaly significant. Median OS of patients with ISS stage 3 was 23.6 months, with ISS stage 2 57.5 months, and with ISS stage 1 72.8 months. Patients with ISS stage 3 had significantly shorter PFS and OS than others (p=0.007, p=0.005) in our group of patients. The differences in PFS and OS between ISS stages 1 and 2 were not significant.

Conclusions. In our group of patients the survival after ASCT correlated with the stage according to ISS. Our patients with ISS stage 3 had poor prognosis. We found no significant correlations between DS staging system and survival after transplantation.

References

1. Greipp PR, Miguel JF, Durie BG et al. New International Staging System (ISS) for multiple myeloma from the International Myeloma Working Group. Blood 2003; 102: 190a (abstr. 664).

P0.312

VALIDATION OF THE INTERNATIONAL STAGING SYSTEM FOR MULTIPLE MYELOMA: A RETROSPECTIVE ANALYSIS OF 172 PATIENTS AT TWO BRAZILIAN CENTERS

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Introduction. The survival of patients with multiple myeloma varies from a few months to more than 10 years. This heterogeneity is related to the characteristics of the myeloma itself and of the host. The identification of the factors which influence the prognosis is very important to predict the result, assist in the choice of the treatment and adequately stratify the patients in clinical studies. Many prognostic factors have been identified in patients with multiple myeloma, such as anemia, renal failure, β_2 microglobulin, albumin and chromossomic alterations. Some authors have combined prognostic factors and proposed various systems of staging. However, none of them have yet substituted the Durie-Salmon staging system. Recently, the International Myeloma Working Group, with the objective of developing a simple and reliable staging system, which can be internationally applied to classify and stratify patients with multiple myeloma, identified 3 risk grooups. This new system of staging, the *International Staging System* (ISS), consists of stage I: β_2 microglobulin < 3.5 mg/L plus albumin ≥3.5 g/dL (median survival: 62 months); stage II: neither I nor III (median 44 months); stage III: β_2 microglobulin > 5.5 mg/L (median 29 months).

Objective. To validate the ISS in patients with multiple myeloma at Brazilian centers.

Patients and Methods. One hundred and seventy-two patients with the diagnosis of multiple myeloma within the period of 1998 to 2002 at Santa Casa de São Paulo Hospital and Hospital das Clínicas de São Paulo, with available data on albumin and ß2 microglobulin, were stratified according to the ISS. A total of 150 patients received standard therapy and 22 received high-dose therapy as initial therapy. The survival was estimated using the Kaplan-Meier method with differences in survival examined using the logrank test.

Results. The median age of the patients was 63 years, 52% female and 48% male. In Stage I (n=23), the global median survival was 53 months, in stage II (n=73), 43 months and in stage III (n=76), 23 months (p<0.001).

Conclusions. The new system of staging for multiple myeloma (ISS) is simple, based on variables easy to be performed and was possible to be validated in patients with multiple myeloma at two Brazilian centers.

P0.313

NEW INTERNATIONAL STAGING SYSTEM APPLIED IN 588 PATIENTS: EXPERIENCE OF THE CZECH MYELOMA GROUP BASED ON ANALYSIS OF THE ONE MULTICENTER TRIAL (4W) AND TWO SINGLE CENTER EXPERIENCES

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Background. Recently new International Staging System (ISS) was presented by Greipp *et al.* It has shown promise in patients treated by conventional as well as high-dose chemotherapy and is based on a simple combination of

serum β_2-microglobulin and albumin values. We compared ISS with Durie-Salmon staging system (DS) in the 3 large cohorts of patients.

Methods and Results. We have retrospectively analyzed 270 pts. who underwent conventional treatment in Olomouc (OL) and 133 pts. who underwent autologous transplantation (AT) in Brno (BR). These two cohorts represented single center experience. Also 185 pts. generated from multicenter trial 4W of Czech Myeloma Group were evaluated. All patients from AT groups (BR and 4W) were pts. with newly diagnosed MM and were transplanted no later than one year after diagnosis. The aim of our analysis was to evaluate ISS in our set of patients. Clinical stages according to DS were as follows: OL/BR/4W trial stage I – 16%/12%/11%, stage II -41%/13%/20%, stage III – 43%/75%/69%. Clinical stages according to ISS were the following: OL/BR/4W stage 1 – 21%/38%/43%, stage 2 -31%/45%/36%, stage 3 – 48%/17%/21%. No ISS stage 3 occurred in the clinical stage I and II in both AT groups except 10% of ISS stage 3 in DS II of the trial 4W. Differences in survival among patients with clinical stages according to DS system were not statistically significant in both AT groups. Using ISS medians of OS were as follows (OL/BR/4W): for pts. with ISS stage 3 was 20.0/23.6/45.7 months, with ISS stage 2 – 31.1/57.5/77.7 months and with ISS stage 1-77.3/ 72.8 months/not reached in 4W trial. Patients with ISS stage 3 had shorter OS than others and this was statistically significant for both single center analyses (Olomouc; p=0.005 ISS 2 vs. ISS 3, p<0.001 ISS 1 vs. ISS 3; Brno; p=0005). In multicentric trial 4W strong trend for shortening of OS in pts. with ISS stage 3 was observed (p=0.07) with significance if evaluated for event free survival (p=0.0039). The differences in OS between ISS stages 1 and 2 were not significant in both AT groups (BR and 4W) but in conventional group (OL; p<0.001 ISS 1 vs. 2).

Conclusions. We have shown that in the hands of a single centers and a single trial group, the outcome of AT as well as of conventional treatment correlated with the stage according to ISS, especially with the category of ISS stage 3.

References

1. References: Greipp PR, Miguel JF, Durie BG et al. New International Staging System (ISS) for multiple myeloma from the International Myeloma Working Group. Blood 2003; 102: 190a (abstr. 664)

P0.314

PROGNOSTIC FACTORS AND OUTCOME OF OLDER PATIENTS WITH SYMPTOMATIC MULTIPLE MYELOMA: A STUDY OF THE GREEK MYELOMA STUDY GROUP

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Introduction. Prospective randomized trials have shown that high dose therapy with autologous stem cell transplantation may improve the survival of patients with multiple myeloma (MM). This procedure is usually applied to patients ≤70 years of age. Nevertheless, many patients with MM are diagnosed at an older age and are usually treated with conventional chemotherapy. We performed a retrospective analysis in order to compare the prognostic factors and outcome of younger patients with myeloma (≤70 years) with those of older patients (>70 years). We also applied the recently proposed International Staging System (ISS) for multiple myeloma in the two groups of patients.

Patients and Methods. Since 1987, 1162 patients with symptomatic myeloma requiring treatment have been included in the data base of GMSG. Patients with asymptomatic myeloma were not included. 357 patients (31%) were > 70 years at the time of initial treatment. Multiple clinical and laboratory variables were evaluated in patients >70 years of age and in younger patients. Furthermore, the same variables were assessed for possible correlation with prognosis in patients >70 years of age.

Results. The only variable that was different between the two groups of patients was the ISS with older patients presenting more often with advanced ISS (p=0.02). Older patients received more often primary treatment with standard alkylating agents - corticosteroid combinations whereas younger patients received more often primary treatment based on high dose pulse dexamethasone (VAD etc) (p=0.001). Response to treatment was similar but patients ≥70 years had a median survival of 40 months versus 29 months for older patients (p<0.001). Variables associated with shorter survival for the group of older patients were: anemia, thrombocytopenia, hypercalcemia, renal impairment, more extensive bone marrow plasmacytosis, elevated serum LDH and advanced ISS (2+3). A Cox regression analysis showed, that adjusting for the effects of other factors, elevated LDH, bone marrow plasmacytosis and advanced ISS retained prognostic significance in this group of older patients (median survival of 12 months, 22 months and 21 months respectively). However patients >70 years of age with ISS 1 had a relatively long median survival of 55 months.

Conclusions. The clinical and laboratory characteristics of older patients with MM are similar to those of younger patients but older patients tend to present with more advanced ISS. The survival of older patients is shorter than that of younger patients. Elevated serum LDH, advanced ISS, and increased bone marrow plasmacytosis may identify older patients with particularly bad prognosis. Older patients with poor prognosis should be entered into clinical trials in order to see if treatment with new agents or combinations would prolong their survival. The application of ISS in patients >70 years of age permitted the identification at diagnosis of a subgroup of patients with ISS 1 who had a relatively good prognosis.

P0.315

INTERNATIONAL PROGNOSTIC INDEX – A CRITICAL COMPARISON WITH FIVE OTHER MULTIPLE MYELOMA STAGING SYSTEMS IN PATIENTS TREATED BY CONVENTIONAL CHEMOTHERAPY

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Background. Multiple myeloma (MM) is a very heterogeneous disease requiring an individual choice of therapy based on the staging results at the time of diagnosis.

Patients and Methods. In the group of 271 patients with MM treated between 1991-2004 by conventional chemotherapy prognostic value and practical utility of IPI in comparison with five selected multiple myeloma staging systems were assessed.

Methods and Results. The prognostic significance was assessed using the overall survival curves constructed according to Kaplan-Meier and the log rank test (p<0.05). The practical utility and prognostic significance of Durie-Salmon system were confirmed by different overall survival

(OS) medians (stage I-III: OS medians 76.8, 41.0 and 14.7 months, $p<0.001$; substages A-B: OS medians 44.3 and 11.0 months, $p<0.001$). The simple staging systems based on measurement of S-beta2 microglobulin and S-albumin levels were proved to be advantageous e.g. Bataille system (stages 1-3: OS medians 69.0, 23.8 and 11.0 months, $p<0.001$) and Hussein system (stages 1-4: OS medians 76.4, 63.1, 21.0 and 8.2 months, $p<0.001$). Modified scoring system after San Miguel, which uses apart from the age and S-beta$_2$microglobulin level also the propidium-iodide proliferative index (PI/CD$_{138}$) of plasmocytes, seems to be very promising (stages 1-3: OS median of stage 1 was not up to now achieved, of the stages 2-3 were 32.3 and 5.6 months, $p<0.001$). If the examination of PI/CD$_{138}$ is not accesible, we suggest to use our Olomouc's simple staging system based on measurement of S-beta$_2$microglobulin and S-thymidinekinase levels (stages 1-3: OS medians 68.5, 24.9 and 5.0 months, $p<0.001$). In our study we confirmed a good prognostic significance of IPI (stage 1-3: OS medians 77.3, 31.1 and 20.0 months, $p<0.001$). But in comparison with five other analyzed systems (stages 3 OS medians: 14.7, 11.0, 8.2, 5.6 and 5.0 months) relatively long OS median of stage 3 (20.0 months) was observed.

Conclusions. This study has confirmed the prognostic significance of standard staging systems after Durie-Salmon, Bataille, Hussein and substantially the advantageous results of staging systems based on proliferative properties of myeloma cells measurement after San Miguel and Olomouc. Our analysis confirmed generally good prognostic significance of IPI, but it failed to identify the group of patients with very bad prognosis with very short OS in comparison with five other analyzed systems.

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PO.316

NEW INTERNATIONAL STAGING SYSTEM EVALUATED IN PATIENTS UNDERGOING AUTOLOGOUS TRANSPLANTATION: EXPERIENCE OF THE CZECH MYELOMA GROUP

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Background: Recently a new International Staging System (ISS) was presented by Greipp et al. It has shown promise in patients treated by conventional as well as high-dose chemotherapy and is based on a simple combination of serum β2-microglobulin and albumin values. We compared ISS with Durie-Salmon staging system (DS) in the two large cohorts of patients who under underwent autologous transplantation (AT).

Methods and Results: We have retrospectively analyzed group of 133 pts. represented single center experience (BRNO) and 185 pts. generated from multicentric trial 4W of Czech Myeloma Group. All patients with newly diagnosed MM had the same therapy and were transplanted to one year after diagnosis. The aim of our analysis was to evaluate both ISS and DS systems in our set of patients. Clinical stages according to DS were as follows: BRNO/4W trial stage I - 12%/11%, stage II -13%/20%, stage III - 75%/ 69%. Clinical stages according to ISS were the following: BRNO/4W stage 1 - 38%/43%, stage 2 - 45%/36%, stage 3 - 17%/21%. Initial values of β2-microglobulin and albumin were not available for 6% pts. from Brno, 13% of pts. from trial 4W. No ISS stage 3 occurred in the clinical stage I and II in both groups except 10% of ISS stage 3 in DS II of the trial 4W. Median OS of pts. was: for single center experience BRNO/4W trial DS stage I - 67.6/76,6 months, DS stage II - 71.0/82.5 months, DS stage III - 71.3/67.3 months. Differences in survival among patients with clinical stages according to DS system were not statistically significant in both groups. Medians of OS were as follows (BRNO/4W): for pts. with ISS stage 3 was 23.6/45.7 months, with ISS stage 2 - 57.5/77.7 months and with ISS stage 1 72.8 months/not reached in 4W trial. Patients with ISS stage 3 had significantly shorter EFS and OS than others and this was statistically significant for single center (BRNO; p=0007 for EFS; p=0005 for OS) as well as multicentric trial (4W; p=0.0039 for EFS; p=0.07 for OS). The differences in EFS and OS between ISS stages 1 and 2 were not significant.

Conclusions. We have shown that in the hands of a single center and a single trial group, the outcome of AT correlated with the stage according to ISS. Especially, the cathegory of ISS stage III was very useful to define subgroup with poor prognosis. We have not found any significant correlation between DS staging system and survival after transplantation.

References

1. Greipp PR, Miguel JF, Durie BG et al. New International Staging System (ISS) for multiple myeloma from the International Myeloma Working Group. Blood 2003; 102: 190a (abstr. 664).

PO.317

SOLUBLE SYNDECAN-1 (CD138) LEVEL AT DIAGNOSIS IS AN INDEPENDENT PROGNOSTIC MARKER IN 324 MULTIPLE MYELOMA PATIENTS AND THE EXTENT OF FALL FROM DIAGNOSIS TO PLATEAU PREDICTS OUTCOME

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Syndecan-1 (CD138) is a heparin sulphate proteoglycan that is overexpressed on the surface of malignant plasma cells and actively shed from the cell surface. It is involved in cell-cell adhesion, cell-matrix adhesion and in modulating the signalling of heparin binding growth factors. A study of 138 myeloma patients (Seidel *et al.,* 2000 Blood 95, 388-392) showed that soluble syndecan-1 (sCD138) was a new independent prognostic marker and split their patients into two groups (above and below 1170 units/mL) with highly significant survival differences. Using a myeloma mouse model, sCD138 was shown to have biological activity, supporting myeloma tumour growth, indicating that cCD138 was not just a marker of tumour bulk. Its role in prognostic classification systems needed further clarification. We have retrospectively measured sCD138 in 324 presentation samples and 247 plateau phase samples from the UK Medical Research Council Myeloma VI trial using a commercial CD138 Elisa kit (Diaclone Research, Besancon, France). Log-rank analysis showed that presentation value of sCD138 is a highly significant prognostic factor when assessing survival from entry (χ^2=14.92, p=<0.0001) and remains an independent prognostic factor (χ^2 to-remove=6.57, p=0.01) when considered in a Cox regression model with the individual components of the new Medical Research Council 6-factor prognostic index (MRC6PI), i.e. Sβ2m, performance status, age, hospital blood urea, platelets and calcium, and again with the calculated MRC6PI (χ^2 to-remove=5.76, p=0.016). The magnitude of fall in sCD138 from presentation to

plateau also had prognostic value when assessing duration of plateau (χ^2=4.47, p=0.03), in contrast to most myeloma studies that have shown no correlation between the magnitude of response to induction chemotherapy and outcome.

In conclusion this large study indicates that sCD138 levels is a powerful independent prognostic factor that needs to be explored in future prognostic classification systems.

PO.318

FACTORS PREDICTING DURATION OF FIRST REMISSION AND SURVIVAL FROM RELAPSE FOR PATIENTS RECEIVING NON-INTENSIVE CHEMOTHERAPY IN UK MEDICAL RESEARCH COUNCIL MYELOMA TRIALS

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Introduction. For newly diagnosed patients with multiple myeloma, overall survival is the most important endpoint together with a good quality of life. The duration of first stable remission/plateau phase provides an early assessment of efficacy of first line treatment but when analysing randomised trials using this endpoint it is important to assess prognostic factors for this patient group. There are increasing numbers of randomised trials to assess the efficacy of second line therapy in patients relapsing from first plateau phase; this necessitates identification of prognostic factors to determine survival from relapse. The aim was to identify prognostic factors that predict duration of plateau and survival from relapse as well as assessing the value of re-measuring prognostic factors at first plateau and relapse.

Methods. 2734 patients randomised to MRC trials 1980-2002 received melphalan (856 patients) or ABCM (1869 patients) based first line therapies and 1586 patients (58%) achieved plateau phase with an overall survival of 3.9 years (IQR 2.4-6.1 years). Clinical and laboratory factors were recorded at entry to the trial, plateau and relapse. Log-rank and Cox regression analyses were used to identify important prognostic factors predicting duration of plateau and survival from relapse. The newly developed MRC 6-factor prognostic index (MRC6PI) comprising Sβ2m, age, performance status, blood urea, platelets and corrected serum calcium was also considered.

Results. Log-rank analyses identified performance status, blood urea, Sβ2m, lytic lesion, bone pain and bone marrow plasma cells (log-rank p<0.0001) having the most significant association with duration of plateau; paraprotein level, urinary free light chain, platelets, haemoglobin, corrected serum calcium and serum creatinine having a weaker association (log-rank p<0.01). Cox regression identified performance status, bone marrow plasma cells and serum creatinine as independent factors but when MRC6PI was considered it was the single most important factor. Sβ2m, performance status, serum creatinine, bone pain and paraprotein levels at the time of plateau were assessed for their value in predicting duration of plateau, only performance status was of any value (p=0.001). The most influential factors associated with survival from relapse were performance status, Sβ2m and paraprotein class distribution (log-rank p<0.01) with MRC6PI again being the most significant factor in a Cox regression. Sβ2m, performance status and bone-pain were measured at relapse and all found to be significantly more informative that their corresponding values at entry and plateau.

Conclusions. The newly developed MRC6PI was the single most important factor when assessing both duration of plateau and survival from relapse; the individual components should be recorded in all future clinical trials. Sβ2m, performance status and bone pain measurements at relapse were significantly associated with prediction of survival from relapse and performed much better than their corresponding entry and plateau values.

PO.319

PROGNOSTIC IMPACT OF SERUM YKL-40 LEVEL IN PATIENTS YOUNGER THAN 60 YEARS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background. YKL-40 is a lectin secreted by inflammatory cells and cancer cell lines. Gene expression is found in several different types of solid carcinomas and in some of these an elevated serum YKL-40 is an adverse prognostic factor, but the biological function in cancer is not known. YKL-40 secretion in haematopoietic cancers has not been investigated.

Materials, Methods and Results. Serum YKL-40 (sYKL-40) was determined by ELISA in 82 patients (pts) aged less than 60 years with newly diagnosed, symptomatic multiple myeloma (MM) eligible for intensive treatment. Of these pts 24% had an elevated sYKL-40, and there was a relation to the International Staging System ISS (13% of pts in stage I, 22% of pts in stage II, and 50% of pts in stage III; p =.02). The group of pts with elevated sYKL-40 had a higher median s-β_2-microglobulin (s-β_2M) and fewer pts with M-protein class IgA, but did not differ significantly from the group of pts with normal sYKL-40 in any other of the registered baseline characteristics, treatment actually received or response to intensive treatment. sYKL-40 was correlated to age, s-creatinine, s-C-reactive protein and especially s-β_2M. Median follow-up for surviving pts was 63 months (mths). Median overall survival (OS) for pts with normal sYKL-40 was 74 mths versus 44 mths for pts with elevated sYKL-40. The difference in OS was not significant (p=.07). Event free survival (EFS) was significantly shorter for pts with elevated sYKL-40 (p=.04) with a median EFS of 17 mths versus 33 mths for pts with normal sYKL-40. Baseline characteristics significant for survival in univariate analysis were entered together with sYKL-40 level and ISS stage in a multivariate Cox analysis. Independent predictors of OS were s-β_2M and s-lactate-dehydrogenase, but the only independent predictor of EFS was s-β_2M. In order to identify YKL-40 secreting cells in MM cDNA archives were generated by global RT-PCR from bone marrow (BM) plasma cells (PC) in 7 controls (nonMM), from BM myeloma cells (MC) in 7 pts with MGUS, 46 pts with intramedullary MM and 8 pts with extramedullary MM (exMM), from MC in the extramedullary manifestation (EM) in 6 of the 8 pts with exMM, from 7 different human myeloma cell lines (HMCL), and from BM stromal cells (SC) in 7 pts with MM and 3 controls. PC and MC had been identified and sorted by flow cytometry. YKL-40 gene expression was analysed by QRT-PCR and found in MC from EM in 3 pts with exMM, in 6 HMCL and in SC from all investigated. The level of expression in SC did not differ in MM and nonMM. Further studies to identify YKL-40 secreting cells in MM are ongoing.

Conclusions. Abnormal amounts of YKL-40 are secreted in a subset of pts with newly diagnosed multiple myeloma, and sYKL-40 is correlated to s-β_2M. In this study the sYKL-40 level had prognostic impact on event free survival. YKL-40 is not secreted by intramedullary MC, but possibly by extramedullary MC or by SC in the bone marrow microenvironment.

P0.320

PROGNOSTIC EFFECT OF LABORATORY CORRELATES IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH THALIDOMIDE

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Background. The level of bone marrow angiogenesis has been reported to be an important prognostic factor for survival in patients (pts) with multiple myeloma (MM). The mechanism of action of thalidomide (thal) in treating MM is multi-factorial but is thought to include an anti-angiogenic effect. We aimed to assess the effect of thal treatment on various laboratory correlates involved in angiogenesis and other aspects of MM biology.

Methods. 75 pts with relapsed/refractory MM were enrolled in a prospective multicenter phase 2 trial using thal ±interferon-alpha-2B. Platelet poor plasma (PPP) plus a bone marrow aspirate and trephine (BMATx) was collected prior to trial entry and then 3 monthly during thal treatment. Each BMATx was stained using immunohistochemistry for CD34, mast cell tryptase and CD57. Microvessel density (MVD) was assessed as the average number of vessels counted in 3 high-powered fields. ELISA analysis was performed on PPP for vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin-6 (IL-6) and hepatocyte growth factor (HGF). Objectives were to examine the effect of these parameters on response rate (RR), progression-free (PFS) and overall survival (OS) and to determine how these parameters changed during thal treatment.

Results. Overall RR was 28% with 55% stable disease. The only factor predictive of response was VEGF, with zero responses seen in pts with a level of 0 vs 34% in those with a level > 0 (p=0.015). MVD did not predict for response to thal, PFS or OS. However, pts achieving a response or SD had higher baseline MVD than those who progressed, with a median CD34 count of 22 (0-89) vs 12 (1-20); p=0.01. The level of MVD fell significantly in responding pts from a median baseline count of 21 (7-71) to 10 (4-20) at best response (p=0.002), and rose in pts who progressed on study from 10 (1-130) to 20 (1-113) (p = 0.027). VEGF levels also fell in responding pts from a median of 65.8 pg/mL (9.2 –562.4) to 43.3 (0–208.1) at best response (p=0.024). Levels of bone marrow mast cells increased significantly in responding pts. There was no significant change in numbers of CD57 positive cells over time. Univariate analysis demonstrated inferior PFS in pts with low baseline VEGF (p = 0.027); and inferior OS in pts with raised baseline levels of IL-6 (p=0.014), raised baseline HGF (p=0.016) and zero baseline CD57 positive cells (p=0.003). Multivariate analysis for OS, including relevant clinical parameters, demonstrated age > 65 years (p=0.009), raised serum lactate dehydrogenase (p=0.008) and zero baseline CD57 positive cells (p=0.011) predicted inferior outcomes.

Conclusions. Levels of VEGF and MVD decline with successful thal treatment, suggesting an anti-angiogenic effect. However, high baseline angiogenic activity was not necessary to obtain a response. Inferior PFS was seen in pts with low baseline levels of VEGF. The clinical parameters of increased age and LDH remain important predictors of OS, in addition of levels of IL-6, HGF and numbers of CD57 positive cells in the bone marrow.

P0.321

PROGNOSTIC INFLUENCE OF ANTIGENIC MARKERS IN 587 MULTIPLE MYELOMA PATIENTS UNIFORMLY TREATED WITH HIGH DOSE THERAPY

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Conflicting results have been reported on the prognostic impact of antigenic markers in multiple myeloma (MM). These discrepancies may be due to technical pitfalls (eg. use of single vs multiparametric labelling, differences in the clones of monoclonal antibodies (MoAb) and fluorochromes, criteria for definition of positivity) and the heterogeneity of treatments used. In order to analyse the prognostic influence of antigenic markers and their correlation with other disease characteristics we have analysed, in a large series of 587 newly diagnosed MM patients, the antigenic profile of plasma cells (PC), using a panel of MoAb in quadruple antigenic combinations. CD38 and CD138 were included in all combinations for identification of PC, together with the following markers: CD19, CD20, CD28, CD33, CD45, CD56 and CD117. All patients were uniformly treated according to the GEM-2000 protocol that included six courses of VBMCP/VBAD followed by high dose melphalan (200 mg) with stem cell support. The median of event-free survival (EFS) and overall survival (OS) of the whole series of 587 patients was 37 and 58 months, respectively. The frequency of the different markers analysed was as follows: CD19 (present in 7% of patients), CD20 (18%), CD28 (38%), CD33 (20%), CD45 (15%), CD56 (70%) and CD117 (34%). Patients lacking CD56 antigen showed an adverse prognosis defined by both a shorter EFS (31 vs 40 months, p=0.05) and OS (50 vs 58m, p=0.01). The expression of CD117 was associated with a favourable outcome (EFS: 41 vs 31m, p=0.008, and OS: NR vs 58m). The opposite situation was observed for CD28 expression, associated with a shorter EFS (31 vs 41m, p=0.04) but no significant differences for OS (51 vs 58m). Patients with CD19 expression also showed shorter EFS (p=0.01) and OS (p=0.01). CD56 and CD117 negative patients showed higher BM infiltration, B2M, hypercalcemia, thrombocytopenia, non-hyperdiploid DNA PC content and advanced ISS. Using multivariate analysis the following variables were selected: ISS, CD19, ploidy status and S-phase. In summary, our results, obtained in a uniform and large series of patients treated with high dose therapy, show that the antigenic profile of PC may have more influence on disease outcome than previously supposed.

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P0.322

PROGNOSTIC FACTORS FOR PROGRESSION OF SOLITARY PLASMACYTOMA: A LARGE MULTI-CENTER STUDY

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Prognostic factors for patients with plasmacytoma are not fully understood. This study aims to define factors predicting outcome for patients diagnosed with a solitary plasmacytoma. A large cohort of patients was identified from 3 different regional referral centers in the UK. Sixty-five patients were studied retrospectively, including 49 with solitary bone plasmacytoma (SBP) and 16 with solitary extramedullary plasmacytoma (SEP). Median age was 53 years (range 21 to 81) and 60 years (range 33 to 88) respectively. There was a male predominance in both groups. Twenty-six patients (53%)with SBP had axial disease, 12 patients (75%) with SEP had head and neck disease. Forty-eight patients with SBP received radiotherapy (median dose 40 Gy range 20 to 50 Gy), 23 had prior surgery and 14 chemotherapy. Fourteen patients with SEP received radiotherapy (median dose 40 Gy range 27 to 50 Gy), 5 patients had surgery and 4 had chemotherapy.

Results. SBP patients: Serum paraprotein was present in 27 of 47, Bence Jones protein (BJP) in 7 of 30 and immuneparesis in 5 of 30 evaluable patients. Local relapse occurred in 6 patients (at a median of 24 months), relapse at another site occurred in 7 patients (at a median of 30 months). Twenty-four patients (49%) progressed to multiple myeloma (MM) at a median of 28 months (range 5 to 220 months), including 5 patients who had previous isolated relapses. Median follow up from progression to MM is 8 months. Thirty-seven patients remain alive with a median follow up of 34 months (range 2 to 226 months). Seven patients died of disease related causes. Immuneparesis at diagnosis predicted progression to MM (p=0.032). Five patients with immuneparesis at diagnosis progressed to MM, whereas no immuneparesis was found in those patients that did not progress to MM. Although not statistically significant there is a suggestion that disease in the axial skeleton at diagnosis predicts progression to MM (p=0.061). Age, paraprotein level at diagnosis, radiotherapy dose, use of surgery or chemotherapy and disappearance of paraprotein at 1 year did not predict for progression to MM. All patients with isolated relapse were less than 55 years old at diagnosis. No factors predicted relapse at original or distant site.

SEP patients: Paraprotein was present in 5 patients, 1 with BJP only. There was no immuneparesis at diagnosis. Four patients had isolated relapses (2 local, 2 at distant sites). Three patients progressed to MM at a median of 18 months (range 16 to 23 months). Eleven patients remain alive with a median follow up of 23 months (range 3 to 252 months). Two patients died of disease related causes. No factors were found to predict disease relapse or progression to MM.

Conclusions. Data are presented on a large cohort of patients. Our results suggest immuneparesis at diagnosis in a patient with SBP predicts progression to MM.

PO.323

PROGNOSTIC SIGNIFICANCE OF THE PLASMA CELLS PROPIDIUM IODIDE, ANNEXIN-V/CD138 INDICES AND THEIR MUTUAL RATIO IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is a clonal neoplastic lymphoproliferative disease affecting terminally differentiated B-cells, i.e. plasmocytes, which typically display a slow proliferative activity and different resistance to apoptosis with latent accumulation of myeloma cells in the bone marrow. The results of the past years studies imply, that in the evaluation of valid prognosis of the multiple myeloma patients the most important role play those indices, which have immediate relationship to the biological properties of myeloma cells.

Aim. The aim of submitted study was to evaluate the prognostic importance i.e. the medians (M) of the overall survival (OS) of the kinetic parameters of the myeloma cells, i.e. the proliferation and apoptotic indices.

Methods. Analyzed group consists of 122 myeloma patients examined at the time of MM diagnosis before the start of conventional chemotherapy. Plasma cell proliferative activity was measured by means of a propidium iodide index (PI) examined by flow cytometry using a DNA/CD_{138} double staining technique. For detection of plasma cells entering apoptosis (AI) flow cytometry method with annexin-V FITC and MoAb CD_{138} was used. The statistical significance was evaluated using the Kaplan-Meier method and log rank test (p<0.05).

Results. In our analyzed group there was found optimum of the PI/CD138 index cut off value 2.9%, divided our patients into two different prognostic groups: for PI/CD_{138} ≥2.9% - OS median was 17 months, for <2.9% - OS median was not in the time of statistical analysis achieved yet, p=0.031). In the case when the cut off of the AI/CD_{138} was 4.4% we obtained also two different prognostic groups: for AI/CD_{138} ≤4.4% - OS median was 23 months, for >4.4% - OS median was not up to now achieved, p=0.022). The most optimum cut off of the PI/AI ratio index was 0.71. This summary kinetic index of the myeloma cells also enables the differentiation of our patients into two groups with different prognosis: for PI/AI index ≥0.71 – OS median was16 months, for <0.71 – OS median was not achieved so far, p=0.032).

Conclusions. These results suggest, that in clinical practice not only proliferative but also the apoptotic properties and PI/AI index expressing the comprehensive proliferative and apoptotic properties of the myeloma cells are important for the prognosis of multiple myeloma patients.

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PO.324

A REVIEW OF THE CYTOKINE NETWORK IN MULTIPLE MYELOMA: DIAGNOSTIC, PROGNOSTIC, AND THERAPEUTIC IMPLICATIONS

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Because many studies have focused on growth factors in multiple myeloma, the study of the cytokine network appears to be useful for this purpose. Interleukin-6 (IL-6) and IL-2 with their soluble receptors IL-3, IL-4, IL-10, IL-11 have been examined. Plasma cells may produce IL-6 by an autocrine mechanism whereas a paracrine mechanism is believed to be involved in the production of IL-6 by bone marrow stromal cells through an interaction between adhesion molecules present on myeloma plasma cells and their respective receptors that are present on bone marrow stromal cells. Control over production of IL-6 may be exerted by other Ils such as IL-1β and IL-10. Evaluation of the serum level of IL-6, C reactive protein, soluble IL-6 receptor (sIL-6R), and soluble IL-2 receptor (sIL-2R), together with the activity exerted by IL-3 and IL-4 on some cellular subsets, may constitute an element in the differential diagnosis between multiple myeloma and other limphoproliferative disorders. Serum levels of IL-6, sIL-6R, sIL-2R and the expression of membrane-bound IL-2 receptors, both on bone marrow

plasma cells and on peripheral blood mononuclear cells, are correlated with disease activity and disease stage. IL-6 and IL-6R serum levels are believed to be correlated with the duration of disease-free survival because a high serum level at the time of diagnosis is believed to be correlated with a short duration of survival. Hence, the real advantage of the prognostic evaluation of cytokines is reserved for patients who do not exhibit uniform results with regard to β_2 microglobulin and LDH serum levels, or, better, for borderline cases. With regard to differential diagnosis, all immunologic parameters should be evaluated concomitantly rather than separately to confer a real prognostic value to results. A particular relation was found between a high sIL-6R serum level and a poor response to chemotherapy, therefore suggesting the possibility of identifying in advance a subset of patients with a high risk of treatment failure, as has already been demonstrated in other hematologic malignancies. Finally, the majority of studies indicate that interferons are used mainly in the immunotherapy for multiple mieloma, whereas many clinical trials should still be required for the effectiveness of anti-IL6 antibodies or antiidio-typic vaccines in reference to the eligible patients for these particular therapies.

PO.325

CONTRIBUTION OF CLINICAL SIGNIFICANCE OF MONITORING PLASMA CELLS PROPIDIUM IODIDE AND ANNEXIN-V/CD138 INDICES IN THE COURSE OF MULTIPLE MYELOMA

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Background: Multiple myeloma is a clonal B-lymphoproliferative neoplastic disease characterized by slow proliferative activity and different resistance to apoptosis with accumulation of myeloma cells in the bone marrow.

Aim: The aim of this study is focused on the monitoring of the proliferative and apoptotic indices in the course of multiple myeloma (MM), e.g. during smoldering (SMM), plateau (PMM) and active i.e. progressive/relapsing (AMM) phase.

Methods: Analyzed group consists of 14 SMM, 32 myeloma patients examined at the time of MM diagnosis (DMM) and 57 patients analyzed during various phases of this disease. Plasma cell proliferative activity was measured by means of a propidium iodide index (PI) examined by flow cytometry using a DNA/CD138 double staining technique. For detection of plasma cells entering apoptosis (AI) flow cytometry method with annexin-V FITC and MoAb CD_{138} was used.

Results: In 3 patients with transformation from SMM into AMM the increase of PI/CD_{138} values was found (M: 2.0-2.8%) while AI/CD138 remained at the medium levels (M: 6.2-5.2%), but in 11 patients with persisting smoldering phase any significant change of both markers was not recorded (1.1 vs. 1.8 and 10.9 vs. 9.7%). In 3 patients with primary chemoresistance and with signs of laboratory and clinical progression the increase in proliferative (M: 2.4-2.8%) and significant decrease in apoptotic indices (M: 9.2-3.4%) were observed, while in 3 patients the decrease in PI/CD_{138} (M: 3.0-2.5%), together with stable AI/CD_{138} indices (M: 5.0-5.5%) were present. In 18 MM patients analyzed at the time of laboratory and clinical remission lasting for 4-27 months after the induction therapy the significant decrease in PI/CD138 (M: 3.0-2.1%) and increase of AI/CD_{138} (M: 2.1-5.1%) were observed. In 25 patients examined during various phases of the disease it was found, that in the active phase (progression/relaps after 2-22 months) the proliferative indices were elevated (M: 2.5-3.0) and apoptotic activity fell down (M: 7.1-4.1%), while the patients in long-standing remission had stable values of PI/CD138 indices (M: 2.1-2.2%) and some rise in apoptotic indices (M: 5.8-8.2%).

Conclusion: Measurement of kinetic parameters i.e. proliferative and apoptotic indices contributes to deeper insight into the changes of myeloma cells compartment in individual patients, widens the possibilities of individual follow up of the disease course and contributes to the individual flexibility in treatment options.

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PO.326

HAZARD FUNCTION – A USEFUL TOOL FOR EXPLORING SURVIVAL DATA

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Plotting Hazard functions is often ignored in the analysis of clinical trial survival data. The most common technique is to look at the Kaplan-Meier survival plots to examine the survival distributions, which can be stratified to explore certain prognostic subgroups. In many circumstances this is enough information to explain the survival distribution of the population of interest, particularly when survival is the primary endpoint. Log-rank analysis and Cox Regression analysis are used to identify and test the potential prognostic factors. However, there may be circumstances when exploring the hazard function is more informative than using the cumulative survival function. A hazard is calculated for specified time bands (e.g. every month or year) and the smoothed hazard function plots examined to indicate changing hazards over the whole course of the disease, giving a more useful graphical description of the risk of failure at any time point. Multiple Myeloma is one example where hazard functions are more informative about the whole disease process. We compared the survival distribution and hazard function plots for the 5th UK Medical Research Council myeloma trial comparing ABCM combination chemotherapy versus melphalan and the 6th UK Medical Research Council myeloma trial comparing ABCM +/- prednisone. Six hundred and thirthy patients were randomised between 1982 and 1986 into the 5th MRC myeloma trial. There was a significant treatment effect in favour of ABCM combination chemotherapy (MacLennan et al. 1991), which remains significant with long-term follow-up (χ^2=9.22, p=0.002). The cumulative treatment survival curves separate immediately and remain apart until around 5 years when both curves eventually come together. The hazard function plots for these treatments indicate differing hazards across the disease phases. For patients in the 6th MRC myeloma trial, there was no significant difference in the overall survival between the two treatments (χ^2=1.34, p=0.25). However, plotting the hazard function it can be seen that there are early differences between the two treatments, with the ABCMP patients reaching a stable disease/plateau phase quicker but then have a quicker time to first relapse. The hazard functions plots identify certain time points in the disease state where the hazards change; i.e. at first plateau and relapse. These changes would have been missed if the data were explored by cumulative survival distributions alone.

MacLennan et al. 1991, Lancet; 339: 200-205.

POSTER SESSION 4: NEW MODALITIES IN ASSESSMENT AND MONITORING OF MYELOMA

P0.401

DYNAMIC FLUORO-DESOXY-GLUCOSE POSITRON EMISSION TOMOGRAPHY STUDIES FOR THE PREDICTION OF CHEMOTHERAPY RESPONSE IN MULTIPLE MYELOMA

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Objectives: There is no optimal staging procedure for multiple myeloma. Especially extramedullary myeloma is usually not detected by standard staging. Fluoro-desoxy-glucose positron emission tomography (FDG-PET) is able to provide detailed information on tumour localisation and metabolism. We evaluated the FDG-PET in staging of multiple myeloma in comparison to standard radiological bone scans. Secondly, we examined the biological behaviour of myeloma during the course of chemotherapy, as the metabolic changes of a tumour under influence of cytotoxic substances appear to have predictive value.

Methods: We investigated a group of nine patients with plasmocytoma or multiple myeloma. All patients received an anthracycline-based chemotherapy. Three FDG-PET-studies were carried out (1. prior to the chemotherapy, 2. after the first course of chemotherapy, 3. after the third course). The clinical follow-up data and the EBMT-criteria for progressive disease (PD), stable disease (SD), partial remission (PR) and complete remission (CR) served as reference for the PET-data. The following parameters were retrieved from the dynamic PET studies: standard uptake value (SUV), fractal dimension (FD), two compartment model with computation of the kinetic parameters k1, k2, k3, k4 and the vessel density (VB). Furthermore, the FDG-influx according to Patlak was calculated using the rates of the two-compartment model and the formula (k1 x k3)/(k2 + k3). Discriminant analysis was used for data analysis. Due to the limited number of patients we dichotomised the patients into two groups, namely PD and SD/PR. Furthermore, we evaluated each parameter separately with regard to response.

Results. Eight patients presented themselves with multiple myeloma and one patient with extramedullary myeloma. Two patients had been previously treated. One patient has just ended the first circle and has not yet been re-evaluated. Best parameters for the discrimination between SD and PD were the influx and k3 of the first study with an overall correct classification rate (CCR) of 83%, followed by the distribution volume VB and the SUV with an overall CCR of 80%. A high VB, a high SUV and a high FD were associated with PD. The other kinetic parameters demonstrated an overlap of the classes and were not helpful as single parameters.

Conclusions. These data of our ongoing study demonstrate the value of the FDG-PET in the staging of multiple myeloma. Our analysis revealed a combination of parameters to be helpful for the prediction of therapy outcome in these patients.

P0.402

MAGNETIC RESONANCE IMAGING DEFINED FOCAL LESIONS IN MULTIPLE MYELOMA AT RELAPSE ARE OFTEN NEW SITES OF DISEASE

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Observation of macrofocal lesions (FL) on MRI scans in the medullary space of patients with multiple myeloma commonly occurs. These MRI-FL present at baseline as well as later, including at relapse. We evaluated MRI exams of 25 patients to determine if each MRI-FL present after remission of ≥180 days (CR or nCR based on M-protein response and bone marrow biopsy) represented FL present at baseline, new FL, or both. We also evaluated FL size at baseline vs. relapse to determine if size was related to persistence. All patients were enrolled in Total Therapy II (UARK 98-026). Baseline MRI examination (axial skeletal including skull, vertebral column, pelvis, and proximal femora) and relapse MRI exam of the same regions within 2 weeks before to 4 weeks after relapse date were analysed from patients who had ≥1 FL on relapse MRI. In terms of MRI FL, no patient achieved a normal MRI examination before relapse. On relapse, 17/25 (68%) had fewer number of FL, 5/25 (20%) had the same number of FL, and 3/25 (12%) had a greater number of FL on relapse compared to baseline exam. Despite the trend to fewer focal lesions on relapse, 11/25 (44%) had new FL at relapse. Importantly, FL size was not a determining factor, with max baseline size of resolved FL being 7.0 cm and max size of a new FL on relapse being 6.0 cm. Importantly, 4/25 (16%) presented with new extramedullary disease (EMD) at relapse. These data establish that despite a trend to fewer MRI-FL at relapse versus baseline, 44% of patients on relapse present with new areas of macrofocal disease not present at diagnosis.

	n	*% of patients*		*Max Size (cm)*
Fewer # FL at Relapse	17	68 %	Resolved FL	7.0
Same # FL at Relapse	5	20 %	New FL @ Relapse	6.0
Greater # FL at Relapse	3	12 %	New EMD @ relapse	4/15 (16%)
New FL @ relapse	11	44%		
Same # FL, Same Sites	2	9%		
Fewer # FL, Same Sites	5	23%		

MRI-FL at Time of Relapse (n=25)

Size (cm) at Relapse	*# FL of Baseline FL Persistent at Relapse*	*% of Baseline FL Present*
< 0.5	1/2	50 %
0.5 - 1.0	8/10	80 %
1.1 - 2.0	13/18	72 %
> 2.0	10/16	63 %
All Sizes	26/46	57 %

MRI-FL Persisting from Baseline to Relapse (n=25)

PO.403

NORMALIZATION OF SERUM FREE LIGHT CHAINS AND NEGATIVE IMMUNOFIXATION ELECTROPHORESIS MAY BE PREDICTIVE OF PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL FOLLOWING HIGH DOSE MELPHALAN

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Introduction. Measurement of the serum free light chain (sFLC) kappa/lambda levels and ratio is more sensitive than immunofixation electrophoresis (IFE) in detection and monitoring of nonsecretory multiple myeloma and light chain multiple myeloma. sFLC measurements are also applicable to monitoring myeloma associated with an intact immunoglobulin. The aims of this study are to ascertain whether complete serological response to treatment as assessed by IFE and sFLC levels and ratio is predictive of progression free survival (PFS) and overall survival (OS).

Methods. Patients who received intensive therapy in the UK MRC VIIth Myelomatosis trial and in whom serum samples were available at diagnosis, maximal response and at 3 month intervals thereafter were chosen for this study. These patients had known maximal response to therapy by conventional testing. sFLC ratios were performed on an Olympus AU series analyser, with normalisation of the K/L ratio defining complete response. Overall and progression free survival times were measured from randomisation. Kaplan-Meier plots and Cox regression models were used to further explore the data.

Results. Patients who were IFE negative had improved PFS and OS than those who were IFE positive at all time points. 10 (22%), 13 (36%) and 13 (27%) of patients had an abnormal sFLC ratio associated with negative IFE at 6, 9 and 12 months respectively. Patients with deeper serological response defined by negative IFE and normalisation of sFLC ratio appeared to have improved OS and PFS when compared to patients with either result abnormal. This was assessed at 6, 9 and 12 months post high dose melphalan (n=67, 54 and 61 respectively). The combination of abnormal sFLC ratio and positive IFE confers a poor outcome at these time points although there are small numbers in this group of patients.

Discussion. Improved OS and PFS are seen for patients who are IFE negative and achieving this state remains an important goal of therapy. However this study showed that approximately 30% of these patients with negative IFE have an abnormal sFLC ratio, indicative of residual disease. Moreover this preliminary data is suggestive of inferior outcome for patients with an abnormal IFE or sFLC following high dose therapy. These results await confirmation in the MRC IXth myelomatosis trial where sFLC ratios are being measured prospectively. If these results are confirmed, patients with abnormal sFLC levels post high dose melphalan might be considered for further therapy.

PO.404

SERUM FREE LIGHT CHAIN ASSAYS AS A REPLACEMENT FOR URINE ELECTROPHORESIS

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For the diagnosis of light chain multiple myeloma (LCMM) serum protein electrophoresis is insensitive so analysis of 24h-urine specimens is usually required. We believe there is now sufficient evidence that urinalysis can be replaced by serum measurements of monoclonal free light chains (mFLC). This is based on four pieces of evidence. First, urine light chain excretion is restricted by renal clearance so there can be significant levels of mFLC in the serum but none in the urine. Only when the absorptive capacity of the renal tubules is exceeded do FLC pass into the urine. This problem is avoided by measuring mFLC in serum. Second, techniques for measuring urine mFLC are cumbersome. Protein electrophoresis is a manual technique and interpretation of gels is difficult in the presence of heavy proteinuria or when the mFLC concentrations are low. Also, reproducibility is poor and 24h-urine collections are unreliable. In contrast, serum FLC assays are quantitative and fully automated. Third, serum mFLC tests were clinically satisfactory in the following diagnostic studies: (1) Presentation sera from 224 LCMM patients were all abnormal for serum mFLC. (2) In nonsecretory MM, 68% of 28 presentation sera had elevated levels of mFLC. (3) In AL amyloidosis, serum mFLC were abnormal in 95% of 263 patients and provided an easily measurable disease marker for monitoring. Urine mFLC tests were much less sensitive for nonsecretory MM and AL amyloidosis. In studies monitoring LCMM, good concordance was observed between the changes in serum and urine mFLC while the serum mFLC was more sensitive for disease response and remission. In a study of 80 LCMM patients during treatment, 32% achieved complete remission (CR) by urinalysis compared with only 11% for serum. Thus, urine mFLC analysis gave a false indication of CR, presumably due to the proximal tubule absorption of small amounts of mFLC. Other studies published recently have reported similar findings. Fourth, on a cost/benefit analysis, serum mFLC immunoassays are cheaper than urine electrophoresis tests. Urine electrophoresis tests have higher material costs, more technical and interpretation time and collection, storage and handling of urine is relatively expensive. Comparative costs of urine and serum FLC assays were ~ £12 and £10 respectively, per patient in a study from the Christie Hospital in Manchester, UK. There is now overwhelming evidence showing that clinical information gained from serum FLC assay is better than from urinalysis and the test is simpler. Re-assessment of the standard practice of analysing urine samples for mFLC in multiple myeloma is justified.

PO.405

THE USE OF SERUM FREE LIGHT CHAIN ASSAY IN CLINICAL PRACTICE

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An assay for serum free light chains (SFLC) is available commercially in kit form (FreeliteTM) produced by the Binding Site. Its use is perhaps not yet as widespread as its potential shows. SFLC assays were carried out on 73 patients over a period of 2 years. Data is presented on these patients, some of whom were followed up serially. The patients were 29

with multiple Myeloma at various stages from diagnosis to stable complete remission (4 of these patients also had associated AL amyloidosis), 13 with smouldering multiple myeloma (SMM), 15 with MGUS, 4 with primary AL amyloidosis, 2 with CLL and 2 with a lymphoplasmacytic lymphoma and 7 patients who were being investigated to exclude a plasma cell dyscrasia. The serum free light chain assay proved an indispensable test for monitoring AL amyloidosis and Bence Jones Myeloma as is well established. In addition it was extremely useful in monitoring patients with SMM although the interpatient variation was considerable. It also proved valuable in predicting relapse in some patients and also response to treatment in which case falls in SFLC levels predate the fall in paraprotein in those with intact immunoglobulin myeloma by several weeks. Two patients with intact IgG Myeloma developed renal impairment during the course of treatment, one requiring dialysis. Both these patients had a elevated levels of SFLC, one of K light chains of 350mg/L and the other with lambda light chains of 1324 mg/L. In these two patients and in others with intact and LCM the serum levels were not always reflected in the levels of the BJP even predating the renal failure. One patient with non-secretory (NS) Myeloma relapsed with increasing anaemia and rising µ2 microglobulin but without any rise in SFLC. This patient has not had SFLC levels measured prior to treatment and it is therefore advisable not to rely entirely on this test to predict relapse in NS Myeloma unless it is known beforehand that they had LC imbalance at diagnosis. In conclusion, other than the well documented value of SFLC assays in the management of AL amyloidosis and BJ Myeloma, we believe that the test has a useful role in the management and diagnosis of all cases of PCD. Further work is needed to determine whether this assay may be useful in predicting the likelihood of development of renal failure in patients with intact immunoglubin MM. It is useful in monitoring patients with SMM and MGUS and in predicting early relapse and response to treatment in those with LC imbalance.

P0.406

COMPARISON OF SERUM FREE LIGHT CHAIN MEASUREMENT AND URINE ELECTROPHORESIS FOR DETECTION OF B-CELL PROLIFERATIVE DISORDERS

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Introduction. Serum free light chain (FLC) measurement has been established in the retrospective diagnosis of light chain myeloma, nonsecretory myeloma and management of patients with B cell proliferative disorders. Here we report a prospective study of the use of FLC assays alongside serum protein electrophoresis (SPE) and urine electrophoresis, as a first line screen to identify patients with possible disorders of B-cell lineage.

Methods. SPE, serum FLC and urine electrophoresis were utilised as a screen, to exclude multiple myeloma or other B cell proliferative disorders in 94 patients from whom paired urine and serum samples were obtained. The results of the urine electrophoresis and serum FLC were compared for these patients. Serum FLC were measured by immunoturbidimetric immunoassay (The Binding Site, Birmingham UK) and urine electrophoresis and immunofixation was performed on a Sebia Hydrasys Electrophoresis system (Sebia, France).

Results. As the table shows, the FLC ratio was abnormal in 7 out of 8 patients in whom Bence Jones Protein (BJP) was identified. The 8th patient had polymyositis with oligoclonal bands present in the serum and a normal ratio, but the individual FLC concentrations were greater than 10 times the upper limit of normal. Subsequent investigation did not confirm the persistence of urine BJP in this patient, indicating that it had been a *false positive*. In six patients FLC ratios were abnormal but no free light chains were identified by serum or urine immunofixation which is consistent with the poorer sensitivity of electrophoretic methods. Follow-up samples have been requested from these patients.

		Free light chain ratio	
		NEG	POS
Urine BJP	NEG	80	6
	POS	1	7

Conclusions. From this preliminary prospective study we suggest the serum FLC ratio has the potential to replace urine electrophoresis as one of the first line-screening test for patients with a suspected B cell proliferative disorder. The automated FLC assay rapidly identified the 80 negative patients, allowing resources to be concentrated on the 13 with abnormal ratios.

P0.407

COMPARISON OF SERUM AND URINE FREE LIGHT CHAIN MEASUREMENTS WITH BONE MARROW ASSESSMENTS IN MULTIPLE MYELOMA

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Introduction. Diagnostic criteria for multiple myeloma include abnormal plasma cell infiltration of the bone marrow plus the presence of monoclonal intact immunoglobulin in the serum and/or monoclonal free light chains in the urine. Recent studies have indicated, however, that measurement of free light chains in the serum (sFLC), is more sensitive than urine assays for the diagnosis and monitoring of patients with multiple myeloma. The aim of this study was to compare the relative sensitivity and specificity of serum free light chain (sFLC) measurement, urine free light chain measurement (uFLC) and serum immunofixation (sIFE) with bone marrow analysis.

Patients and Methods. All patients were enrolled in the UK, Medical Research Council's Myeloma VII trial. One hundred and five archived serum samples from 64 patients were selected for sFLC measurement and sIFE. The sera had been collected at various times before, during and after treatment but all within 1 week of a bone marrow assessment and uFLC measurement (by radial immunodiffusion assay). sFLC results were classified as abnormal when the kappa/lambda ratio was outside the normal range and for uFLC, when there was >40 mg/L. The bone marrow assessment was called abnormal if 5% or greater, plasma cell infiltration was recorded.

Results. The relative sensitivities of the sFLC and sIFE assays were similar at 90% and 92% respectively but the uFLC assay was considerably less sensitive at 58%. sIFE, sFLC and uFLC results were abnormal in some patients who had normal bone marrow assessments (18, 10 and 7 respectively).

Conclusions. sFLC measurement showed a good degree of correlation with bone marrow assessments of myeloma while uFLC assays were considerably less sensitive. This can be explained by the reabsorption of light chains in the kidneys which results in a *threshold* concentration of sFLC, beneath which light chains do not enter the urine in meas-

urable quantities. sFLC, sIFE and uFLC assays appeared to identify disease in a number of patients who had normal bone marrow assessments. This was probably because the paraprotein assays sample monoclonal protein produced throughout the body while distribution of the disease in the bone marrow is occasionally *patchy*.

PO.408

CONTRIBUTION OF THE QUANTITATION OF FREE LIGHT CHAINS IN 273 PATIENTS PRESENTING WITH A NEWLY DISCOVERED MONOCLONAL GAMMOPATHY

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Introduction. Quantitation of κ and λ free light chains (FLC) has become an important step in the diagnosis and follow up of patients with lymphoproliferative disease and plasma cells diseases. We report here our experience in the contribution of the quantitation of serum FLC in a cohort of 273 patients attending a general hospital and presenting with a newly discovered monoclonal gammopathy which was to be investigated.

Methods. In 273 sera a monoclonal gammopathy was diagnosed in our laboratory using capillary electrophoresis, electrophoresis-immunofixation (IFE) and a nephelometric measurement of FLC. In 84 cases the serum was associated with a sample of a 24h-urine collection which after concentration was submitted to electrophoresis and IEP. Patients were then grouped according to the concentrations of FLC and the value of κ_f/λ_f ratio. Comparisons taking into account the group and the pathological conditions were made.

Results. Four groups could be distinguished with 158 patients (57.8%) belonging to group III (one or both FLC falling outwards the reference range and an abnormal Îf/Ïf ratio) and 63 (23.07%) to group I (both FLC and κ_f/λ_f ratio within the reference range). When pathological conditions were taken into account, it appeared a highly significant difference (p<10-6) between patients with myeloma (79) and patients with monoclonal gammopathy of undetermined significance (MGUS) (99):74 patients with myeloma (93.7%) were in group III versus only 35 (35.3%) patients with MGUS. Thus, this group might contain patients liable to undergo a malignant evolution of their monoclonal gammopathy. Of 22 cases showing no evidence of monoclonal gammopathy on the beta and /or the gamma fraction of the electrophoregram 17 (77.3%) were in group III and thus could have been detected by the quantitation of FLC. In 84 patients urines were studied in the same time as the serum. In 66 cases, a urine BJP was detected while 58 patients (87.9%) happened to be in group III and thus could also have been detected by quantifying serum FLC. This preliminary study also revealed intriguing facts such as the lack of correlation between the measurement of a high concentration of a light chain of a given type and the detection of a BJP in the corresponding serum by conventional techniques. For κ FLC, the lowest value associated with a λBJP detectable in the serum was 28.7 mg/L while the highest value associated with an undetectable kBJP was 880 mg/L. Likewise for λFLC the lowest concentration with a detectable λBJP was 75.5 mg/l while the highest concentration with an undetectable λBJP was 293 mg/L.

Conclusions. The preliminary results of this study encounter previous results obtained by others showing how valuable FLC quantitation may be notably when there is no evidence of monoclonal gammopathies on the electrophoregram. They also suggest that an abnormal κ_f/λ_f ratio and one or both FLC falling outwards the reference range might be the first sign of a malignant evolution for a MGUS.

PO.409

APPARENT DISCREPANCIES IN THE QUANTITATION OF FREE LIGHT CHAINS IN SERUM OF PATIENTS PRESENTING WITH A MONOCLONAL GAMMOPATHY

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Introduction. With the development of a new sensitive technique, measurement of κ and λ free light chains (FLC) has become an interesting option specially when no M-component can be detected by conventional tests. However, while using FLC quantitation to follow up patients with monoclonal gammopathy of undetermined significance (MGUS) or plasma cells diseases, sometimes conflicting results emerged between the immunodetection step showing the lack of free monoclonal light chains (FMLC) in the serum and the quantitation step showing a high level of one type of FLC. We report here the results of a study undertaken in a cohort of 112 patients presenting with a monoclonal gammopathy in order to determine the frequency of these phenomenom and in an attempt to explain them .

Methods. In a cohort of 112 patients presenting with a monoclonal gammopathy we measured serum FLC. When the concentration was outwards the reference range, we performed a conventional electrophoresis-immunofixation (IFE) and a higly sensitive technique of immunoelectrophoresis (IEP) using home-made ultrathin layers of agarose with precut troughs , minute amounts of antisera directed against κ or λ FLC and alpha-1-antitrypsin. According to the characteristics of the samples, various volumes of serum (1, 5 and 10 µL) were applied and in order to overcome the limitations of using weakly titered antisera anti-FLC, the troughs of IEP were filled twice with the antisera.

Results: In 65 cases (55%) the measured FLC were outwards the reference range and in 33 cases (50.7%), no bands could be detected with IFE while with IEP at least one arch was clearly visible either with anti-κ_f (23 cases) or λ_f (10 cases).The lowest values measured for κ and λ FLC were respectively 25.4 and 23.8 mg/l and the highest ones, 536 and 100 mg/L. However in most cases, the arch was either located at the same place as or in the vicinity of the intact monoclonal immunoglobulin and thus FMLC were hidden by the M-component. When even with IEP no FMLC could be detected (8 κ and 2 λ) the mean concentration of the concerned light chain was 36.5 mg/L (extremes, 20.5- 40.9) for Îf and for the ratio k_f/l_f, 6.8 (extremes, 2.34-19.9) and for λ_f , 40.5 mg/L and the ratio, 14.9. Concerning κ FMLC in 4 cases there was clearly an overestimation of the concentration (2860 mg/L, 4200 mg/L, 5370 mg/L and 16600 mg/L) and in all the cases there were 3 arches, 2 reflecting the presence of monomeric and dimeric FLC and one arch revealing a complex between alpha-1-antitrypsin and κ FLC.

Conclusions. Discrepancies between measured FLC and FMLC detected by IFE are partly due to the mobility of the FMLC which happen to often migrate close to the intact monoclonal immunoglobulin and on the fact that antisera directed against FLC have a an insufficient titer to unable the detection of FMLC with IFE. As to the overestimation of κ FLC it is the result of a mixture of several molecular forms, a monomere, a dimere and a complex between alpha-1-antitrypsin and κ FLC acting as multiantigenic targets.

P0.410

SERUM FREE LIGHT CHAIN IMMUNOASSAYS FOR MONITORING SOLITARY BONE PLASMACYTOMA

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Solitary bone plasmacytoma (SBP) is a single bone lesion owing to a monoclonal plasma cell infiltrate with no evidence of Multiple Myeloma (MM) elsewhere. SBP usually become symptom-free with local radiotherapy (RT). After RT, patients with SBP may experience local recurrence within the RT fields, develop MM, or remain free of disease for many years and thus may be considered cured. However, the majority of patients will develop distant focal recurrence or MM at a median time of 2-4 years. The 10-year disease free survival is approximately 30%. No prognostic factor has been identified. Nevertheless, although not statistically significant, the prognosis of patients with secretory SBP compared to non-secretory tumours appears to be better, as does the disappearance of the monoclonal protein after RT. We measured the serum free light chains (sFLC) in serum collected and stored from patients with SBP. Sera were available from 13 patients at diagnosis and 10 had a subsequent sample later in the disease course. Eight patients had a second sample taken in the months following the RT treatment and 3 at progression to MM. The levels from SBP patients were compared to 8 MM patients diagnosed with a plasmacytoma lesion. The median age was 60 years (±12) for SBP patients and 57 years (±10) for the MM patients, respectively. For the SBP subgroup, the M/F ratio was 0.53. M-component heavy chain isotypes were 38% IgG, 15% IgA, 23% light chain and 15% non secretory. Thirty eight percent of the patients had a kappa light chain. None of the patients had renal failure. The median (min-max) sFLC was 32.4 mg/L (13.4-1371.5) for SBP patients and 24.9 mg/L (9.6-527.5) for MM patients. One of the two non-secretory SBP patients could be detected by sFLC assay. Levels of sFLC did not correlate with any of the usual biological values. In 7 cases, levels of sFLC were reduced after RT with a median value of 61% (range, 6-98). 5/7 patients are in persistent response/plateau with a median overall survival of 80 months (range, 12-119) and 2 patients evolved toward MM at 68 and 98 months. Three patients had no change in sFLC and progressed with a median of 31 months (range, 9-27). Three patients for technical reasons were delayed to their RT, 2 remained stable and one had an increase in SFLC values. SFLC measurements can be used to detect and monitor patients with SBP. It may provide a more accurate marker of response after RT. Further studies on a larger population are ongoing to confirm the role of sequential SFLC survey to predict MM development.

P0.411

MODEL FOR ASSESSING FREE LIGHT CHAIN KINETICS WHEN MONITORING PATIENTS WITH MULTIPLE MYELOMA

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Introduction. Cancer markers with short half-lives more closely reflect changing tumour size than those with long half-lives. For serum free light chains, the dominating clearance mechanism is filtration through the renal glomerular pores. At 25kDa, monomeric free light chain molecules (typically kappa) have a half-life of approximately 2 hours while dimeric molecules (typically lambda) have a half-life of 4-6 hours. In contrast, IgG molecules have a half-life of 20-25. The 200-300 fold shorter serum half-life of free light chains compared with IgG, allows a much more sensitive evaluation of changing monoclonal protein production and hence changing tumour mass, during treatment. Tumour kill rates, relapse rates and tumour marker half-lives are different for each patient and their interplay is complex.

Methods. A mathematical model that incorporates half-life kinetics has been constructed to simulate changes in serum free light chains and intact immunoglobulins under different clinical conditions. Serial sampling from myeloma patients were used to compare with the predictions of the model

Results and conclusion. The model indicates that the half-life of serum free light chains adds only a few hours to the true picture of the tumour cell kill rate. Hence, tumour responses and complete remission can be rapidly identified using serum free light chain analysis. In contrast, the 21-day serum half-life of IgG is so long that differences in rates of tumour destruction are largely obscured. The short serum half-life of free light chains should allow reliable assessments of tumour cell killing rates and identification of tumour remission many months earlier than using IgG. Production of the monoclonal free light chain and intact immunoglobulin usually return when multiple myeloma relapses and both types of molecule typically become re-detectable at the same time. However, when tumour re-growth occurs before all the monoclonal IgG has disappeared, then free light chain measurements can detect tumour recurrence many months earlier. This is because the falling concentrations of monoclonal IgG from chemotherapy are superimposed on rising concentrations produced by the relapsing tumour, thereby obscuring an early IgG increase. In contrast, because serum free light chains normalise early, increasing production by the relapsing tumour is not hidden by residual levels. The model clearly identifies situations where repeated serum free light chain measurements are of assistance in patient management. Its predictions are supported by results in 30 patients that have had multiple immunoglobulin measurements.

P0.412

UTILITY OF SERUM FREE LIGHT CHAINS FOR MONITORING MYELOMA POST AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. Serum free light chains (FLC) are useful in the diagnosis and monitoring of non-secretory and light chain myeloma (LCMM). We aimed to determine if the serum kappa to lambda (K/L) FLC ratio was similarly useful for monitoring patients with intact immunoglobulin myeloma (IIMM) following autologous stem cell transplantation (ASCT).

Methods and Materials. FLC concentration was measured in the serum of patients with IIMM or LCMM who underwent ASCT (n=34) and in healthy subjects (n=11) using a kit assay (The Binding Site Ltd., Birmingham, UK) and the IMMAGETM (Beckman Coulter, Brea, CA) protein system. Monoclonal paraprotein was detected by serum protein electrophoresis (Beckman Coulter) and/or immunofixation (IFE). The definitions of myeloma response and relapse were according to international criteria (BJH 1998:102:1115).

Results. K/L FLC ratios were within the manufacturer's reference interval for healthy subjects (0.51-1.00 vs. quoted 0.26-1.65) and for IIMM and LCMM patients in complete remission (0.41-1.59; n=15) except for one LCMM patient (K/L ratio 0.15) with no Bence Jones protein detected on IFE and 2% polyclonal plasma cells on bone marrow biopsy. Seventy-eight percent (14/18) of IIMM and LCMM patients in plateau phase (i.e. residual serum paraprotein ranging from <1 to 19 g/L or trace Bence Jones proteinuria) had a normalised FLC ratio (0.33-1.49) with 4 patients having abnormal ratios (0.23, 0.24, 3.4, 5.5). Whereas the FLC ratio and monoclonal FLC concentration reflected changes in disease progression in LCMM, the same did not apply to IIMM. Of 11 relapsed IIMM patients, four had persisting, normal FLC concentration and ratio despite rising paraprotein concentrations of 10-36 g/L and abnormal bone marrow biopsy (plasma cells 9-100%). In two other patients increasing paraprotein concentration (IgA lambda 1.7 to 23 g/L; IgG kappa 11 to 27 g/L) preceded increasing monoclonal lambda FLC concentration (14 to 35 mg/L, ratio 0.96 to 0.29) and kappa FLC concentration (60 to 75 mg/L, ratio 5.5 to 11.9) by 8 and 4 months, respectively. In another, increasing lambda FLC (40 to 179 mg/L; ratio 0.49 to 0.05) preceded increasing paraprotein (19 to 31 g/L) by 11 months, and in four others, serum paraprotein and monoclonal FLC concentration increased simultaneously. Thus, 55% (6/11) of our transplanted group failed to give an abnormal ratio or elevated FLC concentration at or prior to disease relapse.

Conclusions. Serum FLC measurement does not appear useful as an early predictor of disease reoccurrence compared with the serum paraprotein in patients with intact immunoglobulin myeloma.

P0.413

CHANGES IN SERUM FREE LIGHT CHAIN CONCENTRATIONS AS A MARKER OF CHEMOSENSITIVITY AFTER HIGH-DOSE MELPHALAN AND AUTOLOGOUS STEM CELL TRANSPLANT IN MYELOMA PATIENTS

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Immunoassays specific for free immunoglobulin light chains (FLC) in sera, are useful for the diagnosis and monitoring of Bence Jones myeloma, nonsecretory myeloma and AL amyloidosis and are several hundred fold more sensitive than immunofixation or serum protein electrophoresis. Changes in serum FLC will be a more rapid indicator of treatment response than intact immunoglobulin due to the short half-life of serum FLC and will rapidly mirror the kinetics of tumour kill. This study analysed the changes in serum FLC after autologous peripheral blood stem cell transplantation (PBSCT) in 20 patients. Before transplant, 10/20 patients had elevated levels of tumour FLC/abnormal FLC ratio, 2/20 patients had elevated FLC/normal FLC ratio, 2/20 had suppression of the non-tumour FLC with an abnormal FLC ratio and 6/20 had normal FLC/normal FLC ratio. All patients with raised tumour FLC showed a rapid fall within 48 hours. In all patients with monoclonal paraproteins, the tumour FLC fell quicker (median half-life 4.2 days) than the monoclonal paraprotein (median half-life 14 days). The rate of fall and range of reduction of FLC varied between individual patients indicating different tumour killing rates and chemosensitivity. FLC recovery occurred either after (13/19 patients) or around the time of neutrophil engraftment (6/19 patients). Early recovery of FLC production is a marker of lymphocyte recovery and early lymphocyte recovery has been associated with a more favourable outcome. With a median follow up of 210 days post transplant, 12/19 patients have normal FLC concentrations and ratios, 4/19 have normal FLC ratios with slightly elevated FLC and 3/19 have elevated tumour FLC/abnormal ratios. FLC assays provided a sensitive monitor of changes in the numbers of tumour and non-tumour plasma cells after PBSCT and predicted the intact immunoglobulin response. Further follow up is required to ascertain whether differences in the kinetics of FLC responses have any prognostic clinical utility.

P0.414

IMMUNOTYPING OF SERUM MONOCLONAL PROTEINS ON A SEBIA CAPILLARY ELECTROPHORESIS SYSTEM

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Background. The Sebia capillary electrophoresis system CAPILLARYS™ is designed for routine analysis of serum proteins in clinical chemistry laboratories. The key features of this system are complete automation, high sample throughput (up to 90 samples per hour) and high sensitivity. We describe now an automated method for immunotyping of monoclonal proteins that includes positive ID from direct tube sampling. Standard reagents, CAPILLARYS Immunotyping kit, are designed for the typing of G, A, M, kappa and lambda immunoglobulin classes in conjunction with the CAPILLARYS ?1?2+ separation buffer; the reagents for the less common cases are designed for the typing of D, E and free light chain classes.

Prodecure. Each well of the CAPILLARYS dilution segments is filled with antiserum comprising specific antibodies against one immunoglobulin class. Thereafter, CAPILLARYS performs the mixing of the serum sample with appropriate antisera, and the mixtures are directly injected into capillaries. After migration and detection at 200 nm, overlay of the resulting electrophoregrams with a reference profile, i.e., the same sample untreated with antiserum, identifies the previously detected monoclonal abnormalities. The reaction between antibodies and antigens is performed in a liquid state and is very rapid. This procedure requires no extra incubation time and no immune complex separation step.

Results. About 200 sera were analyzed using the CAPILLARYS Immunotyping kit and compared with the standard agarose gel electrophoresis - immunofixation method (Hydragel 2 IF and 4 IF). The abnormal sera contained monoclonal components of various types migrating in gamma, beta-2 or beta-1 zones, oligoclonal and biclonal components, as well as hypo- or hyper-polyclonal background. The identification of abnormalities was identical by both methods. The dilution of a monoclonal component into a normal serum gave the detection limit of the system: the lowest concentration detected was 30 mg/dL. Actual throughput: 9-10 samples per hour.

Conclusions. The main advantages of this new method, CAPILLARYS Immunotyping, for the identification of monoclonal components (gammopathies) are: (i) full automation with positive sample ID, (ii) no incubation time with the antisera and no separation of the immune complexes, (iii) high sample throughput and (iv) high sensitivity.

POSTER SESSION 5: MYELOMA MANAGEMENT (NON-TRANSPLANT) AND EPIDEMIOLOGY

P0.501

SURVIVAL OF PATIENTS IN THE UK MEDICAL RESEARCH COUNCIL MYELOMA TRIALS IN WHOM NON-INTENSIVE CHEMOTHERAPY ACHIEVED A STABLE REMISSION; COURSE OF DISEASE FROM RELAPSE AND THE EFFICACY OF SECOND LINE THERAPIES

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Introduction. There are few large studies of the efficacy of different second line cytotoxic drug regimes and the few randomised trials mostly concern new agents like thalidomide and velcade. There is a bias to giving a different second line therapy rather than reintroducing the first line treatment, particularly if the duration of stable remission/plateau phase was short, but there is little evidence base for this in myeloma. Similarly there is a need to establish from previous experience what relapse therapy should be expected to achieve in relation to stable remissions and their duration as well as overall survival.

Methods. 1372 of 2528 patients (54%) receiving melphalan (845 patients), ABCM (1622 patients) or cyclophosphamide (61 patients) based non-intensive chemotherapy in the MRC trials between 1980 and 1997 achieved a stable plateau phase. Only patients entered before 1997 were chosen so that there would be long-term follow-up on all patients. These 1372 patients were used to assess the course of disease from relapse. In 639 of 2528 patients the nature of second line therapy (non-randomised) was known and allowed comparison of efficacy between treatments and between identity and non-identity with first line therapy. These 639 patients were grouped according to ABCM (182 patients), melphalan (224 patients), VAD (103 patients) and cyclophosphamide (130 patients) based therapies.

Results. The 1372 patients achieving plateau phase had an overall median survival of 3.9 years (95% confidence interval 3.7-4.0). 225 patients (16%) survived >7.5 years; of these 135 (60%) patients achieved a second plateau phase and, having relapsed a second time, 50 (37%) achieved a third plateau. The duration of plateau became significantly shorter between first, second and third plateaus. Median survival from relapse, for the 1151 patients who had a recorded date of relapse, was 1.2 years (95% confidence interval 1.1- 1.4 years). In 247 patients the treatment used as first line therapy was reintroduced at relapse and survival from relapse was better for these patients when compared to the 392 patients who received second line therapy different to that used for first line (χ^2=8.03, p=0.005); this was still the case when the patients were stratified by β2-microglobulin line (χ^2=7.85, p=0.005). Duration of plateau phase (<1 yrs, 1-3 yrs and >3 yrs) was associated with median survivals from relapse of 11, 17 and 21 months, respectively, with those patients achieving a longer duration of plateau also associated with a longer survival from relapse (χ^2=44.73, p<0.0001). However, when the two second line treatment groups were stratified by duration of plateau there was a borderline difference in survival from relapse (χ^2=4.45, p=0.04).

Conclusions. Relapse from first plateau is often followed by further but shorter plateau phases and performance status during the post relapse phase is usually good. Choice of second line cytotoxic chemotherapy has an impact on survival post relapse.

P0.502

LONG TERM FOLLOW-UP OF HIGH DOSE MELPHALAN VERSUS INTERMEDIATE DOSE MELPHALAN CONSOLIDATION THERAPY AFTER VAMP/VAD FOR NEWLY DIAGNOSED MYELOMA

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Introduction: VAD/VAMP plus high dose melphalan is standard therapy for patients with myeloma <65 years. However it is not curative and is dependent on successful harvest of stem cells. Further therapy is always required and few patients tolerate several intensive regimens. This randomised multicentre study compares high dose melphalan (HDM) and intermediate dose melphalan (IDM) as consolidation of an initial response to VAMP/VAD.

Methods: 60 newly diagnosed patients with myeloma aged <65 years who had been treated with 4-6 cycles of VAMP or VAD were randomised to HDM or IDM after stem cell mobilisation with cyclophosphamide (CTX; 1.5-4 g/m^2). Patients were treated with either HDM (200mg/m^2) and stem cell re-infusion or IDM (80mg/m^2) and G-CSF (Lenograstim; Chugai Pharma UK Ltd). Maintenance interferon-alpha (Intron-A, 3MU/m^2 three times weekly; Schering-Plough Ltd UK) was offered to patients in both arms.

Results. 30 patients received HDM; 28 patients received IDM. 2 patients randomised to IDM did not receive any consolidation. Median age was 55 (range 39-64). 21/37 male patients received HDM. 3 patients had Durie-Salmon Stage I myeloma; others were stage II / III. All but 2 received 4-6 x VAMP (44) or VAD (12). These received 1 or 7 courses of VAMP respectively. All but 2 received 1.5-4 g/m^2 CTX. These were mobilised with G-CSF alone or 8 g/m^2 CTX respectively. Most patients had IgG (HDM 19; IDM 18) or IgA (HDM 8; IDM 4) myeloma. Median beta-2 MG levels were comparable (HDM 3.6; IDM 4.3). Median inpatient stays were comparable (24 v 23d) but HDM was associated with shorter neutropenia (10 vs 14d). HDM achieved more CR's (3/26 vs 1/24). 17/30 (57%) HDM patients have progressed (median 24.6 mo) vs 22/28 (79%) IDM patients (20.3 mo). 17/30 HDM patients remain alive (median 70 mo; range 10-111 mo) vs 14/28 after IDM (53 mo; 14-120 mo). 10/13 HDM and 14/14 IDM deaths are due to myeloma.

Conclusions: HDM offers a higher chance of achieving CR, a more durable response and longer survival but IDM offers a reasonable alternative for patients in whom stem cell mobilisation is unsuccessful.

P0.503

THE UK MYELOMA FORUM ELDERLY STUDY: A COMPARISON BETWEEN ORAL MELPHALAN AND INTRAVENOUS INTERMEDIATE DOSE MELPHALAN FOR *DE NOVO* DISEASE IN PATIENTS AGED >65 YEARS

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Multiple myeloma is a disease of the elderly that despite advances in chemotherapy and supportive therapy remains incurable. In a recent population based study in the south of England the median age was 72 years with a median survival of 18 months for those older than 65 years. Treatment is indicated for symptomatic patients and for elderly patients oral melphalan remains the gold standard. But there are wide variations in response rates to this approach and unpredictable absorption from the gastrointestinal tract may account for this. We have previously demonstrated that intravenous intermediate dose melphalan (IDM) is well tolerated with response rates comparable to oral melphalan (OM). The UK Myeloma Forum has undertaken a randomised trial to compare oral and intravenous melphalan. *Methods:* This was a multi-centre, open, randomised, prospective study of patients aged>65 years with symptomatic untreated disease. Patients were randomised to receive oral melphalan (7 mg/m^2 od for 4 days) or intravenous melphalan (25 mg/m^2 od for 1 day) every 28 days. Patients also received prednisolone (40mg od for 4 days) or dexamethasone (20 mg od for 4 days). Patients continued treatment until maximum response or disease progression. *Results.* 53 patients were entered into the study (29 male: 24 female). The median age at entry was 73 years (range 63-88). 50 patients had a serum paraprotein, 2 had light chain disease and the paraprotein was unknown in 1 patient. The median WHO performance status at start of treatment was 1. 26 patients received IDM and 27 patients received OM. The median time from diagnosis to start of treatment was 16 days and the median follow-up is 20 months. IDM patients received a median of 5 courses (1-7) and those receiving OM received 6 courses (1-8). Grade 3/4 neutropenia developed in 21/26 patient receiving IDM and in 5/27 receiving OM. Treatment was withdrawn early in 8/26 patients receiving IDM (3/8 patients due to infection, 1/8 following a perforated duodenal ulcer, 1/8 tolerated treatment poorly, 1/8 had a poor response, 1/8 was poorly compliant and 1/8 withdrew consent) and in 12/27 patients receiving OM (due to infection in 2/12, poor response in 5/12, poor compliance in 3/12, unable to tolerate treatment in 1/12 and pelvic fracture in 1/12). There were 8 treatment related deaths (5 in patients receiving IDM – infection in 4 patients and perforated duodenal ulcer in 1, and 3 in patients receiving OM – infection in 2 patients and post operative complications in 1 patient). Response rate were as follows: IDM (2/26 CR, 15/26 PR, 1/26 MR, 2/26 SD and 6/26 unknown), OM (1/27 CR, 9/27 PR, 5/27 MR, 9/27 SD, 1/27 PD and 2/27 unknown). 16/26 and 20/27 patients that received IDM or OM respectively have died. There was a trend towards improved survival with IDM (IDM median OS 36.5 months, OM median OS 28 months) but this did not reach statistical significance. At 36 months the survival in the IDM group was 51% (29-70%; 95% CI) and 28% (12-47%; 95%CI) in the OM group. *Conclusions.* IDM can be safely delivered in the outpatient setting with superior response and survival than OM and compares favourably with population studies.

PO.504

PRIMARY TREATMENT WITH PULSED MELPHALAN, DEXAMETHASONE, THALIDOMIDE FOR SYMPTOMATIC PATIENTS WITH MYELOMA ≥75 YEARS OF AGE

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Introduction. Thalidomide is usually administered orally continuously once daily and has shown activity in about 30% of patients with heavily pretreated multiple myeloma (MM). Moreover, there is considerable interest in the administration of thalidomide-containing combinations as primary treatment of MM. At least 60% of patient achieve an objective response. Thalidomide can cause a variety of side effects whose incidence and severity may be related to the maximum dose and duration of thalidomide treatment. Furthermore, this drug may be poorly tolerated by older patients. We designed a phase II study for the primary treatment of elderly patients (≥75 years of age) with MM which was based on intermittent oral administration of melphalan, thalidomide and dexamethasone.

Patients and Methods. This study was initiated in February 2003 and includes patients with symptomatic myeloma ≥75 years of age regardless of performance status, renal function, and comorbidities. Treatment consists of melphalan (M) 8 mg/m^2 days 1-4, dexamethasone (D) 12 mg/m^2 p.o. after breakfast on days 1-4 and 14-18 and thalidomide (T) 300 mg p.o. at bedtime on days 1-4 and 14-18. This regimen is repeated every 5 weeks for 3 courses. Patients without evidence of progression are scheduled to receive 9 additional courses of MDT but without DT on days 14-18, every 5 weeks.

Results. 40 patients have been enrolled so far; median age is 78 years (range: 75-85 years). Features of advanced myeloma are frequent and include ISS 3 in 58%, hemoglobin <8.5g/dL in 13%, calcium >11.5 in 15%, creatinine > 2 mg/dL in 28% and elevated serum LDH in 11%. On an intent-to-treat basis, 72% of patients achieved at least a partial response (EBMT criteria) including 10% of patients who achieved complete response. Median time to 50% reduction of monoclonal protein was 2 months (range 0.5-5.5). Grade 3 or 4 granylocytopenia occurred in 15% and 10% of patients. Twelve episodes of infections, one fatal were noted. Several patients developed thalidomide-related side effects, usually of mild or moderate, such as constipation (30%), somnolence (35%), tremor (25%), xerostomia (15%), headache (10%). Deep venous thromosis (DVT) and peripheral neuropathy occurred in 10% of patients each. With a mean follow-up of 15 months 88% of patients remain alive.

Conclusions. This is one of few prospective studies designed for myeloma patients with advanced age (≥75 years) ie patients who are frequently excluded from trials. The pulsed MDT regimen appears to be a well tolerated and active primary treatment for elderly patients with multiple myeloma. The incidence of DVT and of peripheral neuropathy appears lower than that seen when thalidomide is administered continuously. Further patient accrual and follow-up is needed to assess the impact of this regimen on response duration and survival.

P0.505

VAD VERSUS CY-DEX AS INITIAL TREATMENT IN NEWLY DIAGNOSED MYELOMA – A RANDOMIZED TRIAL

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Background. Intensive therapy (IT) including high dose therapy with autologous stem cell transplantation (ASCT) is today considered to be standard therapy in younger newly diagnosed myeloma patients. VAD has been a common induction therapy preceding ASCT. In a previous NMSG study (#5/94) where newly diagnosed patients younger than 60 years were treated with IT, 15% did not receive ASCT due to disease progression and/or treatment related mortality or morbidity. Most of these patients dropped out during the induction therapy of three to four cycles of VAD. The contribution of vincristine and doxorubicin in tumor burden reduction is not clear and both substances are potentially toxic. In addition, VAD is a relatively complicated therapy to administer. In an attempt to increase the fraction of patients who received ASCT by reducing toxicity and to simplify the induction therapy, NMSG initiated a prospective randomized study comparing conventional 3 courses of VAD with experimental 2 courses of Cy-Dex as induction. The primary end-point was proportion of patients receiving ASCT. Secondary end-points were response and survival.

Methods. From Nov 2001 to Oct 2003, 314 patients younger than 65 years with symptomatic newly diagnosed myeloma were randomized to receive 3-4 cycles of VAD (157 pts) or 2-3 cycles of Cy-Dex (cyclophosphamide 1000 mg/sqm day 1 + dexamethasone 40 mg days 1-4 and 9-12; new cycle day 22) (157 pts) as initial treatment. Thereafter, both groups received cyclophosphamide 2 g/sqm i.v. plus rhG-SCF (filgrastim) s.c. as mobilization therapy followed by stem cell harvest and subsequent high dose melphalan 200 mg/sqm supported by autologous stem cell infusion (ASCT).

Results. No difference was observed in proportion of patients receiving ASCT, 132/157 (84%) in the VAD group compared to 136/157 (87%) in the Cy-Dex group. Major responses (CR+PR) three months after ASCT were 91/114 (80%) in the VAD group and 96/114 (84%) in the Cy-Dex group with a non-significant trend towards a higher CR rate for VAD, 37 versus 27%. Seven patients in the VAD group died within 3 months from randomization versus 2 patients in the CY-Dex group. However, bearing in mind that the trial was not powered for evaluation of survival and that the follow-up time is too short for any safe conclusions, no difference in survival was seen, the 2 years overall survival probability being 81% (95%CI 72-87%) for VAD and 77% (95%CI 69-84%) for Cy-Dex.

Conclusions. Comparing conventional VAD with an experimental CY-Dex induction therapy in newly diagnosed myeloma patients this randomised study did not identify any difference in the proportion of patients actually receiving ASCT. The response rate and overall survival were comparable for the 2 groups. Cy-Dex is an effective alternative to VAD and is simpler as initial therapy with no need for hospitalization and central venous access. Being a less complicated initial treatment, Cy-Dex also offers the advantage of being easier to combine with new experimental drugs.

P0.506

DEVELOPMENT OF UK-NORDIC GUIDELINES FOR MANAGEMENT OF MYELOMA

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Clinical research in myeloma has generally been limited to specific areas or endpoints. Until the publication of the British Society for Haematology (BSH)/UK Myeloma Forum (UKMF) guidelines in 2001 there was limited published information on its overall management. National and international feedback on the UK guidelines was favourable. They have since been published in other languages. The Nordic Myeloma Study Group (NMSG) also developed guidelines around the same time as the UK group; these were published in Scandinavian languages and available on the NMSG website. Following informal contact between the British and Nordic groups it was agreed that a jointly written update of diagnostic and management guidelines would be appropriate. Approval was obtained from the BSH, NMSG, & UKMF. The work has followed established principles for guideline development in terms of rigorous review of literature, Cochrane data etc. Contributors and authors mainly comprise clinicians with active involvement in myeloma diagnosis and treatment, with a balance towards those engaged in clinical service as opposed to mainly academic roles The working group included specialist nurses in myeloma and patient advocacy, represented by the IMF (UK). Input was also obtained from specialists in nephrology, clinical oncology and orthopaedics. The remit was to produce a document encompassing the whole care pathway experienced by the myeloma patient. To achieve this, subgroups with representation from the UK and Nordic countries addressed specific topics, e.g. indications for treatment, primary treatment, management of relapse etc.& drafted their respective sections. Work was undertaken electronically and then reviewed at a joint 2-day meeting of the whole project group. Sections had been previously circulated and reviewed by all beforehand. Rigorous discussion and review of material took place. A consensus of recommendations and evidence grading was achieved, to be incorporated into a final document for review by the "sounding board" of clinical opinion of the BSH and NMSG regional coordinators before publication in peer-reviewed literature and on the internet. The work was undertaken voluntarily by the various contributors and proved highly educational for all those involved. Administrative costs were minimised by working electronically and networking at established international meetings. The IMF (UK) provided independent financial support for the consensus meeting and also provided key help with administrative tasks. The process could form a model for the development of internationally based guidelines for myeloma or other diseases. Clinical problems in myeloma are similar in all countries. There would seem benefit in international consensus on care pathways and treatments in an attempt to offer evidence based treatment to all patients, as well as identifying areas where further research is needed.

P0.507

NEWLY DIAGNOSED MYELOMA PATIENTS ARE AT RISK OF VENOUS THROMBOTIC EVENTS – HIGH RISK PATIENTS SHOULD RECEIVE THROMBOPROPHYLAXIS: THE MRC EXPERIENCE

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Standard treatment for younger presenting myeloma patients is VAD followed by high-dose melphalan with stem cell support. However this regimen requires a central venous catheter introducing problems with line infections and central venous thrombus. A number of oral alternatives have been used including dexamethasone and thalidomide, although to date no randomized control trial has directly compared iv with oral induction therapy. In older patients melphalan/prednisolone remains the standard approach. Thal combinations improve response rates and providing side effects can be managed effectively may also be appropriate for an elderly population. Patients with myeloma have an increased risk of venous thrombotic events (VTEs), and presenting patients receiving Thal may be at increased risk due to bulk disease. Combination with anthracyclines may also exacerbate this risk. The MRC Myeloma IX trial has been designed to address some of these issues. Younger patients are randomized to receive iv CVAD or an oral Thal containing regimen, CTD prior to autologous transplantation; older patients are randomised to MP or the Thal containing regimen, CTDa. At the initiation of the trial physicians were advised that patients should start low-dose Thal and slowly dose escalate to 200 mg. Patients at high risk of VTE should be considered for full anticoagulation (warfarin or LMWH). As of Aug 2004 420 patients (239 intensive, 181 non intensive) have been randomized and a total of 30 VTEs in 28 patients have been reported (22 intensive, 6 non-intensive). In the intensive arm there were 8 DVT, 9 PE and 7 line-related thromboses. In the non-intensive arm there were 4 DVT and 2 PE.

	CVAD	CTD	CTDa	MP
DVT	4.2%	2.5%	4.4%	0%
PE	1.7%	5.8%	2.2%	0%
Line related	5.0%	0.8%	NA	NA
Total	10.9%	9.1%	6.6%	0%

The median time from randomization to DVT/PE was 54.5 days (range 15-113). 4 patients could be identified who had additional risk factors. Only 1 patient was receiving prophylaxis having previously suffered a DVT. There was 1 PE-related death. Importantly 2 PE and 5 DVT occurred in patients not receiving Thal therapy. In the non-intensive arm the addition of Thal increased VTE risk compared to MP. In conclusion myeloma patients have an increased incidence of VTE with patients receiving infusional regimes also at increased risk of line-related thrombosis. The addition of Thal only marginally increased DVT/PE risk over and above the risk seen in patients with infusional regimens. Even in a large study such as this the number of events are too small to make firm recommendations and our current advice remains unchanged: ALL high-risk patients should receive thromboprophylaxis.

P0.508

RISK FACTORS FOR VENOUS THROMBOEMBOLISM IN MYELOMA AND RELATED DISORDERS; A PREVALENCE STUDY OF ELEVATED LEVELS OF FACTOR VIII AND VON WILLEBRAND FACTOR ANTIGEN

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Venous thromboembolism (VTE) is a common complication of disseminated adenocarcinomas, but is less common in patients with haematological malignancy. Clinical trials of thalidomide and anthracycline chemotherapy (thal/CTX) in refractory multiple myeloma (MM) have shown a markedly increased rate of VTE (incidence 25-30%). The prothrombotic mechanism(s) of thal/CTX remain to be defined, but elevated procoagulant Factor VIII (FVIII:c), von Willebrand Factor antigen (vWFAg) and acquired resistance to activated Protein C (aPC-R) have been implicated in studies to date. Elevated FVIII:c (>150%) has been associated both with VTE and recurrent VTE in patients without malignancy. We have observed elevated pretreatment levels of FVIII:c and vWFAg in patients with mesothelioma who developed VTE following therapy with thalidomide and chemotherapy. To provide a baseline for measuring the effect of thal/CTX on these parameters, we assayed FVIIIC and vWFAg in currently untreated patients without a history of thrombosis. We compared MM patients (n=34) with monoclonal gammopathy of uncertain significance (MGUS; n=26) and chronic lymphocytic leukaemia (CLL; n=31). MM patients showed significantly increased mean FVIII:c compared to the MGUS/CLL group (p=0.001) and 74% of MM patients had elevated levels compared to 19% of MGUS patients. Correlations between FVIII:c levels and Durie-Salmon MM Staging established that for indolent disease the mean FVIII:c was 1.57 IU/dL compared to a mean of 2.14 IU/dL for aggressive disease (Stage 2 and above). Mean plasma levels of prothrombin fragment 1+2 were increased in aggressive myeloma compared to indolent and MGUS cases (p=0.034), indicating increased thrombin turnover. VWFAg was within the reference range for most MM patients, showing no difference with the CLL/MGUS group. This unexpected finding could be explained by MM-related cytokines which have been reported to increase FVIII:c independently of vWFAg. Activated Protein C resistance was seen in a small subset (<13%) of patients, with no significant difference between disease groups. This prevalence study demonstrates that FVIII:c, but not vWFAg, is routinely elevated in patients with MM compared to MGUS and CLL, and that the degree of FVIII:c elevation is related to myeloma activity. Elevated FVIII:c levels may reflect the effect of myeloma-related cytokines, and may be implicated in treatment-associated thrombosis. We propose that MM patients with elevated FVIII: c are at higher risk of VTE when treated with prothrombotic combinations such as thal/CTX. Thal/CTX therapy appears to trigger acute thrombosis through additional effects on coagulation factors, including vWFAg and on endothelial function. Defining these mechanisms will be important in improving patient outcomes in refractory myeloma.

P0.509

TOXICITY IN STANDARD MELPHALAN-PREDNISONE THERAPY AMONG MYELOMA PATIENTS WITH RENAL FAILURE – A RETROSPECTIVE ANALYSIS AND RECOMMENDATIONS FOR DOSE ADJUSTMENT

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Introduction. For myeloma patients not eligible for high dose therapy MP po is still widely used as first line therapy. Whether the melphalan dose used in this setting should be routinely reduced or not in patients with renal dysfunction has not been settled and recommendations in myeloma guidelines differ between nations. In this retrospective analysis we have studied the influence of renal function on melphalan toxicity in patients treated with per oral MP.

Patients and Methods. From July 1990 until November 1992 275 patients, 155 men and 120 women, were randomised to receive MP alone in a randomised study comparing MP with MP+IFN. Patient median age was 66 (37-87) years. Ig subtypes were IgG 175, IgA 56 and only light chains 44. Twenty-eight were in stage I, 91 in stage II and 156 in stage III. The MP schedule used was oral melphalan 0.25 mg/kg and prednisone 100 mg/day on days 1-4 every 6 weeks. The melphalan dose in the first course was not adjusted with regard to renal function or age. All patients were evaluated for haematological and infectious toxicity according to WHO criteria after the first MP course. In subsequent courses the melphalan dose was adjusted according to toxicity. Creatinine clearance was calculated according to Cockcroft and Gault.

Results. At diagnosis median serum creatinine was 103 (39-1004) µmol/L, and median creatinine clearance 55 (3-148) ml/min with clearance <30 mL/min in 43, 30-50 mL/min in 68 and >50 mL/min in 164 patients. Only 4 patients had clearance >10 mL/min. Grade 4 infectious toxicity was seen in eight patients with lethal outcome in four. Two additional patients died within 6 weeks from the first MP course. The causes of death in these two patients were gastrointestinal bleeding associated with thrombocytopenia in one and sudden cardiac death in the other. Besides this lethal bleeding no other severe bleedings (grade 3-4) were seen. Out of the six deaths within 6 weeks from the first MP course two occurred in patients with creatinine clearance <30 mL/min; one infectious death and the sudden cardiac death. A negative correlation was found between creatinine clearance and haematological toxicity with after the first MP course haematological toxicity WHO grades 3-4 in 18%, 28% and 36% of patients with a creatinine clearance of >50 ml/min, of 30-50 mL/min, and of < 30 mL/min, respectively. Infections WHO grades 3-4 occurred in 6% and were not significantly related to renal function.

Conclusions. We conclude that po MP therapy can be used for initial therapy in myeloma patients with renal impairment but suggest that reduction of the melphalan dose should be considered in patients with a GFR of <30 mL/min. Since only 2% of our patients had a clearance of >10 mL/min no conclusions can be drawn for this subgroup.

P0.510

IMPLICATON OF RENAL FAILURE IN PATIENTS WITH NEWLY-DIAGNOSED MULTIPLE MYELOMA

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Background. The presentation serum creatinine value in MM is used for staging and assessment of prognosis. However, renal function can change with hydration and correction of hypercalcemia, but a proportion of patients have persistently compromised kidneys when chemotherapy is started despite correction of dehydration and metabolic abnormalities.

Aims. We propose ourselves to study the impact of RF on immediate and medium term evolution in patients with MM.

Methods. We reviewed the outcome of 31 patients with serum creatinine >200 micromole/L at start of chemotherapy with VAD or Dexametazone high dose (Dex).

Results. 7 patients died early (1-9 weeks, median 3) after starting therapy due to renal failure, infections or other medical complications. 5 of 18 receiving VAD died compared with 2 of 13 getting Dex. Of the remaining 24 patients, 3 attained CR, 13 PR, and 8 did not respond after median 4 course of therapy. On an intent-to-treat basis, overall response rate to induction therapy was 59%. Creatinine declined with therapy in 84% of 27 evaluable patients, and the change in creatinine from cycle 1 to 2 was on an average of 32%. The extent of the change in creatinine was not predictive of eventual survival suggesting that intensive therapy should be continued even if renal function does not improve after 1 cycle of chemotherapy. 6 patients had creatinine >200 micromole/L (including 2 on dialysis). Median survival of the whole group was 21 months, with one patient alive with disease at 4 years. Not surprisingly, 15/24 patients who died had creatinine values >200 micromole/L at death vs 2/9 living patients at last follow-up (p=0.03) suggesting that kidneys are compromised and regardless of cause of death, they fail as terminal event.

Conclusions. While renal dysfunction at initiation of induction therapy is indicative of relatively poor outcome, it's largely due to early mortality - which can be reduced with intensified supportive therapy. In patients surviving the first 2-3 months, long-term outcome is comparable to those with normal renal function if treated similarly.

P0.511

HYPERCALCEMIA IN PATIENTS WITH PLASMA CELL DYSCRASIA

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Background. Hypercalcemia (about 30%) and skeletal lesions (about 60%) are common diagnostic findings in patients with multiple myeloma (MM). As far as we have seen there has be no report of the simultaneous finding of hypercalcemia and normal radiographic survey of the skeleton. This question was raised in three patients with M-component and hypercalcemia but without skeletal pathology. We report these three cases and present the Ca-concentration in 945 patients with MM in relation with the radiographic skeletal findings.

Materials. a. Three females with M-component and hypercalcemia (see Table below): b. 945 patients with MM were analyzed at time of diagnosis with regard to radiographic skeletal lesions and hypercalcemia.

pat	s-M-protein g/L	u-M-protein g/24 h	Corr s-Ca mmol/L	Plasma-cell %	X-ray	Preliminary diagnosis
1	-	0.6	2.88	9	Neg.	MGUS
2	IgG⁻ 20	-	3.79	7	Neg.	MGUS
3	IgG⁻ 35	-	2.94	13	Neg.	MM stage I

Results. a. Two of three patients did not fulfilled the diagnostic criteria of MM and the third was except the s-Ca-value diagnosed as MM stage 1. The follow-up showed the etiology of hypercalcemia to be due to: pat 1: D-vitamin treatment for hypoparathyroidism; pat 2: hyperparathyroidism; pat 3: Ca-D-vitamin treatment for osteoporosis; b. The finding of increased corrected s-Ca was more common in patients with skeletal lesions.

Skeletal changes	n	Corr. s-Ca < 2.65 mmol/L	Corr. s-ca> 2.65 mmol/L
None	190	162 (85 %)	28 (15%)
Moderate	426	322 (76 %)	104 (24 %)
Extensive	329	160 (49 %)	169 (51 %)

Conclusions. Hypercalcemia is seldom seen in patients without radiographic skeletal changes but seen in half of the patients with extensive skeletal lesions. In patients with monoclonal gammopathy and an increased corrected s-Ca but without skeletal changes other explanations than the plasma cell dyscrasia must be considered.

P0.512

EFFICIENT REMOVAL OF SERUM FREE LIGHT CHAINS BY HEMODIALYSIS

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Introduction. Excess monoclonal light chain production in multiple myeloma (MM) and AL amyloidosis can lead to impaired renal function which is associated with a poor prognosis. In MM approximately 25% of patients have renal failure at presentation and almost 50% have some renal pathology. In AL amyloidosis, in addition to chemotherapy, removal of the circulating amyloidogenic light chain would also be of benefit. Light chains could be removed using plasma exchange (PE); however, this involves the replacement of patients' plasma with saline and human plasma products. This study evaluated the ability of peritoneal dialysis (PD) and haemodialysis (HD) to remove excess light chains from the serum of patients with renal failure.

Methods. Blood samples were taken from 22 HD patients before and after dialysis, 11 patients undergoing PD and 19 patients with chronic renal failure (CRF) not requiring dialysis. Serum free kappa and lambda light chain levels were examined using a nephelometric serum free light chain (sFLC) assay (The Binding Site).

Results. Before HD, patients had elevated mean serum free kappa and lambda levels of 87.9 mg/L and 102.6 mg/L respectively with a kappa/lambda (κ/λ) ratio of 0.99 (normal means: κ 7.3 mg/L, λ 12.7 mg/L and κ/λ ratio 0.6). Following 3 hours of HD there was a significant reduction in sFLC levels. Serum free κ and λ levels fell to means of 36.8 mg/L and 60.9 mg/L respectively, representing a mean fall of 55% (p<0.0001) and 35% (p<0.0001). The mean κ/λ ratio fell to 0.65 (p<0.001). Serum free kappa and lambda levels in 11 PD patients were 73.1 mg/L, 88.4 mg/L respectively (mean κ/λ ratio 0.83). CRF patients had mean sFLC levels of 27.2 mg/L for κ and 26.8 mg/L for λ (ratio 0.94). Conclusions: These data indicate that 3 hours of HD effectively removes sFLC from the circulation. Free λ chains are usually found as 50 kDa dimers which may account for the smaller fall in λ concentrations, due to retention by the dialysis membrane. Levels of sFLC in PD were similar to those found in HD patients before dialysis, indicating that PD is less efficient for light chain removal. CRF patients had lower levels of sFLC, reflecting the varying levels of renal function observed in this group. These results suggest that HD is a potential option for the removal of excess light chains from MM and AL amyloid patients.

	Mean Kappa (mg/L)	Mean Lambda (mg/L)	Mean Ratio
Normal sera	7.3 (range 3.3-19.4)	12.7 (5.7-26.3)	0.6 (0.26-1.65)
Pre-haemodialysis	87.9	102.6	0.99
Post-haemodialysis	36.8	60.9	0.65
Peritoneal dialysis	71.3	88.4	0.83
Chronic renal failure	27.2	26.8	0.94

P0.513

RESULTS OF A RANDOMIZED CONTROLLED TRIAL OF PLASMA EXCHANGE IN ACUTE RENAL FAILURE OF MYELOMA

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Background. Plasma exchange has been suggested to be of theoretical benefit in the treatment of acute renal failure at the unset of multiple myeloma. Two small-randomized trials provide conflicting evidence.

Methods. We tested the hypothesis that 5 to 7 plasma exchanges for acute renal failure at the onset of multiple myeloma reduce the primary composite outcome of death, dialysis dependence or creatinine clearance <30 mL/min at 6 months. We conducted a randomized controlled trial in which 97 patients were randomly assigned to receive plasma exchange (58 patients) or no plasma exchange (39 patients). Randomization was stratified by 4 strata (chemotherapy and dialysis dependence) recruiting physicians were blinded to assigned treatment allocation.

Results. The baseline characteristics of the plasma exchange and control group were similar for dialysis dependence, chemotherapy, gender, age, serum calcium, serum albumin, 24-hour urine for protein, serum creatinine clearance and Durie-Salmon staging. The incidence of the primary composite end point – death, dialysis dependence or creatinine clearance < 30 ml/min at 6 months was 68.6% in the plasma exchange group compared with a 61.5% in the control group (hazard ratio in the plasma exchange group adjusted for baseline characteristics was 0.92; 95 percent confidence interval, 0.34 to 2.46; p=0.86).

Conclusions. In patients with acute renal failure at the onset of multiple myeloma, this study suggests plasma exchange provides no further benefit in reducing death, dialysis dependence or creatinine clearance <30 mL/min at 6 months follow-up.

P0.514

PERCUTANEOUS VERTEBROPLASTY FOR PAIN RELIEF AND STABILIZATION OF A PATHOLOGICAL C2 AND DENS FRACTURE IN A PATIENT WITH MULTIPLE MYELOMA: A CASE REPORT

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Background. Percutaneous injection of bone cement in the vertebral body (PVP) gives pain relief and reinforce the vertebra in >80% of patients with malign destruction of the spine due to myeloma. We describe a patient with neck pain and an unstable fracture in the vertebral body of C2 dens due to myeloma. Pain relief and stabilization of the pathological fracture was obtained after PVP.

Case report. A 47 year old woman was admitted to our hospital with a history of neck pain for 6 weeks, but without focal neurological symptoms. The radiological work up showed an extensive destruction and a pathological, unstable C2 and dens fracture. Several vertebral biopsies were inconclusive. The bone marrow aspirate showed 20% plasma cells and she excreted 3 g/24 h χ-chains in the urine fulfilling the diagnosis of multiple myeloma. She immediately received a cervical collar as the risk for development of neurological deficit from the instable C2 fracture was considered very high. After discussion it was decided to start with cytotoxic treatment and radiation against C2 and underwent high-dose chemotherapy with autologous stem cell support. After this treatment there was still a massive C2 osteolytic destruction at CT scan. Due to the unstable situation she continued to wear a cervical collar for 9 months. During this time she had a lot of discomfort, which increased her need for pain relief and sedatives. After she had recovered from cytotoxic treatment PVP was carried out on the patient without any clinical complications. A CT-scan with flexion and extension provocation was performed a short time after PVP and it revealed stable conditions. Ten months after the diagnosis the cervical collar was removed, and she has no neck pain. Her myeloma is still in remission.

Discussion. Chemotherapy and local irradiation was initially considered to be the best treatment for this patient. However, the problem with an unstable pathological C2 fracture remained unsolved. This caused the patient a great deal of discomfort from the neck region even after the myeloma was in partial response. In cases where there is a risk of dangerous fractures threatening the spine, one will therefore have to add stabilizing treatments as e.g. operation or PVP. In this case, the minimal invasive PVP was considered the best alternative compared to a much more extensive operative stabilization procedure.

P0.515

ERYTHROPOIETIN INCREASES THE SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA

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Erythropoietin (Procrit®) is effective in the treatment of anemia in patients with multiple myeloma. Mittelman *et al.* reported on the anti-myeloma effect of Procrit®. We reviewed the prospectively entered data of 104 patients with multiple myeloma enrolled on a phase II study with liposomal Doxorubicin, Vincristine, decreased frequency Dexamethasone and Thalidomide (DVd-T) and an additional 72 non study patients treated at the Cleveland Clinic Foundation Myeloma Program with complete Procrit® use data from October 1996 to November 2003. Thirty percent of patients had SWOG stage I, 38% stage II, 21% stage III, and 11% stage IV. Mean serum hemoglobin was 10.9 g/dL (S.D. 1.9), mean beta-2 microglobulin was 5.9 mg/L (S.D. 6.3). Eighty-six patients (49%) used Procrit®, of which 31 patients had longer than 6 month use and 55 had less than 6 month use. Twenty-nine patients (33% of Procrit® users) received higher dosages (defined as greater than 60,000 units per week). Median Procrit® use was 3.5 month (range 1 to 48 month). While no difference in overall survival was noted among patients using Procrit® compared to non-users (OS 825 versus 838 days p=0.8), high dose users (>60,000 units weekly) had an increase overall survival compared to lose dose Procrit® users (OS 1097 versus 686 days, p=0.01). Patients with longer than 6 month use had an increased overall survival compared to patients using Procrit® less than 6 month (OS 1035 versus 707 days, p=0.01). Procrit® users had lower serum hemoglobin compared to non users (10.4 versus 11.4 g/dL, p<0.001), higher serum beta-2 microglobulin (6.7 versus 5.0 mg/L, p=0.08), and lower serum albumin 3.4 versus 3.7 g/dL, p=0.003). The mean baseline serum hemoglobin among high dose Procrit® users was 10.3 g/dL (compared to 10.4 g/dL among low dose Procrit® users p=0.8) and the mean baseline serum hemoglobin among long term users was 10.5 g/dL (compared to 10.3 g/dL among short term users, p=0.5). There were no differences in baseline serum beta-2 microglobulin, albumin and creatinine among low dose Procrit® users versus high dose users, long term users Procrit® versus short term users. High dose and longer Procrit® use is associated with increased survival in patients with multiple myeloma. While survival among Procrit® users was similar to non-users, the former patients had worse prognostic parameters (lower hemoglobin, higher serum beta-2 microglobulin and lower serum albumin). Postulated mechanisms include, CD8 T-cell modulation, a direct cytotoxic effect to malignant plasma cell, a change in marrow microenvironment or possibly an improvement in performance status of patients resulting in increased tolerance to cytotoxic agents. We are in the process of combining the data from all our studies and initiating a randomized trial of low versus high dose Procrit® therapy in multiple myeloma patients receiving active therapy.

P0.516

WEEKLY EPOETIN BETA IMPROVES HEMOGLOBIN LEVELS AND IMPROVES QUALITY OF LIFE IN PATIENTS WITH MULTIPLE MYELOMA

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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 multiple myeloma (MM).

Methods: Anaemic patients (Hb 9-11 g/dL) with MM received treatment with epoetin beta 30 000 IU once weekly. Patients had a WHO performance status (PS) of 0-2 and life expectancy of >6 months. Hb response was defined as an increase of more 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 more 2 g/dl). There were compared the results of this lot with

those one similar to a lot where only antineoplastic chemotherapy was administrated.

Results. A total of 41 patients received epoetin beta once a week and a number of 52 patients were treated only with antineoplastic chemotherapy. There are not important differences between the 2 lots concerning the rate on performance status, on patients in stage III of disease, or those ones who get antineoplastic chemotherapy. Most patients had advanced tumour stage at baseline (std III aprox 84%) and the vast majority of patients (97%) received concurrent chemotherapy. Hb response rate in patients treated with epoetin beta once weekly was high 72% vs 45% in patients treated only with chemotherapy (chi2=4,75 p< 0,045). Also patients with a Hb response were more likely than patients without an adequate response to have an improvement in PS (29% vs 11%) and less likely to have a deterioration in PS (epoetin lot, 10% vs 29%) chi2= 3,68; p<0,057. Over half of patients showed a complete, partial or stable response to chemotherapy by study end (epoetin lot, 68% vs 55% in only chemotherapy lot (Chi2 doesn't attain the significative limit). Overall, 81% of epoetin patients who answer to chemotherapy had an Hb increase of 2 g/dl and only 68% of the lot without epoetin present this characteristic. Chi2 =3, 98 p<0,052.

Conclusions. Epoetin beta once weekly results in a high Hb response in patients with multiple myeloma. Hb response with epoetin beta is associated with an improvement in PS and improved response to chemotherapy. If, anaemia correction is associated with treatment response in patients with multiple myeloma is to be fully explained by larger studies.

PO.517

THE QUALITY OF LIFE BENEFIT ASSOCIATED WITH EPOETIN TREATMENT IN MULTIPLE MYELOMA AND LYMPHOMA IS SMALL

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Epoetin alfa, epoetin beta or darbepoetin alfa (EPO) is widely prescribed for cancer patients with anemia on the assumption that it will increase hemoglobin, reduce transfusion needs and improve quality of life (QOL). As an expensive drug, its merits need to be examined closely. We evaluated the clinical significance of the quality of life benefits reported in the three largest randomized, using two approaches. Effect size (ES) (score difference divided by the standard deviation (SD)) were calculated, using the population derived SDs for the FACT-An questionnaire reported by Cella (2003). According to Cohen (1977), ESs of 0.2-0.5 are considered small, 0.5-0.8 moderate and above 0.8 large. We also used the results obtained by Osoba (1998), where breast cancer patients evaluated score changes (EORTC QLQ-C30 questionnaire) of 5-10 on the 0-100 scale as *a little* change, 10-20 as *moderate change* and above 20 as *very much* change. To facilitate comparison, the score differences reported in the three EPO trials with the FACT-An and its subscales were transformed to a 0-100 scale. The table shows the maximum score differences between EPO and placebo groups, and their ESs.

	FACT-An Total Score diff (ES)	*FACT-G Score diff (ES)*	*Fatigue subscale Score diff (ES)*	*Anemia subscale Score diff (ES)*
Littlewood (2001)	NDR	4.5 (0.27)	10.0 (0.50)	7.9 (0.45)
Osterborg(2002)	3.1 (0.19)	2.9 (0.18)	4.2 (0.21)	1.8*
Hedenus (2003)	NDR	NDR	4.4** (0.22)	NDR

NDR=no data reported; * 7 items only, no SD available; ** calculated from Fig 3 of that paper, no numerical data reported

The ES in Littlewood's paper are small for the general part of the questionnaire (FACT-G) and somewhat larger, but still small for the fatigue and anemia subscales. The ESs in the Osterborg trial barely reach the level of *small* for the fatigue subscale while the score differences for the entire FACT-An, the FACT-G and the anemia subscale are unlikely to be clinically meaningful. In the Hedenus paper, only the results obtained with the fatigue subscale are reported, with a small ES. The score differences for the Fatigue and Anemia subscales in Littlewood's paper are the only ones that would be considered meaningful (but small) according the subjective significance rating of Osoba. Whether these results reflect differences between the erythropoietin products is impossible to determine since none of the results has been confirmed in independent trials and no comparisons have been performed. Although there are obvious caveats (e.g. the importance of score differences may vary across scales and between patient groups, the interpretation of ESs is a matter of debate, results obtained with the EORTC QLQ-C30 may not be transferable to the FACT-An), these data do suggest that the average QOL benefit obtained with EPO in hematological malignancies is small and of uncertain clinical significance. Fundamental issues as to who should be treated and how need to be resolved.

PO.518

A PILOT PROGRAM OF INDIVIDUALIZED HOME-BASED AND HOSPITAL-BASED EXERCISE FOR PATIENTS WITH MULTIPLE MYELOMA

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Cancer-related fatigue, anemia, bone pain, treatment toxicities, concurrent depression, and even advanced age itself can contribute to debilitation in patients with multiple myeloma. Many myeloma patients do not take part in a regular exercise routine and may, in fact, be fearful of activities that can cause harm. A disease-modified, supervised exercise program is needed. We, at our institution, have initiated a pilot program of two exercise programs tailored to the individual myeloma patient. Using the Duke Activity Status Index (DASI) as a screening tool, approximately 45% of myeloma patients seen at our institution are determined to have a maximum MET (metabolic equivalent) ≥6 or 21 mL O_2 consumption/kg/minute, a functional capacity deemed sufficient to undergo a regular exercise program. These patients are offered access to either a home-based individualized exercise program or a supervised group exercise program. Patients anticipating admission to hospital for stem cell transplantation within 4 months are not routinely included due to anticipated interruptions in the program. Exercises in both programs are based on general recommendations of the American College of Sports Medicine (ACSM) as modified to cancer patients by Lucia et al (Lancet Oncology 2003;4:616). Exercise activities include: 1) low-impact aerobic (using treadmills, track walking and recumbent cycle ergometers to 45-75% maximum heart rate); 2) resistance training (using resistance bands or body weight as appropriate); and 3) flexibility training. Patients in the home-based program undergo an initial 90 minute consultation with exercise specialists outlining the individualized schedule, with subsequent telephone follow-up every 2 weeks and a review consultation at 12 weeks. Patients in the group program undergo once weekly 90 minute sessions, during which group and individual exercises are supervised, for a total period of 12 weeks. Patients in both groups maintain exercise diaries and complete the Godin Leisure-Time Exercise Questionnaire every 2 weeks to monitor exercise outside of the program. All patients undergo multi-dimension-

al assessments with baseline and 12 week evaluations of: 1) body composition (body weight, body mass index, bioelectrical impedence); 2) physiologic parameters (resting heart rate, peak oxygen consumption with a 6 minute walk test, maximum grip strength using a hand grip dynamometer, muscular endurance with wall push-up/sit-to-stand repetitions, and flexibility measurements); 3) fatigue (FACT-F quality of life questionnaire); 4) pain (Brief pain inventory); 5) mental health (Hospital Anxiety and Depression Scale). Preliminary findings of our pilot exercise program with 20 patients in the home-based program and 21 patients in the group program will be presented. This pilot program will serve as the basis for feasibility of a randomized controlled trial between the two exercise approaches.

P0.519

AN EVALUATION OF QUALITY OF LIFE IN LONG-TERM SURVIVORS WITH MULTIPLE MYELOMA

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Achieving optimal Quality of Life (QoL) is a key objective of clinical practice, especially in the management of malignant disease. Measurement of QoL provides important information to the clinician about the patient's level of function in all aspects of daily life, and may also highlight gaps in current service provision. This study examines the QoL in patients with multiple myeloma. The treatment of multiple myeloma is intensive and, at times, invasive. Many studies have been carried out to examine the *patient experience* at times of diagnosis, treatment and palliation, but very little has been explored with regard to the period of survival.

Objectives. To assess the QoL in patients considered to be clinically stable who had undergone treatment for multiple myeloma. To identify specific areas of concern in this group of patients, and the factors that influence their day-to-day QoL.

Methods: Patients who were at least two years from the time of diagnosis, and one year since the conclusion of active treatment were identified from the out patient lists at Southampton University Hospitals NHS Trust. Each patient was contacted, and once consent had been obtained, a semi-structured interview using the HADS, SF-36 and SEIQoL was conducted. Data were then analysed using the technique specific to each questionnaire.

Results. 27 of the 35 consenting patients were interviewed. HADS showed a significantly lower occurrence of anxiety symptoms compared to the reference population (p=<0.05), but there was no significant difference in the level of depression symptoms. SF-36 showed a significantly decreased QoL with regard to physical functioning, physical role, general health and emotional role (p=<0.05, <0.005, <0.0001 and <0.05 respectively) compared to a reference population. There was no significant difference between the SEIQoL index of the study group compared to the reference population.

Conclusions. The questionnaires give conflicting views of the QoL of patients with multiple myeloma. HADS suggests that it is better than the healthy population, SF-36 that it is worse, and SEIQoL that there is no difference. Patients described a perceived increase in the symptoms of anxiety when a hospital appointment was due, this requires further examination to ensure that the follow up process is not detrimental. SEIQoL identified many issues affecting the QoL of this group. A greater understnding and appreciation of these factors is required if we are to enhnace the experience of long-term survivorship. are we are to enhance the exoperience of long-term survivorshuip.

P0.520

EPIDEMIOLOGY, CLINICAL PROFILE AND TREATMENT OUTCOME OF MULTIPLE MYELOMA IN INDIA - A HOSPITAL BASED STUDY

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The data on the presentation and results of treatment on multiple myeloma in India patients are scarce. We reviewed the clinical profile and treatment outcome of patients with multiple myeloma seen at our centre over the last 15 years. A total of 32 proved cases of multiple myeloma were observed during 15 years (1989-2004). There were 20 males and 12 females. The median age was 53 years. 60% of patients presented with body aches/bone pains, 20% with generalized weakness and 8% with pathological fractures. Anemia was the common finding in these patients (80%). It was normochromic to microcytic. All the patients received (Melphan and prednisolone M.P.) regimen (6-8 cycles). Five patients received VAD therapy in addition to M.P. and 2 patients received Bone Marrow transplant. Most of the patients responded initially but later the disease progressed and 15 patients died during follow up due to progressive myeloma. 3 cases died due to infections and 2 due to renal failure. The median survival was 3 years. The study highlights that while the clinical profile of multiple myeloma was similar to that seen in the west, the outcome was poor in Indian patients.

P0.521

SOUTH AMERICAN MULTIPLE MYELOMA STUDY: EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF 751 PATIENTS

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Background. The incidence of multiple myeloma varies substancially and its geographic distribution in the world may be distinct. The clinical characteristics, including the behavior of the disease, can be different in diverse regions. There are few statistics on this disease in South America.

Objective: To evaluate the profile of multiple myeloma patients in South America and to observe if the characteristics in these patients are similar to those verified in patients in other regions.

Patients and Methods. A retrospective analysis of the available data on patients with multiple myeloma, diagnosed between 1998 and 2002 in Brazil, at Santa Casa de São Paulo Hospital (n=138), Hospital Universitário Clementino Fraga Filho do Rio de Janeiro (n=127), Hospital das Clínicas de São Paulo (n=126), HEMOPE, Recife (n=62), Hospital de Clínicas de Porto Alegre (n=44) and Hospital de Clínicas da Unicamp, Campinas (n=44); in Argentina, Hospital Italiano (n=83) and La Plata (n=32); in Venezuela, at Banco Metropolitano de Sangre, Caracas (n=40) and Hospital Ruiz y Paez, Bolívar (n=20) and; in Chile, Sociedad Chilena de Hematologia (n=35). The data of these patients was revised and the age, sex, race, monoclonal component type, creatinine, hypercalcemia, presence of anemia and lytic lesions and Durie & Salmon staging were evaluated.

Results. The median age of the patients was 60.5 years; 51% female and 49% male; 87% white/non-white and 13% black; monoclonal component type IgG 60%, IgA 22%, light chains 11% and non-secretor 4%; with hypercalcemia (calcium>10.5 mg/dL) 25%; creatinine>2.0 mg/dL 22%; 81% with lytic lesions; 57% with anemia (Hb<10g/dL); Durie & Salmon staging: IA 8%, IIA 11%, IIB 1%, IIIA 61% and IIIB 19%.

Conclusions. The median age, component monoclonal type, hypercalcemia and creatinine were not different from the others series. Related to sex, most series show a slight male preponderance, however, we observed a slight female predominance. This data shows that the majority of the patients with multiple myeloma at these centers in South America present an advanced stage of the disease upon diagnosis.

P0.522

CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF MULTIPLE MYELOMA PATIENTS IN RIO DE JANEIRO, BRAZIL

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Introduction. The epidemiological characteristics of multiple myeloma patients may show significant variation from one country to another. Such variation can impact the clinical aspects of the disease onset. In order to determine the epidemiological characteristics of Multiple myeloma patients, we reviewed a large series in a single Brazilian institution. Our findings were compared with the results in the international literature.

Patients and Methods. We reviewed the records of all multiple myeloma patients, diagnosed in HEMORIO, Rio de Janeiro, Brazil, from January 1st, 2000 to September 20th, 2004.

Results: Our study involved 173 patients, the median age was 66 years (22-87), 87.8% were 50 or older, 38.7% were 70 or older, 2.3% were below 40. The male/female ratio was 1:1.27 (55.7% were female). Hypercalcemia (calcium level >11 mg/dL) was present, initially in 12% of the patients, anemia (hemoglobin level <10 g/dL) in 66.4% and an increased serum creatinine level (>2 mg/dL) in 21.5%. Immunoelectrophoresis showed a monoclonal protein in 88.5% of the patients (75.8% were IgG and 24.2% were IgA).

Conclusions. Except for the preponderance of female patients in our series (male/female ratio 1:1.27), the characteristics were very similar to those published in the literature. Female preponderance was also found in other Brazilian series.

P0.523

MULTIPLE MYELOMA IN AN ASYMPTOMATIC BLOOD DONOR: A CASE REPORT

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Abstract. The authors report on a multiple myeloma diagnosis while investigating a 31-year old black female patient who had been declared unsuitable for blood donation. The patient's young age and hardly any symptoms reported in her records showed it was an exceptional situation, while illustrating the relevance of meticulous, systematic clinical investigation in all patients reporting anemia with no known disease associated.

Case Report. A 31-year-old black woman had been considered unsuitable for blood donation two months earlier was sent to HEMORIO for investigation. Having initially declared herself asymptomatic, the patient later admitted, in her history, that she had been feeling tired, with moderate joint pain, and body weight loss for the past two months. Initial clinical investigation revealed: HCT, 20.6%; RBC, 2,32 milion/uL; MCV, 89 fl, Hemoglobin, 6,7 g/dL, WBC, 4,500/mm^3 (eosinophils, 1.9%, basophils, 2.6%, neutrophils, 34.8%; lymphocytes, 58%; monocytes, 3.7%); platelets, 250,000 /mm^3; Glucose 96 mg/dL; Urea 21 mg/dL; creatinine 1,1 mg/dL; Uric acid 4.0 mg/dL; Total Protein 10.0 g/L (Albumin 3.7 g/dL, Globulin 6.3 g/dL), Serum Glutamic-Oxaloacetic Transaminase (SGOT) 27U/L; Serum Glutamic Pyruvic Transaminase (SGPT)-26U/L; Total Bilirubin 0.3 mg/dL (Indirect - 0.2 mg/dL) and serum calcium 8.1 mg/dL. The normochromic normocytic anemia condition - of unknown origin - indicated the need for additional exams that included electrophoresis of serum and urine immunoglobulin that revealed monoclonal bands. The serum IgG level was raised at 13,281 mg/dL, IgM-359 mg/dL and IgA-39 mg/dL. The bone marrow biopsy showed plasmocytosis (70%). Those findings - among others - led to the multiple myeloma diagnosis.

Discussion: Multiple myeloma is not usually included in the differential diagnosis of anemia in young patients. This case report aims at pointing out the relevance of rigorous investigation based on appropriate routine. The relevance is associated to defining an algorithm for basic exams - as those carried out in this case report - so that diagnosis can be reached at an early stage.

P0.524

MULTIPLE MYELOMA IN A 22-YEAR-OLD PATIENT: A CASE REPORT

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Introduction. Multiple myeloma is a condition typically reported in advanced age patients (in average 66 years old), and seen as rare for middle aged individuals (around 40 years old), and exceptional for anyone under 30. The purpose of this paper is to report the case of a young, asymptomatic woman (22 years old) whose diagnosis of multiple myeloma at an advanced stage was reached while investigating an anemia condition.

Case Report. The patient was a 22-year-old white woman, housekeeper, birthplace Rio de Janeiro. In March, 2004 she was sent for anemia investigation after having been declared unsuitable for blood donation. The patient reported no symptoms. Laboratory data on admission, included the following findings: HCT 20.6%; RBC 2,0 milion/uL; Hemoglobin 7.7 g/dL; WBC 5,700/mm^3; Rouleaux formation in erythrocytes; Platelets 250,000/mm^3; Creatinine 0.8 mg/dL; Serum calcium 10.3 mg/dL; Total Protein – 12.2 g/dL (Albumin 4.2 g/dL, Globulin 8.1 g/dL). Those findings indicated the need for additional exams which included serum protein electrophoresis – which reported monoclonal gamma globulin peak – serum immunoglobulin levels – which reported IgG - 9840 mg/dL; IgA - 28.6 mg/dL; IgM-359 mg/dL. A bone marrow biopsy showed dense plasmocyte infiltration with aberrant plasmocytes, and radiograms revealed lytic lesions in right humeral diaphysis. The cytogenetic study did not report abnormalities. The findings were compatible with multiple myeloma diagnosis, stage IIIA (Durie and Salmon stage system).

Discussion. The literature available worldwide shows that median age for multiple myeloma prevalence is the 6th decade in life, being rare below 40 (approximately 2%), and considered exceptional in the 20's (0.3%). This report presents a patient with multiple myeloma diagnosed at 22 years.

Additionally, patient's advanced stage of the condition is to be pointed out (stage IIIA) at the time of diagnosis, despite the fact that no symptoms were referred to. The combination of factors build up case relevance.

P0.525

UK – NORDIC GUIDELINES FOR MANAGEMENT OF MYELOMA: ESSENTIAL NEW RECOMMENDATIONS

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Key changes from the guidelines published by the British Society for Haematology/UK Myeloma Forum and those issued by the NMSG in the Scandinavian languages in 2001 are presented. The modifications reflect progress during the last four years in the treatment and care of multiple myeloma patients.

Chemotherapy. The new diagnostic criteria of the International Myeloma Working Group and the new International Prognostic Index based on serum concentrations of β2 microglobulin and albumin are recommended. High dose therapy with autologous stem cell transplantation should be part of the treatment strategy in newly diagnosed patients up to the age of 65 (grade A recommendation, level Ib evidence). It may be considered in patients >65 with good performance status (grade B, level IIa). Double autologous transplantation cannot be recommended on current evidence but may be performed within the context of clinical trials. It is recommended, however, that enough stem cells are collected to support two high dose procedures (grade C, level IV). Patients <50 years who have achieved at least a partial remission after initial therapy may be considered for HLA matched sibling allogeneic stem cell transplantation, preferably as a part of a clinical trial (grade B, level IIb). Reduced-intensity conditioning allografts early in the disease may be considered in patients up to 65 years with an HLA-matched sibling as part of a clinical trial (grade B, level IIb/grade C, level IV). Thalidomide with or without dexamethasone is recommended for relapsed/refractory patients, particularly those known to be melphalan resistant (grade B, level IIa).
Supportive Therapy. A therapeutic trial of EPO may be considered in patients with symptomatic anaemia, whether (grade A, level Ib) or not (grade B, level IIa) they are receiving chemotherapy. In newly diagnosed patients, the initial response to chemotherapy should be reviewed before considering EPO (grade C, level IV). The new guidelines contain an expanded chapter on pain management based on the *analgesic ladder* principle. The newer techniques of vertebroplasty and balloon kyphoplasty are recommended as additional measures to manage refractory back pain, where a competent multidisciplinary specialist team is available. The current joint guidelines recommend bisphosphonate therapy for all patients with myeloma requiring chemotherapy (grade A, level Ib). Treatment should be continued for at least two years (grade A, level Ib). Given the lack of randomized trials comparing intravenous to oral bisphosphonates, the choice of bisphosphonate is left to patient and physician preference. These guidelines are intended to support physicians to offer evidence based treatment to multiple myeloma patients in the UK and Nordic countries as well as abroad.

POSTER SESSION 6
HIGH DOSE THERAPY AND ALLOGENEIC TRANSPLANTATION

P0.601

LONG-TERM FOLLOW-UP (5 YEARS) OF A PROSPECTIVE, RANDOMIZED MULTICENTER STUDY COMPARING A STANDARD VERSUS AN INTENSIFIED CONDITIONING REGIMEN FOR HIGH-DOSE CHEMOTHERAPY IN PATIENTS WITH MULTIPLE MYELOMA

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High-dose chemotherapy (HDT) improves the outcome of patients with multiple myeloma (MM) in comparison to conventional chemotherapy. Dose-escalating strategies including tandem HDT are currently evaluated to further improve remission rates and survival of patients. Therefore we conducted a randomized multicenter trial to compare an intensified conditioning regimen with the current standard high-dose melphalan. The primary study endpoint was response rate, with overall survival (OS), event-free survival (EFS) and toxicity analysed as secondary endpoints. Between 1997 and 1999 a total of 56 patients with stage II and III disease, who were matched for the number of previous therapies (median time from diagnosis to transplant 7 months) and different risk factors (beta2-microglobulin, LDH, CRP, cytogentic abnormalities, chemosensitive disease) were randomized. All patients received 2 courses of oral idarubicine/dexamethasone and 2 courses of intravenous cyclophosphamide/adriamycin in combination with G-CSF followed by peripheral stem cell collection. Thirty patients were treated with melphalan 200 mg/m^2 (HD-M) whereas 26 patients received idarubicine 42 mg/m^2, melphalan 200 mg/m^2 and cyclophosphamide 120 mg/kg (HD-IMC) followed by autologous blood stem cell transplantation. Acute toxicity was higher with HD-IMC, including 5 (20%) treatment-related deaths due to infections versus none (0%) in the HD-M group. This lead to early termination of the study. Severity of mucositis (grade III-IV 80 vs. 27%, p=0.001), maximal CRP-level (20 vs. 7 mg/dL, p<0.001), days of fever (11 vs. 3, p<0.001), days with iv-antibiotics (13 vs. 4, p<0.001), number of erythrocyte-transfusions (6 vs. 2, p<0.001) and number of platelet-transfusions (16 vs. 4, p<0.001) were significantly higher after HD-IMC. Response rates did not differ significantly between both treatment groups (HD-IMC vs HD-M: CR+vgPR: 50% (95%CI 26-74%) vs. 33% (95%CI 17-55%), PR 35% (95%CI 16-61%) vs. 50% (95%CI 30-70%, p=0.3). After a follow-up of 5 years median OS for all patients was 46 months in the HD-IMC arm in comparison to 66 month in the HD-M arm (p=0.2). EFS for all patients was 20 months for HD-IMC and 15 month for HD-M (p=0.8). Even analysis restricted to patients surviving the first 100 days after HDT showed no significant differences in time-to-progression (p=0.1) or OS (p=0.6) in the HD-IMC treatment arm in comparison to HD-M. Thus, even patients surviving intensified HDT had no better outcome than patients treated with standard HDT conditioning. In conclusion, intensified conditioning for HDT had an intolerable high treatment-related mortality and did not improve EFS and OS in patients with multiple myeloma.

PO.602

HIGH-DOSE THERAPY IN MYELOMA: A META-ANALYSIS OF PATIENTS' DATA

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Background. The Myeloma Trialists' Collaborative group (MTCG) has published two previous meta-analyses on the treatment of myeloma (1998, 2001). High-dose therapy (HDT) with supporting autologous stem-cell transplantation has increasingly been adopted as first-line treatment for multiple myeloma, despite the lack of conclusive evidence from individual randomised controlled trials (RCTs) of a survival benefit over conventional chemotherapy.

Objectives. A systematic review identified 13 RCTs investigating the use of high-dose therapy. This meta-analysis, using individual patient data where available, will combine data from all identified RCTs (including more than 3000 patients) in order to synthesize the evidence for use of HDT in terms of overall survival and progression-free survival.

Methods. Trialists from the identified RCTs were contacted and, if not already members, have been invited to join the MTCG with the aim of combining the results of individual trials in a meta-analysis. Estimates of treatment effect from each trial will be pooled using fixed effect methods and odds ratios with 95% confidence intervals presented in forest plots, along with survival curves to show absolute differences. Tests for heterogeneity of trend will be used to investigate whether treatment effect differs in different patient and disease subgroups.

Results. A recent limited published data overview of three trials suggested a beneficial survival effect of HDT (p=0.01) when compared with conventional treatment (Child 2003). These data will be updated using individual patient data to allow extended follow up and the inclusion of additional trials. Analysis will be presented to compare HDT versus conventional-dose chemotherapy, early versus late HDT and single versus double transplants.

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PO.603

COMBINED THERAPY WITH TWICE-MONTHLY PAMIDRONATE AND AUTOLOGOUS PERIPHERAL BLOOD STEN CELL TRANSPLANT FOR ADVANCED MULTIPLE MYELOMA: A PILOT STUDY – A SINGLE INSTITUTION EXPERIENCE FROM JAPAN

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Introduction. Bisphosphonates (BPs) have been reported to have several activities against bone resorption as well as anti-myeloma effects through induction of apoptosis and potentiation of effector cells. In 1998, we could observe a drastic decrease of IgG monoclonal protein from 7020 mg/dL to 5010 mg/dL within ten days after single injection of pamidronate 45 mg in a Japanese female myeloma patient at resistant phase, which was associated several laboratory abnormalities compatible with tumor lysis syndrome. Since this experience, we had started a pilot study where pamidronate 45 mg was administered twice monthly throughout our treatment protocol. In this report, we summarized the results of this pilot study, where the median survival from the initiation of therapy was 65 months.

Patients and Methods. Our treatment protocol was consisted of initial induction phase (full VAD 2-4 cycles, mainly), followed by PBSCH phase (cyclophosphamide 4/m^2 + G-CSF) and final phase of single or tandem autologous unmanipulated PBSCT (L-PAM 200 mg/m^2 + 2x10^6 CD34+ cell infusion). Pamidronate 45 mg was given twice monthly throughout this protocol. During and after PBSCT, pamidronate was given at the same dose. Thirty-two patients with D/S stage 2 or 3, who completed phase 1 and phase 2, could receive autologous PBSCT from July 1998 to December 2003. Single PBSCT or tandem PBSCT was not randomised. In single PBSCT group (n=12), 3 patients were in D/S stage 2 and 9 patients were in stage 3. These 12 patients were younger than 60 years (median 49.6 years). In tandem PBSCT group (n=20), 2 patients were in D/S stage and 18 patients were in stage 3. Four patients of tandem PBSCT patients were older than 60years (median 52.5 years). The average albumin/beta-2-microglobulin level of single PBSCT and tandem PBSCT were 3.9/2.5 mg/dL and 3.9/2.3 mg/dL. ISS (1/2/3) of single PBSCT was 10/2/0, and ISS of tandem PBSCT was 14/5/1. The following statistical analysis was performed using Stat View-J 5.0.

Results. Transplant-related mortality (within 100 days) was 0% during these consecutively transplanted 32 patients. The median survival from the initiation of therapy was 65 months. Among 32 patients, CR was obtained in 7 patients. All patients who achieved CR were alive during observation period. Patients with Hb>10 g/dL just before phase 1 therapy showed significantly longer survival compared with Hb<10 g/dL.

Discussion. Through this pilot study, it was shown that the combined therapy of twice monthly pamidronate 45 mg infusion and single or tandem autologous PBSCT might be one of the promising strategies for D/S stage 2 or 3 myeloma. Further study must be undertaken to determine the optimal dose or schedule of several bisphosphonates reported to be effective in myeloma treatment, using prospective randomised trial.

PO.604

RESULTS OF THE CLINICAL TRIALS OF THE CZECH MYELOMA GROUP USING AUTOLOGOUS TRANSPLANTATION OR AUTOLOGOUS RETRANSPLANTATION

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Background. High-dose chemotherapy followed by autologous stem cell transplantation (AT) is accepted as first-line therapy for patients (pts.) with multiple myeloma. The trials of the czech myeloma group (CMG) are focused on evaluation of posttransplant maintenance therapy (MT) (trial 4W) and/or consolidation therapy (CMG 2002) in the randomized fashion. Retransplantation (RE-AT) in the case of the first relapse is one of the best treatment options and all

pts. in our program have stored stem cells for this event. RE-AT has positive influence on OS.

Methods and Results. In the trial 4W totally of 185 newly diagnosed pts. were randomized after AT to interferon (IFN) arm and IFN/dexametazon (ID) arm. Almost 25% (61/245) have been lost before randomization which was done after AT. In recent analysis (August 31, 2004), with median follow up of 63.3 months, total of 120 (120/185; 66%) pts. already relapsed and 80 pts. (44%) died. Median of event free survival (EFS) and overall survival for all group of randomized pts. (185) are 33,9 and 77,1 months respectively. We did not be able to detect statistically significant differences between I and ID arm (p=0.905 for OS; p=0.943 for EFS). Consequently CMG 2002 trial was activated in 2002 to evaluate benefit of consolidation therapy in the same model as in the trial 4W. Pts. were randomized after AT to IFN MT versus consolidation therapy (4xCED during 1,5 year after AT) followed with the same IFN MT. 280 pts. were enrolled during 2.5 year and the trial is open until 400 pts. will be randomized. Enrollment in CMG 2002 is almost twice higher than in the trial 4W. TRM is similar for both trials (2.35% vs. 3.4%). Recent data will be presented but it is still to early for evaluation of benefit of consolidation therapy after AT. Finally between 1997 and 2004, 42 pts with relapsing/progressing MM after the 1st AT from this trials (mainly 4W) were retransplanted and receive some of experimental therapies. Sensitivity to C-VAD reinduction chemotherapy (4 cycles) was 74.4 % (80% to 4 VAD induction), the response to the 2nd AT compared to the 1st one was 87.5 % versus 95.2 % to the 1st one. Toxicity of the 1st and 2nd transplantation was similar and usually did not exceed grade II (SWOG criteria), there were no significant differences instead of clinically irrelevant hematological toxicity (longer thrombocytopenia in the 2nd AT). Transplant-related mortality of AT II was 9.5 % (4/42). EFS II is known in 30/42 pts; 8 pts. (19%) have achieved prolongation of EFS II versus EFS I, which should be been effect of experimental therapy. Four out of 8 pts. with EFS 2 > EFS 1 was in thalidomide MT group.

Conclusions. AT is a mainstay of our program and of the trials of the Czech Myeloma Group. IFN MT after AT is unfortunately only of limited benefit and the ID combination MT after AT is not better. Consolidation therapy with 4xCED is well-tolerated and its benefit after AT remains to be established. Repeated transplantation is a successful strategy in the treatment of relapsing MM and has a positive influence on OS. Thalidomide has shown promising effects in relapsed myeloma after AT, but longer follow-up is necessary.

PO.605

AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA 1989-2003: DATA FROM THE AUSTRIAN STEM CELL REGISTRY

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Background. High dose chemotherapy with autologous stem-cell transplantation has shown superiority over conventional chemotherapy in newly diagnosed patients aged 65 or younger. Here we report the results of all multiple myeloma patients transplanted between 1989 and 2003 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. Total body irradiation (TBI) was given to 33 of 136 evaluable patients. 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.

Conclusions. 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 has been enrolled 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.

PO.606

AUTOLOGOUS HAEMOPOIETIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA – THE CANBERRA HOSPITAL, AUSTRALIAN CAPITAL TERRITORY EXPERIENCE

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The natural outcome of patients with multiple myeloma (MM) is unsatisfactory, with a median survival of less than 3 years. The prospects for survival at 10 years are poor with conventional chemotherapy. Autologous peripheral blood stem cell (PBSC) – supported high-dose Melphalan is now considered standard therapy for patients with Multiple Myeloma less than 65 years of age. It attempts to achieve a greater tumour reduction with longer disease-free and overall survival.

Objectives. A retrospective review over 9 years of PBSC autologous transplants for patients with Multiple Myeloma, attending The Canberra Hospital. All patients received a high dose Melphalan conditioning regimen.

Methods. Patient characteristics, diagnostic criteria and staging for MM, time to transplant from diagnosis, response

and survival rate, and time to relapse were determined. The number of stem cells collected and infused at the time of transplant, as well as platelet and neutrophil engraftment were analysed.

Results. Data on 64 patients diagnosed over a 16 year period and receiving an autologous transplant over a 9 year period (since 1995), were analysed. Mean age at transplant was 56.5 years and mean time to transplant from diagnosis of MM was 22.5 months. 67% (n=43) had an IgG paraprotein, 14% (n=9) had an IgA paraprotein and the remainder had free light chains in the urine. The mean number of stem cells infused at time of transplant was $3.42x10^6$ cells. The mean number of days to platelet and neutrophil engraftment post transplant was 19.1 and 11.4 days respectively. 5 year survival data was only available for 26 patients and was 50%. Rates of complete and partial response and median survival rates were comparable to registry data. There were no transplant related deaths in the first 3 months post-autologous transplant.

Conclusions. High dose Melphalan supported by autologous peripheral blood stem cells (PBSC) is well tolerated with low toxicity and procedure related mortality. Median survival data are comparable with those of the Australian Bone Marrow Transplant registry data and other published reports. This therapy should be considered in all patients diagnosed with this condition who are less than 65 years of age, without any other specific contraindication.

PO.607

VINCRISTINE, DOXORUBICIN AND DEXAMETHASONE CHEMOTHERAPY AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION AS FIRST LINE TREATMENT FOR ADVANCED MULTIPLE MYELOMA: RESULTS OF A SINGLE CENTER PROTOCOL

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Introduction. Chemotherapy followed by autologous stem cell transplantation (ASCT) is considered for many authors as first line treatment for advanced multiple myeloma.

Objectives. To evaluate overall response, complete and partial response after induction chemotherapy and the same after autologous transplantation. To evaluate overall survival and event-free survival. Secondary, to evaluate the feasibility to mobilise hematopoietic progenitors after VAD chemotherapy.

Patients. 34 consecutive patients diagnosed of symptomatic multiple myeloma, Durie-Salmon stage II and III, were included in the protocol from January 1998 to January 2004. Gender: 22 (65%) male and 12 (35%) female with a median age of 56 (41-66). M-Protein: Ig G: 15 (44%); Ig A: 9 (26%); Light chain: 4 (12%); Non secretor: 4 (12%) and Ig D: 2 (6%). At the moment to start the treatment, 29 patients (85%) were in Salmon-Durie stage III and 5 (15%) in stage II. All the patients received VAD chemotherapy for 6 courses and pamidronate every month. Fourteen patients (41%) had received radiotherapy previously or concomitantly with chemotherapy. Mobilisation of stem cells consist on Cyclophosphamide 4 g/m^2 + G-CSF 5 mcg/kg/d s.c. Conditioning regimen for autologous transplantation consist on Melphalan 140 mg/m^2 + TBI in 8 patients (23%) and Melphalan 200 mg/m^2 in 26 patients (77%).

Results. Evaluation after VAD chemotherapy: overall response: 30/34 (88%). Complete remission (Immunofixation negative): 9/34 (26%). Mobilisation of hematopoietic progenitors: 34/34 (100%), median of CD34 criopreserved: 12.0 x 106/kg. In 23 patients (67%) one apheresis procedure was enough. Evaluation after ASCT: 16 patients (47%) were in CR. Overall survival: 61%; event-free survival: 46% with a median follow-up of 45.2 months from diagnosis.

Conclusions. Our results confirm that VAD regimen obtains a good rate of overall response and that ASCT increases the rate of CR after induction chemotherapy. Induction chemotherapy with VAD does not compromise mobilisation of stem cells. However, the number of patients is still short and we need a longer follow-up to demonstrate an increase in overall survival and event-free survival.

PO.608

CYCLICAL HIGH-DOSE ALKYLATING CHEMOTHERAPHY IN PREVIOUSLY UNTREATED PATIENTS WITH MULTIPLE MYELOMA FOLLOWING Z-DEX INDUCTION TREATMENT IS ASSOCIATED WITH IMPROVED DISEASE CONTROL

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A phase II study of high dose alkylating agents in newly diagnosed patients with multiple myeloma after maximum response to Z-Dex therapy and DHAP stem cell mobilisation is reported. Twenty-six patients, median age 56 yrs (range 42-66) completed Z-Dex chemotherapy and PBSC were mobilised with DHAP. Patients then preceded to high dose cyclophosphamide (HD Cy: 6 g/m^2) supported with G-CSF. This was followed by High Dose Busulphan-Melphalan (HD BuMel) supported by re-infusion of un-manipulated autologous PBSC. IFNα was introduced at 3 months post-transplant as maintenance therapy. Six patients failed to complete the full protocol. Median time from diagnosis to transplantation was 8 months (range 6-12). Mean CD34+ cell dose collected was $15.8\ x10^6$/kg (CI 11.8, 19.8). Median time from DHAP to HD-Cy was 6 weeks (range 4-12) and median time from HD-Cy to HD BuMel was 8 weeks (range 6-12). The median follow up is 65 months (range 42-104). On an intent to treat basis, the response rates were, 3 CR (12%) 21 PR (80%) 2 SD (8%) post Z-Dex, 5CR (19%) and 21 PR (81%) post HD-CY and 14 CR (54%) and 12 PR (46%) post-transplant. The treatment related mortality (TRM) was 4% (1 patient). The actual median progression-free survival is 48 months with an estimated Median OS of 60 months (actual median OS not yet reached). The 3yr OS and PFS were 72% and 62%. Actuarial 5yr OS and EFS are 49% and 32%. Sequential high dose Alkylating chemotherapy supported by haematopoietic growth factors and autologous stem cell rescue in patients who received Z-Dex as induction therapy offers significant durable disease response rates with acceptable TRM.

PO.609

THE CAPE TOWN COMPREHENSIVE THERAPY PROGRAM FOR PATIENTS WITH MULTIPLE MYELOMA

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Background. In selected patients with multiple myeloma (MM) the use of successive cytotoxic dose intensive courses appears valuable to improve survival. However, poor performance status may limit the eligibility of patients for myeloablative conditioning. This pilot study analyzed the effectiveness of an outpatient program of sub-myeloablative conditioning fol-

lowed by infusion of a graft. At 6 months transplantation with fully myeloablative doses was then undertaken.

Patients and Methods. Patients with myeloma were first treated with standard schedules followed by a single mobilization course with etoposide (2 g/m^2) and filgrastim (10 ug/kg/day x 10 days). A minimum of 4x10^6/kg CD34+ cells were collected, the graft was split into 2 aliquots and cryopreserved. Patients then received intravenous melphalan 100 mg/m^2 followed by the infusion of the first aliquot of stem cells 24 hours later. The aims of the study were to determine the effectiveness and toxicity of outpatient transplantation. The secondary endpoints included need for admissions to hospital, response to therapy and the rate of CR (on immunofixation). Six months later a second inpatient transplant was performed after conditioning with melphalan 200 mg/m^2.

Results. 25 patients with advanced stage myeloma with a median age of 50 years (40-63 years) and median presentation Karnovsky score (KS) of 30% were entered into the study. Mobilisation with etoposide was well tolerated with no toxicities >2 WHO reported, although one individual died at home of a cardiac event. After conditioning with melphalan 100 mg/m2, the median duration of grade 4 neutropenia was 4 days and thrombocytopenia 2 days. 6/25 patients required admission for neutropenic fever and spent a median of 5 days in hospital. Extramedullary toxicities were minimal. All patients showed improvement of all the parameters tested. The median change in the paraprotein (45 to 0 g/dL; $p< 0.00001$), bone marrow plasmacytosis (55 to 0%; $p<0.00001$), albumin (33 to 41 g/L; $p< 0.01$), Hb (9.25 to 13 g/dL; $p<0.01$) and serum calcium levels (2.8 to 2.35 mmol; $p<0.01$) were all significant. There was a remarkable improvement of the median KS to 90% ($p<0.0001$). Sixteen patients have also completed the second (fully myeloablative) graft. One patient died of disease progression. For the 25 individuals who were evaluable for response and survival the median follow-up time is 29 months (7-73 months) and 23/25 (92%) survive, 74% disease free.

Conclusions. Sub-myeloablative therapy with melphalan followed by stem cell support is well tolerated and is a feasible outpatient strategy as it induces correction of most of the abnormal parameters in MM. The remarkable improvement in the performance of all patients, allow more individuals to benefit from the second, myeloablative component of this protocol.

P0.610

THE EFFECT OF PARAPROTEIN LEVELS ON STEM CELL APHERESIS IN MYELOMA

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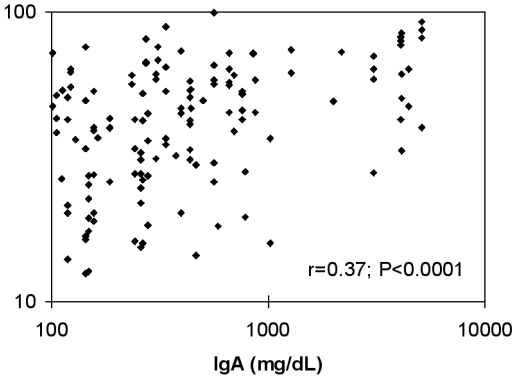
CD34+ cell collection efficiency (CE) reflects the proportion of CD34+ cells contained in the blood passing through the cell separator during leukapheresis that is harvested. Higher CE results in a higher number of stem cells being collected per procedure reducing the number of procedures required to reach the target. Whole blood and plasma viscosity would be expected to affect the mechanics of the apheresis procedure which is based on centrifugal force-induced separation of blood constituents. As a result, factors influencing viscosity (e.g. protein levels in the blood) could also affect the CD34+ cell CE. We retrospectively studied 381 leukaphereses (15-20 L) done in 141 patients with myeloma to examine the effect of pre-apheresis immunoglobulin levels and protein fraction quantities on CD34+ cell CE. The pre-apheresis WBC count was 0.5-116x10^9/L (median 17.1), and peripheral blood CD34 count was 1-1036/mm^3 (median 23). The total number of CD34+ cells collected was 4-4955x10^6 (median 179); corresponding to 0.1-91.1x10^6 (median 2.6) per kg. As expected, there was strong correlation between peripheral blood CD34 and the number of CD34+ cells collected (r=0.9; $p<10^{-100}$). CD34+ cell CE was 4-262% (median 43). There was negative correlation between CE and the WBC count (r=0.24; $p<0.0001$) and the peripheral blood CD34 count (r=0.19; $p=0.0002$). There was no correlation between albumin, IgG, IgM, total (IgG+IgA+IgM) immunoglobulins, M protein, or $\alpha1$, $\alpha2$, β, γ, or total ($\alpha1+\alpha2+\beta+\gamma$) globulin and CD34+ cell CE. There was a modest positive correlation between the IgA level and CE (r=0.15; $p=0.004$).

IgA level	n	r	P
>100	142	0.37	<0.0001
>200	100	0.38	0.0001
>300	77	0.34	0.003
>400	62	0.39	0.002
>500	51	0.34	0.02

IgA levels were too low to affect viscosity (<100 mg/dL) in 239 instances, these were eliminated from further analysis. The table shows positive correlation between IgA levels and CD34+ CE for various IgA values. The figure illustrates the correlation between IgA and CE for IgA values >100 mg/dL. On multiple regression analysis of the entire population, lower WBC count ($p<0.0001$), lower peripheral blood CD34 ($p=0.001$), and higher IgA levels ($p=0.02$) were associated with higher CD34+ cell CE. When multiple regression analysis was confined to the 142 procedures with IgA levels of >100 mg/dL, only lower WBC ($p=0.007$) and higher IgA ($p=0.0003$) were associated with higher CD34+ cell CE. IgG levels did not influence CE even when analysis was confined to procedures with elevated IgG. We conclude that protein and paraprotein abnormalities seen in myeloma do not interfere with the process of leukapheresis as measured by CD34+ cell CE. Curiously, increased IgA levels are associated with improved CE; a finding compatible with the clinical observation that hyperviscosity syndrome is more frequent with IgA myeloma than with IgG myeloma because IgA molecules affect viscosity more powerfully than IgG molecules (*Mehta J, Singhal S. Hyperviscosity syndrome in plasma cell dyscrasias. Semin Hemost Thromb 2003; 29:467-71*).



P0.611

INTERIM RESULTS OF A PHASE 2 STUDY TO EXPLORE THE EFFICACY AND TOLERABILITY OF THALIDOMIDE CONTAINING REGIMENS TO REDUCE TUMOR CELL LOAD PRIOR TO HEMATOPOIETIC STEN CELL (MSC) TRANSPLANT IN MULTIPLE MYELOMA AND THE FEASIBILITY OF HARVESTING HSC FOLLOWING THALIDOMIDE CONTAINING REGIMENS

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Aim. To explore role of thalidomide as part of first line treatment in multiple myeloma.

Patients and Methods. 27 patients with advanced de-novo multiple myeloma, median age 55 median β-2-microglobulin 4.2. 10 of 17 patients with FISH or metaphase cytogenetics had Δ13q. The regimen included TDx3 (thalidomide 400mg/d x 21, pulse dexamethasone 32mg TDS x 5d), followed by DT-PACEx2 (thalidomide 400mg/d, dexamethasone 40 mg/d x 4, cisplatin 10 mg/m^2/d, doxorubicin 10 mg/m^2/d, cyclophosphamide 400 mg/m^2/d, etoposide 40 mg/m^2/d 4 day infusion). Stem cells were harvested off the second cycle of DT-PACE.

Results. 14/27 patients completed study treatment, 12 had successful stem cell harvests, 8 were withdrawn, 1 died of sepsis and 4 were still to complete planned therapy. 2 failed stem cell harvest. Median tumour bulk at end of TD x 3 and DT-PACE x2 was 13% and 5% respectively. A historical group of 52 patients treated with VAD and HD cyclophosphamide, had a median tumour bulk at the end of VAD and cyclophosphamide 5G/m^2 of 32 and 25% respectively (p=0.025 at end of induction). The median stem cell harvest was 4.3x10^6/kg CD34$^+$ cells, after median of 3 aphereses. After VAD/cyclophosphamide median harvest and median aphereses were 11.6x10^6/kg and 2 (p=0.000 and 0.004 respectively). Commonest adverse events were fever 11, infection 6 (1 fatal), rash and neutropenia 4 each. Self assessed overall health showed a trend (p=0.056) toward improvement from entry to completion of study treatment. There was no trend towards improvement in self assessed quality of life (p=0.222).

Conclusions. 1. Thalidomide containing combination appears to be as efficacious as VAD/HD cyclophosphamide as first line therapy in advanced myeloma. 2. Thalidomide containing combinations are associated with tolerable but not insignificant toxicity. 3. Thalidomide containing combinations may reduce stem cell harvests. Findings need to be tested in a randomised study.

P0.612

FACTORS EFFECTING PERIPHERAL STEM CELL COLLECTION IN MULTIPLE MYELOMA PATIENTS

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High dose chemotherapy and autologous peripheral stem cell transplantation (APSCT) is agreed to be one of the standard treatment procedures in multiple myeloma (MM) patients. Ongoing studies showed that in these patients mobilization procedure is difficult and stem cell product collected can be insufficient (*Br J Haematol 2003; 120: 413-23*). In this study, we aimed to determine the factors effecting number of CD34+ cells collected in MM patients for APSCT. Between February 1999 and May 2004 median 2 (1-6), totally 70 procedures were done for 28 male, 10 female, totally 38 MM patients who had undergone autologous stem cell collection procedure. The disease subtype distribution was 15 IgGκ (39.5%), 9 IgGλ (23.7%) and the stages were 3A 55.3%, 3B 18.4%, 1A 13.2%. Chemotherapy regimens before stem cell collection procedures were, 11 melphalan-prednisone (MEL-P) (28,9%), median 6 (1-12) cycles, 27 vincristine-adriamycin-dexamethasone (VAD) (97.4%), median 4.5 (2-8) cycles, 2 thalidomide (5.3%), and 3 radiotherapy (7.9%). Time from diagnosis to apheresis was 8.8 (2.1-51.1) months and most commonly used mobilization regimens were cyclophosphamide (CY) + etoposide (28.9%), CY (26.3%), G-CSF following VAD (15.8%) and G-CSF alone (21.1%). Target CD34+ cell count was 4-5x10^6/kg for single, 8-10x10^6/kg for tandem transplantation and collection procedure was performed to patients with peripheral CD34 count (pCD34) over 5/μL. In 47.4% of patients enough stem cell product (CD34 ≥4x10^6/kg) was collected in one apheresis cycle. Mobilization failure (pCD34 <5/μL) was seen in 5.3% of the cases. Enough CD34+ cells have been collected in 64.9% of the cases (CD34+ >4x10^6). Patients using MEL treatment which recently was shown to reduce efficiency of the procedure was analyzed separately. In these 9 patients pCD34 cell count has a mean of 39±37 /μL, whereas in the 23 patients who did not received MEL it was 128±183.1/μL (p=0.035). In patients who received MEL, possibility of reaching the satisfactory amount of product was lower (p=0.05). MEL chemotherapy is a negative risk factor (RR 2.76% 95CI 0.95-8.07). Type of mobilization regimen did not have a significant effect on stem cell amount. Total CD34+ cell count is positively related to the basal pCD34 count (r=0.639 p=0.0001). As a result, in the treatment of MM patients who are candidates for APSCT, alkylating agents like melphalan should be avoided. For determination of a accurate and cost effective apheresis time, monitorisation of the basal CD34 cell count is important.

P0.613

COMPARISON OF THREE DIFFERENT CYCLOPHOSPHAMIDE REGIMENS FOR PERIPHERAL BLOOD STEM CELL MOBILIZATION IN MULTIPLE MYELOMA PATIENTS

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Introduction. Autologous peripheral blood stem cell transplantation currently has an established role in the treatment of multiple myeloma (MM). Various chemotherapy regimens combined with recombinant human granulocyte colony-stimulating factor (G-CSF) are used in MM patients to mobilize and collect peripheral blood stem cells (PBSC). However the best mobilization schedule is not yet generally accepted.

Aim of study. We have studied three different cyclophosphamide regimens for mobilizing of PBSC in multiple myeloma patients, to compare their efficacy, toxicity and costs.

Patients and Methods. We have retrospectively analysed 53 patients (27F/26M) with MM who were transplanted between 1999 and 2004 in a single center, median of age was 55 years (28-74). Mobilization schemes were as follows: Group A (n=18) – Cyclophosphamide 6 g/m^2 + G-CSF 10

mcrg/kg/day, Group B (n=15) – regimen CED (Cyclophosphamide 3 g/m^2 day 1 + Etoposide 100 mg/m^2 day 1-3 + Dexamethason 40 mg/m^2 day 1-4) + G-CSF 5 µg/kg/day and Group C (n=20) – Cyclophosphamide 2.5 g/m^2 + G-CSF 5 mcrg/kg/day. Aphereses were started after leucocyte recovery (>5x10^9/L). All three cohorts of patients were comparable for age, sex, immunoglobulin subtype, stage of disease, time from diagnosis, response to initial chemotherapy and extension of previous therapy. The parameters observed were: maximal reached number of CD34+ cells in peripheral blood, number of collected CD34+ cells, number of required aphereses, therapeutic efficacy (we compared pre- and post-therapeutic immunoglobulin levels), G-CSF utilization and side effects.

Results. We observed statistically significant higher maximal reached number of CD34+ cells in peripheral blood in group A (median 1.23%) and group B (median 1.05%) than in group C (median 0.59%). There were no statistically differences in number of aphereses between all the groups, but statistically significant reduction in G-CSF utilization was observed in group B and C as compared to group A. Failure of mobilization was observed only in 1 patient of the group A. Therapeutic efficacy of all used mobilizing regimens was similar. No major side effects were observed in all three groups, but hematologic toxicity was significantly higher in group A (median 3,8; fever >38°C in 33% of pts.) than in group B (median 2,5; fever >38°C in 20% of pts) and C (median 1,2; no fever). All patients of the groups A and B were hospitalised. We did not observe significant difference in the engraftment parameters.

Conclusions. In this study we confirmed that all three retrospectively assessed cyclophosphamide schemes are effective mobilizing regimens for collecting of PBSC in MM patients. Because of similar therapeutic efficacy, much lower toxicity and for the relevant economic implications we currently prefer cyclophosphamide 2.5 g/m^2 + G-CSF 5 mcrg/kg/day to historically used high-dosed cyclophosphamide and CED regimen.

P0.614

MOBILIZATION AND COLLECTION OF PERIPHERAL BLOOD STEM CELLS AFTER FIRST–LINE CHEMOTHERAPY WITH ALKYLATING AGENTS IN PATIENTS WITH MULTIPLE MYELOMA: RESULTS OF THE SPANISH MYELOMA GROUP

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Background. It has been suggested that front-line therapy with alquilating agents could adversely affect peripheral blood stem cell mobilization in patients with multiple myeloma (MM) planned to undergo autologous stem cell transplantation (ASCT) due to the deleterious effect of these drugs on bone marrow hematopoietic progenitor cells. We have analyzed the impact of first-line chemotherapy with alkylating agents on stem cell collection (measured as CD34+ cells) in patients with MM undergoing/ASCT).

Patients and Methods. Six-hundred and twenty four patients included in the Spanish multicenter protocol GEM-MM2000 form part of the study. Stem collection was performed after a median of 2 cycles of alternating chemotherapy consisting of VBMCP/VBAD in those patients with normal renal function (creatinine <2 mg/dL) or 3 cycles of VAD chemotherapy in case of creatinine >2 mg/dL. According to the number of CD34+ cells collected, patients were divided into Group A (CD34+ cells <2x10^6/kg) and Group B (CD34+ cells >2 x10^6/kg).

Results. Mean number of CD34+ cells collected in the whole series was 5.46x10^6/kg (range, 0.34->90) and median (range) number of aphereses was 2 (1-9). Overall, 78 patients (12%) were in Group A and 564 (88%) in Group B. Ninety-six percent of the patients received therapy with alquilating agents before stem cell collection. Median number of cycles of chemotherapy before transplant was 1, and median time from diagnosis to transplant was 7 months. Mobilization regimens more frequently used consisted of G-CSF alone (69%) or combined with chemotherapy (26%). Overall, 82 patients required a second cycle of mobilization to collect enough number of CD34+ cells and 12 needed a third cycle. By univariate analysis, patients receiving more than one chemotherapy regimen before collection (p=0.02) and advanced age (>60 years) (p=0.04) were the two variables associated with a lower CD34+ cell collection. Besides, there was a trend towards a lower mobilization capacity in women (p=0.08). Finally, no differences were observed in terms of the number of CD34+ cells collected according to the type of chemotherapy administered (VBMCP/VBAD vs. VAD). By multivariate analysis, patient's age was the only variable significantly affecting the number of CD34+ cells collected, being elderly patients less able to mobilize appropriately (p=0.0003). Among those patients undergoing the planned autologous transplant, median time to recover 0.5 neutrophils x10^9/L was similar in both groups of patients (12 and 11 days, respectively). Time to reach 20 platelets x10^9/L was slightly shorter in Group B patients (12 vs. 13 days, respectively; p 0.05). Finally, no differences in overall survival were observed according to the number of CD34+ cells infused.

Conclusions. Therapy with alquilating agents as it is planned in the Spanish GEM protocol does not affect collection of CD34+ cells in patients with MM, and the majority of patients achieved enough number for ASCT. In this series, only advanced age was associated with a lower mobilization capacity. Finally, the number of CD34+ cells collected had a minor impact on hematopoietic recovery after transplant in this group of patients.

P0.615

INCIDENCE OF SINUSOIDAL OBSTRUCTION SYNDROME IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS TRANSPLANTATION AND BUMEL CONDITIONING

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One of the primary objectives of the present GEM-2000 protocol for multiple myeloma (MM) was to increase response rate. The first line chemotherapy (M2/VBAD) was selected based on the combination therapy that in previous Pethema studies afforded the highest CR. For high dose intensification, BUMEL (busulphan 12 mg/m^2 + melphalan 140 mg/m^2) was chosen as the conditioning regimen for the

first autologous transplantation procedure, based on the result of two GEM retrospective studies showing that BUMEL, compared to 200 mg/m^2 melphalan (MEL200) or MEL140+TBI, had achieved a better global response rate and a slight, but not significant, advantage in survival rates. However, the report of some cases of sinusoidal obstruction syndrome (SOS) prompted us to perform an interim analysis on the incidence of SOS in our trial. At that moment 249 patients had been already transplanted with BUMEL. Subsequently the study was amended and the conditioning regime switched from BUMEL to MEL200.

Patients and Methods. Post-transplantation SOS was analyzed among the patients included in the GEM-MM2000 protocol. All patients received a first line treatment consisting in 3 VBMCP cycles (vincristine, carmustine, melphalan, cyclophosphamide, prednisone) alternating with 3 VBAD cycles (vincristine, BCNU, doxorubicine and high dose dexametasone). The severity of the SOS was graded as Minimum (resolution within 100 days without treatment), Moderate (resolution within 100 days with treatment) or Severe (death due to SOS or resolution in more than 100 days despite treatment). The day of symptoms appearance in relation to the day of CSSP reinfusion was also analyzed. In April 2004, 619 patients included in the MM2000 protocol had been subjected to autotransplantation; 249 had received BUMEL conditioning and 370 MEL200.

Results. Among the 619 cases there have been 23 SOS events, 21 (8.4%) in patients treated with BUMEL and 2 (0.5%) in patients receiving MEL200 as conditioning regimens (p<0.00000). One of the MEL200 patients presented severe SOS on day +3, in the other patient SOS began on day +9 and it was moderate. One-third (33%; 7 cases) of SOS cases conditioned with BUMEL presented the first symptoms before day +22 (3 mild, 1 moderate, 3 severe) while in two-thirds (14 cases, 66%), SOS began between days +22 and +57 (4 mild, 5 moderate and 5 severe). Of the 9 patients with severe SOS, 8 died due to this complication.

Conclusions. Although the use of busulphan in autologous or allogeneic transplant conditioning regimens is a known SOS risk factor, none of the available BUMEL studies on MM patients had, until now, reported the incidence of SOS that we observed in our series. The late onset of most of the cases is another characteristic that is singular to our series. Perhaps the prior use of alkylating agents may have influenced the frequency of this complication.

PO.616

COMPARISON OF EARLY AND LATE AUTOLOGOUS STEM CELL TRANSPLANTS FOR MULTIPLE MYELOMA: A SINGLE INSTITUTION EXPERIENCE

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Background. High dose chemotherapy and autologous stem cell transplant (SCT) remains the preferred therapy for eligible patients with multiple myeloma (MM) since it improves overall survival. Patients with MM may undergo SCT immediately following 4-6 cycles of induction therapy or at the time of relapse from the initial plateau phase. Available evidence does not suggest a survival difference between the two approaches.

Methods. We retrospectively evaluated our experience with autologous single SCT for MM to compare the results of delayed SCT to early SCT. We identified from our transplant database, 202 patients (pts) with MM, who underwent SCT between October 1992 and November 2002. 101 pts, who responded to induction chemotherapy, had stem cells collected in plateau phase, received maintenance chemotherapy and had SCT at the time of first relapse (delayed SCT group). The remaining 101 pts, after initially achieving a response underwent upfront SCT (early SCT group). Patients refractory to initial therapy were excluded from this study. Most of the early transplants were done in the recent years and hence this group has a shorter follow up.

Results. The study cohort had a median age of 55 years (range 29 72) at diagnosis consisting of 126 males (62%), with no significant demographic difference between the two groups. As expected measures of tumor burden (M protein, B2M and marrow plasma cell percentage) were significantly higher in the delayed group. Plasma cell labeling indices and presence of abnormal cytogenetics were higher in the delayed SCT group as well at transplant. TBI containing regimens were used more often in the delayed group reflecting a difference in the time periods of transplant. There was no difference between the groups in terms of overall response to transplant though complete response rate was higher for the early transplants (see Table below). There was no difference in the time to response between the groups (p=0.13, Kaplan Meier estimate). Though the median progression free survival (PFS) from transplant was shorter for the delayed SCT group, the overall survival (OS) from diagnosis of MM was comparable (see Table). The OS estimate at 5 year from diagnosis was 60% for both groups. Conclusions: Review of our experience demonstrates comparable OS for an early SCT compared to SCT at the time of relapse. Although there is an advantage for early SCT in terms of more chemosensitive disease as shown by a longer PFS from SCT, there is no benefit in OS from time of diagnosis. Patient preference and other co-morbidities should play a role in the decision regarding timing of transplant.

	Early (n=101)	*Delayed (n=101)*	*p*
Overall Response	100 (99%)	94 (94%)	0.07
Complete Response	43 (42%)	28 (27%)	0.04
Median PFS (From transplant)	26.7 months	14.8 months	<0.0001
Median OS (From diagnosis)	67 months	70 months	0.1

PO.617

HIGH-DOSE THERAPY/STEM CELL SUPPORT, INCLUDING TANDEM TRANSPLANT, FOR PRIMARY REFRACTORY MULTIPLE MYELOMA: RESULTS FROM THE SPANISH MYELOMA GROUP (PETHEMA/GEM) IN 49 PATIENTS

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Spanish Myeloma Group (PETHEMA/GEM)

Background. It is assumed that patients with primary refractory myeloma are the most likely to benefit from early HDT. In four reported series the CR rate was between 8 and 40% and the overall survival ranged from 4 to 6 years. However, the number of patients with progressive disease versus those with no change or stable disease was not given in published series.

Objective. To investigate the efficacy in terms of response up-grading and survival of early HDT in patients with primary refractory myeloma.

Patients and Methods. From October 1999 to December 2003 patients with MM younger than 70 years were given 6 courses of VBMCP/VBAD chemotherapy. Patients with refractory disease were planned to receive a tandem trans-

plant, the first procedure intensified with busulphan-12 mg/kg-/melphalan-140 or melphalan-200 and the second procedure intensified with CVB –cyclophosphamide, etoposide and BCNU- or with a dose-reduced intensity *allo* conditioned with fludarabine/melphalan-140, depending on sibling donor availability. Response and progression were defined according to the EBMT criteria. Forty-nine patients (30M,19F, median age 57 yrs) from the GEM trial with primary refractory disease were identified.

Results. Twenty patients showed progression after their initial chemotherapy while 29 patients had *non-responding, non-progressive disease* or *no-change*. Eighty per cent of the patients achieved some degree of response after the first autologous transplant (CR or near-CR: 8%, PR: 56%, MR: 16%) while 10% did not respond and 10% died from the procedure. In 25 patients a second transplant was not performed due to different reasons. Twenty-four patients were given a second transplant (autologous: 17, allogeneic: 7). Forty-six percent of the 17 patients who received a second autologous transplant up-graded their response (CR: 6%, PR: 17%, MR: 23%) while 41% had progressive disease or *no-change* and 13% died from the procedure. Three of the seven patients who underwent a dose-reduced intensity *allo* responded (2 CR, 1 PR) while two had progressive disease and two died from transplant-related complications. The median survival of the whole series of 49 patients was 32 months. Patients who had progressive disease after the initial chemotherapy had a significantly shorter survival than those who showed *non-responsive, non-progressive disease* (median 21 months vs. not reached, p=0.003). Finally, patients with *non-responding, non-progressive disease* had similar survival than those with chemosensitive disease intensified with HDT in the GEM trial.

Conclusions. 1) A high-dose therapy approach in patients with primary refractory myeloma results in a high overall response, but the CR rate is low, 2) patients with progressive disease to the initial chemotherapy have a short survival despite the intensive therapy, and 3) patients with *non-responding, non-progressive disease* have similar survival than chemosensitive patients. Whether the good outcome of the latter patients is mainly due the effect of HDT or to the natural history of a more indolent disease remains to be determined.

P0.618

PHASE I/II STUDY OF TANDEM HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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Objective: To investigate the safety and efficacy of tandem high-dose chemotherapy with tandem autologous peripheral blood stem cell transplantation (APBSCT) for treatment of advanced multiple myeloma.

Patients and Methods. Patient eligibility included confirmed diagnosis of stage II/III untreated multiple myeloma and patient ages between 15-64. The eligible patients were consequtively enrolled to the protocol and received 2-4 courses of VAD regimen. Those with progressive disease after VAD therapy were excluded. The responding patients underwent PBSC harvest following high dose cyclophosphamide and filgrastim. The first APBSCT following high dose melphalan (100 mg/m^2 for 2 days) was performed within 2 months of PBSC harvest. 3-6months later, the second APBSCT was scheduled. The primary endpoints were feasibility, safety including treatment-related toxicity and protocol completion. The secondary endpoints were response rate, overall survival and progression-free survival.

Results. Forty patients entered the protocol. A male: female ratio was 5:3. Their median age was 55 (42-64). Sixteen of 40 patients had a stage II disease, and 24 of them a stage III disease. Immunoglobulin classes of M protein were IgG in 23 patients, IgA in 9, IgD in 1, Bence-Jones in 6, and non-secretory type in 1. Of these patients, 32 underwent the first APBSCT and 28 did the second APBSCT; the completion rate of the protocol was 70%. Treatment-related mortality was 2.5% (n=1) throughout the protocol. Severe toxicity (non-hematologic grade 4 toxicity) occurred in 12.5% and 14.3% in the first and the second APBSCT, respectively. All but one who died achieved hematopoietic recovery, suggesting the feasibility and safety of the protocol. Among 28 patients who completed the second APBSCT, the results were favorable with a response rate of 65% (complete response rate 27.5%, n=11); 3-year overall survival was 89%, and 3-year progression-free survival 34% at a median follow-up of 562 days.

Conclusions. Tandem high-dose chemotherapy with APBSCT is feasible and safe with favorable response rates in the treatment of advanced multiple myeloma. These observations suggest that this combined modality may be further investigated.

P0.619

PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN FIRST-LINE AND RELAPSE TREATMENT OF MULTIPLE MYELOMA PATIENTS WITH DIALYSIS-DEPENDENT RENAL FAILURE

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Purpose. The objective of our analysis was to compare the toxicity of high-dose therapy (HDT) followed by autologous stem cell transplantation (ABSCT) in patients with MM on haemodialysis versus patients without renal insufficiency.

Patients and Methods. 79 patients (pts) received a single course of HDT followed by ABSCT after conventional pretreatment. 21 pts were on maintenance haemodialysis due to chronic renal failure. 16 pts had MM, whereas 5 pts suffered from light-chain amyloidosis. 5 out of the 21 dialysis pts relapsed after a median of 11 months and received a second ABSCT. In a control group 58 pts without signs of renal insufficiency were treated with a single course of HDT followed by ABSCT. The conditioning regimen of this group comprised melphalan 200 mg/m^2, while the haemodialysis group received 100 mg/m^2 of melphalan. Patients in the latter group were slightly older (median, 60 vs. 57 years) and had less previous cycles of therapy (median, 4 vs. 8 cycles). Dialysis-patients at relapse therapy had a median age of 61 years, 8 cycles of pre-treatment, and 14 months between 1st and 2nd ABSCT.

Results. Transplant-related mortality was 1 of 5 relapsed dialysis-patients after 2nd ABSCT due to sepsis, while none of 21 pts died after 1st ABSCT in the haemodialysis group and 0/58 in the control group. Following 1st and 2nd ABSCT, haemodialysis pts required extended i.v. antibiotic treatment (median, 10 vs. 7 days). In 5 of 21 cases (19%) i.v. antimy-

cotic treatment was required after 1st ABSCT compared to 7% in the control group, while it was not required in any case after 2nd ABSCT. Relevant differences were seen neither in the number of platelet or erythrocyte transfusions nor in the days with fever, total parenteral nutrition, hospitalisation or until white-blood cell count > 1/nl. A first survival analysis revealed an estimated median event free survival after 1st ABSCT of 18 months.

Conclusions. In our analysis, HDT of 21 patients with MM and light-chain amyloidosis on chronic haemodialysis showed no increased toxicity in comparison to a control group. Even in relapse treatment, haemodialysis should not constitute a criterion for exclusion from high-dose therapy and autologous stem cell transplantation.

*Median (range)	First-line treatment, on hemodialysis (n=21)	Relapse treatment, on hemodialysis (n=5)	Control group (n=58)
Days until white-blood cell count > 1/nl*	12 (8 - 24)	13 (12-14)	14 (10 - 24)
Days until platelet count > 20/nl*	11 (1 - 28)	13 (7-14)	12 (4 - 59)
No. of platelet transfusions*	3 (1 - 21)	2 (1-6)	2 (1 - 50)
No. of erythrocyte transfusions*	4 (0 - 16)	3 (0-16)	2 (0 - 12)
Days with total parenteral nutrition*	4 (0 - 30)	2 (0-19)	3 (0 - 36)
Fever days*	3 (0 - 20)	3 (2-3)	2 (0 - 17)
Days on i.v. antibiotics*	10 (0 - 37)	10 (8-15)	7 (0 - 41)
Cases requiring i.v. antimycotic treatment	5/21	0/5	4/58
Days in hospital*	18 (12 - 63)	17 (13-39)	17 (12 - 58)

P0.620

SINGLE HIGH-DOSE CHEMORADIOTHERAPY VERSUS TANDEM HIGH DOSE MELPHALAN FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION: PRELIMINARY ANALYSIS

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The overall survival of patients with advanced multiple myeloma (MM) undergoing high-dose chemotherapy and autologous stem cell transplantation (SCT) depends mainly on the quality of response. Thus, to improve the response rate, different modifications of high dose chemotherapy and autologous stem cell transplantation have been evaluated with the final goal to prolong survival after high dose therapy for multiple myeloma. In this randomised phase III study 2 strategies to improve response rates were compared: tandem high dose melphalan and an intensified single chemoradiotherapy regimen. Tandem high dose chemotherapy followed by auto-PBSCT was shown to be superior to single high dose chemotherapy in a recently published French multicenter study (IFM94). In a multicenter phase I/II study we showed TMI/Bu/Cy – modified total body irradiation, high dose busulfan and cyclophosphamide to induce a high CR rate (48%) in patients with de novo multiple myeloma. Thus, in this phase III study, tandem high dose melphalan followed by autologous SCT was compared to a single autologous SCT after radiochemotherapy with TMI/Bu//Cy. 294 patients with age 60 years or less withde novo multiple myeloma or with <6 cycles of MP were registered for the study, 160 of them underwent an induction therapy with idarubicin/dexamethasone (4 cycles ID). Following 4 courses of ID 6% of patients achieved a CR, 62% a PR, 14% a MR, 12% had stable disease and 6% PD. After achieving at least SD and when > $4x10^6$ CD34+ cells were collected 198 patients (Intention-to-Treat Population) proceeded to high-dose therapy followed by autologous SCT. Toxicity was higher in patients treated with TMI/Bu/Cy vs patients treated with Mel 200 with more mucositis grade III/IV (93 vs 66%) and infections (79% vs 68%). But TRM was not different with 3% following TMI/Bu/Cy vs 4% after tandem-high dose melphalan therapy. The ORR 3 months after TMI/Bu/Cy and Tandem-Mel 200 (94 vs 99%). The maximal ORR 6-24 months after TMI/Bu/Cy and Tandem-Mel 200 was 80 vs. 86%. The accrual progression free survivals were median PFS 25.9 months (Tandem-Mel) vs. 31.2 month (TMI/Bu/Cy) (p=0.258). Thus, an intensified conditioning therapy and a single autologous stem cell transplantation and tandem high dose chemotherapy followed by autologous PBSCT can be safely applied to patients with advanced multiple myeloma. The higher organ toxicity following the intensified conditioning regimen does not result in a higher TRM when compared to two cycles of high dose melphalan. With a still short follow up of 4 years no significant difference in PFS and OS can be demonstrated between a single course of high dose chemoradiotherapy and tandem high dose melphalan therapy.

P0.621

ROLE AND TIMING OF SECOND TRANSPLANT IN MYELOMA: AN APPLICATION OF STATISTICAL MULTI-STATE MODELS

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Autologous transplantation is seen as a standard of care for younger patients with myeloma. While an improvement in survival duration is seen, most patients still relapse. In an effort to improve survival times, a significant number of patients have been given second transplants while the patient is still in remission in an attempt to reduce the relapse risk. This treatment strategy is characterised by further tox-

icity with increased risk of non-relapse mortality. It is therefore of primary importance to evaluate the effect of second transplantation and in particular to provide indications on *optimal* or *safe* time to transplant. Our Group has recently investigated retrospectively 7452 myeloma patients treated with autologous stem cell transplantation of whom 1725 received a second transplant while in remission. Since an analysis based on the intention-to-treat was hampered due to a lack of reliable information, a multi-state approach was applied to provide a description of the course of the disease with respect to the different modalities of administration of a second transplant. The complex course of the disease was simplified to fit a *disease-death* scheme, considering relapse or death in remission as final event, and second transplant as intermediate event. The Cox model was used to estimate the transition hazards, introducing time dependent co-variants for second transplantation and its timing. The calculation of transition probabilities allowed an interesting visualisation of the results, facilitating the interpretation, as shown in the figure below. From our data it is clear that second transplants if given, are best given early after the first transplant, i.e. 3-6 months and that transplantation at more than 24 months after the first transplant may be detrimental to the patients wellbeing.

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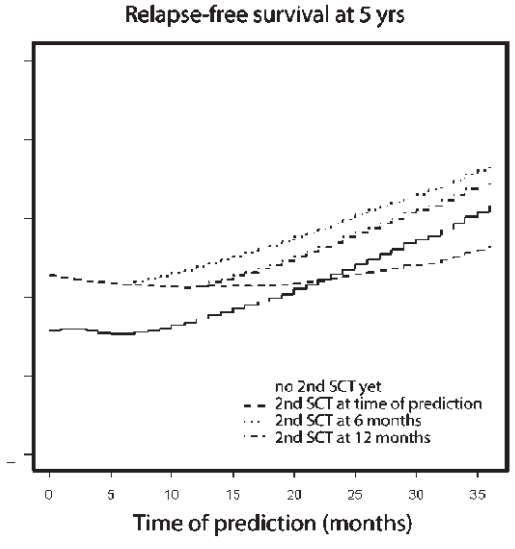

PO.622

MAINTENANCE WITH LOW-DOSE THALIDOMIDE IN MULTIPLE MYELOMA PATIENTS AFTER AUTOTRANSPLANTATION

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High dose therapy with single or double transplantation (auto-SCT) has improved prognosis of multiple myeloma (MM). New drugs are promising in upfront therapy while the role of maintenance is still debated. Thalidomide (thal) is an active drug in the treatment of myeloma, and is been investigated as first line therapy. It could be useful in the control of minimal residual disease. We used thal as maintenance after autologous transplantation (single or double) and compare the outcome with other maintenance or none. From January 2001 to June 2004 20 patients (10 males and 10 females) with MM have been treated in our institution. Median age was 58 years (range 51-72). 9 were IgG, 6 IgA, 2 light chains and 2 plasma-cell leukaemia. Treatment was 4 cycles of VAD regimen followed by auto-SCT. 10/20 performed double auto-SCT. 3 months after SCT, 10 patients (5 single and 5 double SCT) began thal 50 mg/die as maintenance therapy. 10 patients (5 single and 5 double SCT) received IFN-γ (3/10), dexa (3/10) or no therapy (4/10). The 2 groups were regarding the type of myeloma: 5 IgG, 2 IgA, 2 light chains and 1 plasma-cell leukaemia the thal group; 4 IgG, 4 IgA, 1 IgD and 1 plasmacell leukaemia the other. Response to SCT: 2 CR and 8 PR in the thal group; 5 CR, 4 PR and 1 NR in the other. In the thal group only 1/10 patient relapsed (plasma-cell leukaemia) after suspension of thal. Median follow up from the beginning of maintenance therapy was 25.8 months (range 7-30) with 9/10 patients in CR or stable disease, with a progression free survival (PFS) and overall survival (OS) projected at 30 months of 83%.

In the other group, 8/10 patients relapsed. Median follow up was 35 months (range 5-46) with a PFS of 15% and OS of 47%. The difference between the 2 groups is statistically significant for PFS (p:0,003), and not significant for OS (p: 0,13) even if difference (83% vs. 47%) appears clear. (Tab. 1-2). Thal was administered for a median period of 6 months, being neurological toxicity the main reason of suspension (3/10 patients). Neurological toxicity grade I-III was present in 65% of patients, while haematological toxicity grade I occur in 55% of patients. In conclusion, in a small number of patients low dose thal as maintenance after auto-SCT resulted in an improved PFS and OS when compared with other or none maintenance, with acceptable toxicity. Further studies in larger cohorts and randomized trials are needed to confirm this experience.

PO.623

THALIDOMIDE IN RELAPSING MULTIPLE MYELOMA AFTER THE FIRST AUTOGRAFT: A PILOT STUDY FOR THE EVALUATION OF EXPERIMENTAL MAINTENANCE THERAPIES

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Background. Therapy of relapsing multiple myeloma (MM) is still considered to be experimental. Thalidomide continues to show benefit in MM treatment.

Design of study. We tested maintenance therapy using thalidomide (MT-THAL) 100 mg daily after repeated autologous transplantation (AT) in patients (pts) with MM relapsing/progressing after the first AT. Results were compared using intra-individual analyses [the comparison of event free survival I (EFS I) (after the 1st AT) and EFS II (after the 2nd AT) in one patient], therefore inter-individual differences are excluded (T2 model).

Patients and Methods: Between January 1997 and July 2004, 42 pts with relapsing/progressing MM after the 1st AT were included in the study, 23 of them received THAL. Median follow-up in whole group was 86.8 months, in MT-THAL 73.5 months.

Results. Sensitivity to C-VAD reinduction chemotherapy (4 cycles) was 74.4% (80% to 4 VAD induction), the response to the 2nd AT compared to the 1st one was 87.5% versus 95.2% to the 1st one. Toxicity of the 1st and 2nd transplantation was similar and usually did not exceed grade II (SWOG criteria), there were no significant differences instead of clinically irrelevant hematological toxicity (longer thrombocytopenia in the 2nd AT). Transplant-related mortality of AT II was 9.5% (4/42). EFS II is known in 30/42 (71%) in all pts.; in 12/23 (52%) pts. in THAL group. In the whole group median of EFS I was 21.4 months, median of EFS II was 13.6 months, median of overall survival (OS) was 78.5 months; 18/42 patients were alive at the time of analysis. Total of 10 pts. (24%; 10/42) have achieved prolongation of EFS II versus EFS I, 4 of them (33%; 4/12 with known EFS II) were in MT-THAL group. EFS I for MT-THAL group is 26.4, respectively EFS II 15.5 months. In thalidomide treatment was mouth dryness (6), polyneuropathy (3), sleepiness (3), constipation (3) and neutropenia (3) the most often but acceptable toxicity. No patient had to discontinue thalidomide therapy due to side effects.

Conclusions. Thalidomide has shown benefit in relapsing myeloma post auto transplant and in the running pilot cohort of pts. 33% patients have prolonged EFS II interval. Importantly, MT-THAL dose 100 mg had acceptable toxicity and could be used for long-term period.

P0.624

MAINTENANCE THERAPY (INTERFERON ALPHA OR SEQUENTIAL INTERFERON ALPHA AND DEXAMETHASONE) AFTER HIGH-DOSE CHEMOTHERAPY AND PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN NEWLY DIAGNOSED PATIENTS WITH MULTIPLE MYELOMA. A RANDOMIZED TRIAL OF THE CZECH MYELOMA GROUP

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Background. Optimal maintenance therapy after autologous transplantation is unclear. In order to assess the role of interferon alfa (IFN) and IFN plus dexamethasone (IFN/DEX) as the maintenance therapy after ASCT, 246 previously untreated patients with multiple myeloma (MM) were enrolled to trial 4W from 1996 to 2002. In recent analysis (August 31, 2004), with median follow up of 63.3 months, total of 120 (120/185; 66%) pts. already relapsed and 80 pts. (44%) died; also median of overall survival was reached.

Patients and Methods. Patients received primary therapy with four cycles of VAD regimen (vincristine, doxorubicin, dexamethasone) followed by mobilization with cyclophosphamide 5g/m^2 and G-CSF 10 μg/kg. A conditioning regimen with melphalan 200 mg/m^2 was used for first and second transplantation. The later was indicated only in patients who did not achieve a good remission (>75% reduction of MIG). After transplantation patients were randomized to two arms of maintenance therapy: IFN 3x10^6 units three times weekly s.c. or the same dose of IFN in alternating cycles with dexamethasone 40 mg in days 1-4, 10-13, 20-23.

Results. Data of 185 randomized patients were evaluated in this analysis. One month after transplantation 6% of pts. achieved complete remission (M-component 0% and negative immunofixation), overall response rate was 88% (>50% reduction of M-component). Transplant related mortality at day +100 was 2.35%. All 185 randomized pts. received the maintenance therapy, 95 pts. in the IFN arm and 90 pts. in the IFN/DEX arm. Median of event free survival (EFS) and overall survival for all groups of randomized pts. (185) are 33.9 and 77.1 months respectively. We did not be able to detect statistically significant differences between I and ID arm for EFS (p=0.905) as well as for OS (p=0.942).

Conclusions. In analysis, the OS and EFS curves have shown no significant statistical differences between the IFN and IFN/DEX groups. Substantial number of pts. in IFN group (23% vs. 13% in ID group) premature finished maintenance therapy due to IFN toxicity. Interferon maintenance therapy after AT is unfortunately only of limited benefit and the ID combination after AT is not better.

P0.625

INTERIM SAFETY ANALYSIS OF THE RANDOMIZED MULTICENTER ALLG MM6 TRIAL OF POST-AUTOLOGOUS STEM CELL TRANSPLA TRIPLE MAINTENANCE THERAPY

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Since May 2002 the Australasian Leukaemia and Lymphoma Group (ALLG) has been conducting a randomised trial (ALLG MM6) of thalidomide maintenance therapy in patients with Multiple Myeloma undergoing an initial autologous stem cell transplant (ASCT). All patients had baseline serum creatinine (Cr) values within 2 x upper limit of normal and were conditioned with melphalan 200 mg/m^2. Those showing no disease progression at 6 weeks post-ASCT were randomised to zoledronic acid (ZA) 4 mg IV by 15 minute infusion Q4 weekly and alternate day prednisolone 50 mg, with (ARM 1) or without (ARM 2) thalidomide (maximum dose of 200 mg daily for a maximum of 12 months). Cr was measured prior to each ZA infusion and the ZA was withheld based on criteria used in prior Phase III trials (Cr increase >44 μmol/L for baseline Cr <124 μmol/L or >89 μmol/L for baseline Cr >124 μmol/L). Additionally, ZA Cmax and AUC (0-48) were determined for the day 1 and day 29 ZA infusions in a subgroup of patients. Based on prior concerns about potential interaction between thalidomide and ZA, we performed a prospective, comparative (ARM 1 vs ARM 2), evaluation of renal function. Univariate analysis was conducted using student t-tests and chi-square tests for equal proportion. Multivariate analysis was conducted using a repeat measures analysis of variance. Differences in Cr between arms over time were determined by fitting an interaction between dose and arm, with dose treated as a linear predictor. At present 173 patients (ARM 1=82; ARM 2=91) have been randomised to maintenance therapy and 24 patients (ARM 1=12; ARM 2 = 12) have undergone pharmacokinetic evaluation. The arms were well matched for gender, age, pre-ASCT β2-microglobulin and baseline Cr. To-date 42 serious adverse events have occurred (ARM 1=25; ARM 2=17), principally infective, with 2 deep vein thromboses in each arm. The median dose of thalidomide at the completion of the first 12 months of maintenance is 100 mg with dose reductions being predominantly for peripheral neuropathy. The majority of patients remain on full doses of alternate day prednisolone at 12 months. The median number of doses of ZA administered is 11 for both ARM1 and ARM 2. Seven patients (4%) have had ZA withheld following Cr rises. In 2 cases these rises were temporally associated with disease progression and in the remaining 5 cases (ARM 1 = 3; ARM 2=2) Cr stabilised or improved following cessation of ZA. Overall, mean rises in Cr of 0.4

µmol/L (ARM 1) and 0.8 µmol/L (ARM 2) per ZA dose have occurred (p=.22). ZA pharmacokinetics demonstrated no significant differences between those patients who were or were not concomitantly receiving thalidomide. We conclude that post-ASCT triple maintenance with ZA-prednisolone-thalidomide is feasible and that there is no evidence for a pharmacokinetic, nor, based on serial measurements of renal function, a clinically adverse interaction between ZA and thalidomide.

P0.626

SIGNIFICANCE OF ABNORMAL PROTEIN BANDS IN PATIENTS WITH MULTIPLE MYELOMA FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. The appearance of small APB post-ASCT for myeloma is not infrequent and can pose problems in patient surveillance and management as they may be mistaken for disease relapse. Traditional serum electrophoresis and/or immunofixation may be unable to determine the nature of these small bands, whereas isoelectric focussing can distinguish between oligoclonal banding (OB) and new apparent monoclonal bands (MB).

Methods. A retrospective analysis of patients with myeloma undergoing ASCT was performed. Paraprotein identity and quantification was performed using standard immunofixation electrophoresis. The nature of any new bands was determined by isoelectric focussing. We studied the characteristics and prognostic significance of these small APB (OB and MB).

Results. Forty-nine cases were reviewed: median age at transplant 57yrs (range, 32-75); female 40%; stage I (8%), stage II (2%) and stage III disease (90%); 45% of patients achieved CR and 49% PR. With a median follow-up of 34 months, 5yr survival is 76% (95%CI, 59-86%) and 24 patients have relapsed at a median of 15 months (range, 3-65). Thirty-six (73%) patients developed APB with a median time to development of 1.8 months post-transplant (range, 0-29). Similar proportions of patients in CR and PR developed APB (85% and 69%, respectively). Bands of up to 5g/L were observed. Twenty-two patients had more than one episode of APB resulting in a total of 69 episodes of APB observed post-transplant. Isoelectric focussing demonstrated that 54 of these APB were oligoclonal and 15 appeared monoclonal. The median duration of OB and MB was 4 months (range, 0.5-42+) and 3 months (range, 1-22+), respectively. Of the 15 episodes of MB, ten resolved and five still persist with only one representing true disease progression. The presence of an APB (i.e. OB or MB) in the post-transplant period was associated with significantly improved event free survival (5 yr EFS 43% vs 0%, p<0.05) and a trend to better overall survival (5 yr OS 83% vs 57%, p=0.12). The presence of MB had no impact on survival.

Conclusions: Small APB are very common post ASCT and isoelectric focussing appears a useful technique to distinguish between OB and MB. These small bands are not indicative of relapse and are in fact associated with improved outcomes. As such, these small bands must be reported and interpreted in a cautious manner.

P0.627

SIGNIFICANT DIFFERENCE IN PROGNOSIS BETWEEN PATIENTS WHO REACHED AND DID NOT REACH AUTOLOGOUS TRANSPLANTATION IF THIS TREATMENT WAS INDICATED

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Background. In our first trial 4W total of 246 newly diagnosed pts. were enrolled from whom 185 pts. were undergo AT. In recent analysis (August 31, 2004), with median follow up of 63.3 months median of overall survival (OS) for group of pts. randomized (R) after AT (185) was 77.1 months. Almost 25% (61/246) have been lost during induction part of the treatment due to several reasons. Prognosis of this cohort of pts. was very pure and our data confirmed, that AT is key factor for prognosis of pts.

Methods and Results. Two groups of pts. enrolled to the trial 4W were compared: randomized pts. who underwent AT as required (4W-R; n=185) vs. non-randomized pts. (4W-nonR; n=61) who were dropped out from the trial before transplantation during induction (90%) or stimulation (7%) treatment. Median follow-up of was 65.3 vs. 69.6 for 4W-nonR group. From standard parameters only age (53.4 vs. 57 for 4W-nonR; p=0.01), renal failure (5.4% vs. 23% for 4W-nonR; p=<0.001) and LDH (5.3 vs. 7.0 for 4W-nonR; p=0.003). changed significantly. Albumin and CRP were similar in both groups, beta2M (4.3 vs. 6.4; p=0.073) had trend reflected higher number of pts. with renal failure in 4W-nonR group. Distribution of pts. based on Durie-Salmon and International Staging System (stage 1: 43% vs. 29%; stage 2: 36% vs. 35%; stage 3: 21% vs. 35%) did not reach significance despite trend to higher frequency of stage 3 ISS in 4W-nonR group. Distribution of response on VAD therapy was significantly changed (p=0.01) with 6% of progressions in 4W-nonR group. From total of 61 pts. 56% were lost during VAD induction treatment, 20% during stimulation and graft collection. Main reasons of dropping out from the trial were early death due to complication or resistance on therapy during VAD treatment (23% pts.; 14/61). Also deep venous thrombosis or pulmonary embolism were significant factors for contraindication of AT. Finally EFS and OS were compared. For all pts. median of EFS was 40.6 and OS was 64.4 moths. 4W-R group has median of EFS 33.9 and OS 77.1 moths but for 4W-nonR group median of OS was dramatically lower (12.8 moths). Based on the statistic modeling was suggested that *keeping patients on board of transplantation* avoiding loss of pts. during induction treatment could significantly improve overall results. For example decrease number of pts. who did not reached AT about 20% will prolong OS of all group of 6 moths and in the case of 50% (from 61 pts. to 30 pts.) median of OS would be prolonged in our group of pts. from 64 months to 73 moths, which represent almost one year benefit.

Conclusions. There is a significant difference in prognosis between pts. who reached and did not reach AT if this treatment is indicated. 25% drop-out from the transplantation program is common but unacceptable high. The analysis of the reasons for the loss the pts. concluded that better supportive care and especially earlier diagnosis and better induction treatment of MM is essential in order to bring the benefit of AT to more pts.

P0.628

SUBMYELOABLATIVE ALLOGENEIC TRANSPLANTATION IN MYELOMA: COMPARISON OF OUTCOMES WITH OTHER HEMATOLOGIC MALIGNANCIES

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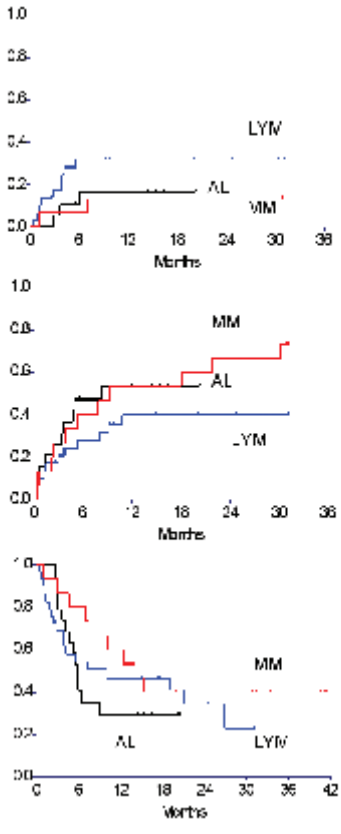
Results of conventional allografts in MM are poorer than in other diseases. It is unclear if this is so with submyeloablative allogeneic transplantation (SMAT). 63 adults underwent SMAT for MM (n=15), acute leukemia (n=19; AL) or lymphoma (n=29; LYM) after 100 mg/m^2 melphalan without (n=42) or with (n=21; prior autograft) 50 mg/kg cyclophosphamide from HLA-matched sibling (n=37; MSD), 10 allele-matched unrelated (n=19; MUD), or 1-locus/allele-mismatched (n=7; MMD) donors. Cyclosporine (MSD) or tacrolimus (MUD/MMD) were given with mycophenolate to prevent GVHD. Characteristics known or likely to affect outcome are shown in Table 1. 14 patients died of toxicity (TRM), and 32 relapsed (25 dead, 7 alive in CR/PR). In Cox analysis, adverse factors (RR; P) were: TRM – HLA mismatch (0.16; 0.02), age >55 (0.27; 0.09), performance status (PS) 2-3 (0.31; 0.09). Relapse – refractory disease (0.19; 0.002), donor age >45 (0.33; 0.02). DFS – refractory disease (0.21; 0.0009), donor age >45 (0.33; 0.005), PS 2-3 (0.43; 0.03), abnormal LDH (0.54; 0.08), HLA mismatch (0.44; 0.09). OS – poor performance status (PS) (0.27; 0.001), abnormal LDH (0.29; 0.002), refractory disease (0.20; 0.004), HLA mismatch (0.33; 0.03), donor age >45 (0.38; 0.02), age >55 (0.45; 0.06). The diagnosis (MM vs AL vs LYM) did not affect outcome in Cox analysis. The figures show the effect of the diagnosis on TRM, relapse, and OS. Our data suggest that the benefit of SMAT in MM is similar to other malignant hematologic disorders. Results can be improved by performing SMAT when the disease is chemosensitive, when the patient's general condition is good, and with HLA-identical and younger donors. The latter suggests that a young 10-allele-matched unrelated donor may be preferable to an old 1-antigen-matched sibling, but this question needs to be explored in a larger patient population.

Table 1.

	MM	AL	LYM	P
Age >55	27%	42%	24%	0.39
Refractory disease	47%	53%	72%	0.18
PS 0-1	80%	84%	66%	0.29
HLA mismatch	13%	21%	3%	0.16
Donor age >45	80%	47%	41%	0.05
Prior autograft	53%	16%	53%	0.07
Abnormal LDH	40%	47%	59%	0.47



P0.629

MINI ALLOGRAFT IN MULTIPLE MYELOMA: THE ROYAL ADELAIDE HOSPITAL EXPERIENCE

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Multiple myeloma is refractory to chemotherapy alone with relapse common after autologous PBSCTx. Allogeneic stem cell transplantation can result in a durable graft versus myeloma effect, but with 40-50% TRM in the first 100 days.

Aim/Methods. Retrospective assessment of 8 patients undergoing non myeloablative allogeneic transplantation at the RAH using Badros/Barlogie or Seattle protocol. Median age was 54 years. Six underwent sibling allogeneic transplants and 2 mini MUD's. Each patient had poor prognosis cytogenetics; B2M 4 or relapse after autograft.

Results. Three were in CR at transplantation and 5 PR. 6 patients had undergone 1 autologous stem cell transplant, and 2 patients 2 previous transplants. No TRM occurred in the first 100 days. 7/8 patients engrafted. By D+100 engrafted patients had 90-100% donor chimerism. Autologous recovery occurred in the non engrafted patient. GVHD developed in 7/8 patients, 3 acute GVHD, 2 progressing to chronic GVHD. Four patients developed chronic GVHD only. CMV antigenaemia occured in 3/4 donor positive to recipient positive transplants. 3/8 patients developed peri-transplant septicaemia. No VOD occurred. The 3 patients transplanted in CR have remained in CR. Two patients transplanted in PR are currently in CR, one having responded after relapse to decreased immunosuppression. Both relapsed patients and the patient who failed to engraft were transplanted in PR. 1 relapsed patient died 17 months post mini MUD in full donor chimerism. Median time since

transplant is 13 months.

Conclusions. Non myeloablative allogeneic bone marrow transplantation is well tolerated and exerts Graft versus Myeloma effect, in high risk patients transplanted both in CR and PR.

PO.630

GRAFT-VERSUS-MYELOMA EFFECT FOLLOWING REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

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Extensive clinical and experimental data support an important role for a graft-versus-tumor effect in eradicating a number of different tumor cells in patients who receive allogeneic stem cell transplantation (allo-SCT). Specifically, it has been shown that a graft-versus-myeloma (GVM) effect can be induced against multiple myeloma (MM) even in patients who have been heavily pretreated or relapsed after high dose therapy. However, these promising results are often achieved at the cost of high treatment-related mortality (TRM), considered as a contraindication to the use of standard myeloablative allo-SCT as a classical treatment strategy for MM patients. The application of reduced intensity conditioning regimens (RIC) before allo-SCT, may temper the frequency of transplant-related toxicities. This report describes the results obtained in 56 consecutive MM patients (median age, 52 y.) who received a RIC allo-SCT using fludarabine, busulfan and antithymocyte globulin from HLA-identical siblings. Fourty-two patients (75%) were in partial remission, one patient in complete remission, and 13 (27%) with progressive disease at the time of allo-SCT. Median time between diagnosis and allo-SCT was 26 months. 48 patients (86%) received at least one autologous transplantation prior to allo-SCT, but this was not part of a systematic auto/allo strategy. Engraftment occurred in all 56 patients at a median of 18 (range, 0-29) days for ANC>500/μL and 0 (range, 0-32) days for platelets >20000/μL. The cumulative incidences of grade II-IV and grade III-IV acute graft-versus-host disease (GVHD) were 41% (95%CI, 28-54%) and 14% (95%CI, 5-23%) respectively. 14 patients developed limited chronic GVHD, whereas 10 developed an extensive form (cumulative incidence, 43% (95%CI, 30-56%)). With a median follow-up of 16 months, the overall cumulative incidence of TRM was 18% (95%CI, 8-28%). 18 patients (32%) could achieve a continuous complete remission (according to EBMT criteria), and the Kaplan-Meier estimates of overall survival (OS) at 4 years was 45% (95%CI, 28-62%). OS was significantly higher in patients with chronic GVHD as compared to patients without chronic GVHD (81% vs. 19%; p=0.0001). Collectively, these data demonstrate that RIC allo-SCT can mediate a potentially curative GVM effect. We are currently developing a *global* RIC approach (auto/allo/maintenance therapy) designed to enhance the GVM effect of allo-SCT. As part of this strategy, assessment of the potential morbidity associated with chronic GVHD adjusted for quality of life will be crucial determinants for the ultimate outcome and are obvious targets of investigations into improving the safety of RIC allo-SCT approaches for MM.

PO.631

T-CELL DEPLETION OF ALLOGENEIC GRAFTS SEEMS TO BE ASSOCIATED WITH A FAVORABLE OUTCOME IN MULTIPLE MYELOMA

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Introduction. Although allogeneic stem cell transplantation remains the only curative approach in patients with multiple myeloma (MM), its role remains controversial. High treatment related mortality associated particularly from graft vs. host disease (GvHD) and disease recurrence remained substantial challenges in this group of predominantly older patients. This study reviewed the outcome of patients who received T-cell depleted grafts from HLA identical siblings.

Patients and Methods. Patients with symptomatic multiple myeloma who had an HLA identical sibling were initially treated with anthracycline containing combinations (VAD, C-VAMP, etc) until response was obtained according to ECOG criteria. Conditioning for transplantation included TBI, TNI and Melphalan 140 mg/m^2. In addition patients unable to get radiation received Busulphan, 12 mg/kg, melphalan 140 mg/m^2 and cyclophosphamide 120 mg/kg. GvHD prophylaxis consisted of T-cell depletion with CAMPATH-1 G or H antibodies. Patients receiving BMT had no further immunosuppression. Individuals who received PBPC grafts (mobilized with filgrastim 5-10 ug/kg x 5 days) had, in addition, therapeutic doses of cyclosporin for 90 days. The objective of the study was to determine transplant related mortality OS, and DFS after adequate follow up.

Results. Seventeen patients with a median age of 44 (range 37-56) years who had responded to chemotherapy received myeloablation with radiation (n=10) of chemotherapy (n=7) based conditioning. The source of stem cells was the marrow in 6 and PBPC in the remainder. Seven patients had significant organ dysfunction from effects of the disease (amyloid, nephrotic syndrome). Patients received a median of 23 x 10^4/kg CFU-GM in 0.87 bone marrow mononuclear cells and 9.22 x 10^8/kg PBPC (CD34+: 8.46 x 10^6/kg). Median time to 0.5 and > 20 x10^9/L polymorphs and platelets was 13 days. Bone marrows were all treated with CAMPATH-1G (median 10 mg) while in 8 patients PBPC were incubated with CAMPATH-1H (median 15 mg). Five patients died, 2 of infection within 100 days. Four patients had disease recurrence. Two patients developed GvHD and died of infection or relapse, respectively. One of two responded to DLI and remains in remission. Death occurred at a median of 152 (range 39-1221) days. Eleven (65%) patients survive at a median of 1623 days (range 385-5309) disease free.

Conclusions. In patients with symptomatic MM who had responded to standard chemotherapy allogeneic stem cell transplantation with T-cell depleted grafts was associated with 18% mortality, mainly due to good protection from GvHD. This effect seems to have had a favourable impact in the overall long-term survival.

P0.632

GRAFT-VERSUS-HOST DISEASE PREVENTION BY SELECTIVE DEPLETION OF ALLOREACTIVE DONOR T CELLS

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Allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice for many hematological malignancies. Graft-versus-host disease (GVHD) is a leading cause of morbidity and mortality occurring in 50-80% of patients after HSCT. GVHD is mediated by a subpopulation of the T cells in the stem cell graft. Ex vivo T cell depletion of all T cells of the graft can prevent development of GVHD but can lead to a delay in immune reconstitution and an increase of potentially lethal opportunistic infections and tumor relapse. Hypothetically, an approach that enables a selective depletion of the alloreactive donor T cells that cause GVHD while preserving anti-leukemic and anti-microbial reactivity would be optimal for recipients of HSCT. Our preliminary data demonstrate that an anti-CD25 immunotoxin (IT), which reacts with a cell surface activation antigen, can selectively deplete alloreactive donor T cells activated by non-leukemic recipient white blood cells while preserving the beneficial third-party (anti-leukemia and anti-microbial) reactivity in vitro. In adult patients with hematological malignancies, we were able to demonstrate that the alloreactive donor-derived T cell clones causing GVHD could be eliminated ex vivo before HSCT with the anti-CD25 IT immunotoxin and patients could receive an infusion of stem cells depleted of alloreactive T cells. We evaluated 5 HLA-mismatched healthy volunteer pairs in a mixed lymphocyte reaction (MLR) using 10^8 responder cells. Responder cells were activated by allogeneic stimulator cells in a cell ratio 1:1 and their peak activation (19.6-26.5%, mean 23.1% of CD3+CD25+ responder cells) occurred after 72 hours. If the IT and IT enhancer NH4Cl were added for 24 hours after the first 24 hours of the MLR, the remaining population represented 0.15-0.98% (mean 0.44%) of CD3+CD25+ responder cells. The percent recovery of viable responder cells was 62.3-89.7% (mean 78.2%). The depletion of alloreactive cells increased following another 24 hour exposure to the IT and NH4Cl resulting in 0.00-0.24% (mean 0.10%) of CD3+ CD25+ responder cells with 64.7-86.5% (mean 75.4%) viability. Third party reactivity was preserved, reaching 10.3-19.6% (mean 15.5%) on Day 7 when activated on Day 3 with HLA-mismatched PBMCs from another healthy donor. Thus, using the anti-CD25 IT for selective depletion of alloreactive donor T cells while minimizing GVHD and preserving third party reactivity should lead to a safer allogeneic HSCT.

POSTER SESSION 7: NEW THERAPEUTIC AGENTS

P0.701

LONG-TERM FOLLOW-UP OF COMBINATION THERAPY WITH THALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA NOT UNDERGOING UPFRONT AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. Thalidomide plus dexamethasone (Thal/Dex) has emerged as an effective alternative to dexamethasone (Dex), and vincristine, doxorubicin and dexamethasone (VAD) as a pre-transplant induction therapy for newly diagnosed multiple myeloma. We have previously reported the response to Thal/Dex as initial therapy in a phase II clinical trial conducted at Mayo Clinic (JCO 20:4319-4323, 2002). Although the regimen was developed as a pre-transplant induction regimen, many patients on this study did not undergo upfront autologous stem cell transplantation (ASCT). In this study we present data on the long-term outcome of patients with newly diagnosed MM treated with Thal/Dex who did not undergo upfront ASCT.

Patients and Methods. We identified 24 patients with newly diagnosed MM who were treated with Thal/Dex but did not undergo autologous stem cell transplantation. Patients were treated with thalidomide at a dose of 200 mg/day orally and dexamethasone 40 mg daily on days 1-4, 9-12, 17–20 on odd months. During even months, the dexamethasone dose was 40 mg daily for 4 days. Each cycle was repeated every month.

Results. Of the 24 patients, 14 were male and 10 female. The median age of the patients at the time of diagnosis was 65.5 years (36-78). Based on the International Staging System (ISS), 11 (46%) patients had Stage I disease, 7 (29%) were in Stage II and 6 (25%) had Stage III disease. Patients were treated for a median of 5 months (range 1-42 months) and the median duration of follow-up was 21 months (1-52). Two (8%) patients achieved a complete response (CR), 11 (46%) had a partial response (PR) (overall CR+PR=57%), and 7 (29%) patients had stable disease. One patient did not respond to the combination and 3 patients died while on therapy. The disease progressed in 6 patients while on treatment. Nine patients did not complete therapy due to various reasons other than progressive disease. In 3 patients, therapy was stopped due to adverse effects, 3 patients died while on therapy, 2 patients refused further treatment and in 1 patient, therapy was stopped for other medical condition. The overall survival (OS) was 30 months with a progression free survival (PFS) of 19 months. The median time to progression was 20.5 months.

Conclusions. In patients who do not undergo stem cell transplantation for MM, the combination of Thal/Dex is effective with a time to progression of 20.5 months and median OS of 30 months.

P0.702

LOW-DOSE THALIDOMIDE IN COMBINATION WITH STANDARD VINCRISTINE, ADRIAMYCIN AND DEXAMETHASONE YIELDS HIGHER RESPONSE RATES IN TREATMENT NAÏVE MULTIPLE MYELOMA: PRELIMINARY RESULTS OF AN ONGOING PHASE II CLINICAL STUDY

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Introduction: Multiple myeloma MM remains an incurable disease with median survival of 3 years. Understanding the biology of this disorder has helped in drug development as well as rationale new drug combinations with promising results. Tumor microenvironment (ME) plays a key role in growth and dissemination of malignant MM cells. Also, interaction of the MM cells with the bone marrow stromal cells promotes drug resistance. Thus, targeting the ME early on in the disease may prevent development of resistance and improve response to therapy. Thalidomide (T) is a novel agent that alters the tumor ME through down regulation of essential cytokines (IL-6, TNF-alpha and VEGF) and has shown encouraging clinical results in patients with MM. We have previously shown that vincristine, adriamycin and dexamethasone (VAD) plus low dose T (t) is an effective salvage regimen for VAD refractory pts. We now report the preliminary results of an ongoing phase II clinical trial using a combined chemoimmunotherapeutic approach with standard dose VAD and t, targeting concurrently the tumor and its ME.

Patients and Methods. Treatment naïve MM pts (≥ stage I) were eligible for study. Monthly infusional VAD (standard dose) was given for 4 cycles every 28 days concurrently with t for 4 months. t(100 mg) was started 1 wk prior to VAD and was escalated in 1-2 wks to 200 mg. Pts were evaluated for response after each cycle and after completing 4 cycles using the SWOG response criteria. Pts characteristics include: enrolled n=16, 8M, 8F; median age 58 yrs range 46-77; Stage III disease n=11, Stage II, n=3 and Stage 1, n=1; and Median B2M, 1.9 mg/L (range 1.2-11.5). Low dose coumadin (1-2 mg) was used for prophylaxis of venous thromboembolism (VTE).

Results. All pts are evaluable for toxicity and 11 are evaluable for response after completing 4 cyc. 10/11 (91%) responded clinically; CR, n=3, PR, n=7. 1 pt had progressive disease. 3 pts with responding disease were removed from study for Grade III toxicity (1 adriamycin associated cardiomyopathy, 1 pulmonary hypertension and 1 recurrent infections) but are not included in response evaluation. Toxicities: Fatigue (Gr. II) was the most common side effect noted (25%) and DVT was noted in 2 (12%). All pts (n=9) who were eligible for stem cell (SC) transplant, were able to collect adequate amount of SC.

Conclusions. Preliminary results suggests that VAD-t is well tolerated and is an effective regimen with improved ORR when compared to standard VAD regimen in treatment naïve MM pts. No increase in incidence of VTE was noted with the combination. Adequate SC collection was achieved in transplant eligible pts. Accrual is ongoing on this study and updated results will be presented at the meeting.

P0.703

DOXORUBICIN AND DEXAMETHASONE FOLLOWED BY THALIDOMIDE AND DEXAMETHASONE AS INITIAL THERAPY FOR SYMPTOMATIC PATIENTS WITH MULTIPLE MYELOMA

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Several drug combinations have been designed for the initial treatment of multiple myeloma. Although none has been shown to be superior, a regimen that leads to a high response rate in association with low incidence of major adverse events is highly desirable. We report response rates and complications - specifically thromboembolic complications- with the combination of doxorubicin, thalidomide and dexamethasone for patients with Durie-Salmon stage II and III symptomatic multiple myeloma.

Methods. In this regimen, the drugs are used in a sequential fashion with the intent to reduce the high incidence of venous thromboembolic complications known to be associated with this combination of drugs (*NEJM 2001;344:1951-2; Blood 2001;98:1614-5; Blood 2002;100:1168-71*). Doxorubicin and dexamethasone (AD; A=9 mg/m^2/day, Days 1-4; D=40 mg/day, Days 1-4, 9-12, 17-20) are given for 3 months followed by thalidomide and dexamethasone (TD; T=200 mg nightly; D=as above) for 2 months with prophylactic antibiotics and daily aspirin (81 mg/day). At any point during therapy patients achieving a complete response (immunofixation negative) are permitted to forgo further induction therapy and proceed with autologous stem cell transplantation.

Results. As of 11/04, we have enrolled 40 patients (24 men, 16 women) with a median age of 59 years (range, 35-82). Median β2 microglobulin level was 2.5 mg/L (ND-12.5) and median albumin level 3.95 g/dL. Fluorescent *in situ* hybridization (FISH) studies of baseline bone marrows, searching for abnormalities of chromosomes 11, 13 and 14, are available for 36 patients. Among those, 22 patients have abnormal findings. Three patients have been removed from study, one for a DVT that occurred during cycle 5, one for a myocardial infarction after cycle 1, and one for refusing further therapy. Seven patients are currently receiving treatment. Therefore response data are available for 30 patients. Among those, 26 have responded to therapy (86.6%), including 6 complete responses (20%), 8 very good partial responses (26.6%) and 12 partial responses (40%). Two patients (6.6%) have stable disease while two patients (6.6%) have progression of disease. When patients are stratified according to the International Staging System using β2 microglobulin and albumin levels, the response rate is not influenced by stage, as overall response rate is 81% for stage I (n=22), 100% for stage II (n=7) and 100 % for stage III (n=1). Likewise, the presence of 13 does not appear to affect overall response rate (82% for patients with no 13 and 100% for patients with 13). Among patients who completed the treatment and those removed from study because of DVT, only two developed DVT (2/31; 6.4%). All other patients tolerated the treatment well and completed therapy with no major adverse event.

Conclusions. These results indicate that the regimen consisting of doxorubicin, dexamethasone, and thalidomide used in a schedule that allows sequential administration of the drugs as described and DVT prophylaxis with low dose aspirin is well tolerated and results in a high response rate with a low incidence of therapy-related thromboembolic complications.

P0.704

THALIDOMIDE-DEXAMETHASONE VERSUS MELPHALAN-PREDNISOLONE AS FIRST LINE TREATMENT IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA: AN INTERIM ANALYSIS

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Thalidomide-Dexamethasone (TD) has shown significant activity in patients with relapsing or refractory myeloma. Recent phase II studies revealed an even higher response rate in previously untreated patients. In the present trial we compare TD with standard Melphalan-Prednisone (MP) in previously untreated elderly patients with multiple myeloma.

Patients and Methods. The trial is designed to include 350 patients with multiple myeloma, 116 patients have been enrolled so far (median age: 72 years, stage I: 5 (4%), stage II: 43 37%), stage III: 68 (59%). Patients are randomised to either Thalidomide 200 mg/day and Dexamethasone 40 mg, days 1-4 and 15-18 (on odd cycles) and days 1-4 (on even cycles) or Melphalan 2.5 mg/kg day 1-4 and Prednisone 2 mg/kg days 1-4, q 4-6 weeks. The dose of Thalidomide should be increased to 400 mg/day if feasible. Patients achieving response or stabilisation are randomised to maintenance treatment either with Thalidomide(maximal dose 200 mg/day)-Interferon alpha-2b (3Mega U, TIW) or interferon alpha-2b (3Mega U/TIW). All patients are scheduled for monthly Zometa (4mg) during the entire study period. Response is defined according Blade's criteria, statistical results are given by intend to treatment analysis.

Results: 93 patients are evaluable for response as yet. Best response to TD was: CR 6 (18%), NCR 4 (12%), VGPR 5 (15%) PR 8 (24%), MR 7 (21%) yielding an ORR of 63%. Three patients had SD (6%) and 15 PD or failure (31%). The respective results in patients on MP were: CR 2 (4%), NCR 5 (11%), VGPR 5 (11%), PR 8 (18%), MR 8 (18%), ORR 62% (no significant differences). Analysis per protocol revealed on overall response rate of 88% in the TD and of 68% in the MP group ($p<0.05$). Time to response and time to best response was significantly shorter in the TD (8, 11 weeks, respectively) compared to the MP group (10, 39 weeks, respectively; $p<0.01$, $p<0.0047$, respectively). Due to the interim nature of the analysis, data on progression-free and overall survival will be presented only for both groups combined. Grade III-IV hematological toxicity was significantly more frequent in patients on MP. Leukopenia 19 (38%) vs 1 (2%), thrombocytopenia 4 (18%) vs. 1 (2%), $p<0.00001$, $p<0.028$ respectively). Patients on TD had more grade II-III neuropathy 10 (22%), psychological toxicity 9 (19%) and, skin toxicity 5 (10%) compared to those on MP (0 (0%), 4 (8%), 1 (2%), respectively). Thromboembolic complications were seen in 4 (8%) patients on TD and in 2 (4%) patients on MP. In conclusion, time to response and time to best response was significantly shorter in patients on TD in relation to those on MP. Per protocol analysis showed a higher response rate in TD treated patients (88% vs 68%, $p<0.05$) while intend to treatment analysis revealed similar response rates (63% vs 62%). Leukopenia and thrombocytopenia were significantly more frequent in patients on MP, while neuropathy, psychological disturbances and skin toxicity were significantly associated with TD. Updated data will be presented at the meeting.

P0.705

THALIDOMIDE MAINTENANCE FOLLOWING HIGH DOSE THERAPY IN MULTIPLE MYELOMA: A UK MYELOMA FORUM PHASE 2 STUDY

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Thalidomide has an established role in the therapy of multiple myeloma but available data refers mainly to induction or consolidation treatments, with almost no information on its potential use in maintenance. High dose autografting produces good disease control and extends survival. Attempts to prolong survival have focused mainly on the use of interferon but this has met with limited success and significant toxicity. We reasoned that continuous oral thalidomide might act as a tolerable and effective maintenance agent. This hypothesis is being tested prospectively in the UK MRC Myeloma IX trial. As a prelude to that study, we set up this pilot investigation. The study examines thalidomide mono-therapy as maintenance in stable patients; commencing 3 months post high dose melphalan with peripheral blood stem cell autograft . We are evaluating the long-term tolerance of thalidomide at 5 dose levels; 50 mg, 100 mg, 200 mg, 250 mg and 300 mg. A hundred patients were recruited by February 2004, 20 patients in each cohort. End points are disease progression and toxicity. Information is being collected for tolerance, side effects, disease status and quality of life. The median age is 58 (36-69). Most (69%) had received only one prior modality of treatment and the median time from diagnosis to autograft was 8 months (3-122). Thirty-five of patients were in CR when thalidomide was started, 55% in PR, 5% minimal responders and 1% no change. Median follow-up is 19 months (9-35). So far, 60 patients have stopped thalidomide, 20 following disease progression and 40 due to side effects. These include neuropathy (61 cases overall, 29 of these had to stop the drug), general lethargy and somnolence (39), constipation (30), rashes (17), dry skin and pruritus (14), dyspnoea (4), hypothyroidism (2) and only one deep vein thrombosis despite no routine anticoagulation. Median progression free survival has not been reached yet with a non-significant trend of better survival in patients with early (stage 1) disease in comparison to stage 2 or 3. The 18 months progression free survival is 79% for patients with intended dose of 200 mg or greater and 63% for patients on intended dose less than 200 mg. 22 patients achieving PR or MR at the start of thalidomide improved their disease status with a greater than 5 g/L reduction in paraprotein. twelve patients however converted to CR at 6 months with no detectable paraprotein. Patients intended to get 200 mg thalidomide or more had a shorter duration on the intended dose (median 7 months)

than patients intended to receive less than 200 mg thalidomide (median 10 months on intended dose) (p=0.03). In summary, thalidomide can be used in maintenance but is difficult to tolerate long-term due to side effects. Lower doses of thalidomide seem to be better tolerated (less than 200 mg). Peripheral neuropathy is the main adverse event. Thrombosis is not a risk in this setting. Further follow-up is needed to establish the proportion of patients who are able to tolerate the drug long-term at different dose levels and the randomisation in the UK Myeloma IX trial will ultimately help to answer the efficacy question.

P0.706

LONG-TERM TREATMENT WITH LOW DOSE THALIDOMIDE IN MULTIPLE MYELOMA

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Introduction. Thalidomide has showed a relevant antitumoral effect in patients with multiple myeloma refractory to previous therapy or in progression. Its role in iductio as first line is being investigated in clinical trials. Other potential role of thalidomide is as maintenance at low dose to prolong duration of response acting on different pathogenic mechanisms of myeloma. However, the potential toxic effect of this agent has limited its application and a precise monitorizacion of long term therapy is needed to originate the less toxicity with the maximum effect. We report here our experience in a group of patients who have received low dose of thalidomide for a long period.

Patients and Methods. 25 patients with MM who have received thalidomide as rescue treatment for relapse after autologous transplant and that showed favourable response maintened the thalidomide treatment at low dose to prolong duration of response. Initial treatment was at dose of oral 100 mg/d that were escalated according tolerance with a median of 200 mg/d. After observing the higher stable response, the dose was reduced to 100 mg/d for long term therapy adjusting inclusive to 50 mg/d or alternate days therapy according tolerance. Toxicity studies were performed including neurological exams and thiroyd tests.

Results. 23 patients were evaluable for long term follow up with at least 8 months of treatment. One patient on dialysis recovered renal function after 24 months of treatment. Median duration of treatment was 19 months (8-50). 2 patients showed CR maintained after more than 2 years of treatment, 11 cases (47%) showed PR and 10 patients (43%) progressed after more than 6 months of tratment. Tolerance to this regimen was acceptable. More frequent adverse effect were somnolence, constipation, cutaneous rash, and mild neuropathy (40-60%). No alteration of thryroid function was observed. Neurological symptoms were reversible with dose adjust or temporary suspension. Only one case of venous thrombosis was observed on a patient with V Leyden mutation.

Conclusions and Comments. Long term treatment with oral thalidomide is effective and presents an acceptable tolerance. The stability and long duration of response observed in this group of patients, suggest a potential role of this agent as maintenance therapy alone or associated to other drugs. More experienced and controlled clinical trials comparing this scheme with other maintenance regimens are necessary to evaluate the real impact of this strategy.

P0.707

THALIDOMIDE IN REFRACTORY AND RELAPSED MULTIPLE MYELOMA: DURATION OF RESPONSE

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Introduction. Thalidomide administered as a single agent produces a response rate of about 40% in patients with refractory or relapsed multiple myeloma (MM). However, there is little information on the duration of such responses.

Objective. To determine the quality and duration of responses to thalidomide in patients with refractory or relapsed MM.

Patients and Methods. From October 1999 to December 2003, fourty-two consecutive patients (22M/20F, median age 61 years) with refractory/relapsed MM were given thalidomide as single agent at our institution. This series included 22 patients with relapsed MM and 20 with refractory disease. Most of these patients (70%) had previously received two or more lines of therapy (median: 2; range: 1-4). Sixteen of them (38%) had undergone an autologous stem cell transplantation (ASCT). The median daily dose of thalidomide was 400 mg (range: 200-800).

Results. Nineteen of the 42 patients (45%) responded to thalidomide. Eleven (26%) achieved a minimal response (MR) and 8 (19%) a partial response (PR) according to the stringent EBMT criteria. The median time from the onset of thalidomide to the achievement of response was 3 months and the median duration of therapy in responding patients was 11.7 months. Treatment with thalidomide was discontinued due to toxicity in 11 of the 19 patients, progression in 4, early death due to infectious complications in 2, and in one patient when he received an ASCT. The toxicity consisted of: peripheral neuropathy (5 patients), severe fatigue (4), ataxia (1), and syncope due to atria-ventricular block of 2nd grade (Mobitz I) (1). At the time of this analysis, 11 of the 19 patients had relapsed. The median time from the onset of thalidomide to relapse was 15.8 months (range: 1.3-52.6). The median time to relapse for patients who had achieved a PR was 22.6 months versus 11.2 months for those who had only achieved a MR (p= 0.09). The median duration of response was 10.3 months (range: 1-50) (19.3 months for PR vs 8.3 months for MR, p=0.11).

Conclusions. These results show that the effect of thalidomide in refractory / relapsed MM is durable, particularly in those patients who achieve a greater degree of response and support the investigation of the role of this drug as maintenance therapy.

P0.708

THALIDOMIDE THERAPY IN RELAPSED/REFRACTORY LIGHT CHAIN MYELOMA

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Light chain myeloma is found in 15% of patients with multiple myeloma. We investigated the response to Thalidomide therapy in a series of 15 light chain myeloma patients (LCM). Serial measurements of serum free light chains was used to monitor the response to therapy. The 15 patients included 12 men and 3 woman with a median age of 65. The diagnosis of LCM was made by bone marrow plasma cell concentration of > 20% and the presence of monoclonal

light chain in serum or urine. Five of the patients had significant renal impairment (creatinine over 300 mmol/L) and one patient was on peritoneal dialysis. Three of the younger patients were initially treated with VAD and an autologous transplant but subsequently relapsed. The other 12 patients were initially treated with VAD or standard Melphalan and Prednisone therapy prior to the use of Thalidomide. Patients were given Thalidomide at standard doses of 100 to 200 mg as tolerated. Monthly measurements of the serum free light chains (FLC) were done on a Dade-Behring nephelometer, using Binding-Site reagents. The results were expressed as kappa/lambda ratio. The duration of the Thalidomide therapy ranged from 6 months to 25 months with the majority of patients on therapy for over a year. All 15 patients responded to Thalidomide therapy as indicated by a reduction in the serum free light chains. Only one patient normalised his SFLC levels and was considered to be a complete response. The responses were quite rapid and were often evident only after several weeks to therapy. Four patients have died and the rest are still on treatment so OS not yet evaluated. There were no thrombotic complications in this group and no antithrombotic prophylaxis was used. In summary we found a high response rate to Thalidomide in this group of refractory light chain myeloma patients. The serial use of SLC measurements allowed monitoring of responses with recourse to bone marrow examinations.

PO.709

THALIDOMIDE IN THE TREATMENT OF PLASMA CELL DYSCRASIAS A SINGLE INSTITUTION'S EXPERIENCE IN 43 PATIENTS

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Thalidomide, alone or in combination with other drugs, has now become established as a therapy for relapsed and resistant multiple myeloma. We report our experience in using this drug in our patient population. Since 1999, 43 patients with plasma cell dyscrasias received thalidomide with or without other drugs. The most commonly used drug combination was thalidomide and dexamethasone followed by thalidomide and cyclophosphamide with or without dexamethasone. Only one patient was treated with melphalan dexamethasone and thalidomide. The aim was to use thalidomide in the minimal effective dose and to escalate the dose only there was no response and the patient tolerated the treatment. The dose varied between 25 mg and 300 mg daily. 5 patients (reported separately) had AL amyloidosis, two without and three with an underlying light chain secreting multiple myeloma. One patient had primary IgG cryoglobulinaemuia. The other 37 patients had multiple myeloma (MM). 21 of the 43 are alive and the range of presentation is from 1997 to January 2004. 2 patients showed a complete response and immunofixation negative. A good partial response, >75 reduction of PP, was obtained in another 6, and partial response, >50% reduction in another 13 patients. 6 patients had stable disease on thalidomide. The remainder 22 patients had resistant disease, insufficient trial of thalidomide or were lost to follow up. Patients experienced the usual range of side effects associated with thalidomide, including somnolence, lethargy and peripheral neuropathy but none were dose limiting. Four patients had thromboembolism, one of whom had a pulmonary embolus. The time to achieve maximum response was between 16 and 100 weeks. In patients who responded, the shortest continuos duration of therapy with thalidomide was 26 weeks and the longest was 108 weeks. Most of the patients discontinued thalidomide between 9-12 months. 5 patients responded to thalidomide, with other drug combinations, on second administration after relapse following a period of discontinuation of thalidomide of between 26 and 74 weeks. All five patients responded with a reduction in M proteins by more than 50% on the second occasion. Two patients have been off thalidomide and remained in plateau phase. One diagnosed in January 1999 has been without treatment for myeloma for 3 years after taking thalidomide for 58 weeks and has a low level of paraproteinaemia of 7 g/L, the other remains in complete remission, immunofixation negative 2 years after 56 weeks of thalidomide treatment. The conclusions from this study is that in some patient, plateau phase can be maintained after stopping thalidomide and that further responses can be obtained from a second administration of thalidomide, usually with other drugs. This approach leads to less exposure to the drug with the benefit of reduced side effects. Adverse events include three patients with thromboembolism, one with a patient with suboptimal disease control when the dose was increased to 200 mg daily, another at a time when she was in plateau phase following a good response and has been off treatment for 2 years with CR. A third a had a relapse with fractured hip and that was started with dex soon after a THR. Constipation and neuropathy as reported by others was trghe commonest manifestation. Another common adverse effect is a tremor which is controlled with propranolol. In general however pts tolerated the treatment well. X discont, y had thal for less than 4 weeks. In conclusion we have used low dose thalidomide than most others with successful results We believe that the dose of thalidomide should be tailored individually to patients as there are some patients who show remarkable responses with minimal doses. Our strategy now is to give thalidomide for 8 weeks at 100 mg, if there is no or minimal repose, the patients are either offered an increase of dose or dex, if nor response, an increase in dose or cyclo. Most prefer added drug than increase dose.

PO.710

LOW DOSE DEXAMETHASONE AND THALIDOMIDE WITH HIGHER FREQUENCY ZOLEDRONIC ACID FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Patients with relapsed/refractory multiple myeloma (MM) are frequently unable to tolerate further doses of standard chemotherapy. Moreover, disease- and/or therapy- induced toxicities, including peripheral neuropathy, may preclude the use of newer agents such as thalidomide (Thal) and bortezomib. We evaluated the activity of a novel regimen combining low dose dexamethasone (Dex) and Thal plus higher frequency zoledronic acid (Zol) - dtZ - in patients with symptomatic MM who were unable to tolerate standard doses of Dex and/or Thal and/or chemotherapy. Twenty-six consecutive patients with symptomatic MM, who had received an average of 21.1 (0.2 to 135.6) months of prior therapy, were treated (on an intention to treat basis) with the low-toxicity dtZ regimen; which comprises weekly Dex 20 mg OM for 4 days, Thal 50 mg ON, and three-weekly Zol

4 mg. Patients were treated for at least 6 months, and for up to 35.1 months. Seven patients had complex karyotypes; including 3 patients with deletion of chromosome 13, del(13). The reasons for intolerance to standard therapy included pancytopenia, peripheral neuropathy, active viral hepatitis B, severe cardiovascular disease and recurrent severe infections. The response rate (RR) was 61.6% (median time to remission 2.9 months among responders), including good responses in 23.1% of patients (Bladè criteria). Two patients with complex karyotypes responded to dtZ. There were however no complete remissions. The median time to progression was 26.9 months and median overall survival was 29.9 months from commencement of dtZ. Grade 2 toxicities included motor neuropathy (2 patients), muscle weakness (2 patients) and infection (2 patients). There were no grade 3 or 4 toxicities, deep venous thromboses or pulmonary embolism. Mild, transient hypocalcemia was reported in 2 patients; and mild, self-limiting hepatitis was observed in 2 patients. Patients with active hepatitis B infection received lamivudine concurrently with no adverse effects. Contrary to expectation, more frequent dosing of Zol was associated with overall improvement in renal function (R=0.23). Moreover cummulative doses of Zol did not have an adverse effect on renal function (R=0.02). Patients also demonstrated accompanying improvements in β2-microglobulin, hypercalcemia, pancytopenia, hypoalbuminemia and normal immunoglobulins. Very few patients required blood component support. All patients reported an improvement in their quality of life and dramatically lower pain scores. There were 7- to 8- fold decreases in hospitalization and infection rates, with accompanying costs savings. Five patients defaulted follow-up but were alive at their final clinic attendance. One patient with del(13) MM died after converting to an aggressive CD138+CD45+ plasmablastic MM. We conclude that the low-toxicity dtZ regimen was effective and well tolerated in patients with symptomatic MM who were unable to tolerate conventional doses of Dex and/or Thal and/or chemotherapy. Since dtZ was associated with a better RR than the melphalan/prednisolone combination, dtZ could be considered for use as a palliative regimen for patients with relapsed/refractory MM.

P0.711

LONG TERM USE OF THALIDOMIDE: SAFE AND EFFECTIVE

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Background and purpose: Thalidomide has emerged as one of the important drug over the last few years for the treatment of not only relapsed/refractory myeloma but also as front line therapy. Even though enough data is available on short term use of and toxicity of thalidomide not many reports describing its efficacy and toxicity over long term uses are available. It is not clearly known whether use of thalidomide as a maintenance therapy in myeloma is effective. At our center thalidomide is being used since year 2000. This report describes efficacy and safety of long term use of thalidomide in relapsed/refractory myeloma patients.

Materials and methods: All the myeloma patients who are using thalidomide for more than 2 years and who have received 600 or higher dose for at least 2 months and currently are alive were included in this study. Response assessment has been done as per standard criteria. Patients received thalidomide beginning at a dose of 200 mg/day with fortnightly increments of 200 mg to a maximum tolerated dose of 800 mg/day.

Results: Twelve patients fulfilled the criteria for inclusion in this study. Seven were males, median age was 55 years (range 40-68 years). Median duration of thalidomide therapy was 38 months (range 26-48 months). Six patients received 800 mg dose for period of 4-12 months and remaining received 600 mg for a period of 6-12 months. There after dose of thalidomide has been reduced in a planned way to minimum required dose to maintain the best response. Currently 6 patients in CR are maintaining their response with 200 mg dose and another 6 (Stable disease) with 100 mg daily dose of thalidomide. Side effects: constipation and mild neuropathy has been seen in all patients requiring use of laxative and high dose of pyridoxine. One patient had an episode of DVT requiring anti coagulant therapy.

Conclusion: Long term use of thalidomide is safe and helps in maintaining the response in myeloma patients. However, a larger trial should address the question of use of thalidomide as a maintenance therapy.

P0.712

THALIDOMIDE-INDUCED PERIPHERAL NEUROPATHY IN NEWLY DIAGNOSED AND PRE-TREATED MULTIPLE MYELOMA PATIENTS

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Thalidomide has shown remarkable activity in advanced, relapsed or refractory multiple myeloma (MM), so that its use has been recently proposed either in newly diagnosed patients or as maintenance treatment after conventional or high-dose therapy. This latter therapeutic approach has risen the concern of side effects occurring after long-term therapy with this drug. In order to investigate this issue we analyzed the outcome of 74 patients who have been treated with thalidomide + dexamethasone for longer than 8 months at our Institution. Thirty-four patients (18M, 16F, median age=55 years) had newly diagnosed symptomatic MM and were treated with four monthly courses of thalidomide 200 mg/day + dexamethasone 40 mg on days 1 to 4 (even cycles), or on days 1 to 4, 9 to 12 and 17 to 20 (odd cycles), followed by cyclophosphamide 7 g/m^2 + G-CSF, peripheral blood stem cell (PBSC) collection, and double autologous PBSC transplant. Thalidomide + dexamethasone was administered throughout the whole treatment program. Forty patients (27M, 13F, median age = 61 years) were treated with thalidomide 200 mg/day +dexamehasone 40 mg on days 1-4 every four weeks as salvage therapy for relapsed (n=14) or progressive (n=26) MM. Neurotoxicity was the most troublesome and frequent toxic effect that was observed after > 8 months treatment, the incidence averaging 74% in newly diagnosed patients and 75% in pretreated ones. Symptoms included paresthesias, tremor and dizziness; serial electrophysiological studies revealed a symmetrical, mainly sensory peripheral neuropathy, with minor motor involvement. The severity of neuropathy, graded according the NCI-CTC 2.0 scoring system, varied greatly in the two groups of patients, as pretreated patients showed grade 2 and 3 toxicity in 32.5% and 27.5% of the cases, respectively, while the majority of newly diagnosed patients complained about grade 1 toxicity (57%), and none of them experienced grade 3 toxicity. In both groups thalidomide neurotoxicity was not related to sex, M protein isotype, and thalidomide daily dose. In pretreated patients, a significant

correlation between grade 2+3 neurotoxicty and longer disease duration was found, thus suggesting that subclinical MM-related neurotoxicity could favour drug-induced toxic effects. These results suggest that long-term thalidomide therapy in MM may be hampered by the remarkable neurotoxicity of the drug, and that a neurological evaluation should be mandatory prior to thalidomide treatment, in order to identify patients at risk of developing a periperal neuropathy.

P0.713

DEVELOPMENT OF HIGH-GRADE B-CELL NEOPLASMS FOLLOWING THALIDOMIDE THERAPY

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Thalidomide is an effective drug in patients with advanced multiple myeloma. Multiple mechanisms of action have been proposed including effects on the neoplasm microenvironment such as anti-angiogenesis, direct inhibition of myeloma cell growth and modulation of adhesion molecules. We and others have reported that aggressive extramedullary disease (EMD) may appear after thalidomide treatment, and while the reasons for this phenomenon remain unclear, considerations include: a) selective inhibition of mature plasma cell clones; b) clonal evolution; or c) a change in the natural history of the disease given the lengthening of overall patient survival. We therefore initiated an exhaustive review of our patient population and the literature regarding this issue, and performed a comprehensive analysis of the clinical and laboratory parameters of 6 more patients. All patients examined had stage III multiple myeloma and developed anaplastic EMD of various sites, including skin, epidura, frontal sinus, chest wall, psoas muscle and lung following thalidomide therapy. Demographic information included 2 patients with IgG kappa multiple myeloma, one with IgA kappa, one with IgA lambda, one with IgG lambda and one with kappa free light chain myeloma. The median age was 59.5 years (range 45-71), the median albumin was 3.15 g/dL (range 0.3-4.1), the median LDH was 193 U/L (range 164-964), the median bone marrow plasmacytosis was 32% (range 5-80), the median plasma cell proliferation index was 44.4% (range 1-98.2) and the median time to progression to EMD was 11.5 months (range 2-24). Our observations suggest that thalidomide is effective in initially reducing a more mature plasma cell compartment confined to the marrow, and allows a relatively immature myeloma cell compartment to escape the marrow microenvironment. These studies serve to further elucidate the molecular role thalidomide plays in the development of EMD.

P0.714

PROSPECTIVE EVALUATION OF LOW-DOSE COUMADIN FOR PROPHYLAXIS OF VENOUS THROMBOEMBOLISM IN MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE-BASED REGIMENS

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Introduction. Thalidomide (THAL) is an immunomodulatory agent currently used for pts with multiple myeloma (MM). It is often combined with dexamethasone (dex) and or chemotherapy for improved clinical responses. An important and serious side effect of Thal therapy is venous thromboembolism (VTE), the incidence of which increases when it is combined with dex or adriamycin. As the underlying mechanism of VTE with Thal is unknown, the optimal method for prophylaxis is also undetermined. Various prophylactic measures including therapeutic doses of coumadin, low molecular heparin, and/or aspirin have been advocated with variable risk and cost/benefit association. To date there is no definitive consensus as to the best and safest method of prophylaxis for Thal induced VTE. At our institute we prospectively studied the prophylactic effect of low dose coumadin (1 or 2 mg) in pts treated with Thal based therapies.

Methods and Patients: All pts treated with thal-based therapies since November 2003, on clinical trials were prescribed low-dose coumadin (1mg for <70kg, 2mg for ≥70kg body weight) in the absence of underlying hypercoagulable disorder. The 2 studies included VAD + low dose thal (VAD-t) and velcade/doxil/thal (VDT). The incidence of VTE is recorded throughout the duration of therapy. All pts are tested for underlying hypercoagulable disorders (Factor V Leiden, protein C, S or antithrombin III deficiency). To date 33 pts (19F, 14M; median age 57, range 43-80 years) are available for VTE evaluation. Of these, 18 pts had relapsed/refractory MM and were treated on VDT combination and 15 treatment naïve pts were treated on the VAD-t regimen.

Results: None of the 33 pts had an underlying hypercoagulable disorder. All pts therefore received prophylaxis with low-dose coumadin. Two pts developed deep vein thrombosis (DVT) and 1was incidentally noted to have asymptomatic pulmonary embolism. None of the pts on the VDT regimen developed VTE. Cumulative incidence of VTE among all pts enrolled was 9%.

Conclusion: In our experience low-dose coumadin is easier to manage and can be safely given to patients treated on Thal based therapies. We did not observe an increased incidence of VTE despite using combination of Thal with dex and/or adriamycin when low-dose coumadin was used as prophylaxis. Interesting, no VTE was noted among pts treated so far on the Doxil based regimen-VDT. Updated results will be presented at the meeting.

P0.715

A MULTI-CENTRE PHASE-II TRIAL OF THALIDOMIDE AND CELECOXIB FOR RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background. The response rate (RR) to single-agent thalidomide (T) in relapsed/refractory myeloma (MM) is approximately 30% and increases to 44-55% with the addition of corticosteroids. Pre-clinical data indicates that Cox-2 inhibition may impair plasma cell growth and be potentially syn-

ergistic with T. Thus we examined the addition of celecoxib (Cxb) to T in patients with relapsed/refractory M.

Methods. We initiated a prospective multi-centre Phase-II trial in patients with relapsed/refractory MM using T up to maximum dose 800 mg/day. Cxb (400 mg bid) was commenced at the time of initiation of T. Patients intolerant of Cxb continued T alone. Thalidomide +/- Cxb was continued until disease progression. Objectives were to determine toxicity, response rate (RR), response duration, progression-free survival (PFS), overall survival (OS), elucidate relevant prognostic factors and perform an exploratory analysis of quality of life scores. Outcomes were compared to a prior trial of T +/- interferon alpha (IFN) using comparable eligibility criteria.

Results. Sixty-six patients, with median age 67 (range 43-83), were enrolled. The median individual-maximum-tolerated dose of T and Cxb was 400 mg/day and 780 mg/day, respectively with median durations of treatment of 6.3 months and 3.6 months, respectively. The most common toxicities associated with premature discontinuation of Cxb (n=29, 57%) were fluid retention and deterioration of renal function. On an intention-to-treat analysis, overall RR was 42% with 48% stable disease (SD). At 20 months median follow-up, the actuarial median PFS and OS were 6.8 and 21.4 months, respectively. Unlike our prior study, age > 65 years was not predictive of inferior RR but this is likely due to the improvement in RR in older patients with the T/Cxb combination (37%) compared to 15% in our prior T study (p=0.08). Predictors for inferior PFS were age >65 years (p= 0.0164) and elevated beta 2 microglobulin (B2M; p=0.0173), with bone marrow plasma cells >50% (p=0.005), elevated B2M (p=0.0134), haemoglobin <110 g/l (p=0.024) and raised creatinine (p=0.048) predictive of poorer OS. For the entire cohort, the outcomes in terms of RR (42% v 29%; p=0.21), response duration, PFS or OS were comparable between the two studies.

Conclusions. We confirm important prognostic factors for patients with MM receiving thalidomide-based therapy. Moreover, the improved response rate in older patients receiving the thalidomide/celecoxib combination warrants further study. Such studies should also examine dose-modifications to reduce celecoxib associated toxicities.

P0.716

CLARITHROMYCIN WITH LOW DOSE DEXAMETHASONE AND THALIDOMIDE (CDT) IS EFFECTIVE THERAPY IN RELAPSED/REFRACTORY MYELOMA

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A previous study has shown that Clarithromycin has a modest anti-myeloma effect[1] and a recent study of the combination of Clarithromycin, Dexamethasone and Thalidomide (CDT) have been shown to have significant activity in patients with myeloma[2]. We report a very high response rate in a Phase II trial of this combination which the object was to minimise the doses of both Thalidomide and Dexamethasone. Thirty patients accrued from December 2001 to July 2004 with relapsed (26) or refractory, (4) myeloma, age range 52-83 years. All of them gave informed consent and were given Clarithromycin 250 mg bd and Thalidomide 50 mg nocte continuously and Dexamethasone 10 mg daily x 4 days, once every 4 weeks with an additional 4 days of 10 mg of Dexamethasone given on days 15-18 for the first 2 cycles. Dose escalation of Thalidomide to 200 mg by 50 mg increments was permitted but only 3 patients had dose escalation to 150 mg and 2 to 100 mg. All patients received IV bisphosphonates once every 4 weeks.

Results. Thirty patients were enrolled and twenty-eight are evaluable. Four patients had complete remission, 6 a very good partial response, 14 partial response, 3 minimal response and there was 1 non-responder whose paraprotein failed to reach the criteria for minimal response. Although there are a number of patients still on therapy, the median survival is in excess of 8 months. Most patients had 1 or more cytopenia at the onset of therapy. Nine patients with thrombocytopenia showed a significant rise in platelet count, 8 to above normal levels. Twelve of 16 anaemic patients had a rise in haemoglobin of >1 g/dl with 5 returning to normal levels. Seven of 9 patients with neutropenia at onset of therapy had their neutrophil counts return to normal levels. In addition we have given CDT therapy to 10 patients off study, some of these at the time of diagnosis because of severe co-morbidity. A similar response rate has been seen in this group illustrating that CDT is a highly effective therapy which may be given to patients with cytopenias or co-morbid disease, even when refractory to other therapies.

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P0.717

THALIDOMIDE, DEXAMETHASONE AND CLARITHROMYCIN AS SALVAGE THERAPY FOR PATIENTS WITH ADVANCED MULTIPLE MYELOMA

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Clarithromycin (C) has variable single-agent efficacy in multiple myeloma but may enhance the effectiveness of Thalidomide (T) and Dexamethasone (D) when used in combination. We report our results of 14 patients with advanced myeloma treated with the combination of T (50-500 mg daily), D (12-40 mg weekly) and C (500 mg bd; TDC). Patients characteristics : median age 62 years (range 36-74), 71% male, IgA/IgG/LC 21%/64%/14% respectively, 44% elevated β_2m, PS ECOG 1 (1-3), 43% transfusion dependent. All patients were heavily pretreated with a median 4.5 (range 2-9) lines of therapy, 71% had received a previous autograft (for median remission duration [RD] of 9 months, range 3-45), and 71%, 71% and 86% had disease refractory to alkylating agents, T and D respectively. Median duration of TDC given was 21 weeks (range 4-100), with the following median doses : T, 200 mg/d; D 40 mg/wk; C, 500 mg bd. 64% of patients required dose reduction of T and/or D for side effects, most notably peripheral neuropathy (64% pts, Grade 3+[G3+] 14%), proximal myopathy (43%, G3+7%), hyperglycaemia (36%, G3+14%) or gastrointestinal tract upset (43%, G3+7%). One patient (7%) developed deep venous thrombosis, and 4 patients experienced an episode of infection during therapy; there were no treatment related deaths. All 14 patients were evaluable for response:

Complete Response (CR)	1/14	7%	}
Very Good Partial Response (VGPR; ≥90% PP reduction)	2/14	14%	} MR or
Partial Response (PR	4/14	29%	} better 71%
Minor Response (MR; 25%-49% PP reduction)	3/14	21%	}
Stable or Progressive Disease (PD)	4/14	29%	

Median RD was 24 weeks for patients who achieved a MR or better. Overall Survival (OS) was 52 weeks, with responders surviving for longer than non-responders (OS 100 weeks for PR+, *vs* 15 weeks for non-responders, p=0.01). Six patients had rapidly progressive disease on T alone and received addition of D and C; 5 (83%) achieved PR or better with median RD of 30 weeks. Four patients had rapidly progressive disease of TD, and received addition of C; only one patient responded (minor response, sustained at 8 weeks). In conclusion C appears to increase the efficacy of D when added to patients failing single-agent T, with response rate of 83% being higher than ~30% expected for addition of D alone. TDC is a potent salvage regimen in patients with advanced and treatment-refractory multiple myeloma, and demonstrates no apparent increase in toxicity over those expected for TD alone.

P0.718

RESULTS OF A RANDOMIZED PHASE III STUDY COMPARING THE STANDARD VAD PROTOCOL (VINCRISTINE, DOXORUBICIN, AND DEXAMETHASONE) TO THE ASSOCIATION OF PEGYLATED LIPOSOMAL DOXORUBICIN (CAELYX/DOXIL) AND DEXAMETHASONE IN FIRST LINE THERAPY FOR 120 PATIENTS WITH MULTIPLE MYELOMA

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The combination of vincristine and doxorubicin administered as a continuous infusion via an indwelling catheter together with intermittent high-dose dexamethasone (standard VAD) is considered as an effective primary treatment giving the more rapid decrease of tumor mass for patients (pts) with symptomatic multiple myeloma (MM). In order to simplify the logistic problems due to the standard VAD, several Phase-II studies have explored the feasibility and efficacy of VAD-like outpatient regimens. In the past, it has been suggested that the benefit of the standard VAD was partly due to the continuous infusion of vincristine and doxorubicin. In one recent randomised Phase-III study comparing VAD bolus to the combination of vincristine 2 mg IV day1, doxil 40 mg/m^2 day1and dexamethasone 40 mg^2 days (D) p.o., no differences were seen between both arms in terms of response and toxicity *(Dimopoulos MA et al., Ann Oncol 2003, 14:1039-44)*. In order to keep the standard way of administration for VAD (i.e. continuous infusion of vincristine and doxorubicin), we compared in a randomised study the standard VAD protocol (arm A) to the association of Doxil (40mg/m^2 IV D1) plus dexamethasone (40 mg/day p.o. D1-4 for all the cycles, and repeated at D9-12 and 17-20, for the 2 first cycles, similarly in the 2 arms) (arm B). After 4 cycles of treatment, pts were evaluated in terms of response and toxicity. Then, pts had mobilization/collection of autologous stem cell support for high dose therapy. All the pts were treated in the 2 centers. 120 pts were included in the study, including 61 pts in arm A and 59 pts in arm B. Median age was 60 years (range 36 to 77). 74 (62%) pts were males and 46 (38%) were females. There were no differences between both arms in terms of clinical characteristics, including age, sex and prognostic parameters (i.e., Durie stage, β2 microglobulin and CRP serum levels, isotypes and percentage of IgA subtype, plasma cell labelling index). At this moment, 95 patients completed their treatment. The overall response rate was not significantly different between the 2 arms, with 44% and 36% respectively in arms A and B [very good partial response (≥90% tumor mass reduction) or CR: 6 pts (10%) in arm A and 3 pts (5%) in arm B; PR: 20 pts (34%) in arm A and 17 pts (30%) in arm B. 14 and 19 pts were in minor response respectively in arm A and B. 4 patients died respectively in both arms. Toxicity were mild to moderate, including 3 pts with resolutive hand-foot syndrome in arm B. Grade 3-4 infections were not different between the two arms (12 pts in arm A and 13 in arm B). 7 pts in arm B and 3 pts in arm A experienced a grade 3-4 neutropenia. Thrombopenia (only at grade 1-2), was rare (1 and 3 pts in arm A and arm B, respectively). Digestive complications were acceptable in both arms, with a majority of Grade 1-2 toxicities. Mucositis was more frequent in arm B, but at grade 1-2. Complete data will be presented. There were no major differences between the two arms allowing the addition of new efficient agents, such as bortezomib or anti-interleukin-6 monoclonal antibodies to the association doxil plus dexamethasone, as developed *in vitro.*

P0.719

ASPIRIN INCREASES THE SURVIVAL FREE FROM DEATH OR DEEP VENOUS THROMBOSIS OF LIPOSOMAL DOXORUBICIN, VINCRISTINE, DECREASED FREQUENCY DEXAMETHASONE AND THALIDOMIDE TREATMENT OF MULTIPLE MYELOMA

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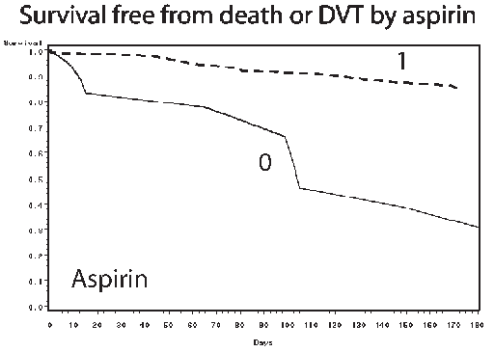
Patients with multiple myeloma (MM) are at risk for deep venous thrombosis (DVT). The risk has been reported to be higher in newly diagnosed (ND) patients treated with Thalidomide and Doxorubicin. Liposomal doxorubicin, vincristine, decreased frequency dexamethasone and thalidomide (DVd-T) is effective in the treatment of MM but carries an increased risk of DVT. We treated 105 patients with DVd-T from 8/2001to 4/2004. After the first 35 patients had a high rate of DVT, the protocol was amended to evaluate coagulation parameters (including Platelet Aggregation studies (PA), factor V leiden (FVL) and Activated protein C resistance (APCR), and von Willebrand factor (vWF)), and to include prophylaxis to patients with 81 mg Aspirin (ASA) daily. Two patients on Warfarin for other indications were excluded from the analysis. Of the 103 patients remaining 54 had ND MM and 49 had Relapsed/Refractory (RR) MM. Mean age was 58.9 years and 56% were males and 26%, 42%, 19% and 13% had SWOG stage I, II, III and IV respectively. Four patients were heterozygous for FVL and did not develop DVT. Fifty eight patients received ASA at the start of treatment while 26 received ASA after the start of treatment and 19 did not receive ASA. Twenty six post treatment (pRx) DVT occurred at a mean 109 days pRx with 15/84 (17.8%) occuring after the start of ASA and 11/19 (57.8%) occurring off ASA (p=0.0002). 2 patients assigned to the ASA group had discontinued their ASA and developed DVT off ASA, hence the pRx DVT on ASA is 13/82 (15.8%) versus 13/21 (61.9% p<0.001). Newly diagnosed status was not associated with an increased risk of pRx DVT. Compared to patient not receiving ASA, patient in the ASA groups had a higher survival free from Death or DVT (32/84 or 38% in the ASA group vs 17/19 or 89% in the no ASA group, p<0.001 figure). Patients in the ASA group enjoyed a higher response rate compared to the no ASA group (combined CR and PR rates of 77% vs 58%, p=0.08; and CR rates

of 29% vs 11% respectively, p=0.09). ASA decreases the incidence of pRx DVT, the combined end point of death or DVT, and is associated with a trend towards increased response rates in patients with MM treated with DVd-T.



PO.720

BORTEZOMIB DEMONSTRATES SUPERIOR EFFICACY COMPARED WITH HIGH-DOSE DEXAMETHASONE, WITH PREDICTABLE TOXICITY

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Introduction: APEX, an international phase 3 randomized study comparing the efficacy and safety of bortezomib (VELCADE®) with high-dose dexamethasone (dex), is the largest study to date in relapsed myeloma.

Methods: Pts who had previously received 1-3 prior therapies were randomized to receive either bortezomib 1.3 mg/m^2 by IV bolus on days 1, 4, 8, and 11 of a 3-week cycle for 8 cycles followed by treatment on days 1, 8, 15, and 22 every 5 weeks for 3 cycles, or dex 40 mg PO on days 1-4, 9-12, and 17-20 every 5 weeks for 4 cycles followed by treatment on days 1-4 every 28 days for 5 cycles. Pts with dex-refractory disease were excluded. Pts with progressive disease on dex were eligible to crossover to bortezomib in a companion study.

Results: 333 pts were randomized to bortezomib and 336 to dex. Baseline demographics were comparable between the groups. In the final efficacy analysis, bortezomib proved superior to dex, demonstrating a highly significant 78% improvement in median time to progression (hazard ratio 0.55, p<0.0001), higher response rates using EBMT criteria (complete + partial response, 38% vs 18%; p<0.0001), and an improvement in 1-year survival (80% vs 66%, p= 0.0025). In this analysis, treatment exposure and safety profiles are reported. Exposure was similar between groups, with 56%, 29%, and 9% of pts in the bortezomib arm completing 5, 8, and 11 cycles, respectively, and 56%, 28%, and 5% of pts in the dex arm completing 3, 5, and 9 cycles, respectively. In the safety population (bortezomib n = 331; dex n = 332), gr 4 adverse events (AE; 14% vs 16%), serious AE (44% vs 43%), and discontinuations due to AE (37% vs 29%) were reported in similar proportions of pts in both arms, respectively. Gr 3/4 AE were reported in 75% of pts receiving bortezomib and 60% of pts receiving dex. Gr 3/4 AE reported by ≥10% of patients receiving bortezomib were thrombocytopenia (29%), neutropenia (15%), and anemia (10%). Thrombocytopenia with bortezomib followed a cyclical pattern, with recovery toward baseline during the rest period. Similarly, a progressive decrease in the mean neutrophil count was observed during the treatment phase, with recovery during the rest period of each treatment cycle. Peripheral neuropathy is a clinically important toxicity of bortezomib and was reported in 36% of pts (≥ gr 3 in 8%). Improvement or resolution of grade 2 or worse peripheral neuropathy was observed in 51% of pts (44 of 87), with a median time to improvement or resolution of 107 days (~3.5 months) from onset. The only ≥ gr 3 AE occurring in ≥10% of dex pts was anemia (11%). Cardiac disorders were reported for 15% of pts in the bortezomib arm and 13% in the dex arm. No single cardiac disorder was reported in >10% of either treatment group: 7 pts (2%) on bortezomib and 8 pts (2%) on dex experienced congestive cardiac failure during the study. Conclusion: APEX demonstrated a superior efficacy advantage of bortezomib over dex in relapsed myeloma. The rates of grade 4 AE, serious AE, and discontinuations were similar in both arms, and the safety profile of bortezomib was predictable and manageable. Bortezomib should be considered an effective treatment option for relapsed myeloma.

PO.721

BORTEZOMIB AT FIRST RELAPSE IS SUPERIOR TO HIGH-DOSE DEXAMETHASONE AND MORE EFFECTIVE THAN WHEN GIVEN LATER IN RELAPSED MULTIPLE MYELOMA

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Introduction: An international phase 3 study (APEX) compared bortezomib (VELCADE®, Vc) vs high-dose dexamethasone (Dex) in patients with relapsed multiple myeloma (MM). At the interim analysis, Vc achieved a significantly

longer median time to progression (TTP) and overall survival (OS) than Dex. A prospective subgroup analysis was performed comparing Vc with Dex as second-line versus ≥ third-line therapy to determine the potential benefit of starting Vc at first relapse.

Methods. Six a hundred and sixty-nine patients who had received 1-3 prior treatments and were not refractory to Dex were randomized to either Vc 1.3 mg/m^2 IV on days 1, 4, 8, and 11 for eight 3-week cycles followed by 1.3 mg/m^2 IV on days 1, 8, 15, and 22 for three 5-week cycles, or Dex 40 mg po on days 1-4, 9-12, and 17-20 for four 5-week cycles followed by treatment on days 1-4 for five 4-week cycles. The European Blood and Marrow Transplantation (EBMT) criteria were used to evaluate response. Randomization was stratified by number of prior therapies (1 versus more than 1).

Results. 251 patients received only 1 prior therapy. Second-line Vc resulted in a significantly longer median TTP and a higher response rate (complete response [CR] + partial response [PR]) versus second-line Dex. OS was significantly improved with second line Vc (hazard ratio 0.42, p=0.0130) versus second-line Dex after a median follow-up of 8.3 months for all patients. The median TTP appeared longer and the RR higher in patients who received Vc earlier as second-line versus as later salvage therapy. Response rates with Vc were higher compared with Dex regardless of type of prior therapy except with prior thalidomide; the response rate to Dex and Vc appeared lower in this subset, and the difference did not reach significance, but the number of patients was low. The median TTP, response rate, and OS (hazard ratio 0.63, p=0.0231) were higher with Vc compared with Dex in patients receiving ≥ third line therapy.

	Vc	Dex	P
Second line, n	132	119	
Median time to progression, months	7.0	5.6	0.0021
CR/PR, % (n/N)	45 (57/128)	26 (29/110)	0.0035
- Prior transplantation, % (n/N)	48 (40/83)	29 (19/66)	0.0039
- Prior thalidomide, % (n/N)	29 (7/24)	12 (3/25)	0.0937
- No prior thalidomide, % (n/N)	48 (50/104)	31 (26/85)	0.0105
- Prior vinca alkaloid, % (n/N)	45 (42/93)	25 (18/71)	0.0053
- Prior anthracycline, % (n/N)	47 (45/95)	26 (19/74)	0.0023
- Prior alkylating agent, % (n/N)	45 (50/110)	29 (26/91)	0.0084
≥ Third line, n	200	217	
Median time to progression, months	4.9	2.9	< 0.0001
CR/PR, % (n/N)	34 (64/187)	13 (27/202)	< 0.0001

Conclusions: Vc provided higher response rates and more favorable time-to-event results than Dex at first relapse and beyond. Vc appeared to result in a longer TTP and higher response rate when used earlier as second-line compared with its use as later salvage therapy. Response to Vc was unaffected by type of prior therapy, except response rates appeared lower in patients who received prior thalidomide. These data warrant the use of Vc during earlier stages of myeloma treatment.

PO.722

BORTEZOMIB INDUCES REMISSIONS IN PATIENTS WITH RELAPSED/REFRACTORY MYELOMA INDEPENDENT OF THEIR CYTOGENETIC RISK PROFILE AND ADDITION OF DEXAMETHASONE OR DEXAMETHASONE PLUS CHEMOTHERAPY CAN RESTORE RESPONSIVENESS

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Background. Bortezomib (B) is the first compound of a new class of agents - the proteasome inhibitors - entering clinical use in multiple myeloma (MM). In a large phase II trial of extensively pretreated patients with MM, B yielded response rates of 35%, with a median response duration longer than that of the preceding chemotherapy. Preliminary observations suggested efficacy of B also in patients with deletion of chromosome 13q [del(13q)] and other unfavorable standard prognostic factors.

Aim. Analysis of clinical activity and toxicity of single agent B in pretreated patients with MM in a routine clinical setting. In patients failing single agent B after an initial response, we tried to restore drug sensitivity by applying dexamethasone (DEX) or DEX plus chemotherapy in combination with B. Activity of B was analyzed in the context of chromosomal aberrations as detected by FISH.

Patients and Methods. Thirty-five patients with the following characteristics were treated with B: Median age 63 years (range, 40-80), M-protein: IgA 13, IgG 17, Bence-Jones-only 1, IgD 1, non-secretory 1. Thirty-three patients had stage III myeloma, 1 patient AL amyloidosis, and 1 patient light-chain deposition disease. Serum creatinine was ≤2 mg/L in 32 patients and ≥2 mg/L in 3 patients (1 patient on dialysis). Prior lines of therapy/number of patients: 1/4, 2/8, 3/10, ≥4/13. Pretreatment included thalidomide in 25 patients, high dose melphalan in 16 and interferon–alpha maintenance treatment in 17 patients. B was given at a dose of 1.3 mg/m^2 on days 1, 4, 8 and 11 (q21 days). In patients failing after initial response to single agent B, DEX (8-20 mg) was added to each B injection. Subsequently, in patients failing the latter regimen, melphalan (10 mg) and then doxorubicin (9 mg/m^2) was added to each B/DEX treatment.

Results. Patients were considered evaluable for analysis after completion of ≥2 cycles. 15 out of 27 (56%) evaluable patients showed objective responses with 3 near-CR (>90% paraprotein reduction), 9 PR (>50%), and 3 MR (>25%). Nine patients had stable disease and 3 presented with progressive disease. Median time to response was 3 weeks (range 3-9 weeks), median duration of response was 27 weeks (range 15-29 weeks). The most prominent toxicity (143 cycles evaluable) was grade 3-4 thrombopenia, seen in 22 out of 143 cycles. Grade 3-4 neutropenia was seen in 8 out of 143 cycles and anemia was encountered in 1 out of 143 cycles. Other toxicities (grade 3-4) were neuropathy (3 patients), infections (5 patients), dyspnea (3 patients), and skin toxicity (2 patients). Addition of DEX to B in patients failing single agent therapy led to responses in 9/15 patients (60%). After failure of the B/DEX combination, additional responses were achieved by a triple combination with either melphalan or doxorubicin in some cases. Among 7 patients with a del(13q) by FISH, 4 patients (57%) achieved an objective response. Notably, a patient with the chromosomal pattern del(13q) plus translocation t(4;14)(p16;q32) responded not only to single agent B with a remission lasting for 7 months, but later also responded to the B/DEX and triple combinations with remissions lasting for several months each.

Conclusion. B is an effective salvage treatment for patients with relapsed/refractory MM (overall response rate of 56%) even in the setting of unfavorable cytogenetics. Addition of DEX ± chemotherapy may render additional remissions in patients failing after initial response to single agent B.

P0.723

BORTEZOMIB IN REFRACTORY MULTIPLE MYELOMA THE FIRST EXPERIENCE IN THE CZECH REPUBLIC

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Chemotherapy itself probably will not substantially improve the prognosis of patients with multiple myeloma (MM) in the future. Therefore new methods are needed – one of the promising options is the direct interference with intracellular metabolism – *targeted* therapy. Proteasome inhibitors are an example of this new approach and bortezomib (Velcade, Millennium Pharmaceuticals) is the first agent in this class to enter clinical trials. We summarize here the first clinical experiences with bortezomib in the therapy of refractory myeloma in hematological centres of Czech republic. Bortezomib was used in 14 patients with refractory MM, eight men and six women, aged 45-80 years (mean 60 years). Monoclonal protein of IgG isotype was identified in nine patients, IgA in three patients and light chains in two patients. All patients had received between two and five previous lines of therapy, and 10 had undergone high-dose therapy with autologous stem cell support (ASCT). Only patients with no evidence of basal exclusion criteria, particularly the absence of serious cytopenia and/or renal failure (creatinine clearance <30 mL/min) were eligible to start treatment with bortezomib. The initial dose of bortezomib was 1.3 mg/m^2 (reduced to 1 mg/m^2 in one patient because of pre-existing renal dysfunction). The dose was reduced during the course of therapy in three other patients, due to worsening of renal function (one patient) and grade 4 hematological toxicity (two patients). In two cases high-dose dexamethasone was added. At time of submitting this abstract, ten patients (76%) had a complete, partial, or minor response. In one patient stabilisation of disease was observed and the disease progressed during therapy in two patients. One patient died immediately after the start of therapy due to concomitant disease (stroke). The response to bortezomib did not appear to depend on the number of previous lines of chemotherapy. The most common adverse events were thrombocytopenia and fatigue, although three patients experienced at least one episode of grade 4 toxicity (thrombocytopenia, leucopenia, or renal failure). One patient developed grade 2 peripheral neuropathic pain but was able to continue therapy after a short interruption and dose reduction. However, in one patient therapy had to be interrupted due to grade 3 neuropathy.

P0.724

BORTEZOMIB PLUS DEXAMETHASONE AS INDUCTION TREATMENT PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA. PRELIMINARY RESULTS OF AN IFM PHASE II STUDY

JL Harousseau, M Attal, X Leleu, R Gressin, C Hulin, JG Fuzibet, J Troncy on behalf of the IFM

When patients (pts) with newly diagnosed multiple myeloma (MM) are treated with autologous stem cell transplantation (ASCT), the standard induction therapy is a dexamethasone (Dex) based regimen. Currently, the use of VAD or, Dex alone results in a) complete remission (CR) rate of <10%. In an ongoing ECOG trial comparing Dex alone to and the combination thalidomideThalidomide plus Dex, preliminary results diddo not show a clear advantage of the combination (Rajkumar ASCO 2004). Since achievement of CR is a major objective in the treatment of MM, better therapeutic regimens are being investigated. Bortezomib is currently approved in the US and in the EU for the treatment of relapsed/refractory MM. Both *in vitro* studies and preliminary clinical experience in relapsed/refractory pts have suggested that the combination of bortezomibVelcade and Dex could further improve the results achieved with bortezomibVelcade alone. The the IFM group initiatedstarted a Phase II open trial assessingtesting the combination of bortezomibVelcade and Dex in pts with previously untreated MM and who are candidates forto ASCT. The regimen consisted of bortezomibVelcade 1.3 mg/m^2 iv on days 1, 4, 8, 11 and Dex 40 mg per os days 1-4, 9-12 (for the first 2 cycles, days 1-4 only for the last 2 cycles), administered on 4 consecutive 21 days cycles. Stem cell collection was performed just before cycle 4 after G-CSF priming. The primary objective of the study was CR rate after 4 cycles. As of August 1, 47 pts have been recruited and data is available for the first 18 pts. The median age is 53 years (38-63) Sixteen/18 pts received 4 cycles : 1 patient progressed after 3 cycles and in one case the last two injections of bortezomibVelcade were not performed because of grade 3 neuropathy. The overall results were as followsthe following: CR (negative electrophoresis) 3 ; very good partial remission (90% reduction of M component) 2; partial remission (50% reduction of the serum M component or 90% reduction of the urine M component) 10; failure (stable disease or progression) 3. The overall response rate was 83% and the CR rate was 17%. Side effect were usually mild (grade 1/2); only). Only one grade 3 neuropathy was recorded. In all cases stem cells could be adequately collected. These preliminary results appear to be very encouraging and the bortezomibVelcade/dex combination appearslooks effective and well tolerated in pts with newly diagnosed MM. The results will be updated at time of presentation.for the meeting. If updated analysis confirms currently available data, the . The IFM will start a large randomized phase III trial comparing VAD and bortezomibVelcade / Dex as induction treatment prior to ASCT in pts with newly diagnosed MM up to the age of 65.

P0.725

BORTEZOMIB THERAPY ALONE AND IN COMBINATION WITH DEXAMETHASONE FOR PATIENTS WITH PREVIOUSLY UNTREATED MULTIPLE MYELOMA

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Introduction. Bortezomib ± dexamethasone (dex) has demonstrated impressive activity in the treatment of relapsed and refractory myeloma. We are conducting a phase 2 trial evaluating bortezomib alone or in combination with dex as first-line therapy in patients (pts) with myeloma.

Methods. Eligible patients had measurable disease and a Karnofsky performance score (KPS) ≥50%. Bortezomib 1.3 mg/m^2 was administered by IV bolus on days 1, 4, 8, and 11 of a 3-week cycle for a maximum of 6 cycles. Oral dex 40 mg was given to pts who achieved < partial response (PR) after 2 cycles or < complete response (CR) after 4 cycles. European Blood and Marrow Transplantation criteria were

used to assess response, with the addition of a near CR (nCR) category defined as nondetectable M-protein by electrophoresis but with positive immunofixation and normal bone marrow.

Results. Of 38 accrued pts, the first 23 have now completed the study. Median age was 63 years, and 44% were men. The major myeloma subtypes were IgG (61%) and IgA (26%). The majority of pts were Durie-Salmon stage II (36%) or IIIA (36%). The major response rate (CR + nCR + PR) was 83% (table). The best response was observed after cycle 2 in 43%, after cycle 4 in 39%, and after cycle 6 in 13% of pts. Dex was added for 14 pts (61%) – 8 pts after cycle 2 and 6 pts after cycle 4. An improved response after combination therapy was observed in 9 pts: 6 pts improved from MR to PR, and 3 pts from stable disease to PR. Five pts underwent stem cell collection, and bone marrow from each pt was successfully harvested; 2 pts have received stem cell reinfusion, and both have had complete hematologic recovery. Ten patients discontinued from the study: 7 due to adverse events, 1 due to progressive disease, and 2 pts withdrew. The most common adverse events (grades 1-3) were neuropathy (56%), fatigue (56%), diarrhea (44%), constipation (38%), and neuropathic pain (12%). Grade 4 neutropenia was reported in 1 pt.

Conclusion. Bortezomib alone or in combination with dex was highly active in the first-line treatment of pts with myeloma. The combination of bortezomib + dex demonstrated additional benefit. Toxicities were manageable and reversible. Although experience has been limited to date, stem cell transplantation was successful in all attempts, indicating that bortezomib-based therapy should be further explored as an induction regimen before transplantation. Accrual is complete, and final results will be presented.

	N = 23
Major response rate, %	83
Complete response, %	13
Near complete response, %	17
Partial response, %	53
Minor response, %	13

P0.726

A PHASE I/II NATIONAL, MULTI-CENTER, OPEN-LABEL STUDY OF VELCADE PLUS MELPHALAN AND PREDNISONE (V-MP) IN ELDERLY UNTREATED MULTIPLE MYELOMA PATIENTS

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Background. For more than 25 years, melphalan and prednisone has remained as the gold standard treatment for elderly multiple myeloma (MM) patients. The proteasome inhibitor velcade has shown significant activity with manageable toxicity in refractory/relapse MM patients. Moreover, in vitro synergy has been reported when velcade is combined with cytotoxic agents such as melphalan.

Aim: To define the appropriate dose of velcade in combination with MP and to analyse the efficacy of velcade plus melphalan and prednisone (V-MP) (CR+PR) in untreated MM patients ≥65 years old.

Methods: This is a dose escalation study with two sequential dose levels (Phase 1) and expansion of the cohort at the MTD up to 60 patients to further refine estimates of efficacy (Phase 2). Patients will receive up to four 6-week treatment cycles of V-MP initially followed by up to five 5-week treatment cycles. In the first cohort, velcade (1 mg/m^2) was administered as a intravenous bolus to 6 consecutive patients on days 1,4,8,11,22,25,29 and 32 followed by a 10 day rest period (days 33 to 42) in combination with oral melphalan, 9 mg/m^2 once daily on days 1 to 4 and oral prednisone, 60 mg/m^2 once daily on days 1 to 4 of the six week cycles. In the 2nd cohort, velcade was administered at 1.3 mg/m^2. DLT was defined as the occurrence of grade 3-4 nonhematological toxicity and grade 4 hematological toxicity thought to be dose-related and occurring during the first 6-week cycle. MTD was defined as that dose level below which 2/6 patients had a DLT.

Patients. 24 patients have been enrolled to date - 12 in the phase I and 12 in the phase II-.

Results. In the first 6-patient cohort at 1 mg/m^2 there was no DLT in the first cycle. Grade 3-4 therapy related included one episode of neutropenia grade 3 (ANC = 0,668x10^9/L). The most common grade 1 or 2 toxicities were nausea, vomiting, rash, constipation, diarrhea, fever, herpes zoster infection, anorexia, neutropenia, anemia and thrombocytopenia. Ocular neuropathic pain (grade 2) was developed *de novo* in 1 patient during the first cycle; one dose of bortezomib was held (D8) and the adverse event resolved with analgesic treatment in 4 days. Treatment was continued at a dose of 0,5 mg/m^2 at days +11 and +22. Afterwards, VELCADE at 1.0 mg/m^2 was restarted. In subsequent cycles no important toxicity has been observed in this first 6-patient cohort who have gone on receiving VELCADE at 1 mg/m^2. In the second 6-patient cohort at 1.3 mg/m^2 there was no DLT during the first cycle. Grade 3-4 therapy-related included one serious adverse event (pulmonary embolism and probable septic shock with death) in one patient at day +11 considered as possible DLT, one episode of neutropenia grade 3 (ANC=0,600 x109/L) and one episode of thrombocytopenia grade 3 at day +32 (platelets=28 x10^9/L). Other grade 1 or 2 toxicities were similar to those observed in the first 6-patient cohort. Therapy-related toxicity in later cycles included one episode of peripheral neuropathy with neuropatic pain grade 3 who has conditioned the delay of the third cycle and one episode of neuropatic pain grade 2 who has conditioned the 25% of dose reduction of VELCADE . In the phase II, 12 patients have been enrolled but only 10 are evaluable because one patient has been a screening failure and other has removed the consent form after 4 doses of VELCADE. They are now receiving or just completed the first cycle, so observations are limited. Regarding response, the 11 patients alive included in the phase I are evaluable for response, with a mean of 4 cycles (2-6) of treatment. There was 2 Complete Remission with immunofixation positive after 2 cycles of treatment, 8 Partial Response (6 out of these 8 patients have experienced > 80% reduction in M-protein) and 1 Minor Response.

Conclusion. According to DLT and MTD definitions, the regimen recommended for phase II was 1.3 mg/m^2 of VELCADE in combination with MP. So far, no patient has developed DLT. The combination of V-MP is well tolerated with a manageable toxicity. Significant M-protein responses have been seen. Results will be updated and presented for the phase II.

P0.727

BORTEZOMIB IN COMBINATION WITH DEXAMETHASONE FOR RELAPSED MULTIPLE MYELOMA

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Single agent bortezomib treatment at the dosage and schedule published by Richardson (2003) stabilizes disease in nearly 60% of patients with relapsed, refractory multiple myeloma (MM). However, only 35% of patients achieve an objective (≥ minor) response (MR). Dexamethasone adds to clinical anti-myeloma activity of bortezomib by inducing 18% responses in patients with either stable or progressive disease on bortezomib alone. In an attempt to improve disease response, we evaluated a primary bortezomib/dexamethasone combination in patients with multiple myeloma in 2nd untreated or refractory relapse. Eligible patients were 18-80 years old, had an ECOG performance status of 0-2 and adequate renal, hepatic, pulmonary and cardiac function. Pre-existing peripheral neuropathy grade 2 or neuropathic pain of any grade were exclusion criteria. However, we made no restrictions in terms of pretreatment blood counts. Fifteen consecutive patients with relapsed multiple myeloma (9/15 with 2nd untreated and 6/15 with refractory relapse; 71% with a chromosome 13 deletion) were scheduled to receive bortezomib 1.3 mg/m^2 IV days 1, 4, 8, 11 q 3 weeks for up to 8 cycles, in combination with dexamethasone 20 mg PO once daily on the day of bortezomib injection and the day thereafter. Treatment was withheld for nonhematologic adverse events (AE) grade 3 and reinitiated at a 25% lower dose after resolution. Treatment was not stopped for myelosuppression of any grade if interim response evaluations precluded myeloma progression as the cause of cytopenia. One patient (7%) achieved a complete response, 10 (67%) a partial response, and 1 (7%) a MR resulting in an overall response rate (≥ MR) of 80% (9/9 with 2nd untreated and 3/6 with refractory relapse; EBMT/IBMTR/ABMTR criteria). Responses occurred after a median of 3 weeks and were independent of conventional prognostic parameters. Importantly, 8/10 patients with a chromosome 13 deletion achieved a PR. Adverse events, mainly myelosuppression (34% grade 3/4 neutropenia; 47% grade 3/4 thrombocytopenia), neuropathy (grade 2/3/4 20%/7%/0%) and fatigue (grade 2/3/4 20%/13%/20%), were manageable. There was no case of neutropenic infection or thrombocytopenic bleeding. Two patients suffered herpes zoster. The percentage of transfusion dependent patients decreased from 44% during the 1st treatment cycle to 23% and 11% after the 2nd and 3rd treatment cycles, respectively. After a median follow-up of 5 months, median event free and overall survival had not yet been reached. Compared with the disease status at study entry, five out of 15 patients showed no response (n=1) or experienced disease progression (n=4). Notably, 2 patients with sustained paraprotein and bone marrow remission (confirmed by biopsy) had extramedullary disease progression, pointing to a bone marrow restricted response to bortezomib in MM. This suggests that bortezomib may be safe even in patients with poor bone marrow reserve, who would not have been candidates for the SUMMIT trial. Though the remission rate was high, remissions often were not durable. This fact underlines the need for consolidating treatment and evaluation of bortezomib combinations with other anti-myeloma agents.

P0.728

BORTEZOMIB IN COMBINATION WITH MELPHALAN IN THE TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA: A PHASE I/II STUDY

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Introduction. Bortezomib (VELCADE®, formerly PS-341) is a selective reversible proteasome inhibitor that has demonstrated durable responses as monotherapy for the treatment of patients with *relapsed and refractory multiple myeloma*. *In vitro*, bortezomib has been shown to restore melphalan sensitivity to melphalan-resistant cell lines (U266-LR7) and to synergize with melphalan in killing myeloma cells, thereby allowing the use of lower concentrations of melphalan (Ma *et al., Clin Cancer Res.* 2003;9:1136). The objective of this dose-escalation phase I/II study was to determine an optimal dose of combination bortezomib + melphalan, starting with doses below those usually recommended for each agent for patients with refractory or relapsed multiple myeloma, *by assessing dose limiting toxicities, general safety and tolerability in a dose-escalation study. Activity was also assessed.*

Methods. Bortezomib 0.7 mg/m^2 was administered by IV push on days 1, 4, 8, and 11 in combination with oral melphalan (0.025, 0.05, 0.1, 0.15, 0.25 mg/kg) on days 1-4 every 4 weeks for up to 8 cycles to 3-patient cohorts with active progressive disease. In the absence of dose-limiting toxicity (DLT), bortezomib was increased to 1.0 mg/m^2 and melphalan co-administered using the original 5 escalating doses to subsequent cohorts.

Results. Twenty-two patients (55% male, median age 55 years, range, 33-77 years) have been accrued to the study. The myeloma subtypes include IgG (15/22), IgA (3/22), IgM (1/22) and light chain only (3/22). The median β2-microglobin level was 5.0 mg/L (range, 2.2-14 mg/L). In this heavily pretreated population (range, 2-7 prior therapies), 8 patients received prior melphalan, 9 prior thalidomide, 12 prior VAD, 2 prior bortezomib, and 7 prior autologous stem cell transplantation. Dose escalation has proceeded into the bortezomib 1.0 mg/m^2 + melphalan 0.025 mg/kg cohort. Toxicities have been manageable. One DLT, grade 4 anemia, was observed at bortezomib 1.0 mg/m^2 + melphalan 0.025 mg/kg, requiring expansion of the cohort. Grade 3 events were predominantly associated with myelosuppression (anemia, neutropenia, and thrombocytopenia) and were observed only among patients with baseline cytopenia. Among the 12 patients with baseline peripheral neuropathy (PN), symptoms worsened transiently in 1 patient, resolved in 1 patient, and remained stable in the other patients. Treatment-related PN (grade 1) developed de novo in 2 patients. Responses were observed in 64% (14/22) of patients: 1 CR, 1 near CR, 6 PR, and 6 MR. The CR and near CR occurred in patients receiving bortezomib 1.0 mg/m2 in combination with melphalan .025 mg/kg. PR or better was independent of prior type of therapy, and was also observed among patients who had previously received melphalan or bortezomib. Median time to progression was 6 mo. Six of 22 patients remain progression-free for 2-15+ mo.

Conclusion. Combination bortezomib plus melphalan is a promising regimen for the treatment of relapsed, refractory myeloma. The responses that were observed in patients who had previously received either drug serve as prelimi-

nary confirmation of preclinical evidence that the combination of low dose bortezomib and melphalan has the capacity for chemosensitization and suggest possible synergy. Dose escalation with melphalan plus a fixed dose of bortezomib 1.0 mg/m^2 is continuing in order to explore the full potential of this combination.

PO.729

BORTEZOMIB AND INTRAVENOUS MELPHALAN FOR RELAPSED MULTIPLE MYELOMA

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Introduction. The efficacy of single agent bortezomib in the treatment of relapsed multiple myeloma is well established. Pre-clinical studies demonstrate potential synergy between bortezomib and a variety of chemotherapeutic agents and in particular has been shown to overcome melphalan resistance in cell lines. This forms the basis of our Phase I/II clinical trial investigating low dose intravenous melphalan with bortezomib (BM).

Aims. The primary objectives were to assess the safety, tolerability and response rates to BM in patients with relapsed myeloma.

Methods. Patients received bortezomib 1.3 mg/m^2 on days 1, 4, 8 and 11 and intravenous melphalan on day 2 of each 28 day cycle. We are currently at level 1 of our Phase I component (melphalan 10 mg/m^2). Patients will receive up to 8 cycles with dexamethasone being added for progressive or stable disease after 2 or 4 cycles respectively.

Patients Characteristics. Currently 5 patients have been enrolled (all male) with a median age of 56 (44-62) and have had a median of 3 (2-5) previous therapies all including high dose melphalan with autologous stem cell rescue. The median β_2 microglobulin was 3 (1-7) and median serum paraprotein was 38 (19-92). The majority (4) were of the IgG subtype with one having light chain only myeloma.

Results. The median number of cycles completed is 3 (1-5) and 80% have achieved at least MR following the first cycle (1 VGPR, 1 PR, 2MR). Tolerability has been reasonable at this dose with no grade 4 toxicities seen to date. 4 have developed grade 3 haematological toxicities with 2 requiring dose reductions due to thrombocytopaenia and 1 withdrawn due to neuropathy.

Conclusions. This early data suggests that BM combination therapy is feasible and is likely to give responses in an otherwise poor prognostic group of patients. Further work is required and we intend to recruit up to 50 patients.

PO.730

BORTEZOMIB AND PEGYLATED LIPOSOMAL DOXORUBICIN AS INITIAL THERAPY FOR ADULT PATIENTS WITH SYMPTOMATIC MULTIPLE MYELOMA: CALGB STUDY 10301

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The ubiquitin-proteasome pathway is responsible for the vast majority of intracellular protein turnover, and contains at its core the multicatalytic proteinase complex, which has been validated as a target for therapy of multiple myeloma. Modulation of proteasome function also appears to be a rational strategy to induce chemosensitization, and to overcome *de novo*, acquired, and inducible drug resistance. Combinations of an anthracycline and a proteasome inhibitor have been documented to show enhanced, possibly synergistic anti-tumor activity in both *in vitro* and *in vivo* model systems. This likely occurs through several mechanisms, including the ability of proteasome inhibitors to suppress anthracycline-mediated activation of anti-apoptotic nuclear factor kappa B and DNA repair enzymes, which may make cells more sensitive to DNA damaging agents like doxorubicin. A phase I study of the combination of the proteasome inhibitor bortezomib (velcade®; formerly PS-341) with pegylated, liposomal doxorubicin (pegLD; Doxil®) found this regimen to be well tolerated. In addition, it induced a complete response (CR) + near-CR rate of 36%, and an overall response rate (CR + near-CR + partial responses (PR)) of 73% in a population of patients with advanced, relapsed and/or refractory multiple myeloma. This prompted us to evaluate this combination in previously untreated adult patients with symptomatic multiple myeloma requiring therapy. Patients enrolled onto Cancer and Leukemia Group B (CALGB) study 10301 are receiving bortezomib at 1.3 mg/m^2 on days 1, 4, 8, and 11 of every 21-day cycle, while pegLD is being given at 30 mg/m^2 on day 4. Our primary objectives are to determine the CR + near-CR rate of bortezomib/pegLD, and also to define the toxicity of this regimen in the up-front setting. Secondary objectives include determining the overall response rate, the ability to collect stem cells for autologous transplantation after therapy, the time to progression, and the effect of bortezomib/pegLD on interleukin-6 and macrophage inflammatory protein-1 alpha levels. Current results of this study, which will complete accrual by April, 2005, will be presented, including data about both the primary and secondary objectives.

PO.731

VELCADE, DOXIL AND LOW-DOSE THALIDOMIDE AS SALVAGE REGIMEN FOR PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA AND WALDENSTROM MACROGLOBULINEMIA: PRELIMINARY RESULTS OF A PHASE II STUDY

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Introduction. Tumor microenvironment (ME) plays an important role in myelomagenesis as well as development of resistance to anti-myeloma therapy. Therefore, targeting the ME concurrently along with the malignant myeloma cells may be an effective way to overcome resistance in pts with rel/ref MM. Velcade (V) is a novel agent that targets myeloma ME by down regulation of IL-6 through inhibitory effect on NFκB, while thalidomide (T), an immunomodulatory agent, exerts its anti-myeloma effect through perturbation of the myeloma ME via down regulation of IL-6, VEGF and TNF-α. Even though these agents have shown impressive clinical activity in multiple myeloma (MM) all pts eventually relapse and become refractory to further therapy. We hypothesize that combining VT (to target the ME) with doxil (D) (to target myeloma cell) may help overcome resistance and enhance clinical efficacy of these agents in pts with relapsed or refractory MM. Here we present the encouraging preliminary results of a phase II trial initiated at our institute, exploring the VDT combination as salvage

therapy for pts with relapsed or refractory MM.

Patients and Methods. Pts with relapsed or refractory disease are eligible for this study. V is given at 1.3 mg/m^2 (D1,4,15,18) and D at 20 mg/m^2 (D1,15) every 4 weeks with daily T (200 mg) for 4-6 cycles. SWOG criterion was used for response evaluation. Low-dose coumadin (1-2 mg) was used for prevention of venous thromboembolism (VTE). 18 pts (7M, 11F; median age 56, range 44-80 yrs; 16MM, 2 WM) are enrolled to date. All MM pts had stage III disease with median β2M of 4.4 (1.7-81.1) and median of 2 prior therapies (range 1-7). Prior therapies included stem cell transplant in (56%), T (50%), adriamycin (A) (55%) and steroid (94%). 11/16 pts (69%) had refractory disease.

Results. Eleven pts are evaluable for response; 8 (72%) showed a clinical response (1 CR, 4PR, 1MR, 2SD) while 3 pts have progressive disease, 1 after 5 and 2 after 2 cycles. All pts were evaluated for toxicity. Two pts developed Gr. I plantar-palmar erythrodysthesia (PPE) and 1 pt had Gr. III cellulitis. Eight episodes of Gr. III neutropenia were noted. No VTE was noted.

Conclusion. VDT is a highly active salvage regimen in pts with relapsed or refractory MM. Responses were noted despite prior failure of steroids, T or A. It is well tolerated without any significant Gr. III/IV toxicity. VTE does not appear to be a problem. Updated results of this phase II study will be presented.

PO.732

COMBINATION THERAPY OF PS-341 (BORTEZOMIB), ADRIAMYCIN AND DEXAMETHASONE (PAD) FOR UNTREATED PATIENTS WITH MULTIPLE MYELOMA

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Introduction. Bortezomib has already been shown to be an effective single agent drug for relapsed myeloma. In addition there is data to suggest synergistic action with other chemotherapeutic drugs and dexamethasone. We have therefore investigated the role of PS-341, adriamycin and dexamethasone (PAD) as front line therapy for patients with multiple myeloma.

Aims. This was a Phase I/II study whose primary objectives were to assess the feasibility of peripheral blood stem cell (PBSC) harvest following PAD, and secondarily to assess safety, tolerability and efficacy.

Methods. Twenty-one patients with previously untreated multiple myeloma were entered into the first cohort where they received 4x21 day cycles of PAD (bortezomib 1.3 mg/m^2 days 1,4,8,11 and dexamethasone 40 mg days1-4, 8-11,15-18 for cycle 1 and days 1-4 for all subsequent cycles. During days 1-4 of each cycle, patients also received 0 mg/m^2, 4.5 mg/m^2 or 9 mg/m^2 of Adriamycin at levels 1, 2 & 3 respectively in the Phase I component). This was followed by stem cell harvest and high dose melphalan (HDM) with PBSC reinfusion. We have currently enrolled a further 11 patients into a second cohort who have received bortezomib at 1.0 mg/m^2.

Results. Of the 21 patients in the first cohort, all completed between 2-4 cycles of PAD with 95% achieving at least PR. 18 received high dose melphalan with median neutrophil (>0.5x10^9) and platelet engraftment (>20x10^9) of 15 (1-24) and 13 (10-33) days respectively. The overall outcome for this cohort was 43% CR, 14% nCR, 24% VGPR (ie 81% at least VGPR) either at 3 months following HDM or following PAD. 20 had a successful PBSC harvest (median collection 3.8x10^6 CD34+ cells/kg (1.6-10.4)). Nine of the 11 patients in the second cohort have completed at least 2 cycles of PAD. All have achieved at least a PR so far (1 CR, 1nCR, 4 VGPR, 3 PR) and 4 have completed HDM with mean neutrophil and platelet engraftments of 16 (13-20) and 14 (11-20) respectively. Toxicities in general have been acceptable in particular in cohort 2. 15 grade 3 events were seen in cohort 1 (6 infections, 4 shingles, 2 nausea & vomiting, 2 neuropathy and 1 postural hypotension), whilst thus far only 1 (abnormal liver function tests) seen in cohort 2 (however there was an isolated case of grade 4 neutropaenia which responded to G-CSF). Also of note the neuropathy profile was better in cohort 2 (only 1 grade 1 sensory neuropathy).

Summary: This preliminary data suggests that PAD chemotherapy is not only well tolerated, but highly efficacious in the front line therapy of multiple myeloma. PBSC collection is not affected. The early results for cohort 2 imply that the toxicity profile is improved with the lower dose of bortezomib, although sufficient response data is not yet available to make conclusions. We recommend further evaluation of PAD as front line therapy in the form of randomised controlled trials.

PO.733

EFFICACY OF BORTEZOMIB (VELCADE) ON PLASMA CELL LEUKEMIAS: CLINICAL RESULTS IN THREE PATIENTS

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Plasma Cell Leukemia (PCL) represents the most aggressive form of monoclonal gammopathy. It is characterized by a high number of plasma cells in peripheral blood (>2 x 10^9/L) and more than 20% of circulating plamocytes on differential count. The prognostic of these patients is very adverse with a median survival below 6 months. For this reason new treatment approaches are needed. We report three clinical cases of secondary PCL that showed favourable response to proteasome inhibitor, Bortezomib.

Cases Reports. First Case. A female, 56 years old, was diagnosed of non secretory stage III/A MM in 1993. She received treatment with VAD followed by autologous transplant conditioned with high dose busulphan+melphalan achieving a complete response (CR). In 1998 presented a relapse with knee plasmacytoma requiring local radiotherapy and CyVAD achieving a new CR. In February 2000 presented a new relapse treated with VBCM. However in May 2001 progression with bone marrow plamacytosis was observed. The patient was treated with oral combination of Thalidomide+Cyclophosphamide+Dexamethasone(TacyDex) obtaining a partial stable response. In December 2003 she developed progresive pancytopenia with Hb: 5.7 g/dL; Plataelets: 20x10^9/L; Leucocytes: 7.8x10^9/L with 60% of circulating plasma cells compatible with PCL. Bortezomib (Velcade) was administered at standard dose 1.3 mg/m^2 iv days 1,4,8,11 evey 21 days. After first cycle circulating plasmacytic cells disappeared and blood counts normalized. The patient have received 8 cycles and continues in CR 11 months after PCL diagnosis. *Second Case:* A female, 57 years old, was diagnosed of MM IgGIII in 1996. The treatment fol-

lowed before developing PCL was: VAD, double autologous transplant and Thalidomide + Cyclophosphamide + Dexamethasone (TacyDex) for relapse. In 2004 the patient progressed with PCL criteria being treated with Bortezomib at standard dose After the first cycle circulating plasmacytic cells disappeared in peripheral blood. *Third Case:* A male 70, years old was diagnosed of MM in June 2004. The patient was refractory to MP and complicated with renal failure being treated with VA. With this scheme no response was observed and the patient developed PCL being treated with Bortezomib. After the first cycle the circualting plasmacytic cells also disappeared.

In vitro studies. Bortezomib reduced PCL numbers in ex vivo evaluation and was more efficient in cell growth inhibition than dexamethasone or doxorubicin. This agent induced pro-Caspase-3 and PARP cleavage, and decreased the amount of Erk1/2 and pErk1/2. However, Bortezomib did not substantially affect the levels of pMEK, p27 and p21 in PCL cells.

Conclusions and Comments. Bortezomib has showed antimyeloma effect by several mechanisms in patients resistant to multiple previous treatments. The favourable response observed in the cases reported here of secondary PCL and the biological ex vivo results on PCL cell lines confirm the potential efficacy of this agent. Although more experienced is necessary these data support the inclusion of Bortezomib alone or associated to other drugs in the treatment of PCL.

PO.734

BORTEZOMIB IS EFFECTIVE IN PLASMA CELL LEUKEMIA

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Plasma cell leukaemia is seen as the end stage or most aggressive form of myeloma with poor outcome from both conventional therapy and autologous or allogeneic transplantation. It is clear that more effective therapy is required. Proteosome inhibition has been shown to be effective in myeloma and it is therefore reasonable to assume that it might also have a role to play in plasma cell leukaemia. We describe four patients with plasma cell leukaemia (2 relapse, 1 refractory, 1 de novo disease) treated with Bortezomib alone or in combination with Dexamethasone +/- Adriamycin. All four patients had some level of response with 2 CRs, one in a previously autografted patient who had relapsed and one in a de novo patient who went on to receive autografting. The third patient who had relapsed after conventional therapy with a large soft tissue mass shown on biopsy to be plasmablastic infiltration, was treated with Bortezomib with little response until Dexamethasone was added where upon she developed acute tumour lysis syndrome with dramatic resolution of the mass. The fourth patient was refractory to conventional VAD therapy with progression during his second course; he achieved stable disease for 1 cycle of Bortezomib plus Dexamethasone plus a further cycle of Bortezomib plus Dexamethasone and Adriamycin before dying of presumed pulmonary embolism. These cases suggest that there is a role for Bortezomib in combination with other agents in the management of plasma cell leukaemia.

PO.735

MULTIPLE PLASMACYTOMAS SUCCESSFULLY TREATED BY VELCADE

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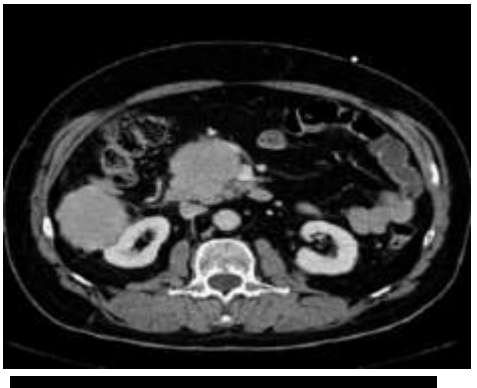
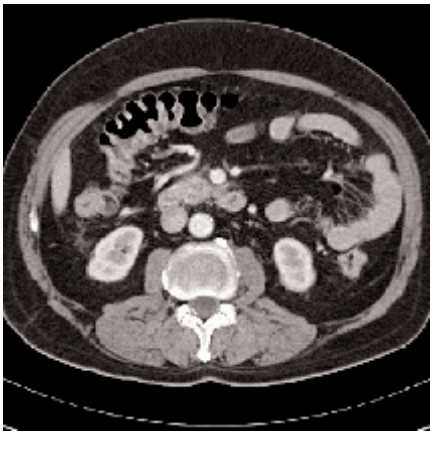
The proteasome inhibitor bortezomib (Velcade) is a novel antineoplastic drug that produces cell-cycle arrest and apoptosis of myeloma cells *in vitro* and *in vivo*. Experimental evidence has demonstrated additive activity when bortezomib is combined with dexamethasone. Velcade is effective to clear bone marrow from malignant plasmacytosis but nothing is known concerning plasmacytomas. A 65 year old man was diagnosed with Multiple Myeloma (MM). He underwent 2 autologous transplantation, relapsed and had a non-myeloablative allogeneic transplantation from his genoidentical brother. He relapsed again a year later with multiple plasmocytomas, one large paracardiac and two subdiaphragmatic masses. He had chemotherapy (DCEP), 8 DLI (last injection 6 months before initiation of Velcade), and Thalidomide successively with no improvements. We started Velcade therapy standard dose, eg 1.3 mg/m^2 day 1,4,8,11 followed by a 10-day rest period in combination with dexamethasone. He received 4 cycles. The main toxicity was postural hypotension. After 4 cycles of Velcade, plasmacytomas have completely disappeared as shown in this abdominal CT scan.





After non-myeloablative allogeneic transplantation, MM patients very often relapsed with multiple plasmocytomas. Studies have shown that Velcade inhibits GVHD while preserving GVT. We demonstrate in one patient that in this setting, Velcade can successfully treat plasmocytomas.

P0.736

DOXIL, VINCRISTINE, REDUCED FREQUENCY DEXAMETHASONE AND REVLIMID A PHASE I/II TRIAL IN ADVANCED RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS

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Doxil, vincristine, and reduced frequency dexamethasone (DVd) in combination with Thalidomide (T) and the appropriate supportive care measures resulted in a high response rate (88%) as well as an improved quality response (50% CR & NCR) similar to what is achieved with high dose therapy. Immunomodulatory drugs (IMiDs) are potent T derivatives. R is 50 to 2000 times more potent than T in stimulating T-cell proliferation triggered via the T-cell receptor, and 50 to 100 times more potent than T in augmenting IL-2 and IFN-α production. A recent phase I trial showed responses of at least 25% reduction in paraprotein in 17 (71%) of 24. We therefore initiated a phase I/II trial to define MTD of revlimid (R) in combination with DVd, then we proceeded to expand the aMTD dose level to evaluate the efficacy and safety of the combination in Pts with relapsed/refractory multiple myeloma (Rmm). SWOG criteria were used to assess response, and NCR was defined as a decrease of the M-Protein by >90%. Refractory Pts were defined as those Pts progressing on active therapy. R was started a week prior to DVd in cycle 1 to evaluate different coagulation parameters, following cycle 1, R was started on day 1 of therapy. The regimen was given as follows: on day 1 D was given at 40 mg/m^2 IVPB; V at 2 mg IVP; d at 40 mg PO daily X 4 days; R was started at 5 mg a day for 21 days with one week off. A standard phase 1 dose escalation of R was performed to identify the MTD. DVd was repeated q 4W, for a minimum of 4 cycles & 2 cycles after best response. Pts were maintained on R +/- prednisone 50 mg QOD. All Pts received amoxicillin, acyclovir and aspirin 81mg prophylactically. 25 pts Rmm pts are enrolled with 21 evaluable for toxicity and mature data available for response on 21 pts (refractory: 16 (76%); relapsed: 5 (24%). 19/21 Pts were stage 3, median age of 62±9 years, baseline β_2 microglobulin level (mean 5.04±2) and serum albumin (mean 3.4±0.7). 10/21 Pts failed T containing regimens. The DLT was sepsis/septic shock that occurred at dose level 3 (R 15 mg) with two of the Pts developing non neutropenic sepsis. The MTD for R was defined at 10mg. Three Pts started therapy with a neutrophil count < 500/μL and or platelet counts < 50 k/μL; all 3 Pts were responders. 8 Pts started therapy with a serum creatinine > 1.4. There was one grade 4 hyper-coagulation event in the form of a PE that has recovered. This event occurred in a refractory patient with renal failure performance status of 3 who achieved CR after 2 cycles. In the expanded cohort there was 2 grade 3 neutropenia & one grade 3 neuropathy requiring dose reductions of R and D in each pt with resolution of the neutropenia and neuropathy. 3/21 (14%) Pts achieved CR, 4/21 (19%) NCR. All CR+NCR Pts (33%) are refractory Pts. An additional 7 pts achieved PR, and 6 SD 5 of whom showing greater than 25% decrease in the m-protein. All Pts except for 4 achieved > 25% reduction of the m-protein after one cycle of therapy and 3/4 after 2 cycles. R at 10mg is the MTD in combination with the DVd in RMM. DVd-R is an extremely effective regimen with a SWOG response rate >66%, CR+NCR of 33% in refractory stage 3 Pts with minimal toxicity.

P0.737

A MULTICENTER, SINGLE-ARM, OPEN-LABEL STUDY TO EVALUATE THE SAFETY AND EFFICACY OF SINGLE-AGENT LENALIDOMIDE IN SUBJECTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Introduction. Lenalidomide is a novel, oral immunomodulatory drug (IMiD) in development for treatment of a wide variety of malignant diseases, including multiple myeloma. In preclinical studies lenalidomide has been shown to decrease binding of multiple myeloma (MM) cells to bone marrow stromal cells, inhibit the production of cytokines mediating growth and survival of MM cells, block angiogenesis, and stimulate host anti-MM natural killer (NK) cell immunity. Phase I studies identified reversible myelosuppression (≥ grade 3 neutropenia and thrombocytopenia) to be the dose-limiting toxicity and the maximum-tolerated dose was 25 mg/day. No significant somnolence, constipation or neuropathy was observed in the first Phase I study. Twenty percent of patients receiving daily doses of 25 mg to 50 mg of lenalidomide achieved a ≥50% reduction in paraprotein levels. In the initial phase II study, 70 patients with relapsed or relapsed and refractory MM were randomized to receive either 15 mg BID or 30 mg QD of lenalidomide. Both treatment arms showed significant anti-myeloma activity with manageable toxicity. An increased incidence of cytopenia was noted in the 15 mg BID group and an additional 30 patients were enrolled into the 30-mg arm, with significant activity and manageable toxicity confirmed upon analysis of the whole cohort (n=102).

Methods. The present Phase II study was designed to further evaluate the effectiveness and safety of single agent lenalidomide administered at a dose of 30 mg QD for 21 days every 28 days (28-day cycle) in patients with relapsed and refractory MM. Patients had to have relapsed after achieving at least stable disease with at least 1 cycle of prior therapy and developed disease progression during salvage therapy. To be eligible for the study patients were required to have adequate renal and bone marrow function. Eligible patients may have been previously treated with thalidomide or bortezomib. The primary endpoint of the study was anti-myeloma response and the secondary endpoints of the study were TTP, duration of response, survival, time to first skeletal-related event (SRE) and safety.

Results. Two a hundred and twenty-two patients were enrolled into the study. All patients had received at least 2 prior anti-myeloma treatments, including bortezomib, thalidomide, and SCT. Thus far at least a 25% reduction in paraprotein level has been achieved in 63 patients (28%). Lenalidomide has been tolerated with acceptable toxicity. Death resulted in study discontinuation in only 2 (1%) patients, and 21 (10%) patients have discontinued the study due to adverse events. The most commonly reported grade 3 or 4 adverse events have been neutropenia (38%) and thrombocytopenia (22%); 4 cases of deep vein thrombosis have been reported (2%).

Conclusion. Accrual to this large multi-center trial of patients with advanced multiple myeloma and poor prognosis is complete and preliminary results are encouraging. Data concerning response, time to tumor progression, duration of response, overall survival and safety will be presented.

P0.738

A MULTICENTER, RANDOMIZED, PARALLEL-GROUP, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF LENALIDOMIDE PLUS DEXAMETHASONE VERSUS DEXAMETHASONE ALONE IN PREVIOUSLY TREATED SUBJECTS WITH MULTIPLE MYELOMA

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Lenalidomide (CC-5013) is a novel oral immunomodulatory drug (IMiD) that has demonstrated anti-myeloma activity, possibly including the following: modulation of the adhesion of myeloma cells to bone marrow stromal cells, cytokine inhibition, anti-angiogenesis, immune modulatory effects and direct effects on myeloma cells and/or bone marrow stromal cells. In preclinical studies, lenalidomide was found to be a more potent inhibitor of inflammatory cytokines and angiogenesis than the parent IMiD, thalidomide, by 50 to 2000 fold. Phase I studies identified reversible ≥ grade 3 neutropenia and thrombocytopenia to be the dose-limiting toxicity and the maximum tolerated dose was 25 mg/day. No significant somnolence, constipation, or neuropathy was observed. Seventeen (71%) of 24 evaluable patients achieved ≥25% reduction of myeloma paraprotein levels. Phase II data confirmed the anti-myeloma activity of lenalidomide and showed that an interrupted daily schedule of administration ameliorated the marrow suppressive affect of lenalidomide. In the current, randomized, double-blind Phase III study study, a phase III design investigated the effectiveness and safety of intermittent dosing of lenalidomide 25mg combined with high-dose dexamethasone (HDD) is compared with placebo and HDD in patients with relapsed or refractory multiple myeloma (MM). Enrollment has been completed (N=354). Lenalidomide and HDD are given in 28-day cycles (Lenalidomide 25 mg once daily on Days 1-21 every 28 days and HDD 40 mg on Days 1-4, 9-12, 17-20 every 28 days. After 4 cycles the HDD schedule is reduced to 40 mg on Days 1-4 every 28 days). The primary endpoint of the study is time to tumor progression (TPP) and the secondary endpoints are response and overall survival. Data concerning response, time to progression, overall survival and safety (an independent committee monitored the safety and efficacy) will be presented. Lenalidomide alone and in combination with HDD shows promising clinical activity in patients with refractory or relapsed MM with an acceptable toxicity profile and offers the convenience of daily oral dosing.

P0.739

ARSENIC TRIOXIDE (TRISENOX®), ASCORBIC ACID AND DEXAMETHASONE AS TREATMENT IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA: PRELIMINARY FINDINGS OF A MULTICENTER, PHASE II STUDY

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Background. Trisenox® (arsenic trioxide-ATO) is highly effective in the treatment of relapsed and refractory acute promyelocytic leukemia. *In vitro* data show that arsenic trioxide induces apoptosis in myeloma cell lines via caspase-9 activation, enhances the myeloma cell apoptosis induced by dexamethasone, and can overcome the anti-apoptotic effects of interleukine-6. Further, arsenic trioxide can induce apoptosis via generation of reactive oxygen species that damage mitochondria. Depleting cellular levels of reduced glutathione (GSH) by ascorbic acid (AA) can enhance the sensibility to arsenic trioxide. A number of clinical trials are investigating the effect of arsenic trioxide in multiple myeloma patients.

Methods. This ongoing study is being performed to evaluate the safety and efficacy of the combination ATO, AA and dexamethasone in myeloma patients with active, progressive disease who have failed at least one line of treatment. If tolerated, the patients receive a minimum of four treatment cycles of 28 days. In the first cycle, during first week: a loading-dose with ATO at 0,25 mg/kg IV over 1-4 hours day 1-5, AA 1000 mg IV over 15 minutes within 30 minutes after each ATO infusion and dexamethasone 40mg orally day 1-5. During the second en third week: a maintenance-dose of twice weekly ATO at 0,25 mg/kg IV, AA 1000 mg IV within 30 minutes after each ATO infusion and dexamethasone 20 mg orally. The fourth week is a rest period. In the second, third and fourth cycle: a maintenance-dose of twice weekly ATO at 0,25 mg/kg IV, AA 1000 mg IV within 30 minutes after each ATO infusion and dexamethasone 20 mg orally. The fourth week is a rest period. Patients with a clinical response after fourth cycle will receive two additional treatment cycles.

Preliminary results. Eleven patients, age 42 to 71 years, with extensive prior chemotherapy have been enrolled in the study. Response evaluation after the fourth cycle was performed in two patients, one had progressive disease and one stable disease. The combination ATO, dexamethasone and ascorbic acid was overall well tolerated. Two SAE were reported, one patient died of a pneumonia during the first cycle and one patient had a grade IV hematological toxicity and was admitted to the hospital because of epistaxis. So far, no prolongation of the QT-interval was reported. An update of the toxicity and response will be presented.

P0.740

PHASE I/II TRIAL OF ARSENIC TRIOXIDE PLUS ASCORBIC ACID FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA (NCI 43/SCCC 200010): INTERIM ANALYSIS OF CLINICAL AND LABORATORY CORRELATES

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We have previously reported that arsenic trioxide (ATO) with concurrent ascorbic acid (AA)-mediated depletion of intracellular glutathione (GSH) was effective *in vitro* against chemotherapy-resistant multiple myeloma (MM), and in the phase I component of this trial could be administered at 0.25 mg/kg/d ATO + 1000 mg/d AA (one cycle consisting of 25 days over a 35 day period) with acceptable toxicity. We now report an interim analysis of 16 patients treated at this dose level. The mean number of previous chemotherapy regimens was 4, with 80% of the patients having relapsed on a thalidomide containing regimen. Toxicity of ATO + AA was generally well tolerated, with rash, nausea (mild-moderate),

and neutropenia (readily managed with intermittent G-CSF) being the most common. 4 patients developed shingles on treatment, which was managed without complications with acyclovir. Twice-weekly EKG monitoring for acute and chronic cardiotoxicity demonstrated a statistically significant increase in the QTc interval over baseline only for cycle 1 (30.1±32.2 msec, $p<0.01$), with no significant increase in cycles 2 through 6. No treatment was held for QTc prolongation, and no arrhythmias were seen. The pharmacokinetic studies of As_20_3 (1 hr post-infusion) revealed no significant variability in arsenic levels over multiple cycles. Pharmacokinetic study of post 1 hr infusion serum AA levels demonstrated no accumulation of AA over the course of a single cycle, nor significant variability over multiple cycles. Depletion of intracellular GSH was seen in most patients, and correlated with treatment response. Eleven of the 16 patients were evaluable for response. Two patients had a greater than 50% reduction in paraprotein and 5 had between a 25%-50% reduction, yielding a response rate of 63.6% (90% CI: 35.0%-86.5%.). The remaining 4 patients had stable disease (0-25% decrease in M protein). Only 3 patients had evidence of disease relapse after achieving their best response, while the remaining patients maintained a plateau in their response. Consistent with this, resistance of patient myeloma cells to ATO + AA over multiple cycles of treatment was not detected in serial *in vitro* studies even when the clinical response plateaued. This suggests that intrinsic resistance to ATO + AA does not develop in the MM cells, but changes in the host allows for some myeloma cells to survival in the face of continuing treatment. In conclusion, we have found that that ATO + AA has acceptable toxicity and evidence of activity in refractory/relapsed myeloma. Importantly, the development of resistance to ATO + AA was uncommon and appears to involve adaptation by the host microenvironment, suggesting that addition of agents that target the microenvironment may further enhance the effectiveness of this combination.

P0.741

ARSENIC TRIOXIDE, ASCORBIC ACID AND DEXAMETHASONE PULSES FOR RELAPSED REFRACTORY PROGRESSIVE MULTIPLE MYELOMA PATIENTS: LONG-TERM FOLLOW UP

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Although several treatment options are available for multiple myeloma patients, no cure exists and 5-year survival rates are about 28%. An important therapeutic goal is to manage myeloma as a chronic disease without compromising quality of life or survival following induction of a clinical response. Agents used in induction and consolidation therapy, as well as subsequent long-term maintenance therapy, must not only be active in potentially resistant tumor cells, but also have an acceptable toxicity profile allowing prolonged exposure. Arsenic trioxide (ATO) is a novel anticancer agent with a unique, multifaceted mechanism of action and has been shown to be active in several hematologic malignancies, including APL, MDS, and MM. Because *in vitro* studies have shown that ATO sensitizes myeloma cells to dexamethasone (Dex), and ascorbic acid (AA) potentiates the effect of ATO, a phase II trial combining ATO with Dex and AA was initiated to investigate long-term responses and tolerance to therapy. This study included 20 evaluable patients, with a mean duration of therapy of 1.9 years. The TAD regimen includes one induction cycle (week 1: ATO at 0.25 mg/kg IV d1-5; Dex 40 mg orally d 1-4; AA 1000mg IV within 30 minutes after each ATO infusion; weeks 2-12: ATO at 0.25 mg/kg IV twice weekly, AA 1000 mg IV within 30 minutes after each ATO infusion, Dex 40 mg orally days 11-14, 29-32, 39-42, 57-60, & 67-70; weeks 13-15: rest), followed by two consolidation cycles in which the Dex frequency is reduced to once a month. Patients achieving SD or better were initiated on maintenance with the regimen administered for five weeks with a 2-month break, and steroids were given once a month. Following the induction cycle, 8 patients achieved >50% reduction of the M-protein, 1 patient achieved CR, 1 NCR, 4 PR, 10 SD, and 4 PD. Only one patient showed further improvement in the M-protein after the first cycle of therapy. Six, 7, 2 and 5 patients received up to three TAD cycles followed by maintenance for a median duration of 58, 151, 351 and 511 days respectively as of day 1 on study. Median survival for the 14 patients alive is 18.3 months (2.5-24.2). The regimen was generally well tolerated with one patient each experiencing Grade 3 hyperglycemia, headaches, burning at IV site, neutropenia, dehydration, syncope, or fatigue. One patient experienced grade 4 painful neuropathy and was taken off study with the event resolving in 4 weeks without any specific therapy. The long-term duration of the responses as well as the ability of most of the patients to tolerate this long-term therapy regimen (1.9 years) is encouraging and further exploration of this strategy to confirm these data is warranted.

P0.742

ARSENIC TRIOXIDE IN REFRACTORY OR RELAPSED MULTIPLE MYELOMA: RATIONALE AND CLINICAL EFFICACY USING THE MAC REGIMEN

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Rationale. Resistance to cytotoxic agents such as melphalan, thalidomide, and the proteome inhibitor bortezomib, is a common occurrence in multiple myeloma (MM) patients. Tumor cells develop resistance by increasing the production of survival factors and becoming insensitive to apoptopic signals. Therefore, effective treatment of refractory or relapsed MM patients requires agents that can restore apoptopic signaling in chemoresistant cells. In MM cells, constitutive activity of transcription factor NF-κB correlates with resistance to chemotherapy[1]. Arsenic trioxide (ATO) inhibits NF-κB activity through inhibition of the NF-κB inhibitor κB[2]. ATO is most active in cells with low levels of the antioxidant glutathione (GSH). Ascorbic acid (AA) decreases GSH levels in MM cells *in vitro* and potentiates ATO-mediated cell death3. A phase I/II trial showed that AA modulates intracellular GSH levels and increases the efficacy of ATO in relapsed/refractory MM patients4. The combination of melphalan, ATO, and AA (MAC regimen) is currently being tested in a multicenter phase II study.

Methods. Relapsed or refractory MM patients are enrolled. Patients received a loading dose of ATO at 0.25 mg/kg IV followed by AA of 1 g IV in days 1-4 of week 1 of each six-week cycle. ATO/AA was given twice-weekly for the next four weeks of each cycle. Low-dose melphalan (0.10 mg/kg) was

administered orally for the first four days of each cycle. Patients received a maximum of six cycles followed by weekly maintenance treatment with weekly ATO followed by AA.

Results: Nine of 17 evaluable patients (53%) had an objective response (3PR, 6MR), an additional four patients achieved stable disease, resulting in a total of 13 patients (77%) with disease control. Since responses were seen after two to five treatment cycles, it is possible that some patients with stable disease may experience additional disease response. All three patients who had failed bortezomib treatment responded to the MAC regimen (2 PR, 1 MR). Of the 13 evaluable patients who enrolled following relapse after thalidomide, six responded to the MAC regimen (2 PR, 4 MR) and an additional four patients had stable disease. Six patients who had relapsed after high dose melphalan in combination with a peripheral stem cell transplant achieved disease control (2 PR, 2 MR, 2 SD).

Conclusion: The preliminary results from this multicenter trial show that the combination of low-dose oral melphalan, IV ATO and RV AA is active in heavily pre-treated MM patients, and has a favorable safety profile. Importantly, this combination is active in some MM patients who are refractory to melphalan, thalidomide, and/or bortezomib. The MAC regimen is generally well tolerated with few significant side effects reported; mild cytopenias were reported as reversible.

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PO.743

ARSENIC TRIOXIDE SHOWS SYNERGISTIC ANTI-MYELOMA EFFECTS *IN VIVO* WHEN COMBINED WITH BORTEZOMIB OR MELPHALAN WHICH IS FURTHER ENHANCED BY THE ADDITION OF ASCORBIC ACID

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Introduction. Melphalan (MEL), bortezomib (BO) and arsenic trioxide (ATO) have been shown to have anti-myeloma effects in clinical trials. In addition, *in vitro* studies show synergistic anti-myeloma effects when ATO is combined with either BO or MEL. Increased glutathione levels are associated with the development of resistance to a variety of anti-myeloma therapies including ATO and MEL. Ascorbic acid (AA) depletes intracellular glutathione levels and AA increases the sensitivity of myeloma cells to ATO and MEL as shown in our previous *in vitro* studies. To better evaluate the potential anti-myeloma effects of ATO in combination with AA, MEL, and BO in vivo, we used our previously established murine SCID-hu xenograft LAGλ-1 model. Each SCID mouse was implanted with a LAGλ-1 tumor fragment (0.5 cm^3) into the left hind limb muscle, and treatment was started 3-4 weeks after implantation.

Single agent therapy. Therapy with MEL intraperitoneally (IP) x1/week at 12.0 mg/kg, 6.0 mg/kg, 0.6 mg/kg, and 0.06 mg/kg showed no anti-myeloma effects in LAGI-1-bearing mice. Mice were also treated with ATO IP alone at 6.0 mg/kg, 1.25 mg/kg, 0.25 mg/kg, and 0.05 mg/kg daily x5/week. Mice receiving the highest dose of ATO showed marked inhibition of tumor growth and reduction of paraprotein levels, whereas no effect was observed in mice receiving lower doses. Finally, a dosing study was conducted with BO IV x2/week for 4 weeks at 0.5 mg/kg and 0.05 mg/kg. Mice receiving 0.5 mg/kg showed tumor growth inhibition and reduced serum paraprotein levels whereas the low dose (0.05 mg/kg) showed no anti-myeloma effects.

Combination therapy. LAGλ-1 mice were treated with ATO (1.25 mg/kg) IP, BO (0.25 mg/kg) IV, or the combination of both drugs, in the schedules outlined above. ATO or BO treatment alone had no anti-myeloma effect at these low doses whereas there was a marked decrease in both tumor volume and paraprotein levels when mice received both. LAGλ-1 mice were also treated with ATO (1.25 mg/kg) IP, MEL (0.6 mg/kg) IP, or the combination of both drugs. ATO or MEL as single agents had no anti-myeloma effect at these doses whereas the combination of ATO and MEL markedly suppressed the growth of the tumor and significantly reduced serum paraprotein levels. We also investigated the combined effects of AA, ATO, and MEL in these mice. LAGλ-1-bearing mice received treatment with either AA (300 mg/kg) daily x5/week, ATO (1.25 mg/kg) daily x5/week, MEL (3.0 mg/kg) x1/week, or the combination of these agents. AA, ATO, and MEL alone had no anti-myeloma effects at these doses, whereas AA+MEL results in significantly decreased tumor burden and paraprotein levels. Notably, the most profound anti-myeloma effects were observed in animals treated with all 3 drugs together.

Conclusion. These data show the potential advantage of combining ATO with either MEL and AA or BO for patients with relapsed/refractory myeloma. Based on these observations, clinical trials using these combinations have started in these patient populations.

PO.744

ARSENIC TRIOXIDE IN MULTIPLE MYELOMA: A SUPPORTIVE ROLE FOR ASCORBIC ACID

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Introduction: Preclinical studies have shown that arsenic trioxide (ATO) induces apoptosis in malignant cells from several hematological diseases, including multiple myeloma (MM). Several pathways have been identified through which ATO is active, including: 1) specific inhibition of pro-apoptotic pathways, and 2) mitochondrial membrane depolarization and activation of downstream apoptotic pathways through the generation of reactive oxygen species (ROS). The cellular redox status is maintained by ROS scavenger enzymes such as reduced glutathione (GSH) and catalase; an imbalance between these intracellular anti-oxidant defense systems and ROS results in oxidative stress and apoptosis. Several *in vitro* studies have established that ATO-induced apoptosis is modulated by the GSH-dependent redox system: malignant cell lines that are very sensitive to ATO have consistently low levels of GSH, while arsenic resistance is associated with increased GSH levels. Agents that down-regulate intracellular levels of GSH, such as L-buthionine-(S,R)-sulfoximine (BSO) and ascorbic acid (AA), potentiate the apoptotic effect of ATO *in vitro*. Several phase I/II studies have shown that ATO has efficacy in MM as a single agent. Based on the described preclinical data, a phase I/II trial was initiated using AA to modulate intracellular GSH levels and increase the efficacy of ATO in MM patients. The phase I component of this trial showed that AA does not

alter the pharmacokinetics of ATO, and that elevated AA blood concentrations correlate with decreased intracellular GSH levels in peripheral blood cells. Interim analysis of the subsequent phase II shows an impressive improvement in response rate (RR) compared to ATO as a single agent (see table 1): RR was 64% (N=11), with the remaining patients having SD, as will be reported at this meeting. Similarly, the addition of AA to a combination regimen including ATO and dexamethasone (Dex) also dramatically improved the RR compared to ATO and Dex alone. Building on these observations, the combination of ATO + AA was subsequently shown to enhance the efficacy of melphalan, even in melphalan refractory patients, and these data became the basis for a multicenter Dex-free phase II trial, the preliminary results of which will be reported at this meeting. The combined data from these studies demonstrate that ATO+ AA is an active combination in patients with MM, and should be further explored in combination with other agents.

Table 1. Response Rate of ATO single agent and ATO combination treatment in MM

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

P0.745

PHASE I STUDY OF SIMVASTATIN COMBINED WITH CHEMOTHERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY MYELOMA AND LYMPHOMA

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We recently described that simvastatin effectively induces apoptosis in myeloma and lymphoma tumor cells by inhibition of proteingeranylgeranylation resulting in the reduction of the anti-apoptosis protein Mcl-1. In addition, low concentrations of simvastatin had a chemosensitizing effect in combination with dexamethasone or doxorubicin. Based on these observations we initiated a Phase I study of dose escalating simvastatin combined with standard chemotherapy in patients with end-stage Myeloma and Lymphoma. Starting dose level of simvastatin was 5 mg/kg/day for 7 days followed by VAD chemotherapy in patients with myeloma and CHOP in patients with lymphoma. Three patients were included per dose level. In the absence of non-haematological side effects WHO grade III/IV in 3 patients the dose of simvastatin was escalated with 2.5 mg/kg. Twenty-one -heavily pre-treated- patients, refractory to at least 2 lines of chemotherapy (13 myeloma patients and 8 lymphoma patients) were included. No non-haematological toxicity beyond WHO 2 was recorded at dose level 1-4 (5 mg-12.5 mg/ kg/day/7 days), but neutropenic fever occurred in four patients, necessitating hospitalisation in 3 patients. One patient treated at dose level 5 (15 mg simva/kg/day/7 days) became severely depressed and performed an unsuccessful suicide attempt. Two patients treated at dose level 6 (17.5 mg/kg/day/7 days had severe gastro-intestinal side effects (WHO 3; vomiting, diarrhoea, dehydration), necessitating interrupting simvastatin after 3 and 4 days respectively. The third patient had moderate gastro-intestinal complaints but died on day 13 (2 days after VAD, deeply neutropenic) from overwhelming septicaemia, although prophylactic antibiotics were given. Three additional patients were then treated at dose level 5 again. One of these three also developed neutropenic fever and gastro-intestinal side effects. The other two had no complaints. None of the patients complained about muscle pains. No signs of rhabdomyolysis were registered. Although response was not the primary endpoint of the study, it could be evaluated in 17 patients who completed at least 1 cycle of simvastatin with chemotherapy. Seven patients (5 myeloma and 2 Non-Hodgkin lymphoma) responded (41 %) including 1 patients with a Complete Response (which is still ongoing, following non myeloablative allogeneic stem cell transplantation), 2 patients with a partial response (>50% reduction) and 4 patients with a minor response. Due to toxicity all 3 patients at dose level 6 were not evaluable for response. In patients treated at dose level 4 and 5 in vivo down regulation of Mcl-1 (>50%) was observed in PBMC collected after 7 days of simvastatin treatment. *In vivo* modulation of Mcl-1 by high dose (simva)statin treatment may be a promising new modality for patients with drug resistant myeloma and lymphoma.

POSTER SESSION 8: EXPERIMENTAL THERAPEUTIC AGENTS

P0.801

PROTEOMIC ANALYSIS OF TARGETED THERAPEUTIC AGENTS IN MULTIPLE MYELOMA

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The objective of this study was to analyze protein changes that occur a the molecular level with novel therapeutic agents used in multiple myeloma (MM) in order to identify potential combinations for clinical trials. We employed antibody protein microarrays (BD Clonetech, CA) to measure changes in the patterns of protein expression in Kas 6/1 MM cell line after treatment with bortezomib, dexamethasone, CC-5013, and 17-AAG. The antibody array is a new technique that assays protein differences directly by hybridizing fluorescently labeled protein mixtures from cell extracts onto glass slides spotted with 512 different monoclonal antibodies specific for human proteins. Induction of apoptosis was assessed by Annexin/Propidium iodide FACS analysis at 24 and 48 hours using different concentrations of the inhibitors. Protein arrays were performed at concentrations and treatment times that did not induce more than 25% apoptosis to ensure adequate analysis of early changes in signaling pathways. Bortezomib 5nM at 10 hours, dexamethasone 100 mM, CC-5013 250nM, and 17-AAG 1mM at 24 hours were used. Control cells were treated with vehicles ETOH or DMSO at the same concentrations as the inhibitors. To assess differential protein expression, the mean of the Cy5/Cy3 ratios for each sample and its control were analyzed using the Clontech software and an internally normalized ratio (INR) was calculated. INR values >1.3 or <0.77 were considered as valid changes in protein abundance for this study. All inhibitors induced the upregulation of apop-

tosis proteins including BAK, BIK, SMAC/DIABLO, and caspase 14. Several proteins were inhibited by all 4 inhibitors indicating common pathways affected by these agents. These included members of the PI3K pathway (PI3K-p85a, p70S6kinase, and cyclin dependent kinases), MAPKinases (ERK1 and 2), antiapoptotic protein Bclx, heat shock proteins Hsp70 and HSP60, and ubiqutin/NFkB pathway (Ubch6, IKKalpha, and IKBe). These results indicate that inhibitors of the PI3K pathway such as mTOR inhibitors, IKK inhibitors or MAPkinase inhibitors may be used in the future in combination with bortezomib, dexamethasone, CC-5013 or 17-AAG. Fatty acid synthetase was downregulated by bortezomib and dexamethasone indicating potential use of fatty acid synthase inhibitors in combination with bortezomib or dexamethasone in future clinical trials. These results suggest that antibody protein arrays may be used in the rational design of combinations of targeted therapies in MM.

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PO.802

CHARACTERIZATION OF A NOVEL PEPTIDYL ALPHA-KETO ALDEHYDE PROTEASOME INHIBITOR

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The proteasome is a multicatalytic protease with three distinct catalytic activities-chymotrypsin-like (CT-L), trypsin-like (T-L) and peptidylglutamyl peptide-hydrolysing (PGPH). Many of the proteins involved in the cell cycle and apoptosis are degraded by the proteasome and proteasome inhibitors represent novel anti-cancer therapeutic agents. The first proteasome inhibitor to enter clinical trials, PS-341 (Velcade), demonstrated marked anti-multiple myeloma (MM) activity and has recently been approved for the treatment of relapsed and refractory MM. Most proteasome inhibitors are directed against the CT-L activity, which is thought to be rate-limiting. Little is known about the involvement of the other two proteolytic activities. The aim of this study was to look at all three activities of the proteasome in MM cell lines and to investigate the effects of commercially available (MG-132) and novel (BZLLL-COCHO) proteasome inhibitors on these activities. Four MM cell lines (U266, OPM-2, KMS-11, KMS-18) and two lymphoma cell lines resistant and sensitive to PS-341 (DHL-4, DHL-6) were studied. Proteasome was extracted from cells by lysis in an extraction buffer containing 5 mM ATP and 0.1 mM DTT. Individual enzyme activities were measured as the rate of turnover of specific fluorogenic substrates and recorded in Arbitrary Fluorescence Units (AFUs). The percentage apoptosis induced by treatment with the different inhibitors was measured using two apoptotic assays (ApoAlert Mitochondrial Membrane Sensor and Hoescht/Propidium Iodide staining). Enzyme assays show that in all of the MM cell lines, the T-L and PGPH activities account for the majority of total proteolytic activity (88±3%). In the PS-341 resistant and sensitive lymphoma cell lines the level of CT-L activity was similar to the MM lines but accounted for 49±2% of total activity. These profiles suggest that the balance of proteolytic activities may be cell type specific. The proteasome inhibitors showed marked differences in their ability to target the proteasome. BZLLL-COCHO showed the greatest specificity to inhibit the enzyme activity of the proteasome but this resulted in modest apoptotic cell death (85% inhibition of all three activities; 40% apoptosis). In contrast MG-132 was less efficient in inhibiting proteasome proteolytic activity but induced significantly more cell death (MG-132 inhibited 47% of CT-L and 24% T-L & PGPH and induced 60% apoptosis). MG-132 induces apoptosis through additional cellular targets such as calpains. It is widely used in biological studies but is considered too toxic for clinical use. The novel inhibitor BZLLL-COCHO is a specific and cell permeable inhibitor of proteasome activity which may have therapuetic potential.

PO.803

ANTI-MYELOMA ACTIVITY OF TWO NOVEL N-SUBSTITUTED AND TETRAFLOURINATED THALIDOMIDE ANALOGS

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The importance of bone marrow angiogenesis in multiple myeloma (MM) provided the initial rationale for using thalidomide in this disease, given its anti-angiogenic properties. Thalidomide (α-N-[phthalimido] glutarimide, $C_{13}H_{10}N_2O_4$), a glutamic acid derivative, has proved useful in the treatment of both newly diagnosed and relapsed/refractory MM. However, its teratogenicity and side effect profile may limit use as a therapeutic agent. Ongoing efforts have been directed towards development of analogues, which retain anti-MM activity without teratogenic effects. A product of cytochrome P450 2C19 isozyme biotransformation of thalidomide, 5'-OH-thalidomide, has been shown to be partly responsible for the anti-angiogenic effect of thalidomide. Based on the structure of this metabolite, several N-substituted and tetraflourinated thalidomide analogues have been synthesized as potential anti-angiogenic agents and have shown pre-clinical activity in prostate cancer. In this study, we demonstrate potent anti-MM activity and delineate potential mechanisms of two such compounds, CPS11 (an N-substituted thalidomide) and CPS49 (a tetraflourinated thalidomide). Both CPS11 and CPS49 inhibited of proliferation of several MM cell lines as well as primary tumor cells from relapsed/refractory MM patients. These drugs had significant activity against MM cell lines resistant to conventional chemotherapy (dexamethasone, adriamycin, mitoxantrone and melphalan). Both the drugs were able to overcome the protective effects of growth factors like IL-6, IGF-1, and VEGF, as well as co-culture with bone marrow stromal cells (BMSCs), demonstrating their activity against MM cells in conditions simulating the natural microenvironment. In most of the cytotoxicity experiments, CPS49 was more potent compared to CPS11. An additive cytotoxic effect was noted when these agents were combined with dexamethasone and melphalan. Apoptosis was noted in MM1.S cells; evidenced by Annexin V and PI staining and a time- and dose-dependent increase in cleavage of poly ADP-ribosepolymerase (PARP) and caspase-8. Additionally, Z-VAD-FMK partially blocked these effects. Importantly, apoptosis triggered by these drugs was associated with down-regulation of anti-apoptotic proteins like Mcl1 and XIAP. In matrigel based angiogenesis assays, both drugs demonstrated ant-angiogenic activity, with CPS49 being more potent than CPS11. To further delineate their molecular mechanism of actions, we evaluated the effects of these

drugs on various intracellular signaling pathways known to be important in myeloma. Both drugs were able inhibit the PI3K/Akt and JAK/STAT pathways in MM1.S cells. These encouraging *in vitro* data will provide the platform for testing these analogues initially in animal models and subsequently in clinical trials.

P0.804

TARGETING FGFR3 IN t(4;14) MULTIPLE MYELOMA: PRE-CLINICAL STUDIES OF PRO-001, A NOVEL ANTI-FGFR3 NEUTRALIZING ANTIBODY

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As with other B-cell malignancies, chromosomal translocations to the immunoglobulin heavy-chain (IgH) locus on chromosome 14q32 are believed to be a hallmark of multiple myeloma (MM), occurring in approximately 50% of patients. Identification of these chromosomal translocations has resulted in the discovery of powerful prognostic tools and novel molecular targets that promise to revolutionize the treatment of this malignancy. Five recurrent translocation partners have been defined, resulting in the dysregulation of the genes encoding cyclin D1 and D3, c-maf, mafB and Fibroblast Growth Factor Receptor 3 (FGFR3) together with MMSET. Genetic analysis of 14q32 translocations in MM has identified distinct groups of patients with separate clinical outcomes supporting a biological correlation of these genes in MM. In particular, the t(4;14) translocation portends a particularly bad prognosis. The association of FGFR3 expression with t(4;14) myeloma and the demonstration of the transforming potential of this receptor tyrosine kinase (RTK), make this a particularly attractive target for drug development for this poor prognosis group. We report here the development of a novel and highly specific anti-FGFR3 neutralizing antibody (PRO-001) isolated from a phage display human combinatorial antibody library. PRO-001 binds with high affinity (Kd=1.3 nM) to FGFR3 in in vitro binding assays and blocks ligand-dependent and independent FGFR3 phosphorylation and signal transduction in cell-based assays. Furthermore, PRO-001 potently inhibits FGFR3-dependent solid tumor growth in mouse xenograft models. We found that PRO-001 bound to, and competed with FGF binding to the surface of FGFR3 on human myeloma cell lines. PRO-001 inhibited FGF-induced phosphorylation of wild-type FGFR3 and downstream ERK phosphorylation in stable B9 cell transfectants (B9-WT) and FGFR3 expressing human myeloma cell lines. The antibody inhibited FGF-mediated growth of B9-WT with an IC50 of 3 g/ml as determined by MTT proliferation assay. Growth of these cells could be rescued by IL-6 demonstrating selectivity of PRO-001 for FGFR3. PRO-001 inhibited the viability of the FGFR3 expressing, human myeloma cell line, UTMC2. Inhibition of viability was still observed when cells were co-cultured with stroma or in the presence of IL-6, a potent growth factor for MM cells. Several myeloma cell lines lacking FGFR3, showed minimal growth inhibition demonstrating selectivity and lack of non-specific toxic at effective dose concentrations. Finally, PRO-001 bound to FGFR3 on the cell surface, inhibited ERK phosphorylation, and induced cytotoxic responses in primary MM samples derived from t(4;14) positive patients. A xenograft mouse model has been established and studies assessing *in vivo* activity of PRO-001 are planned and will be reported. Taken together, the data demonstrate that PRO-001 is a specific and potent inhibitor of FGFR3 and that it deserves further study for targeted therapy in MM.

P0.805

A FULLY HUMAN ANTI-CD40 ANTAGONIST ANTIBODY TRIGGERS SIGNIFICANT ANTITUMOR ACTIVITY AGAINST HUMAN MULTIPLE MYELOMA

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We previously demonstrated that CHIR12.12, a fully human anti-CD40 mAb (IgG_1) generated in XenoMouse mice (Abgenix, Inc), blocks CD40/CD40 ligand (CD40L) interactions and has more potent anti-lymphoma activity than Rituximab both *in vivo* and *in vitro* (abstract #2386, ASH, San Diego, Dec. 2003). In this study, we assess the efficacy of CHIR12.12 against human multiple myeloma (MM) using CD40-expressing MM cell lines and purified $CD138^+$ patient cells. CHIR12.12 binds to Purified $CD138^+$ MM cells in >80% (10/12) of patient samples, as measured by flow cytometry: the mean fluorescence intensity (MFI) range was 1 to 20 for CHIR12.12 vs 0.2-0.9 for control human IgG_1. We next examined the antagonist activity of CHIR12.12 in MM cells. CHIR12.12 blocked CD40L-mediated proliferation of CD40-expressing MM lines and purified $CD138^+$ patient cells from 2 MM patients in a dose-response manner. In contrast, CHIR12.12 alone did not alter constitutive MM cell proliferation. Immunoblotting analysis demonstrated that PI3-K/AKT, NF-κB, and ERK activation induced by hCD40L in 12BM MM cell line was significantly inhibited by CHIR12.12 (5 μg/mL). Adhesion of MM cells to bone marrow stromal cells (BMSCs) confers growth and survival benefit for tumor cells. Since CD40 activation, either by stimulatory mouse anti-CD40 mAb G28.5 or formaldehyde-fixed CHO cells expressing hCD40L, induces MM cell adhesion to fibronectin (FN) or BMSCs, we next asked whether antagonist CHI12.12 abrogates this process. CHIR12.12 inhibited CD40L-induced adhesion of MM cell lines to FN in a dose dependent manner (0.001-10 μg/ml), whereas control human IgG did not. Moreover, CHIR12.12 (1 μg/ml) blocked hCD40L-induced adhesion of freshly isolated patient MM cells to BMSCs. Adhesion of MM cells to BMSCs induces IL-6 secretion, an important growth and survival cytokine for MM cells, and treatment of MM cells with hCD40L further augmented adhesion-induced IL-6 secretion. Conversely, pretreatment of CD40-expressing MM cell lines with CHIR12.12 significantly decreased IL-6 secretion triggered by coculture of MM cells with BMSCs. We next examined whether CHIR12.12 stimulates antibody-dependent cellular cytotoxicity (ADCC) against CD40-expressing MM cells. Human peripheral blood mononuclear cells and purified NK cells (CD56+CD3-) were used as effector cells. CHIR12.12 triggered MM cell lysis in a dose dependent manner, as measured in CD40-expressing MM cell lines. The maximum specific lysis of 20-70% was achieved at 10 μg/mL concentration of CHIR12.12. CHIR12.12 mediated lysis was specific to CD40-expressing MM cells, as CHIR12.12 did not induce ADCC against CD40-negative MM cells. Importantly, CHIR12.12 induced ADCC against $CD138^+$ cells isolated from 2 MM patients. These results provide preclinical rationale for clinical evaluation of CHIR12.12 to improve patient outcome in MM.

P0.806

SYNERGISTIC KILLING OF MYELOMA CELLS TREATED WITH COMBINED PROTEASOME INHIBITION AND FARNESYLTRANSFERASE INHIBITION (LONAFARNIB) IS MEDIATED VIA DOWNREGULATION OF PHOSPHO-AKT

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Introduction. Treatement for multiple myeloma has evolved from alkylator based therapy to the use of novel targeted agents that have the ability to induce durable remissions. Single agent studies demonstrate activity for agents such as Bortezomib and thalidomide, but for maximal efficacy, combinations of novel agents designed with adequate preclinical rationale are needed. We tested 2 such targeted agents, bortezomib and lonafarnib (SCH66366), with the intent of blocking 2 key signaling pathways and inducing synergistic myeloma cell death.

Methods. MM.1S, MM.1R, RPMI 8226 and U266 cell lines were used in addition to fresh unmanipulated human myeloma cells from patients with relapsed myeloma. Cell death was assayed using the MTT assay as well as flow cytometry for Annexin V and PI. Western blots and immunoblots were performed to interrogate the effects of therapy on AKT, Bcl-2, Bcl-XL, Mcl-1, mTOR, Stat/JAK, caspase 3, 8, and 9. siRNA was transfected to inhibit AKT1 and AKT2, while over expression of AKT1 was achieved using cDNA transfection with membrane bound AKT1 using MM.1S, and MM.1R cell lines.

Results. Single agent dose escalation in vitro demonstrated that 8nm bortezomib doses of 8nm, and 20nm represented subtherapeutic and therapeutic doses in vitro. Lonafarnib dose escalation demonstrated minimal activity when used at doses up to 6uM. Lonafarnib doses in excess of 6uM were not used as this cannot be achieved using standard dosing in humans. When both 8nM and 20nM bortezomib is combined with lonafarnib doses up to 6uM, cell death increases significantly. AnnexinV staining demonstrated that used alone, bortezomib (8nm) or lonafarnib (5uM) results in 20% and 10% apoptosis respectively, compared with 88% apoptosis when both agents are given simultaneously. Order of addition may be critical as cell kill is enhanced when bortezomib is administered first followed (3 and 12 hrs later) by lonafarnib. The converse, lonafarnib followed (3 and 12 hrs later) by bortezomib results in less cell death than both agents given simultaneously or sequentially. Combination therapy significantly reduces p-AKT (ser 473) within 24 hrs in MM.1R cell line, a finding not seen when either agent is given individually. In RPMI 8226 cells, the levels of p-AKT (ser 473) and also STAT1 were reduced within 30hrs while the total levels of total AKT remained unaltered. Transfection of 50ng of siRNA, specific for the AKT1 and AKT2, or the use of the AKT specific inhibitor Ly294002 significantly reduced the AKT(ser473) phosphorylation and resulted in cell death similar to that seen when cells are treated with bortezomib and lonafarnib in combination. The addition of exogenous IL-6 (10 ng/mL) and IGF-1 (50 ng/mL) did not abrogate the apoptotic effects of the combination. Downstream targets of AKT show inhibition as well such as a reduction in the phosphorylation of BAD. We have further demonstrated caspase activation, PARP activation, disruption of MDM2 complex, cell-cycle profile, bcl-2, and MCL-1cleavage when lonarfarnib is combined with bortezomib. Similar effects on apoptosis induction, caspase activation, p-AKT down regulation, and BAD are seen when human myeloma cells are treated with the combination of bortezomib and lonafarnib in vitro.

Conclusions. The use of combined FTI and proteasome inhibition represents a novel and synegistic approach to myeloma therapy. This preliminary data supports the combination of these agents as a treatment for relapsed and refractory MM.

P0.807

INDUCTION OF APOPTOSIS BY DIDOX VIA DOWN-REGULATION OF BCL-2 FAMILY PROTEINS IN MULTIPLE MYELOMA

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Ribonucleotide reductase is the rate-limiting enzyme of deoxynucleoside triphosphate synthesis and is therefore an excellent target for cancer chemotherapy. Inhibition of ribonucleotide reductase results in inhibition of DNA synthesis and has anti-neoplastic effects. Here we examine the anti-multiple myeloma(MM) activity of the novel ribonucleotide reductase inhibitors (RRIs) Didox, Imidate, and Trimidox. All three RRIs showed potent cytotoxicity against MM cells in 48-hour cultures. Didox was studied further due to its potential for clinical development. Apoptosis was induced in MM cell lines exposed to Didox (200 μmol/L), evidenced by increased sub-G1 cell fraction. Didox-induced cytotoxicity was associated with caspase-8 and poly ADP-ribosepolymerase (PARP) cleavage, as demonstrated by western blot analysis. Didox induced apoptosis even in cell lines resistant to conventional chemotherapy (doxorubicin, melphalan, dexamethasone) and overcame the proliferation of MM cells triggered by interleukin-6 (IL-6), insulin like growth factor-1 (IGF-1) and tumor cell binding to bone marrow stromal cells (BMSCs). Importantly, apoptosis of MM cells triggered by Didox was accompanied by a down-regulation of anti-apoptotic proteins Bcl-2, Bcl_{xl}, and XIAP. Cell signaling data indicated that Didox induced a time- and dose-dependent inhibition of pSTAT3. Although, Didox has been reported to inhibit radiation-induced NF-ĴB activation in prostate cancer cell lines, here we found that it triggered activation of NF-ĴB, associated with a degradation of IKBα, in MM. Ongoing pre-clinical studies identifying transcriptional signatures will help identify novel therapeutic targets and provide the rationale for studying combinations with didox with the aim of overcoming drug resistance.

P0.808

THE TYROSINE KINASE INHIBITOR ADAPHOSTIN (NSC 680410), BUT NOT IMATINIB MESYLATE, INHIBITS SURVIVAL AND SRC TYROSINE KINASE FAMILY- TRIGGERED SIGNALING PATHWAYS OF MULTIPLE MYELOMA CELLS

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The tyrosine kinase inhibitor adaphostin is a member of the tyrophostin family of small molecules that interfere with peptide binding rather, than targeting the kinase ATP-binding site. Adaphostin has therefore been examined as an alternative to the 2-phenylaminopyrimidine derivate imatinib mesylate, with remarkable efficacy in the treatment of chronic myeloic leukemia (CML). Previous studies show

that adaphostin induces apoptosis: (1) in Bcr/Abl+ cells more rapidly than imatinib mesylate; (2) in imatinib mesylate resistant cells; and (3) in Bcr/ Abl - cells. Imatinib mesylate has minimal, if any activity in MM; the efficacy of adaphostin in multiple myeloma (MM) is unknown. Here we compare the effects of adaphostin and imatinib mesylate against human MM cells. Our results show concentration-dependent apoptosis in MM.1S, U266, OPM-2, INA-6, RPMI8226 and RPMI-Dox40 MM cells after treatment with adaphostin, but not with imatinib mesylate. Imatinib mesylate induced more than 50% apoptosis in K562 cells using concentrations as low as 1μM, which served as a positive control. Moreover, adaphostin, but not imatinib mesylate, induced caspase-9, caspase-8, and PARP cleavage, as well as downregulation of Mcl-1, in MM cells. Further results demonstrated that adaphostin induces peroxide production and DNA strand breaks after long-term treatment. Importantly MM cell proliferation induced by MM cell binding to BMSCs was abrogated by adaphostin- treatment. IL-6 and IGF-1 signaling and sequelae triggered by these cytokines are important growth, survival, and drug resistance factors in MM; conversely, adaphostin but not imatinib mesylate, inhibited phosphorylation of Src tyrosine kinase family, Akt-1, and ERK. Taken together, our studies in MM cells show that (1) adaphostin- inhibits IGF-1- and IL-6- triggered signaling pathways as well as (2) induces reactive oxygen species and apoptosis. These studies therefore provide the preclinical framework for its clinical evaluation to improve patient outcome in MM.

P0.809

EFFECTS OF THE INDAZOLYLPYRIMIDINE GW786034 ON ANGIOGENESIS AND MULTIPLE MYELOMA CELL GROWTH: THERAPEUTIC IMPLICATIONS

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Vascular endothelial growth factor and receptors play an important role in the pathogenesis of MM. Specifically, VEGF present in the MM BM microenvironment: induces neovascularization; triggers tumor cell growth, survival, migration; inhibits dendritic cell maturation; and induces osteoclastogenesis. VEGF therefore provides a potential novel therapeutic target in MM. Our previous studies showed the efficacy of the that the indazolylpyrimidine GW654652 inhibits tumor cell growth and migration in the BM microenvironment. Here we investigated the indazolylpyrimidine GW786034, a derivative of GW654652 to provide the basis for its potential clinical evaluation. As with GW654652, GW786034: inhibits VEGF- triggered Flt-1 phosphorylation and activation of downstream signaling molecules; induces concentration - dependent MM cell and HUVEC apoptosis; and inhibits VEGF- triggered MM cell migration. Specifically, GW786034 triggers caspase-8 and PARP, but not caspase-9, cleavage as well as downregulation of pro-apoptotic molecules survivin, cIAP1,2 and Mcl-1. Furthermore, GW786034 decreases proliferation of MM cells induced by their adhesion to BMSCs, and inhibits VEGF- induced upregulation of ICAM-1 and VCAM-1, thereby abrogating their adhesion to HUVECs. Consequently, both tubuli formation and accumulation of tumor cells at vascular branching points was inhibited by GW786034 treatment. Finally, GW786034 sensitizes both MM cells alone and tumor cells bound to BMSCs or HUVECs to DNA-damaging chemotherapeutic agents (i.e. melphalan) immunomodulatory drugs, and bortezomib. Taken together, these studies provide the preclinical rationale for clinical evaluations of GW786034 either alone or in combination therapies to improve patient outcome in MM.

P0.810

TGF-β RECEPTOR I KINASE INHIBITOR DOWNREGULATES CYTOKINE SECRETION AND MULTIPLE MYELOMA CELL GROWTH IN THE BONE MARROW MICROENVIRONMENT

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Purpose. Transforming growth factors (TGFs) have pleiotropic biologic effects on tumor cells and their environment. In multiple myeloma (MM), we have reported that bone marrow stromal cells (BMSCs) from MM patients produce more TGF-β1 than BMSCs from healthy donors, which in turn induces interleukin-6 (IL-6) secretion. We here show that the TGF-β receptor I kinase inhibitor SD-208 significantly decreases secretion of both IL-6 and vascular endothelial growth factor (VEGF) from BMSCs, as well as tumor cell growth triggered by MM cell adhesion to BMSCs.

Experimental Design. Cytokine production and MM cell proliferation triggered by TGF-β1 or adhesion to BMSCs were examined in the presence or absence of SD-208. Effects of SD-208 on TGF-β1-induced signaling pathways triggering IL-6 and VEGF transcription in BMSCs were also delineated.

Results. SD-208 significantly inhibits not only transcription but also secretion of both IL-6 and VEGF from BMSCs triggered by either TGF-β1 or adhesion of MM cells to BMSCs. Moreover, SD-208 decreased tumor cell growth triggered by MM cell adhesion to BMSCs. SD-208 works, at least in part, by blocking TGF-β1-triggered nuclear accumulation of Smad2/3 and hypoxia-inducible factor 1α, as well as related production of IL-6 and VEGF, respectively. *Conclusions:* These studies indicate that SD-208 inhibits production of cytokines mediating MM cell growth, survival, drug resistance, and migration in the BM milieu, thereby providing the preclinical rationale for clinical evaluation of SD-208 to improve patient outcome in MM.

P0.811

THE ROLE OF CXCR4 INHIBITORS AS NOVEL ANTIANGIOGENESIS AGENTS IN CANCER THERAPY

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Angiogenesis plays an important role in tumor growth. Endothelial cells express the chemokine receptor CXCR4, and interact with its ligand SDF-1/CXCL12. Previous studies have demonstrated that the ERK MAP kinase and the PI3Kinase pathways are activated in response to SDF-1 stimulation. In this study, we investigate the effect of the role of

inhibitors of CXCR4, ERK MAP kinase and PI3Kinase on angiogenesis. The AngioKit (TCS Cellworks, U.K) is a 24 well plate in which human endothelial cells are co-cultured with other human myoblasts and fibroblasts in a specially designed medium. Control wells in the kit include media alone, VEGF (+control) and suramin (-control). Test samples were added on the day the kits arrive, then changed on days 4, 7, and 9, and stained on day 11 with CD31 (PECAM). The wells are then photographed and subjected to image analysis. The software measures angiogenesis as total tubule length per well in microns. Test samples can then be compared to the control wells to determine the drugs affect on angiogenesis *in vitro*. The following drugs were tested in this angiogenesis model system, a human CXCR4 neutralizing antibody (MAB 171, R&D systems, MN), SDF-1, the MAP kinase inhibitor PD098059, and the PI3Kinase inhibitor LY294002. Treatment with the CXCR4 inhibitory antibody, PD098059, and LY294002 caused marked decrease in angiogenesis (below the level of the negative control suramin). Inhibition of angiogenesis below the level of suramin was first detected at 1 mg/mL CXCR4 antibody, and10 mg/mL CXCR4 antibody resulted in complete inhibition of angiogenesis. The effect of PD098059 on angiogenesis was dependent on its concentration; 20 mM PD098059 inhibited angiogenesis while lower concentrations did not. These results are consistent with the drug s known concentration-dependent inhibition of MEK-1 and indicate that the MEK-1 inhibitor leads to angiostasis secondary to its specific inhibitory effect on MEK-1. The lowest level tested of 1 μM LY294002 led to inhibition of angiogenesis, and 50 μM of LY294002 led to complete abrogation of angiogenesis. SDF-1 has been reported to be angiogenic. In this model system, the effect of SDF-1 alone on angiogenesis was subtle. However, the endothelial cells used in this model system may be secreting endogenous SDF-1 leading to the saturation of the CXCR4 receptor and minimal effects of exogenous SDF-1 stimulation. This was demonstrated by the significant effect of the CXCR4 inhibitor on angiogenesis without the addition of exogenous SDF-1. These results indicate that CXCR4 inhibition and its downstream pathways PI3K and ERK MAP kinase lead to significant inhibition of angiogenesis, and suggest that selective inhibitors of CXCR4 may be useful agents to inhibit angiogenesis.

PO.812

THE PROTEASOME INHIBITOR BORTEZOMIB AND HISTONE DEACETYLASE INHIBITOR SAHA REDUCE C-FLIP LEVELS AND ENHANCE APOPTOSIS INDUCED BY HUMAN TRAIL RECEPTOR-1 MONOCLONAL ANTIBODY IN MYELOMA CELLS

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a potent activator of apoptotic pathway in a variety of tumor cells but not in normal cells. However, several myeloma cells were resistant to TRAIL-induced apoptosis by overexpression of Bcl-2 family members. Recent studies have shown that inhibitors of proteasome and histone deacetylase (HDAC) can sensitize neoplastic cells to apoptosis mediated by chemotherapy or death receptor signalling. In this study, we investigated the potential of these inhibitors and human TRAIL receptor monoclonal antibodies (MoAbs) in TRAIL-resistant myeloma cells. Fully human MoAbs that bind specifically to TRAIL-R1 and TRAIL-R2 were generated using KM mice that possess the human chromosome fragments containing human immunoglobulin heavy chain loci and YAC transgene containing human kappa light chain gene. U266 cells were relatively resistant to TRAIL and maximal cytotoxicity was only 27%. In contrast, anti-TRAIL-R1 MoAb (R1-B12) induced apoptosis of U266 cells more effectively in the presence of $F(ab')_2$ goat anti-human IgG with maximal cytotoxicity of 67%. This apoptotic response of myeloma cells was confirmed by activation of caspase-8, -9, and -3, and cleavage of poly ADP-ribose polymerase (PARP). On the other hand, anti-TRAIL-R2 MoAb (R2-E11) failed to induce apoptosis in U266 cells. Flow cytometric analysis demonstrated that TRAIL-R1 was expressed at a higher level than TRAIL-R2 on U266 cells, and specific mean fluorescence intensity (MFI) was 5.8 and 1.6, respectively. Thus, the sensitivity to R1-B12 and R2-E11 was correlated with the expression level of TRAIL-R1 and -R2 on cell surface. Treatment of proteasome inhibitor bortezomib or HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) did not affect the levels of Bcl-2 and Bcl-XL, and cell surface expression of TRAIL-R1 and -R2 in U266 cells. However, these inhibitors significantly induced reduction of cellular FLICE inhibitory protein (c-FLIP) and Mcl-1, activation of caspase-8, and cleavage of PARP. Moreover, bortezomib and SAHA synergistically enhanced the effect of R1-B12 but not of R2-E11 on apoptosis induction of TRAIL-resistant U266 cells. These results suggest that bortezomib and SAHA can sensitize myeloma cells to apoptosis induced by human TRAIL-R1 MoAb and provide a rationale for combination therapy with R1-B12 and bortezomib or HDAC inhibitors in patients with multiple myeloma.

PO.813

COMBINATION OF RECEPTOR TYROSINE KINASE INHIBITON, PROTEASOME INHIBITION AND DEXAMETHASONE ENHANCES APOPTOSIS IN CYTOGENETICALLY DEFINED MULTIPLE MYELOMA SUBGROUPS

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Background: Novel antineoplastic agents have opened perspectives to more selective treatment of multiple myeloma (MM). Target genes include FGFR3 or c-maf expressed in MM subgroups carrying t(4;14)(p16.4;q32) or t(14;16)(q32;q23), respectively. As previously shown [Bisping G, Blood 102, 661, 2003], these MM subgroups are sensitive to induction of apoptosis by treatment with the receptor tyrosine kinase (RTK) inhibitor BIBF1000. In the present study we investigated, whether combinations of highly selective targeted strategies, such as RTK inhibiton, blocking VEGF and FGF signaling, and more unspecific anti-myeloma agents, including proteasome inhibiton and dexamethasone, are capable of enhancing apoptosis in the above mentioned cytogenetically defined MM subgroups.

Methods: The MM cell lines tested included t(4;14) positive, FGFR3 overexpressing OPM-2, KMS-11, KMS-18, t(14;16) MM.1S, expressing VEGFR1, as well as RPMI-8226, U-266 and PBBLs serving as controls. Cells were exposed to either BIBF1000 [0.5–1.0 μM], Velcade [2.0–4.0 nM], dexamethasone [10μM] or the respective combinations for up

to 24 hrs. MM cells were starved for 4 hrs prior to exposure in serum-free RPMI-1640 medium. Apoptosis was quantified by flow cytometry (annexin V/PI-staining). Immunoblots were performed for pMAPK (p44/42), pAkt, pSTAT3, caspase-3, -8 and –9, PARP and, actin.

Results: We found a significant induction of apoptosis in t(4;14) and t(14;16) positve MM by incubation with the receptor tyrosine kinase inhibitor BIBF1000. Coincubation with the proteasome inhibitor bortezomib, or dexamethasone or a combination of these drugs revealed a stepwise increase of apoptosis. In parallel, we found a markedly higher proportion of cleaved caspase-3, caspase-8 and PARP, while caspase-9 was not activated when combining BIBF1000, bortezomib, or dexamethasone. Interleukin-6 partially rescued and the pan-caspase inhibitor z-VAD almost completely reverted induced apoptosis upon exposure to these combinations. Induction of apoptosis by BIBF1000 was associated with inhibition of the phosphorylation of mitogen-activated protein kinase (MAPK p44/42) in t(4;14) cells and of the phosphatidyl-inositol-3 kinase/AKT in t(14;16) MM.1S cells. RPMI-8226, U-266 and PBBLs showed no sensitivity to induction of apoptosis by receptor tyrosine kinase inhibition with BIBF1000.

Conclusions: The data provide the rationale for clinical evaluation of a combination of this class of targeted tyrosine kinase inhibitors, proteasome inhibitors and dexamethasone in MM with focus on defined cytogenetic subgroups.

POSTER SESSION 9: TUMOR MICROENVIRONMENT. ANGIOGENESIS, NOVEL DRUG MECHANISMS

P0.901

BORTEZOMIB TARGETS MULTIPLE MYELOMA ENDOTHELIAL CELLS

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Introduction. Bone marrow (BM) angiogenesis is an important hallmark of multiple myeloma (MM) which correlates with progression. Although MM remains incurable despite conventional and high-dose chemotherapy, the proteasome inhibitor Bortezomib (Velcade, formerly PS-341), can overcome conventional drug resistance *in vitro* and *in vivo* and it has recently been FDA approved for treatment of relapsed and refractory multiple myeloma. Here we evaluated whether anti-angiogenesis may contribute to the anti-MM activity of PS-341. We examined the effect of PS-341 on the angiogenic phenotype of endothelial cells (ECs) isolated from BM of patients with MM.

Methods. MMECs were extracted from BM of patients with active MM using a lectin-based method. The MMEC population contained >95% factor VIII-related antigen (FVIII-RA)$^+$ and CD31$^+$ cells, as assessed by fluorescence activated cell sorting (FACS). Contamination by macrophages and plasma cells was <5%, evaluated by FACS for CD14 and CD38 positivity, respectively, as well as by RT-PCR and Western blot for CD38. Viability, assessed by trypan blue was >90%. MTT assay and [^{3}H] thymidine uptake were used to evaluate the effects of PS-341 on survival and proliferation, respectively, of MMECs. Proliferation of MM.1S cells cocoltured with MMECs was measured by [^{3}H] thymidine uptake. Cytokine (IL-6, VEGF) levels were quantitated by ELISA. Other *in vitro* angiogenesis functions examined included chemotaxis, spreading on fibronectin (FN), and morphogenesis on Matrigel. Ongoing work is looking at the effect of PS-341 on angiogenesis *in vivo* by using a chick embryo chorioallantoic membrane (CAM) model.

Results. PS-341, at concentrations achievable in the plasma of patients, inhibited *in vitro* MMEC and HUVEC functions related to angiogenesis, including proliferation, chemotaxis, spreading on FN, and capillary formation on Matrigel. All these functions were affected in a dose-dependent fashion. A significant concentration-dependent reduction of VEGF and IL-6 production was observed in the presence of PS-341, as demonstrated by ELISA. Importantly, binding of MM.1S cells to MMECs triggers tumor cell proliferation, and PS-341 inhibits proliferation of adherent MM.1S cells in a dose-dependent fashion. Similar data were demonstrated in HUVECs.

Conclusions. These data therefore demonstrate that PS-341 acts both directly and indirectly against MMECs, another mechanism which may contribute to the anti-MM activity of PS-341.

P0.902

MARKERS OF ENDOTHELIAL ACTIVATION, COAGULATION PATHWAY ACTIVATION AND FIBRINOLYSIS IN PATIENTS RECEIVING ACTIMID™ (CC-4047) FOR RELAPSED/REFRACTORY MYELOMA

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The association between multiple myeloma and the development of venous thromboembolic events (VTE) is well recognised. Multiple pro-thrombotic haemostatic effects of myeloma have been described including procoagulant autoantibody formation, paraprotein interference with fibrin structure, acquired protein C resistance and endothelial damage. The use of thalidomide as a single agent for patients with relapsed disease is associated with a low thrombosis risk but patients that receive combination therapy or who have previously untreated disease have a markedly increased risk of VTE. Furthermore the use of thalidomide for other malignant and non-malignant disorders has been associated with VTE. Thalidomide has an observed anti-angiogenic effect via its inhibition of VEGF secretion and modulates expression of adhesion molecules. This may result in endothelial perturbation that induces thrombogenesis. The thalidomide analogues (CC-4047, CC-5013) have a greater anti-TNF and T-cell co-stimulatory activity but similar VEGF inhibition compared to thalidomide. This study evaluated the serum/plasma levels of cytokines and markers of coagulation, fibrinolysis, endothelial and platelet activation during the first four weeks of treatment with the thalidomide analogue Actimid™(CC-4047) in 15 patients with relapsed/refractory myeloma.

Methods. Peripheral blood plasma and serum samples were collected from 15 patients with relapsed/refractory myeloma taking part in a Phase I study of CC-4047 at day 0, 7 and 21 or 28 from start of treatment. Analysis by ELISA was performed in duplicate using standard assays for Factor XIIa, soluble TF (sTF), Interleukin-6, Vascular endothelial growth factor (VEGF), soluble Vascular Cell Adhesion Molecule (sVCAM), p-selectin, prothrombin fragment 1+2 (PF 1+2), D-dimers and TGFβ2.

Results. Median baseline levels of PF1+2, D-dimers and sVCAM were raised and did not change throughout the 28 day study period. Baseline serum levels of IL-6 were within normal range and rose by day 21-28 but this did not reach statistical significance (p=0.09). sTF, VEGF, p-selectin, FXIIa and TGFβ2 remained within the normal range throughout the study period. 10/15 patients had raised baseline D-dimers with no correlation with paraprotein. Four of these patients had D-dimers >500 μg/L (control: 4-52 μg/L) and three of these patients subsequently developed a lower limb deep vein thrombosis (DVT) (3-35 weeks from baseline). One of these patients was subsequently found to have inguinal lymphadenopathy secondary to metastatic melanoma on the same side as the DVT. Development of DVT did not correlate with drug dose, disease response or baseline paraprotein.

Conclusions. Baseline markers of endothelial activation, coagulation pathway activation and fibrinolysis were raised. This suggests that myeloma induces a state of endothelial perturbation that predisposes to the development of VTE. There were no significant changes in markers with the use of CC-4047. Raised D-dimers may predict patients with myeloma most at risk of development of VTE or those patients most at risk of VTE development when treated with CC-4047. A prospective study is planned.

P0.903

INDUCTION OF AUTOPHAGY TO MYELOMA CELLS BY THALIDOMIDE AND CLARITHROMYCIN

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Introduction. Therapeutic benefit of thalidomide (Thal) for myeloma has been well recognized. However, its mechanism of action is not yet fully understood. It has also been reported that clarithromycin (CAM) occasionally exserts anti-myeloma effects in combination with Thal. The aim of the present study is to clarify the mechanisms of anti-myeloma effects of Thal and/or CAM.

Methods. Myeloma cell lines and freshly isolated myeloma cells were cultured either with Thal and/or CAM at a concentration of 10 μg/mL for 24 hours with or without serum and their morphology of these cells were analysed by using light or electron microscopy. A PI-3 kinase inhibitor, 3-Methyladenine, was utilized as an autophagy inhibitor.

Results. Significant induction of vacuoles containing cytoplasmic organelle was seen when myeloma cell lines and fresh myeloma cells were treated with CAM. Induction of vacuoles was more prominent under a serum-free condition. Interestingly, the vacuole formation was substantially enhanced when treated with Thal and CAM accompanying cell death. The vacuolization was significantly inhibited by the presence of 3-Methyladenine regardless the presence of serum. Since PI-3 kinase plays a key role in autophagy, the induction of vacuolization with Thal an/or CAM possibly indicates a feature of autophagy. Electron microscopic analysis confirmed the vacuoles are characteristic of autophagy.

Conclusion. Autophagy is a mechanism of recycling microorganelles when exposed tor various stresses. It is believed that proteasome rapidly degrades unnecessary proteins while some proteins are also degraded by autophagosomes. Although autophagy is necessary for survival of cells under unfavourable conditions, such as nutrient deficiency, it is also suggested autophagy may induce cell death in some situations. This study suggests that either Thal or CAM may mediate autophagy as a single agent. Marked cell death found with the combined treatment with Thal and CAM suggests that the combination of Thal and CAM can lead to cell death through autophagy. Indeed, there is a report showing that this combination is occasionally effective for drug resistant myeloma patients. The present study should provide useful information in the investigation of mechanism of anti-myeloma activity of Thal and CAM.

P0.904

GLUTATHIONE-S-TRANSFERASE PI IS A DETERMINANT OF THE MECHANISM OF ARSENIC TRIOXIDE-INDUCED CELL DEATH IN MULTIPLE MYELOMA

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Arsenic trioxide (Trisenox) has been shown to have remarkable efficacy in the treatment of refractory/relapsed acute promyelocytic leukemia and is currently being tested in clinical trials as a single agent or in combination with oth-

er agents that have activity in multiple myeloma. We and others have demonstrated that glutathione (GSH) levels can influence the ability of ATO to induce cell death in multiple myeloma cell lines/patient samples and based on these data have initiated a trial to test the safety and efficacy of the combination of ATO and ascorbic acid for the treatment of refractory/relapsed multiple myeloma. The interim analysis from this trial has suggested that quality of response correlates with reduction of GSH in PBMCs. Therefore we initiated studies to determine how ATO kills myeloma cell lines/patient samples and the role of GSH in determining the mechanism of killing. In an initial screen of 5 cell lines and 17 patient samples we determined that ATO can kill cells through both caspase-dependent and -independent mechanisms as broad spectrum caspase inhibitors (ZVAD-fmk and BocD-fmk) were effective at inhibiting apoptosis completely in U266 and KMS 18 cells while partially inhibition was achieved in MM.1s and KMS 11 cells. In contrast caspase inhibition was ineffective in 8226 cells as well as several drug resistant variants of this line. Interestingly, in cells where caspases inhibitors could block apoptosis, production of reactive oxygen species was also inhibited. In contrast ROS production was not altered by caspase inhibition in 8226 cells. Moreover ROS production and death were inhibited in all cells tested by the addition of n-acetylcysteine. Taken together these data suggest that GSH functions upstream of caspase activation to block ATO-induced cell death and that production of ROS independent of caspase activation results in caspase-independent cell death. Therefore we investigated the mechanisms by which GSH could be utilized to block ROS production and caspase activation in myeloma cell lines. Two potential mechanisms would be as a reducing agent mediated by Glutathione Peroxidase (GPx) or as conjugation substrate for Glutathione-S-Transferase (GST). We found that in all lines tested GPx basal activity was undetectable and was not induced by treatment with ATO. In contrast GSTpi was expressed in all cell lines except 8226. Additionally MM.1s cells were shown to be homozygous for the GSTpi A allele while the U266, KMS11 and KMS18 lines were heterozygous for both the A and B (Ile105Val) alleles. Transfection of either the GSTpi A or B alleles into 8226 did not alter sensitivity to ATO, however caspase dependence of cell death was detected. These data suggest that GSTpi is not sufficient to induce resistance to ATO, however the mechanism of cell death is altered. The data also suggest that reduction of GSH by agents like ascorbic acid may sensitize cells by converting the mechanism of cell death from a caspase-dependent death to one that is mediated by ROS. This may in part explain the ability of ATO to kill drug resistant cells where altered expression of anti-apoptotic proteins has been previously observed.

P0.905

ANTI-TUMOR EFFECTS OF RISEDRONATE AND ITS PHOSPHONOCARBOXYLATE ANALOGUE NE10790, IN HUMAN MYELOMA CELLS *IN VITRO*

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Bisphosphonates (BPs) are widely used in the treatment of osteolytic bone disease associated with multiple myeloma. In addition to their beneficial effects on bone disease in myeloma, BPs have recently been demonstrated to exert anti-tumour effects both *in vitro* and *in vivo*. Risedronate (RIS) is a potent BP which is in clinical use to treat diseases such as osteoporosis and Paget's disease. NE10790 is a phosphonocarboxylate analogue of RIS, with a lower affinity for bone mineral. This lower affinity for bone mineral may facilitate its release from the bone surface during resorption and increase its bioavailability in the bone microenvironment surrounding the osteoclasts, raising the possibility that it can act more effectively on the tumor cells. In support of this, although NE10790 was found to be less potent *in vitro* in inhibiting proliferation of B02 breast cancer cells as compared to RIS, similar doses of RIS and NE10790 were effective in reducing skeletal tumour burden in vivo in a murine model of breast cancer caused by the same cells. The aim of this study was to examine the effects of RIS and NE10790 on human myeloma cells *in vitro*. Both NE10790 and RIS significantly increased apoptosis in NCI H929, JJN-3, and RPMI 8226 myeloma cells in a dose-dependent manner ($p<0.001$), as determined by characteristic changes in nuclear morphology and by a fluorescence *in situ* nick translation assay. A significant increase in apoptosis was detected following treatment with 1 mM NE10790 ($p<0.01$) and 50 μM RIS ($p<0.05$) in JJN-3 cells. Flow cytometric analysis of propidium iodide (PI)-stained cells demonstrated that RIS induced an increase in the proportion of cells in S-phase of the cell cycle. Double labelling with BrdU and PI showed that DNA replication was inhibited following treatment with RIS. In contrast, NE10790 treatment did not induce accumulation of cells in S-phase, and DNA replication was not completely inhibited even following treatment with the highest concentration, 3 mM, of NE10790. These differences suggest different enzymatic targets of these two compounds. A previous study has demonstrated that NE10790 specifically inhibits the prenylation of small Rab GTPases in macrophages and osteoclasts, in contrast to the N-containing BPs that act by inhibiting FPP synthase, thus disrupting all forms of protein prenylation. In support of this, our study showed that in human myeloma cells, RIS dose-dependently inhibited prenylation of both Rap1a (geranylgeranylated by GGTase I) and Rab6 (geranylgeranylated by GGTase II/Rab GGTase) proteins, which could be prevented by addition of geranylgeraniol (GGOH). In contrast, NE10790 only inhibited the prenylation of Rab6, and this could not be prevented by GGOH. Consequently, GGOH could prevent both apoptosis and cell cycle arrest induced by RIS, while NE10790-induced apoptosis could not be prevented by the addition of GGOH. In conclusion, RIS and its phosphonocarboxylate analogue NE10790 both induce apoptosis in human myeloma cell lines *in vitro*. However, RIS inhibits DNA replication, and causes cell cycle arrest, whereas NE10790 does not. This appears to be related to their different effects on the prenylation of proteins.

P0.906

MICROENVIRONMENTAL CONTROL OF MYELOMA CELL GROWTH AND PLASTICITY

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Progression of myeloma (MM) is considered to be a multistage and dynamic process of cell differentiation, survival, proliferation and dissemination. The aim of this study was to determine the molecular mechanism of osteoclast-induced MM plasma cell (MM PC) survival and the phenotypic plasticity associated with long term survival of MM PCs. Fibroblast-activation protein (FAP) is one of 32 consistently and significantly upregulated genes in osteoclasts after

co-culture with CD138-selected MM PCs (n=19). FAP is a cell surface serine protease with both dipeptidyl peptidase and collagenase activity. To study the role of FAP in MM we initially demonstrated by quantitive real time RT-PCR (qRT-PCR) that FAP was upregulated 3.0 fold ($p<0.01$, n=5) in co-cultured osteoclasts and by >2 folds in myelomatous vs. nonmyelomatous whole human bone marrow in SCID-hu mice ($p<0.05$, n=3). Immunohistochemical staining of myelomatous bone sections from SCID-hu mice revealed expression of FAP by osteoclasts, vascular endothelial cells, osteogenic cells and other stroma elements, but not by MM cells. Addition of FAP siRNA to co-cultures of MM PCs with osteoclasts (n=5) inhibited FAP expression in osteoclasts by >75% and reduced the number of viable MM PCs by 40% ($p<0.05$). To further examine the osteoclast-induced phenotypic changes associated with tumor cell survival, MM cells from 16 patients were co-cultured with osteoclasts for up to 20 weeks. The pre-cultured baseline cells were typically $CD45^{low/inermediate}CD38^{high}$, $CD19^{-}CD34^{-}$. At the end of co-culture (>6 weeks), MM cells were >98% viable and their phenotype consistently shifted to a less mature phenotype, with CD45 expression gradually increasing from $CD45^{low}$ to $CD45^{high}$ and reduced expression of CD38, as determined by flow cytometry and confirmed by qRT-PCR. Further analysis revealed that co-cultured MM cells also expressed low levels of CD19 and CD34, and identified a small subpopulation of $CD138^{low}CD45^{high}$ MM cells. Morphologically, the co-cultured MM cells uniformly gained plasmablastic characteristics when compared to pre-cultured cells. To investigate if the observed phonotypic changes are associated with apoptosis resistance, we determined the effects of 3 days exposure to the pro-apoptotic agent dexamethasone (DEX, 10^{-7} M) on MM cells cultured alone or in co-cultures (n=5), at baseline and after 6 weeks of co-cultures. When baseline MM cells were cultured alone, DEX significantly increased the percent of apoptotic cells over that spontaneous rate ($p<0.01$). In contrast, when MM cells recovered from co-cultures after 6 weeks and cultured alone, they survived and were resistant to DEX-induced apoptosis. Osteoclasts supported survival of MM cells at baseline and after 6 weeks of co-culture ($p<0.01$), and protected MM PCs from DEX-induced apoptosis. Our data identified FAP as a key microenvironmental factor associated with MM cell survival and demonstrated the phenotypic plasticity of tumor cells, as expressed by dedifferentiation of MM PCs into an immature, resilient, apoptosis-resistant phenotype. We hypothesize that in vivo these cells are dormant and could be responsible for relapse.

PO.907

TARGETING OF AN MMP-9-ACTIVATED PRODRUG TO MULTIPLE MYELOMA DISEASED BONE MARROW

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Matrix metalloproteinases (MMPs) are a family of endopeptidases containing approximately 24 members. The enzymes are involved in the cleavage of extracellular matrix components, receptors, growth factors and many other molecules. In multiple myeloma (MM), MMP-9 expression is upregulated *in vivo*. This was demonstrated in the 5T33MM mouse model. The *in vitro* growing MM cells, 5T33MMvt, secrete no MMP-9. When these cells were injected into naïve mice and harvested from the bone marrow an enhanced MMP-9 secretion was observed, both at the mRNA level by real-time PCR and protein level by gelatin zymography. In the 5TMM mouse model, we recently described that MMPs are involved in tumor growth, development of angiogenesis and osteolytic bone disease. In addition to target MMP-9 as a potential therapeutic target, MMP-9 activity might also be exploited to target and activate prodrugs to the MM bone marrow (BM) to obtain a more tumor specific therapy. We investigated this principle in the 5T33MM model. The prodrug EV1 is comprised of an *in vivo* active topoisomerase inhibitor containing an MMP-substrate oligopeptide carrier. EV1 was conjugated to fluorescein isothiocyanate (FITC) and contained an MMP-9 gly-nva cleavage site. Cleavage of the prodrug carrier by MMP-9 results in the release of fluorescence which can be used as a measure of and leads to liberation of the active agent. Addition of EV1-FITC to 5T33MM*vt* and 5T33MMvv cells resulted in a higher release of fluorescence with the MMP-9-producing 5T33MMvv cells than with the non-MMP-9-producing 5T33MM*vt* cells. An MMP-2/MMP-9 specific inhibitor, CTT, was added to the 5T33MMvv cells and resulted in a decrease of fluorescence release compared with the control peptide, STT, whereas the serine proteinase inhibitor, aprotinin, had no effect on the fluorescence release. MMP-9 expression was measured in different organs of the 5T33MM model by gelatin zymography. MMP-9 was present in the lysates from the tumor-bearing organs (BM and spleen), but was not detectable in the non-tumor-bearing organs (heart, lung, kidney). When EV1-FITC was added to the lysates, there was more fluorescence release in the lysates of BM and spleen than in the lysates of heart, lung and kidney. CTT, but not aprotinin, inhibited the fluorescence release when EV1-FITC was added to 5T33MM BM lysates. Addition of EV1-FITC to BM and spleen cells isolated from a naïve and a 5T33MM-bearing mouse demonstrated a higher fluorescence release with the cells of the 5T33MM-bearing mouse than with the cells of the naïve mouse. Furthermore, 5T33MM-bearing animals and naïve animals were injected i.p. with EV1-FITC (50 mg/kg). After 2 hours, BM and spleen cells were isolated and fluorescence was measured. A higher amount of fluorescence could be detected with the cells of the 5T33MM-bearing mice compared to the cells of the naïve mice. In conclusion, these experiments indicate that the strategy of activating prodrugs by subverting the proteolytic action of MMP-9 can be useful in targeting multiple myeloma.

PO.908

LACK OF CORRELATION BETWEEN BONE MARROW ANGIOGENESIS ESTIMATED BY MICROVESSEL DENSITY AND SERUM ANGIOGENIC CYTOKINES IN MULTIPLE MYELOMA

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Introduction. It has been shown that bone marrow angiogenesis is increased in multiple myeloma (MM) and patients with increased neovascularization have a poorer outcome. This was the rationale for the use of thalidomide and other novel agents with new mechanisms of action. Angiogenesis can be assessed by studying the bone marrow microvessel density (MVD) or by measuring serum levels of angiogenic cytokines.

Objective. To assess whether or not bone marrow neovascularization estimated by MVD correlates with serum levels of angiogenic cytokines in patients with MM.

Patients and methods. Bone marrow angiogenesis was estimated by MVD in 31 bone marrow biopsies, obtained from multiple myeloma patients, stained by a standard immunohistochemical technique with anti-CD34 monoclonal antibody to highlight endothelial cells. Moreover, the degree of bone marrow plasma cell infiltration was determined by immunohistochemistry using anti-CD138 antibody. The serum levels of the following angiogenic cytokines, obtained at the time of the bone marrow biopsy, were measured with an enzyme-linked immunosorbent assay (ELISA): vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6).

Results. The MVD estimated in the bone marrow specimens with high degree of plasma cell involvement (>50%) was significantly higher than the MVD observed in biopsies with lower plasma cell infiltration (34.7 vs 18.6, p=0.002). Concerning the possible correlation between the angiogenesis estimated by MVD and by the measurements of serum levels of the angiogenic cytokines (VEGF, bFGF, HGF, TNFα and IL-6), we only found that patients with high MVD (>20) had significantly lower levels of VEGF (p=0.03) and significantly higher levels of TNFα (p=0.03).

Conclusion: There is no relevant correlation between bone marrow microvessel density and serum levels of angiogenic cytokines in multiple myeloma.

PO.909

LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR PREDICT PROGRESSION-FREE SURVIVAL IN MYELOMA PATIENTS WHO UNDERWENT AUTOLOGOUS STEM CELL TRANSPLANTATION

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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 by micro-vascular density in the bone marrow of MM patients plays a significant role in the prognosis of MM. Autologous stem cell transplantation (ASCT) seems not to affect angiogenesis in MM patients. Vascular endothelial growth factor (VEGF) is a potent stimulator of angiogenesis *in vivo* and its levels at diagnosis predict survival in MM. Angiogenin is another angiopoietin that has been found to be increased in MM patients. The aim of this study was to evaluate the role of serum VEGF and angiogenin in a group of patients with MM who underwent ASCT. We studied 37 MM patients (25M/11F, median age: 58 years) who underwent an ASCT after high dose melphalan conditioning. Four patients were transplanted in complete remission, 30 in partial remission, one had progressive disease, and 2 had no response to anti-myeloma treatment prior to transplant, according to EBMT criteria. 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 using ELISA method (R&D Systems, Inc., Minneapolis, MN, USA). The mean VEGF concentration pre-transplant (206.10 ng/mL) did not differ significantly compared with post-ASCT value (189.08 ng/mL). Similarly, the levels of angiogenin before (339,703 ng/mL) and after transplant (385,318 ng/mL) did not alter significantly. VEGF levels of patients who had received thalidomide did not differ significantly compared 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 median follow up was 14 months (ranged from 4 to 29 months). During this period 15 out of 37 patients relapsed. Using the level of 220 ng/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). Other factors, such as age, β_2-microglobulin, CRP, time from diagnosis to transplantation did not influence the progression of the disease in this group of patients. These results suggest that autologous transplantation does not seem to decrease the levels of the circulating VEGF and angiogenin. Higher VEGF levels before ASCT 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.

PO.910

COMPARISON OF THREE METHODS, VASCULAR GRADING, MICRO VESSEL DENSITY AND CHALKLEY, FOR ASSESSMENT OF BONE MARROW ANGIOGENESIS IN MULTIPLE MYELOMA

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Background of the study. The role of angiogenesis has been examined in different plasma cell disorders and patients with advanced myeloma have been shown to have increased bone marrow angiogenesis as compared to patients with monoclonal gammopathy of undetermined significance (MGUS). It has been suggested that bone marrow neovascularization is related to disease progression and may represent a bad prognostic variable in patients with multiple myeloma. If angiogenesis is going to be used clinically in patients with multiple myeloma the reliability of the measurements techniques has to be considered.

The aim of the study was to. evaluate the reliability of three different methods, vascular grading, micro vessel density (MVD) and Chalkley, for estimating bone marrow angiogenesis in patients with newly diagnosed multiple myeloma.

Materials and methods. Thirty bone marrow specimens from patients with newly diagnosed multiple myeloma were examined. Sections of the specimens were stained with haematoxylin and eosin (HE), and immunohistochemically stained for CD34 to identify micro vessels. Bone marrow angiogenesis were examined using three methods; vascular grading, MVD and Chalkley, twice by the same observer and once by a second observer. In the intraobserver as well as the interobserver study the measurements were carried out independently and blinded between the observers. In the intraobserver study at least one month had passed between the first and second measurement.

Results. The reproducibility of the vascular grading method showed that within observer 3 bone marrows were classified in another grade, but none of them had a shift of more

than one group. Between observers 8 bone marrows were classified in another grade, but still none had a shift of more than one group. McNemar test for symmetry showed within observer p=1.00 and between observers p=0.07. The reproducibility of the MVD and Chalkley methods was analysed by regression analysis. Intraobservationally, the slopes with 95% confidence interval (CI) were 0.75 (0.59, 0.92) and 0.76 (0.53, 0.97) of MVD and Chalkley, respectively. Interobservationally, the slopes with 95% CI were 0.84 (0.48, 1.20) and 0.58 (0.33, 0.84) of MVD and Chalkley, respectively. Difference plots were made to illustrate the degree of agreement, distribution of differences and outliners. The coefficient of variation was calculated and the Chalkley method had lesser biologic and interobserver variation than the MVD method.

Conclusion. The vascular grading, MVD and Chalkley methods had moderate reproducibility. The biologic variation among patients was the major contributor of the total variation. The Chalkley method was not better than the MVD method when comparing differences among patients.

PO.911

MYELOMA PLASMA CELL-DERIVED SDF-1 AND RANKL ACT SYNERGISTICALLY TO STIMULATE NEO-ANGIOGENESIS

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Recent evidence highlights the role of angiogenesis in the progression of multiple myeloma (MM). Angiogenesis is a complex, multi-step process characterised by the formation of new capillaries from pre-existing vasculature. Whilst numerous factors, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), have been implicated in this process, recent studies suggest roles for the potent chemokine, stromal-derived factor-1 (SDF-1), and the osteoclastogenic agent, receptor activator of nuclear factor kappaB ligand (RANKL), in endothelial chemotaxis and cell branching morphogenesis. Recent studies from our laboratory and those of others, show that MM patients exhibit higher levels of circulating SDF-1 and RANKL when compared with age-matched normal donors. In fact, we have found that levels of circulating SDF-1 correlate positively with BM microvessel density (MVD), a measure of bone marrow angiogenesis. The role of these factors in the angiogenic process was further investigated by culturing HUVEC in an *in vitro* angiogenesis assay in the presence of plasma cell-line derived conditioned media (RMPI-8226 CM). The contribution of SDF-1 and RANKL in this process, was confirmed using a highly specific small molecule CXCR4 inhibitor, 4F-Benzoyl-TE14011 (T140), and a RANKL antagonist, FcOPG, respectively. Using recombinant factors, we showed that whilst independently failing to stimulate significant increases in angiogenesis *in vitro*, SDF-1 and RANKL were found to act synergistically to increase endothelial cell migration and angiogenesis. Further investigations revealed that this apparent synergy was the result of RANKL-mediated upregulation of CXCR4, the receptor for SDF-1, at both the mRNA and protein level. These findings indicate that myeloma-derived RANKL and SDF-1 play a role in angiogenesis in MM and may therefore provide effective targets for reducing BM angiogenesis and MM disease progression.

PO.912

PLEIOTROPHIN TRANSDIFFERENTIATES HUMAN MONOCYTES AND BONE MARROW STEM CELLS INTO VASCULAR ENDOTHELIAL CELLS FOR ANGIOGENESIS

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Bone marrow angiogenesis is a hallmark of multiple myeloma (MM). We have recently shown that MM patients express pleiotrophin (PTN), a secreted protein that binds syndecan, and it is found at high levels in MM serum. This protein has been shown to stimulate angiogenesis. We have discovered a novel mechanism leading to blood vessel formation by tumor cells. First, we cloned human monocytic THP-1 cells with PTN sense or antisense whole sequencing DNA. We examined expression by RT-PCR of endothelial cell markers (vascular endothelial growth factor receptor-2 (Flk-1), Tie-2, von Willebrand factor (vWf)) and monocyte markers (*c-fms* and CD68). THP-1 cells infected with PTN sense strand expressed high amounts of Flk-1, Tie-2 and vWf similar to that found in human coronary artery endothelial cells and lost expression of *c-fms* and CD68. Endothelial cell marker RNA was not detected in either THP-1 cells infected with PTN anti-sense strand or the GFP control vector but these cells showed the continued presence of monocyte markers. Immunohistochemical studies showed THP-1 cells infected with PTN sense strand expressed endothelial markers but not cells treated with antisense or control cells. We have recently found high levels of PTN in MM serum and expression of PTN by MM cell lines including RPMI8226. Next, we cultured THP1 monocytes with RPMI8226 in transwell cultures, serum derived from MM patients with high serum levels of PTN, cell lines lacking PTN expression, and normal controls lacking serum PTN. The THP-1 cells exposed to the MM cell lines or MM serum showed expression of endothelial markers and lost expression of monocyte markers. The expression of endothelial markers was blocked by adding anti-PTN antibody. In contrast, control serum and cell lines lacking PTN expression did not change monocyte marker expression on the THP1 cells nor induce expression of endothelial markers. We examined whether PTN induced monocytes from human peripheral blood to transdifferentiate. Normal human blood monocytes were highly purified using density centrifugation followed by anti-CD14 micro-bead affinity column selection. These purified monocytes also showed transdifferentiation into endothelial cells in the presence of PTN with m-CSF unlike cells treated with m-CSF-alone or cells without these factors present. We determined whether PTN could also stimulate differentiation of bone marrow stem cells into endothelial cells. The stem cells were derived from bone marrow selected for CD34 using magnetic bead selection, and were stimulated with either m-CSF or PTN alone or a combination of m-CSF and PTN or no treatment for 7 days. Real time PCR analysis showed that the m-CSF and PTN combination markedly increased endothelial cell marker expression and decreased monocyte marker (CD68 and *c-fms*) expression in this stem cell population. When induced with PTN alone, the stem cells exhibited slightly increasing expression of endothelial markers with no change in monocyte marker expression whereas m-CSF alone and no treatment had no effect on either endothelial or monocyte marker expression. These experiments define a previously unrecognized novel mechanism leading to angiogenesis in cancer patients- the

transdifferentiation of monocytes into endothelial cells by a factor highly produced by tumor cells. They also suggest a potential new specific target to inhibit angiogenesis-pleiotrophin which may have profound clinical implications.

P0.913

PLEIOTROPHIN IS A NEW AUTOCRINE GROWTH FACTOR FOR B-CELL MALIGNANCY AND HIGHLY EXPRESSED BY MYELOMA CELLS

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Pleiotrophin (PTN) was originally described as a development regulatory cytokine. This protein regulates the growth of neuroectodermal and mesodermal cell lineages during early embryogenesis but becomes down-regulated during late phase of embryogenesis and shows a very restricted expression pattern in adult neural system. This protein has been recently shown to both induce angiogenesis and bind to syndecan. Thus, we determined whether this protein may be found in tumor cells from myeloma patients and its potential to influence their growth. First, we measured serum levels of PTN in myeloma (n=115) and age-matched controls (n=50) using an ELISA-based technique. PTN levels in the serum of myeloma patients were markedly elevated compared to the normal control group ($p<0.02$). The serum concentration of PTN in MM patients averaged 28 pg (range, 8 to 110 pg). In contrast, the control serum levels averaged only 12 pg (range, 0 to 20 pg). Interestingly, the PTN concentration in two patients with plasma cell leukemia was much higher than other multiple myeloma patients. Next, we analyzed the expression of PTN using RT-PCR on RNA from myeloma cell lines (RPMI8226, U266), and multiple myeloma patients' and normal control bone marrow aspirates. Results showed that the PTN mRNA was strongly expressed in multiple myeloma cell lines, myeloma bone marrow samples but not in normal control bone marrow specimens. In order to determine whether PTN stimulates multiple myeloma growth, we further cloned a whole PTN sense or anti-sense sequencing DNA into the multiple myeloma cell lines RPMI 8226 and U266. Cells transduced with sense PTN showed markedly increased proliferation compared to cells transduced with antisense or vector alone. The role of PTN in hematological malignancies has not been previously defined. Due to the restricted expression pattern of PTN in adults, PTN is suggested as a potential new target for treatment of multiple myeloma. Further investigations are defining the prognostic value of PTN serum levels and the mechanism by which this cytokine drives myeloma growth.

P0.914

EVALUATION OF HEPATOCYTE GROWTH FACTOR AND ENDOSTATIN IN THE BONE MARROW OF PATIENTS WITH MULTIPLE MYELOMA AND THE EFFECT OF PERIPHERAL BLOOD ADMIXTURE

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Background. Angiogenesis plays a key role in a myeloma pathogenesis. It is known a lot of factors which have positive or negative influence on angiogenesis. Key positive regulator of angiogenesis in myeloma is hepatocytar growth factor (HGF) which is produced mainly by bone marrow. But the clinical importance of its levels remains still unclear. One of the most important inhibitors of angiogenesis is endostatin which is produced in parenchymatous organ mainly but its importance in myeloma was not described in detaily yet. The aim our study was to evaluate the concentration HGF and endostatin – during sampling of large bone marrow volumes in patients with multiple myeloma.

Methods and results. We have done sternal puncture in 15 myeloma patients with sampling of 45 ml of bone marrow (BM) and than we have measured HGF and endostatin concentrations were in the 1st ml, the 5th ml, the 19th ml, the 30th ml, and the 45th ml of the sample. The concentrations were measured by ELISA method. Average concentration in different phases are in Figure 1,2. The concentration of HGF was significant highest in the first ml of sampling. Endostatin concentrations did not change significantly during the procedure of sampling.

Conclusion. The concentration of HGF was highest in the first ml of sampling, what can be caused by admixture of the peripheral blood. This could be partly responsible for conflicting results of studies of HGF in MM. Endostatin concentration were not significantly different in during the sampling of BM. Production of endostatin in parenchymatous organs can be a reason that admixture of peripheral blood has no significant influence on endostatin concentration.

Figure 1.

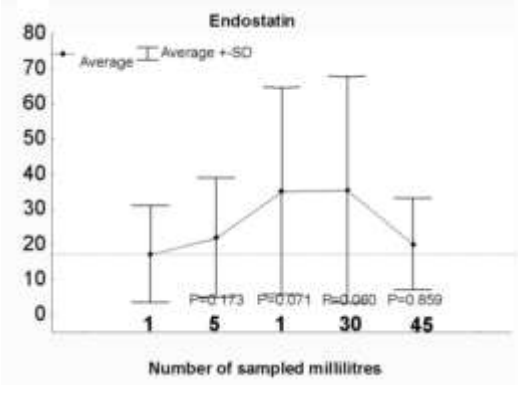
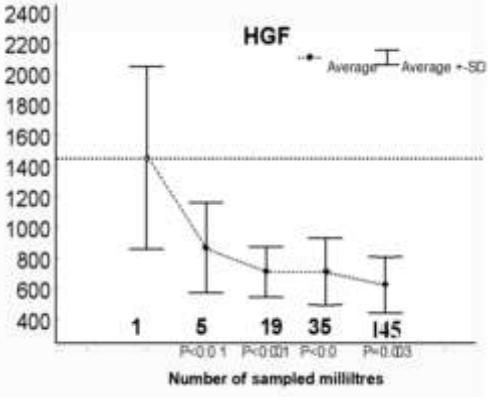


Figure 2.



P0.915

EXPRESSION OF C-MET RECEPTOR IN BONE MARROW OF MULTIPLE MYELOMA PATIENTS FOLLOWING CHEMOTHERAPY

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Despite that the new advances have been made in traditional treatment of multiple myeloma (MM) and high-dose chemotherapy followed by hematopoietic stem cells transplantation has become more wildly use, MM is still incurable disease that accounts for 1% of all cancers and almost 10% of hematological tumors. Because MM is often resistant to conventional therapies the new treatment strategies are necessary. The presence of elevated HGF (Hepatocytic Grow Factor) expression has been well documented in multiple myeloma. The c-met oncogene has been showed to be present in MM cell lines at mRNA and protein level. The most recent data suggests that HGF-c-met axis can be responsible for proliferation and inhibition of apoptosis in MM cells. In this study we looked for the expression of c-met receptor in paraffin section of patients (pts) with MM, before and after antitumour therapy. We have analyzed sample from 10 patients with (MM). This material included samples collected at diagnosis without any prior medical intervention, and after treatment. Five (50%) 5 of these pts responded well and 5 (50%) poorly to therapy employed. All 10 pts were c-met positive before therapy. Bone marrow cellularity of patients who responded well was 76% before (range: 10%-100%) and 46% after treatment (range: 40%-60%). In this group plasmocytes infiltration of bone marrow consisted 59% before (range: 10%-80%) and 9% after chemotherapy (range: 0%-20%). Three (60%) of them had undetectable c-met positive cells among bone marrow cells after treatment. In poor responders group cellularity of bone marrow was 40% (range: 20%-70%) before treatment and 46% (range: 20%-70%) after therapy. Plasmocytes consisted 20% (range: 10%-50%) of bone marrow cells before and 44% (range: 10%-90%) after treatment. All patients in this group had cells positive for c-met receptor after therapeutic regiment. There are published data stating that patients with MM have elevated levels of HGF in comparison to control group. It has been also shown that in patients who do not respond well to treatment this elevated level of HGF continuos. On the other hand level of HGF decreases in patients who respond well to the therapy. In our experiments we have found that also level of c-met expression after treatment correlates with response to the therapy. Patients who respond well had substantially decreased number of c-met positive plasmocytes after chemotherapy on comparison to patients who didn't respond. Based on this data we postulate that c-met receptor could a potential target for therapy in patients who do not respond to first line treatment.

P0.916

THE SEMINAL PLASMA PROTEIN, SEMENOGELIN: A FUNCTIONAL PROGRESSION MARKER OF MULTIPLE MYELOMA

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Multiple myeloma (MM) is a malignancy of plasma cells which localizes to bone marrow. In its terminal phases MM becomes drug-resistant and grows in extramedullary locations. Defining mechanisms that mediate both extramedullary growth and drug-resistance could therefore improve treatment. Recently adhesion to the extracellular matrix protein fibronectin has been shown to generate anti-apoptotic signals imparting drug-resistance in several tumors. Using RT-PCR we identified the fibronectin-binding protein, semenogelin (Sg), in MM cell lines and biopsies derived from extramedullary locations, but not from bone-marrow, indicating Sg may correlate with progression. Comparing sister cell lines KMS-12BM and KMS-12PE, derived from bone marrow and pleural effusion of the same patient, we observed that only KMS-12PE cells expressed Sg, and were dramatically more resistant to mitoxanthrone-induced apoptosis. Sg coagulates semen by binding fibronectin with high affinity and is a target for transglutaminases, enzymes which modify fibronectin to expose functional sites. We hypothesized that Sg expression may increase drug resistance and promote extramedullary growth through generation of a provisional fibronectin matrix on the cell surface. In support we demonstrated by confocal microscopy and mass spectrometry that Sg localizes to signaling domains called lipid rafts, where it associates with fibronectin. Future research will ask whether Sg can assist fibronectin assembly at the surface thereby increasing survival in extramedullary locations and drug-resistance.

P0.917

A NEW INSIGHT INTO PATHOGENESIS OF HEMATOPOIESIS DISTURBANCES IN MULTIPLE MYELOMA

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Bone marrow (b. m.) aspirates obtained from 50 patients (pts) with multiple myeloma (MM) were evaluated by a one step AgNOR technique. The mean number of nucleoli and the mean number of AgNORs in plasma cells (Pc), lymphocytes (lf), pronormoblasts and basophilic normoblasts (Er-I), polychromatic normoblasts (Er-II), promyelocytes (Pm), myelocytes (Mc), monocytes (Mn), and megakaryocytes (Mg) was attained from analysis of at least 100 cells of each type. Similar data from 12 previously healthy subjects were used as a control (C). Induced and basic levels of interleukin (IL) 6, IL-4, and IL-2 in the cells tested were determined in parallel. AgNOR count difference among tested groups was estimated with the Student's test or with an analysis of varience (ANOVA). The association between AgNOR count and other quantitative variables was tested on a linear regression model. Significance was set at $p<0.05$ for all tests. Compared with C the mean number of nucleoli in MM pts did not differ. At the same time the mean number of AgNORs in haematopoietic cells from pts with MM was higher in Er-I, Er-II, and Pm. There were many positive correlations between nucleoli and AgNOR counts in Pc, Lf and those in Er-I, Er-II, and Mc. On the contrary, the relationship between AgNOR number in Pc and Lf and that in Mg was negative (r=-0.72, p=0.001 and r=-0.36, p=0.045, respectively). Additionally the percentage of Pc in b. m. correlated positively with AgNOR numbers of Pc (n=40; r=0.35, p=0.024) but negatively with those in Mg (n=22; r=-0.36, p=0.048). The level of serum IL-6 associated greatly with the mean AgNOR numbers in Pc, Lf, Er-I, Er-II, and Pm, whereas its relationship with AgNOR numbers was inverse in Mg (n=11; r=-0.61, p=0.022). The mean AgNOR number in Mg associated positively with the serum level of IL-2 and IL-4 (n=11;

r=0.65, *p*=0.014 and n=8; r=0.92, *p*=0.001, respectively). The serum IL-2 revealed negative correlations with AgNOR numbers in Pc (n=21; r=-0.42, *p*=0.03) and Er-I (n=21:r=-0.42, *p*=0.03) whereas the serum level of IL-4 associated negatively with the mean number of AgNORs in Pm (n=14; r=-0.58, *p*=0.015) and Mc (n=11; r=-0.55, *p*=0.042). Taken together these findings show that AgNOR numbers in erythroid and granulocytic b. m. precursors increased in parallel with those of Pc and Lf whereas those in Mg declined. On the basis of recently published data the different reactions of erythroid/myeloid and Mg precursors onto various factors of Pc, Lf as well microenvironment elements may be explained hypothetically by overexpression of vessel endothelial growth factor and/or a modulation of protein kinase Cα.

POSTER SESSION 10: SIGNAL TRANSDUCTION AND MECHANISMS OF DRUG RESISTANCE

P0.1001

AKT SIGNALING IS DEPENDENT ON CD45 EXPRESSION IN MYELOMA CELLS

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In Multiple Myeloma, the Akt/PI-3Kinase pathway is involved in the proliferation of myeloma cells. In the current study, we have investigated the impact of the CD45 phosphatase in the control of Akt/PI-3Kinase activation. We selected eight human myeloma cell lines to analyze Akt activation in response to IGF-1, 3 expressing CD45 on 100% of cells (XG-1, XG-6, MDN), one expressing CD45+ on a majority of cells (> 90%) (U266) and 4 lacking CD45 expression (LP-1, NCI-H929, BCN, L363). We show that Thr308 and Ser473 Akt phosphorylation (Akt-P) in response to IGF-1 is highly variable from one myeloma cell line to another. Actually, Akt activation is highly related to whether CD45 is expressed or not. Indeed, both the magnitude and the duration of Akt phosphorylation in response to IGF-1 are more important in CD45- than in CD45+ myeloma cell lines. Pervanadate treatment dramatically increases Akt-P in CD45+HMCL whereas it has no effect in CD45- HMCL. Finally, IL-6 induced CD45 expression in LP-1 on 50% of cells. On these LP-1 CD45+we observed a reduction of both Thr308 and Ser473 Akt phosphorylation by 50% and 25% respectively and Erk-P was also reduced by 32 %. By coimmunoprecipitation, we next demonstrate a physical association between CD45 and IGF-1 receptor suggesting that CD45 could be involved in the dephosphorylation of the IGF-1 receptor. Furthermore, the growth of CD45- myeloma cell lines is mainly or even totally controlled by the PI-3Kinase pathway whereas that of CD45+myeloma cell lines is modestly controlled by it. Indeed, Wortmaninn a specific PI-3kinase inhibitor induced a dramatic growth inhibition of CD45- myeloma cell line (70%, 85% and 75% for LP-1, BCN and NCI-H929 respectively) whereas the inhibition was weaker in CD45+cell lines (21%, 25% and 25% for XG-6, XG-1 and U266 respectively) . The growth inhibition of CD45- myeloma cells is characterized by a G1 growth arrest, a reduction of Bcl-2 expression and an increase of p27(kip1). Altogether, these results suggest that CD45 negatively regulates IGF-1-dependent activation of PI-3Kinase. Thus, strategies that block IGF-1R signaling and consequently the Akt/PI-3Kinase pathway could be a priority in the treatment of patients with multiple myeloma, especially those lacking CD45 expression which have a very poor clinical outcome.

P0.1002

PKC INHIBITION SUPPRESSES PI3K AKT SIGNALING IN MYELOMA CELLS

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PTEN, a cellular phosphatase involved in the regulation of phosphatidylinositol phosphates (PIP s), is mutated in a number of myeloma cell lines. In the absence of gene mutations, phosphorylation of serine and threonine residues in the PTEN C-terminal domain also results in loss of its activity and stability. PIP s are required for phosphorylation of Akt, a key event leading to cellular proliferation and inhibition of apoptosis. Loss of PTEN function results in a failure to de-phosphorylate PIPs and a corresponding increase in Akt kinase activity. We have recently reported that PKCδ inhibition with Rottlerin (3 μM) induces cell death in sensitive and resistant myeloma cell lines (MM1S, MM1R, 8226S and U266) (Blood 2002,100,11,393a). Rottlerin treatment inhibits Thr-505 PKCδ phosphorylation and, more importantly it blocks constitutive as well as IGF-1 induced phosphorylation of Akt abrogating its kinase activity. Furthermore Rottlerin also suppresses the phosphorylation of FKHR, GSK 3αβ and Bad, downstream substrates of Akt, triggerring activation of the intrinsic apoptotic pathway with loss of the mitochondrial membrane potential (Δψ) and cleavage of caspases 9, 3 and PARP. The caspase inhibitor ZVAD-fmk (100 μM) nearly completely reversed Rottlerin induced cell death. Additionally, PKCδ inhibition also suppressed, upstream of Akt, ser 241-PDK1 phosphorylation by PIP's. These findings led us to investigate the PTEN status in human myeloma cell lines (MM1R, 8226S and U266). While normal PTEN expression was detected in these cell lines, PTEN was universally phosphorylated on ser 380 in its C-terminal domain, resulting in loss of its activity. Rottlerin completely suppressed the phosphorylation of this serine residue, restoring PTEN function and dephosphorylating PIP s. In order to verify that Rottlerin exhibited its effects by inhibiting PKCδ, we first stably overexpressed PKCδ in the 8226S cells. PKCδ overexpression partially protected these cells against Rotttlerin cytotoxicity. RNAi-mediated PKCdelta depletion also prevented IGF-1 induced AKT activation. Finally, we examined the effect of Rottlerin on the viability of bone marrow mononuclear cells isolated from patients with relapsed refractory myeloma (n=4). Treatment with Rottlerin (3δ M) for 48 hours induced cell death in 65% of CD138 high cells while having no significant effect on the viability of non-myeloma or CD138 low cells. Similarly, in CFU analysis and compared to control or DMSO vehicle, treatment with Rottlerin had no significant effect on BFU-E and CFU-GM colony formation. In summary, our work provides evidence that PKCδ inhibition restores PTEN function in wild type PTEN expressing myeloma cells, resulting in signaling blockade through the PI3K/AKT cascades and leading to caspase dependent cell death. Our findings reveal PKCδ as a novel biological target for the treatment of multiple myeloma.

P0.1003

HEPOTOCYTE GROWTH FACTOR INDUCES VLA-4-DEPENDENT CELL ADHESION WITHOUT INCREASING VLA-4 EXPRESSION OR CONVERTING VLA-4 TO A HIGH AFFINITY STATE

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Multiple myeloma (MM) is characterized by an accumulation of malignant plasma cells in the bone marrow (BM). Inside the BM, adhesion of myeloma cells to extracellular matrix proteins like fibronectin promotes cell survival and induces drug resistance. Integrins are cell surface adhesion molecules that are often expressed in an inactive form. Cytokines may activate integrins to increase their interaction with extracellular ligands. In this work we examined the effect of hepatocyte growth factor (HGF) on myeloma cell adhesion to fibronectin and the signaling pathways involved. Adhesion experiments were performed with the human myeloma cell line INA-6 and primary MM cells. The HGF signaling pathway and effects on integrins were studied. We found that HGF stimulated myeloma cell adhesion to fibronectin, for INA-6 cells the adhesion increased 6-fold compared to control. Cell proliferation was increased for cells grown on fibronectin compared to cells grown on BSA, when assessed by use of 3H-thymidin incorporation assay. HGF-stimulated adhesion was dependent on the VLA-4 integrin, as neutralizing antibodies toward α4-integrin reduced cell adhesion to control level. By use of flow cytometry we found that HGF did not increase the expression of VLA-4 on the cell surface. Furthermore, in contrast to manganese iones, another stimulus causing VLA-4- dependent adhesion to fibronectin, HGF did not convert the integrin to a high affinity state. Cell adhesion was reduced to control level by PI3K blockers while inhibitors of MAPK had no effect, indicating that HGF-stimulated adhesion was dependent on the PI3K pathway, but not on the MAPK pathway. The signaling pathway downstream of PI3K did not involve mTOR as rapamycin had no effect on cell adhesion. Surprisingly, a significant increase in cell adhesion was found by use of the AKT inhibitors SH-5 and SH-6. Furthermore, inhibition of adhesion by the IκB kinase inhibitor PS-1145, suggests that NF-κB may be involved in HGF-stimulated adhesion. Our work points to HGF as a pro-adhesive factor in cell adherence to the BM matrix protein fibronectin, an event known to promote cancer cell survival and drug resistance. Inhibiting HGF, its receptor c-met or the VLA-4 integrin may be beneficial to the myeloma patient.

P0.1004

NOVEL PURINE ANALOG, 8-AMINO-ADENOSINE, INDUCES LOSS OF PHOSPHORYLATION OF KEY SIGNALING MOLECULES IN MULTIPLE MYELOMA CELLS

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Multiple Myeloma (MM) is a slowly proliferating B-cell malignancy, which accumulates apoptosis-resistant and replication-quiescent cell populations. This poses a challenge for current chemotherapeutics, which target rapidly replicating cells, and myeloma remains an incurable disease in need of new therapeutic approaches. The nucleoside analog, 8-amino-adenosine (8-NH_2-Ado), exhibits potent activity in pre-clinical studies, inducing apoptosis in several MM cell lines. This cytotoxic effect requires phosphorylation of 8-NH_2-Ado to its tri-phosphate form, 8-NH_2-ATP, and results in a concomitant loss of endogenous ATP levels. A novel effect of 8-NH_2-Ado on the phosphorylation status of key cellular signaling molecules is reported here. MM cells treated with 8-NH_2-Ado exhibit a dramatic loss of phosphorylation of several important signaling proteins, including ERK1/2, p38 MAPK and Akt kinase within 30-120 minutes. In addition, pro-caspase 8 and pro-caspase 9 cleavage and activation, and cleavage of the universal caspase substrate, poly (ADP-ribose) polymerase (PARP) occurs in these cells following the loss of phosphorylation of the signaling kinases. This temporal relationship provides evidence for the significance of the de-phosphorylation events in 8-NH_2-Ado-induced apoptosis. Cells depleted of ATP by antimycin A or 2-deoxyglucose treatment to mimic 8-NH_2-Ado-induced ATP depletion do not exhibit the same decrease in phosphorylation of vital cellular proteins. Therefore, the significant shifts in endogenous ATP pools caused by 8-NH_2-Ado treatment cannot account for the changes in phosphorylation levels. Instead, 8-NH_2-Ado may be affecting the activity of protein phosphatases such as PP2A, which are involved in the negative regulation of these signaling molecules. Since inhibition of phosphatase activity with okadaic acid partially blunts the 8-NH_2-Ado-induced decrease in phosphorylation, we hypothesize that 8-NH_2-Ado-mediated activation of phosphatases may contribute to the mechanism of drug action. The distinctive effect of 8-NH_2-Ado on the phosphorylation status of cellular proteins is a novel phenomenon for a nucleoside analog drug and is unique to 8-NH2-Ado among this class of drugs. The kinetics of 8-NH2-Ado-mediated changes in phosphorylation levels of critical pro-survival and apoptosis-regulating proteins suggest that the modulation of these proteins by de-phosphorylation at early time-points may be an important mechanistic step in 8-NH_2-Ado-induced programmed cell death.

P0.1005

PROTEOMIC ANALYSIS OF MULTIPLE MYELOMA IDENTIFIES POTENTIAL TARGETS FOR DRUG THERAPY

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The objective of this study was to investigate the underlying molecular alteration in multiple myeloma at the protein level in order to identify regulators of pathogenesis, discover novel targets of therapy, and compare genetic and proteomic alterations. We employed antibody protein microarrays (BD Clonetech, CA) to measure changes in the patterns of protein expression between MM and normal plasma cells. The antibody array is a new technique enabling protein differences to be assayed directly by hybridizing fluorescently labeled protein mixtures from cell extracts onto glass slides spotted with 512 different monoclonal antibodies. CD138+purified plasma cells were obtained from cryopreserved bone marrow samples of 12 newly diagnosed patients with MM. The labeling index was high (1% cutoff) in 7 samples and low in the other 5. Control plasma cells

were obtained from 9 pooled CD138+purified normal donor bone marrow plasma cells. Interphase FISH analysis for 17p deletion, 13q deletion, and t(11:14) were performed. To assess differential expression, the mean of the ratios of Cy5/Cy3 for each sample were analyzed using the Clontech software to calculate an internally normalized ratio. The normalized data were analyzed by the Genespring software. Unsupervised clustering identified 4 groups of MM. Changes of protein expression ≥2 fold in 70% of the samples as compared to control were identified. There were 6 proteins differentially expressed between all MM samples and control cells including proteins in the ras signaling pathway (KSR-1), the ubiquitin pathway (Ubc-H6), cyclin-dependent kinases (CDK4), cytokines (IL-6), DNA toposisomerase II, and the rho-interacting serine-threonine kinase CRIK. Proteins differentially expressed in MM groups 1 and 2 compared to normal control included cell cycle regulators (cul-2, MCM6, PCNA, TGFβ1), kinases (p70S6K, PKC), and chromatin regulators (Ran, AKAP450, Rad50). Protiens differentially expressed in MM group 3 included cell cycle regulators (CDK2, CLK1, MENA), apoptosis regulators (XIAP, caspase 4, perforin) kinases (IKKa and RAC1 in the Wnt signaling pathway) and P53 regulators, while proteins identified in MM group 4 included NFkB/ubiquitin proteins (IKKa and Ubch6), cell cycle regulators (c-myc, CDK4), p53 pathway proteins (53bp2), ras-signaling proteins (KSR1), and the kinase CRIK. There were no differences in protein expression between the high and low labeling index groups. 13q was identified in 5 (42%), 17 p in 1(8%) and t(11:14) in 1(8%) patients. 80% of the 13 q deletion cases clustered in MM group 1 and 2 patients. Cyclin D-1 was upregulated in 5 (42%) patients including the patient with (11:14) translocation. This is the first proteomic study of patients with MM. The results are consistent with previously identified genetic alterations in MM indicating that this novel technique could be used in identifying molecular changes in MM. It identifies novel proteins dysregulated in MM that differ between the 4 MM groups. These results may be used in the future to individualize therapy based on the proteins dysregulated in each group. For example, IKK inhibitors may be useful in group 3 MM patients, while mTOR inhibitors (upstream of p70S6K) could be used in groups 1 and 2 patients. Future correlations with gene expression arrays and prognosis in a larger cohort of patients is warranted. Supported in part by MMRF.

P0.1006

PROTEASOMAL DEGRADATION OF TOPOISOMERASE I IS PRECEDED BY JNK ACTIVATION, FAS UP-REGULATION AND PARP CLEAVAGE IN SN38-MEDIATED CYTOTOXICITY AGAINST MULTIPLE MYELOMA

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Topoisomerase I inhibitors are effective anti-cancer therapies, and have shown activity in hematological malignancies. Here we show for the first time that SN38, the potent active metabolite of irinotecan, induces JNK activation, Fas up-regulation, and caspase 8 mediated apoptosis in multiple myeloma (MM) cells. Proteasomal degradation of nuclear topoisomerase I has been proposed as a resistance mechanism in solid malignancies. SN38-induced proteasomal degradation of topoisomerase I was observed during SN38-mediated cytotoxicity against MM.1S myeloma cell line, but occurred after JNK activation, Fas up-regulation and PARP cleavage, and failed to protect cells from apoptosis. Differential toxicity was observed against MM cells versus bone marrow stromal cells (BMSCs), and SN38 inhibited adhesion-induced up-regulation of MM cell proliferation when MM cells adhere to BMSCs. In addition, SN38 directly inhibited constitutive and inducible IL-6 and VEGF secretion by BMSCs. Synergy was observed when SN38 was used in combination with doxorubicin, bortezomib, as well as poly-(ADP ribose) polymerase (PARP) inhibitor NU1025 and Fas-activator CH11. These findings have clinical significance because identification of downstream apoptotic signaling following topoisomerase I inhibition will both elucidate mechanisms of resistance and optimize future combination chemotherapy against MM.

P0.1007

PKC412 INHIBITS C-FOS TRANSCRIPTION AND INDUCES APOPTOSIS IN HUMAN MULTIPLE MYELOMA CELL LINES

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PKC412 is a benzoylstaurosporine that inhibits several kinases including Protein Kinase C (PKC) and has been shown to have anti-tumour activity in a variety of *in vitro* and animal models. We have now undertaken an *in vitro* analysis of PKC412 activity against multiple myeloma. Authentic human multiple myeloma cell lines (HMCL) were treated with a range of PKC412 concentrations (0.1microM to 20microM) for 24 and 72 hours and evaluated with an MTS assay to generate dose response curves. Most HMCLs demonstrated anti-proliferative effects with concentrations of ≥0.5microM by 72 hours. Furthermore, variations in HMCL sensitivity were clearly evident at concentrations of ≥5microM with 4 HMCLs being very sensitive to 5microM (OPM2, NCI-H929, KMS-11 and RPMI-8226) with cell viabilities of 2.05%, 3.45%, 28.88% and 14.93%, respectively, and 2 less so (U266 and LP-1) with cell viabilities of 49.21% and 41.99%, respectively. Three HMCLs treated for 72 hours with 1microM PKC412 were analysed by flow cytometry to determine levels of apoptosis using Annexin V and propidium iodide staining. All 3 (OPM2, NCI-H929 and U266) showed an increased percentage of apoptotic cells (52%, 45% and 31%, respectively, compared to untreated controls) that correlated with the viabilities shown by MTS assay. To determine whether HMCL PKC activity was inhibited by PKC412 the cells were pre-treated with Phorbol 12-Myristate 13-Acetate (PMA) and PKC activity was measured using the PepTag® Assay. After 24 and 72 hours PKC412 treatment, PKC activity was decreased in all HMCL tested. Since PKC412 preferentially inhibits the conventional isoforms of PKC we investigated the expression of these isoforms in HMCL by western blot but no relationship was seen between isoform expression and the level of sensitivity to PKC412. However, it was noted that the more PKC412-sensitive HMCL harbour an activating mutation of either *ras* (NCI-H929 and RPMI-8226) or FGFR3 (OPM2 and KMS-11) whereas the less sensitive HMCL (U266 and LP-1) do not. This suggests that sensitivity to PKC412 may be due to activity against common downstream effectors of both the *ras* and FGFR3 signalling pathways. Both pathways act in part through Mitogen-Activated Protein Kinase (MAP kinase) cascades where Extracellular Signal-Regulated Protein Kinase (ERK) 1/2 phosphorylation leads to increased transcription of the proto-oncogene *c-fos*. Semi-quantitative RT-PCR of 2 HMCL (NCI-H929 and OPM2) demonstrated

that baseline *c-fos* transcription was inhibited by 10microM PKC412 after 4 and 20 hours treatment following an initial increase at 30 minutes. Furthermore, pre-treatment with 10microM PKC412 prevented PMA induced stimulation of *c-fos* transcription. Our results suggest that PKC412 may specifically target HMCL harbouring *ras* or FGFR3 activating mutations possibly via modulation of *c-fos* transcription. Further investigation of PKC412 as a treatment for multiple myeloma is warranted.

P0.1008

THE MEK/ERK MODULE IS A POTENTIAL THERAPEUTIC TARGET IN MULTIPLE MYELOMA

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The IL-6R/STAT3 pathway contributes to the pathogenesis of multiple myeloma (MM) and protects MM cells from apoptosis. However, we could show that MM cells survive IL-6R blockade if they are cocultured with bone marrow stromal cells (BMSCs), suggesting that the BM microenvironment stimulates IL-6R-independent pathways that exert a pro-survival effect. Because the MAP kinases ERK1,2 appear strongly activated in our coculture systems, we analyzed the importance of the MEK1,2/ERK1,2 (MAPK) module for proliferation and survival of MM cells. Human MM cells (IL-6-dependent MM cell lines INA-6 and ANBL-6 or primary MM cells) were grown either with or without BMSCs, and with or without blockade of IL-6R/STAT3 signaling. The MEK/ERK module was targeted through two different experimental approaches: pharmacological inhibition of MEK1,2 by PD98059 or downregulation of ERK1,2 expression through highly specific RNA interference. Furthermore, we have evaluated various BM-derived growth factors for their ability to stimulate the MEK/ERK module (in the IL-6-independent MM cell line MM.1s) and to promote the survival of primary MM cells. Abolition of MEK1,2 activity with PD98059, or of ERK1,2 activity through siRNA constructs, was insufficient to induce apoptosis. However, the combined disruption of the IL-6R/STAT3 and MEK1,2/ERK1,2 pathways led to strong induction of apoptosis even in the presence of BMSCs. This effect was observed with MM cell lines and with primary MM cells. Pathway analysis revealed that BMSCs stimulate STAT3 via the IL-6R, and MAPK in parts via IL-6R-independent mechanisms. We could furthermore identify a number of BM-derived cytokines namely, LIF, VEGF, bFGF, MIP-1α, SDF-1α, IL-1β, SCF and IL-3 that in addition to IL-6 redundantly activate ERK1,2 in MM1.s cells, and support survival of primary MM cells. We provide experimental evidence that the MEK/ERK module in addition to its previously-described role in cell proliferation can critically influence the survival of MM cells. The signaling activity of the MEK/ERK module is strongly influenced by the BM microenvironment. We could demonstrate that the BM-mediated activation of MEK/ERK and the IL-6R/STAT3 pathways independently contributes to the protection of MM from apoptosis. Concomitant inhibition of IL-6R-dependent STAT3 and IL-6R-independent ERK activity efficiently induce apoptosis of MM cells. This implicates that for the development of a well directed therapeutic strategy only the combined targeting of different and independently activated signal transduction pathways will be sufficient.

P0.1009

ACTIVE FORMS OF SERUM- AND GLUCOCORTICOID-INDUCED KINASE-1 ARE EXPRESSED ONLY DURING MITOSIS IN MYELOMA CELLS

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Serum- and glucocorticoid-induced kinase (*sgk*) is an early response gene that is expressed when cells are stimulated by serum or glucocorticoids. The corresponding protein, SGK1, is a serine/threonine kinase with close homology to Akt (54% identity in the catalytic domain). The function of SGK1 is still obscure. Several reports suggest that it is involved in regulation of ion channels, and recent reports demonstrate a role for SGK1 in cell proliferation and anti-apoptosis. In gene expression profiling studies of two IL-6-dependent myeloma cell lines stimulated with or without IL-6, IL-21 or TNF, we found that *sgk* expression was induced in both cell lines by all of the mitogenic cytokines. Expression of *sgk* mRNA was confirmed by RT-PCR. By immunoblots, we demonstrated that SGK1 protein expression was induced after stimulation with the same mitogenic cytokines. SGK1 contains at least three phosphorylation sites; ser-78, thr-256 and ser-422. Thr-256 is located in the catalytic domain and phosphorylation of this residue is necessary for catalytic activity. We studied expression of SGK1 in myeloma cells with confocal microscopy after staining with antibodies against total SGK1 and against the three phospho-epitopes. Expression of total SGK1 was strongest in the microtubule-organizing center (MTOC) of cells in interphase. However, neither of the antibodies against phosphorylated SGK1 stained MTOC of interphase cells. Two different antibodies against thr-256 did not stain cells in interphase, but showed strong staining in mitotic cells from prophase through metaphase. This exclusive staining of mitotic cells was seen in several myeloma cell lines as well in primary myeloma cells. In prophase, thr-256 staining was strongest in the centrosomes, where it co-localized with gamma-tubulin, and in the area of the disappearing nucleolemma. In metaphase, thr-256 staining was strong in the centrosomes and at the telomeric end of each chromosome. The two other phospho-epitopes also localized to the centrosomes during mitosis, but did not bind antibodies at the chromosome ends. Interestingly, ser-422 showed strongest staining at the midbody of dividing cells during anaphase and cytokinesis. In conclusion, induction of *sgk* by mitogenic cytokines and the exclusive localization of active forms of the kinase in dividing cells point to an important function of this enzyme during mitosis of myeloma cells.

P0.1010

CAVEOLIN-1 IS REQUIRED FOR VASCULAR ENDOTHELIAL GROWTH FACTOR-TRIGGERED MULTIPLE MYELOMA CELL MIGRATION AND IS TARGETED BY BORTEZOMIB (VELCADE®)

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We recently demonstrated that caveolae, vesicular flask-shaped invaginations of the plasma membrane represent novel therapeutic targets in multiple myeloma (MM). In the present study, we demonstrate that vascular endothelial growth factor (VEGF) triggers Src-dependent phosphorylation of caveolin-1, which is required for $p130^{Cas}$ phosphorylation and MM cell migration. Conversely, depletion of caveolin-1 by antisense methodology abrogates $p130^{Cas}$ phosphorylation and VEGF-triggered MM cell migration. The proteasome inhibitor bortezomib, both inhibited VEGF-triggered caveolin-1 phosphorylation and markedly decreased caveolin-1 expression. Consequently, bortezomib inhibited VEGF- induced MM cell migration. Bortezomib also decreased VEGF secretion in the bone marrow microenvironment and inhibited VEGF-triggered tyrosine phosphorylation of caveolin-1, migration, and survival in HUVECs. Taken together, these studies demonstrate the requirement of caveolae for VEGF-triggered MM cell migration and identify caveolin-1 in MM cells and HUVECs as a molecular target of bortezomib.

P0.1011

ECTOPIC AND INTERFERON-INDUCED EXPRESSION OF FAS OVERCOMES RESISTANCE TO FAS-MEDIATED APOPTOSIS IN MULTIPLE MYELOMA CELLS

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Increased survival *in vitro* as a result of anti-apoptotic signals is a hallmark of multiple myeloma (MM) cells, implying that a therapeutic potential may lie in circumventing such signals. We have previously reported that interferons (IFNs) sensitize cells of the U-266-1970 MM cell line for Fas/CD95-mediated apoptosis (Spets,1998). In the present study, we explore the mechanism underlying this effect. First, we investigated IFN-induced regulation of candidate genes involved in apoptosis. In a wide screening of apoptosis-related genes, Apo2L/TRAIL and Fas were identified as IFN-targets. Blocking Apo2L/TRAIL had no apparent effect on IFN-induced sensitization of Fas-mediated apoptosis, suggesting that the Apo2L/TRAIL up-regulation may not be directly involved in this process In contrast, although U-266-1970 cells already express a considerable basal level of Fas, we found that an increased level of Fas expression *per se* sensitizes U-266-1970 cells to Fas-mediated apoptosis. This was further supported by the finding that IFN-treatment enhanced Fas-mediated caspase-8 activation, one of the earliest signaling events down-stream receptor activation. In addition, we found that IFN treatment attenuated the IL-6 dependent activation of Stat3, interfering with a known survival-pathway in MM that has previously been linked with resistance to Fas-mediated apoptosis. Taken together, our results show that IFN-induced up-regulation of Fas sensitizes MM cells to Fas-mediated apoptosis and suggest that attenuation of Stat3 activation may be a potentially important event in this process.

P0.1012

TRAIL RECEPTOR EXPRESSION IN MULTIPLE MYELOMA CELLS; COULD A DR5 AGONIST OFFER NEW THERAPEUTIC POSSIBILITIES?

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TRAIL (TNF-related apoptosis-inducing ligand) is produced by activated T cells and has been shown to induce myeloma cell apoptosis *in vitro* and to have specific anti-tumour activity *in vivo*. It binds to four membrane-bound receptors, inducing apoptosis by acting through DR4 and DR5, which have cytoplasmic death domains, but also binding to DcR1 and DcR2, decoy receptors lacking death domains. In addition OPG, another member of the TNF-related superfamily, is known to bind TRAIL and may act as a soluble decoy receptor. In support of this, we have previously shown that OPG produced by cells of the bone marrow microenvironment can protect myeloma cells against TRAIL-induced apoptosis, suggesting that OPG may function as a paracrine survival factor in the bone marrow microenvironment. The aim of our study was to investigate the expression of TRAIL receptors by myeloma cells and the effect of an agonist antibody to DR5. Previous studies have failed to show a link between receptor expression and susceptibility to TRAIL-induced apoptosis; however these studies used a variety of methods to analyse expression. Using RT-PCR we found that both the human myeloma cell lines NCI H929 and RPMI 8226 express all four receptors. However, using FACS analysis, we showed that NCI cells express only DR4 and DR5 on the cell surface, but not DcR1 or DcR2, while RPMI cells exhibit cell surface expression of DR4, DR5 and DcR2. Cell surface expression of these receptors was not affected by treatment with rhOPG or with conditioned medium from osteoblast-like cells in either cell line. In contrast the myeloma cell line JJN-3, a human plasma cell leukaemia line, does not express DR4 or DR5 on the cell surface and is not responsive to TRAIL. In NCI H929 and RPMI 8226 cell lines, we have previously shown that rhTRAIL induces apoptosis in a dose-dependent manner (up to 50% at 50 ng/mL) and that this effect can be blocked by the addition of rhOPG. As TRAIL is known to act through the receptor DR5, binding to this receptor by other agonists could also induce apoptosis. We aimed to investigate whether a DR5 agonist could have anti-tumour activity *in vitro*. We treated myeloma cells with an agonist antibody to DR5 and found that at 72 hours there was a dose-dependent increase in apoptosis, up to a maximum of three-fold over control in NCI H929 cells and 2.5-fold in RPMI 8226 cells. A concomitant decrease in cell number in the cultures was observed over the same time period, with cell numbers reduced to 20 to 30% of control at 72 hours. In contrast to the apoptotic effect of TRAIL, the induction of apoptosis by the DR5 agonistic antibody could not be blocked by conditioned medium from osteoblast-like cells. TRAIL has been proposed as a potential anti-tumour therapy but within the confines of the bone marrow microenvironment OPG produced by osteoblastic cells may interfere with the action of TRAIL on multiple myeloma. A specific agonist antibody to an active apoptosis-inducing TRAIL receptor would not be subject to this inhibition and thus offers an alternative possibility for specific anti-myeloma therapy.

P0.1013

MULTIPLE MYELOMA CELLS UNDERGO TRAIL-INDUCED APOPTOSIS VIA TRAIL-R1 AND ARE PROTECTED BY THE BONE MARROW MICROENVIRONMENT

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TNF-alpha related apoptosis inducing ligand (Apo2L/

TRAIL) is a member of the TNF family and has unique potential as an anti-tumour agent. We have previously demonstrated that TRAIL induces apoptosis of primary multiple myeloma (MM) cells. Despite the efficacy exhibited by TRAIL in human myeloma cell lines (HMCL) there was a modest effect elicited on the primary MM cells. In this study we endeavoured to elucidate the mechanisms of TRAIL-induced apoptosis in HMCL and the mechanisms through which primary MM are protected from TRAIL. Four authentic HMCL, RPMI-8226, LP-I, U266 and NCI-H929 which express both TRAIL-R1 and TRAIL-R2 were studied. TRAIL-sensitive (RPMI-8226) and resistant (U266) HMCL were treated with LZ-TRAIL, and agonist antibodies to TRAIL-R1 (TRM-1) and TRAIL-R2 (TRM-2 and KTRM-2) and apoptosis levels were compared. MTS analysis of the treated HMCL indicated that TRM-1 was as effective as LZ-TRAIL whilst TRM-2 and KMTR-2 were not. This was further supported by immunoblot analysis demonstrating that LZ-TRAIL and TRM-1 cleaved PARP to similar levels where agonist antibodies to TRAIL-R2 did not. TRAIL-R1 and TRAIL-R2 levels on all four HMCL were upregulated as a result of 24hr incubation with a sub-lethal dose (5uM) of VP-16. Despite an increase in expression of both receptors, only NCI-H929 exhibited a significant (p-value: 0.01) increase in sensitivity to TRM-2. Treatment of primary myeloma cells with LZ-TRAIL and agonist antibodies for 24hrs exhibited modest efficiency. RPMI-8226 co-cultured with normal bone marrow MNC but not HS-5 condition media exhibited reduced levels of sensitivity to both LZ-TRAIL (50%) and TRM-1 (30%) when compared to treatment of RPMI-8226 alone. Transwell assays were established to identify the mechanism through which primary MM cells are protected from TRAIL-induced apoptosis. Preliminary data indicated that when the RPMI-8226 cells are not in contact with the normal bone marrow MNC the level of protection is reduced. This suggests the existence of cell-adhesion mediated resistance to death receptor ligand mediated apoptosis. We conclude that TRAIL-induced apoptosis in HMCL is driven primarily through TRAIL-R1. *In vivo* resistance to TRAIL-induced apoptosis may be modulated by cell adhesion mediated mechanisms.

PO.1014

HEDGEHOG PATHWAY SIGNALING IN MYELOMA STEM CELLS

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Although a number of agents can induce remissions in multiple myeloma (MM), few therapies are capable of providing a long-term cure. Treatment failure and recurrence is likely due to the persistence of clonogenic myeloma cells that are capable of regenerating the tumor following treatment. We have previously demonstrated that clonogenic cells in MM phenotypically resemble B cells and give rise to terminally differentiated plasma cells. Since self-renewal is a major property of both normal and malignant stem cells, the delineation of cellular processes responsible for self-renewal is critical to the development of curative therapies. The hedgehog (Hh) signaling pathway is essential for axial patterning in embryogenesis, and is necessary and sufficient for maintenance and expansion of stem cells in the developing brain and skin. We have demonstrated aberrant Hh pathway activation in a variety of adult solid tumors, and shown that specific inhibition of Hh signaling inhibits their malignant growth. Given its role in both normal stem cell and cancer biology, we examined Hh signaling in MM stem cells. We found that both MM cell lines and clinical samples expressed components of the Hh signaling pathway. In addition, the treatment of MM cell lines or clinical specimens with either the Hh antagonist cyclopamine or the Hh neutralizing antibody 5E1 inhibited clonogenic growth, suggesting that it plays a role in the self-renewal of MM stem cells. In order to examine the role of Hh signaling specifically in MM stem cells, we studied immature progenitors contained within the MM cell line NCI-H929 and found that they expressed significantly higher levels of all components of the Hh pathway compared to mature plasma cells. Furthermore, the treatment of progenitors induced phenotypic evidence of plasma cell differentiation, suggesting that Hh activity is required to maintain the stem cell phenotype. Hh appears to regulate tumor stem cells in MM and promotes their self-renewal. The inhibition of Hh signaling may represent a novel therapeutic strategy that directly targets MM stem cells.

PO.1015

PROTEASOME INHIBITORS INDUCE THE UNFOLDED PROTEIN RESPONSE IN MULTIPLE MYELOMA CELLS LEADING TO ENDOPLASMIC RETICULUM STRESS-INDUCED APOPTOSIS

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Proteasome inhibitors (PIs) were recently approved by the United States Food and Drug Administration for the treatment of refractory multiple myeloma (MM), however their mechanism of action and the nature of their selectivity remain unknown. MM cells are characterized by a highly developed endoplasmic reticulum (ER) that is required for the production and secretion of immunoglobulin. Conditions that compromise protein folding in the ER trigger a signaling pathway termed the unfolded protein response (UPR), that results in either improved protein folding or apoptosis. The UPR can be further subdivided into a physiological UPR and an ER stress UPR. The physiological UPR is required for the development and function of secretory cells and involves the activation of transcription factors required for the induction of ER chaperones and components of the degradative machinery. The ER stress UPR is activated by the accumulation of misfolded proteins within the ER. In addition to transcription factor activation it also involves the activation of PERK, an ER membrane localized kinase. Activated PERK regulates the transition between reducing the protein load on the ER and the induction of apoptosis. As one of the kinases that can phosphorylate eukaryotic translation initiation factor-2α(eIF-2α), PERK can transiently inhibit most protein synthesis. Following severe or prolonged ER stress, activated PERK causes a paradoxical increase in the translation of CHOP/gadd153, a transcription factor specifically associated with ER stress-induced apoptosis. Importantly, proteasome function is known to be essential for both the removal from and the degradation of misfolded proteins that accumulate within the ER. To investigate whether PIs initiate an ER stress UPR, cell viability and induction of UPR genes were monitored over the course of 24 hours in five different human MM cell lines treated with PIs, known ER stress-inducing agents, or chemotherapeutic agents. Consistent with reports that the physiological UPR is required for mature B cell to plasma cell differentiation, the ER chaperones BiP/grp78 and grp94/gp96, were constitutively expressed at high levels in all five cell lines and their expression did not change over the course of each

treatment condition. Although phosphorylation and caspase-dependent cleavage of eIF-2α were detected in response to all three treatment conditions, CHOP was specifically and rapidly induced in cells treated with PIs and ER stress-inducing agents. These findings suggest that in MM, PIs upset ER homeostasis in cells that already constitutively express ER stress survival factors in order to function as secretory cells. This further stress on the ER leads to the induction of additional UPR components and cell death by an ER stress-induced apoptotic program.

P0.1016

CLINICAL SIGNIFICANCE OF CHEMOKINE RECEPTOR EXPRESSION IN HUMAN MULTIPLE MYELOMA CELLS: A CLOSE ASSOCIATION WITH DISEASE ACTIVITY AND SURVIVAL

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A striking feature of multiple myeloma (MM) is the accumulation and predominant localization of malignant plasma cells (PC) in the bone marrow (BM), in close contact with stromal cells. Although MM cells reside preferentially in the BM, small amounts of tumor cells can also be detected in the blood circulation. It is believed that these circulating MM cells mediate disease spreading to multiple skeletal sites. The capacity of MM cells to circulate and to home to the BM implicates that these cells must be equipped with appropriate adhesion molecules and chemokine receptors to allow their migration across the endothelium and the subendothelial basement membrane. We and others have previously shown that at least 3 chemokine receptors, i.e. CCR1, CCR2 and CXCR4, initially identified for their role in the recruitment and trafficking of leukocyte subsets in both homeostatic and inflammatory conditions, are implicated in MM cell migration. In this study, we analyzed the surface expression of the chemokine receptors CCR1, CCR2 and CXCR4 on primary MM cells from 81 MM patients. Primary MM cells showed a heterogeneous expression pattern of CCR1, CCR2 and CXCR4. MM patients were subsequently stratified into two groups according to there chemokine receptor profile (*chemokine receptor status* (CRS)) : group 1 = no chemokine receptor expression; group 2 = expression of at least one chemokine receptor). The chemokine receptor status was analyzed in relation to clinical and laboratory features and evaluated for prognostic significance. CRS significantly correlated with disease activity; patients with active disease show a significant lower expression of CCR1, CCR2 as well as CXCR4 as compared to patients with non-active disease (22% vs. 81%, (p=0,004) for CCR1, 47% vs. 72% (p=0,007) for CCR2 and 51% vs. 72% (p=0,01) for CXCR4). Furthermore, CRS inversely correlated with markers of disease activity with prognostic significance, i.e. B2 microglobulin (5 mg/L in group 1 vs. 2,5 mg/L in group 2) (p= 0,003), serum CRP (7 vs. 3,2 mg/dL) (p=0,04) and directly correlated with serum albumin concentration (3,4 vs. 3,8 g/dL) (p=0,04). There was a trend towards a higher frequency of del 13 in group 1 (72% vs. 38% in group 2), but it was not statistically significant. There was no significant correlation with paraprotein level, neither with the extent of osteolytic lesions or age, gender, hemoglobin, isotype, plasmacytosis. The survival difference between the two groups was highly significant. Patients expressing at least one chemokine receptor had a median survival of 36 months, whereas patients of whom MM cells did not express any chemokine receptor had a median survival of 16 months (p=0,0003 - log rank test). Multivariate analysis, using the Cox proportional hazard model, identified CRS as an independent prognostic factor. In conclusion, the results obtained in this study indicate that, in addition to the prognostic value of CRS, a loss of expression of at least one of the chemokine receptors CCR1, CCR2 and CXCR4 is associated with increased disease activity in human MM. This observation might reflect an impaired chemoattraction and retention of MM cells within the BM microenvironment, resulting in disease progression.

P0.1017

SPECIFIC INHIBITORS OF GLYCOGEN SYNTHASE KINASE-3 MEDIATE RESCUE FROM APOPTOSIS INDUCED BY DEXAMETHASONE IN MULTIPLE MYELOMA CELLS

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The rational of mapping and targeting the insulin-like growth factor receptor (IGF-1R) pathway in multiple myeloma (MM) rests on the fact that IGF-1 has been established as an important survival factor, while ascribed a modest role in proliferation of normal cells in the adult individual. The IGF-1R and individual components of this signal pathway may therefore be equally important as mutated oncogenes as targets for intervention in improved MM therapy. Based on the hypothesis that abrogating survival circuits may render tumor cells more susceptible to apoptosis, we and others have previously shown that interfering with IGF-1R signaling at the receptor tyrosine kinase level, or use of the blocking anti-IGF-IR antibody (alpha-IR3) will augment the apoptosis induced by serum-starvation, Fas-ligation or Dexamethasone. However, the biological significance of individual anti-apoptotic elements downstream of the IGF-1R are not fully elucidated. Several substrates regulating caspase activity and apoptosis downstream of the PI 3-K/Akt activation have been suggested. In the present study we have examined the role of GSK-3 as a key regulator of survival in MM. The pharmacological blockade of PI 3-K by wortmannin or LY294002 augmented the sensitivity to caspase-3-dependent apoptosis induced by Dexamethasone in MM cell lines and primary tumour cells, similar to the effects observed when interfering at the level of the IGF-1R. We now demonstrate that impairing the function of GSK-3 by use of selective inhibitors CHIR99021and CHIR98014 (Chiron corp. CA), or LiCl will rescue MM cells from the apoptosis induced by Dexamethasone. Implying a critical role of GSK-3 as a key pro-apoptotic molecule in the absence of an exogenic survival signal, the GSK-3 inhibitors also mediated rescue from Dexamethasone-induced apoptosis augmented by blocking the upstream PI 3-K activity. GSK-3 activity is negatively regulated by phosphorylation via several kinases, including Akt, at key regulatory serine residues Ser21 (alpha isoform) and Ser9 (beta isoform). Phosphorylation of GSK-3-beta at Ser9 was indeed reduced by treatment with PI 3-K inhibitors in the presence of Dexamethasone, and increased in response to IGF-I. LiCl inhibits GSK-3 both by direct binding and by indirectly increasing the inhibitory serine phosphorylation, while the selective inhibitors CHIR99021 and CHIR98014 selectively inhibit GSK-3 function by acting as ATP-competitors. Thus, the inactivation of GSK-3 by CHIR99021 and CHIR98014 was demonstrated by the stabilization of the GSK-3 target beta-catenin. The expression of the antiapoptotic gene $cFLIP_S$ was downregulated in response to LY294002, whereas cFLIPL, Bcl-2, and Bcl-xL was not regulated by Dexamethasone,

alpha-IR3 or PI 3-K inhibition. Taken together, inactivation of GSK-3 using LiCl, or CHIR99021 and CHIR98014 mediates rescue from Dexamethasone-induced apoptosis augmented by alpha-IR3, wortmannin or LY294002 and implicates a key role for GSK-3 in the IGF-IR-regulated survival of multiple myeloma.

PO.1018

PROTEOMIC EVALUATION OF PATHWAYS ASSOCIATED WITH DEXAMETHASONE INDUCED APOPTOSIS AND RESISTANCE IN MULTIPLE MYELOMA

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Glucocorticoids are the mainstay of treatment for patients with myeloma (MM). Unfortunately many patients become resistant to therapy and the median survival is approximately 4.5 years. This study has used a global protein-expression approach to further characterise the pathways of dexamethasone (dex)-induced apoptosis and resistance in the sensitive and resistant MM.1 sub-line s (MM.1S and MM.1S respectively). Following dex treatment, protein from MM.1S was separated by 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) using a pH range of 4-7 in the first dimension. Changes in expression pattern were identified between dex-treated and untreated cells with 24 spots downregulated and 3 spots upregulated. Identification by mass spectrometry (4700 Proteomics analyser: MALDI TOF-TOF, Applied Biosystems) demonstrated that 10/24 of the downregulated proteins were involved in cell survival and proliferation whereas the 3 upregulated proteins were involved in post translational modification, protein folding and trafficking. A comparison with gene expression studies identified a number of corresponding genes, as well as a number of proteins/genes highlighted in one study but not the other. Interestingly the upregulation of FKBP51 a key regulatory component of the Hsp90 steroid receptor complex was observed in both gene and protein analysis. Importantly 2D-PAGE of the dex-resistant line MM.1R demonstrated no increase in FKBP51, confirming its role in mediating dex-induced apoptosis. The Hsp90 receptor complex is important in maintaining the glucocorticoid receptor (GR) in a state receptive to dex and comprises the GR, Hsp90, and the immunophilin s FKBP51 and FKBP52. Western and immunoblotting analysis of the complex in the MM.1S cell line identified FKBP51/52 and GR expression to increase in a time dependant manner whilst Hsp90 expression remained constant. No changes were observed in the resistant line post exposure. Overexpression of FKBP51 at resting state or the inability to induce expression following treatment with dex has been associated with glucocorticoid resistance in other disease. Gene array analysis of 30 myeloma cases showed no statistical difference in FKBP51 expression between presenting myeloma cases sensitive to dex and relapsed refractory cases resistant to dex, suggesting in myeloma resistance is not mediated by a resting state upregulation of FKBP51. RQ-PCR of dexamethasone-sensitive cell lines and patient cells showed an increase in FKBP51 post-drug exposure, suggesting this increase may be a surrogate marker for dexamethasone-sensitivity although some resistant cell lines also upregulated FKBP51 following dexamethasone exposure suggesting that other mechanisms down stream to the Hsp90 steroid receptor complex confer resistance. In conclusion these protein profiling studies have identified a number of novel proteins involved in dex-induced apoptosis and resistance, many of which warrant further investigation.

PO.1019

REGULATION OF GLUCOCORTICOID INDUCED LEUCINE ZIPPER IN MULTIPLE MYELOMA CELLS

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Glucocorticoids (GCs) are among the most effective agents for the treatment of multiple myeloma (MM). However, patients ultimately develop resistance to this therapy and despite the application of numerous innovative therapeutic approaches, this disease remains incurable. Understanding the pathway of GC-induced apoptosis in myeloma cells is crucial to understanding the process of drug resistance and to the development of new drug targets for MM treatment. GILZ (Glucocorticoid-Induced Leucine Zipper) was identified in our lab in a cDNA micro-array screen as a gene upregulated by GCs in the well-established MM.1S multiple myeloma cell line. The upregulation of GILZ by the synthetic GC, dexamethasone, (Dex), has been reported in a number of other hematological cell lines and has been confirmed by both Northern Blot and RT-PCR. In this study, we investigated the ability of a panel of GCs, cytokines, and growth factors to also effect GILZ expression as measured by semi-quantitative RT-PCR. GILZ induction by other GCs was shown to correlate with cytotoxicity. GILZ was upregulated by hydrocortisone, prednisolone, methylprednisolone, beclomethasone, beclomethasone DP, triamcinolone, and triamcinolone acetonide. Only prednisone, which is not cytotoxic to MM.1S cells as it requires conversion *in vivo* to an active metabolite, did not upregulate GILZ expression. GILZ mRNA levels were also upregulated in MM.1S cells by 2-methoxyestradiol and the PI3-kinase inhibitor LY294002 and to a lesser extent by βestradiol, IL-10, TGF-β and sonic hedgehog, all which have been reported to upregulate GILZ in other systems. Both the phorbal ester PMA and the cytokine IL-7 reduced Dex induced GILZ upregulation as did the key myeloma growth factors IL-6 and IGF-1. We hypothesize that GILZ is an important mediator in the GC-signaling and GC-induced apoptosis in MM. The interactions between the GC-signaling pathway and these other agents will be investigated further in order to gain a better understanding of the interaction of important signaling pathways in myeloma growth and apoptosis enabling the development of alternative therapeutics. (This research was supported by funding from an MMRF Senior Research Award to NLK).

PO.1020

VASCULAR ENDOTHELIAL GROWTH FACTOR UPREGULATES MCL-1 EXPRESSION AND PROTECTS MULTIPLE MYELOMA CELLS AGAINST STARVATION INDUCED-APOPTOSIS

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Vascular endothelial growth factor (VEGF) induces proliferation of MM cells and induces interleukin-6 (IL-6) secretion in a paracrine loop involving MM cells and bone marrow

stromal cells. In turn, IL-6 triggers multiple myeloma (MM) cell proliferation and also protects against apoptosis by upregulating Myeloid-cell-leukemia 1 (Mcl-1), a critical survival protein in MM cells. The goal of our study was to investigate the role of Mcl-1 in VEGF induced-proliferation and protection against apoptosis. Using two murine embryonic fibroblast cell lines as a model (a Mcl-1 deleted cell line and its control: Mcl-$1^{\Delta/null}$ and Mcl-$1^{control}$ MEFs, respectively), we here demonstrate that deletion of Mcl-1 reduces fetal bovine serum (FBS), VEGF, and IL-6 induced-proliferation. These results demonstrate that Mcl-1 is required to mediate VEGF, Il-6 and FBS-induced-proliferation and cell cycle progression. To highlight the key anti-apoptotic role of Mcl-1 in MM cells, humans MM1s cells were transfected with Mcl-1 siRNA. Specific inhibition of Mcl-1 was associated with decreased proliferation (42% and 61% decreases at 24 and 48 h, respectively) and induction of apoptosis (subG1 peak: 22% and 41% in Mcl-1 siRNA transfected cells versus 15% and 15 % in non-transfected cells at 24 and 48 h, respectively), confirming that Mcl-1 is critical for both proliferation and protection against apoptosis in MM cells. In 3 human MM cell lines (MM1s, U266 and MM1R) and MM patient cells we next showed that Mcl-1 protein expression, but not other bcl-2 family members, is upregulated by VEGF in a time and dose manner; and conversely that the pan-VEGF inhibitor GW654652, blocks VEGF induced-upregulation of Mcl-1. Furthermore using flow cytometry with a double staining (CD38-FITC and Apo 2.7-PE), we demonstrate that VEGF protects MM patient cells from FBS-starvation-induced-apoptosis: the percentage of apoptotic MM patient cells ($CD38^{++}$ and Apo 2.7+) in non starved medium (RPMI 1640 supplemented with 10% FBS) was 15% versus 93% in starved medium (RPMI 1640 supplemented with FBS 2%), and 48% in starved medium supplemented with 25ng/ml VEGF. In conclusion, our study demonstrates that VEGF protects MM cells against apoptosis, and that VEGF-induced MM cell proliferation and survival is mediated via Mcl-1. Our study provides the preclinical framework for novel therapeutics targeting both Mcl-1 and/or VEGF to improve patient outcome in MM.

PO.1021

MOLECULAR MECHANISMS UNDERLYING THE DEVELOPMENT OF DRUG RESISTANCE IN MULTIPLE MYELOMA

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A prominent feature of most cancers including multiple myeloma (MM) is a striking genetic instability, leading to ongoing accrual of mutational changes some of which underlie tumor progression, including development of drug resistance and metastasis. The molecular basis for the generation of genetic diversity in cancer cells has thus emerged as an important focus of investigation and a target for successful eradication. We have previously observed that homologous recombination (HR) is upregulated in MM. Utilizing a genomewide LOH assay based on SNP genotyping (Affymetrix), as a tool to estimate the rate of mutation and genomic instability, we have now observed that over time elevated HR leads to progressive accumulation of genetic variation in MM cell lines and patient cells; and inhibition of HR activity in MM cells by altering components of the HR pathway concordantly affects the acquisition of new genetic changes. As HR activity is dependent on concerted action of number of genes, instead of over expressing single HR related gene, we utilized nickel chloride, a known recombinogen to evaluate effects of increased HR activity on development of genomic diversity. To evaluate whether inhibition or induction of HR can affect the frequency of acquisition of new genetic changes in MM cells, we cultured ARP cells in the presence or absence of MA, *HsRAD51*-siRNA or nickel chloride, over a period of 90 days. Genomewide LOH was evaluated by comparing genotypes before and after the 90-day interval. In three independent experiments treatment of cells with nickel chloride increased the number of new LOH sites by more than 12-fold. We next evaluated the effect of induction of HR and the consequent increase in genetic aberrations, on development of drug resistance in multiple myeloma. Myeloma cells were cultured with nickel chloride as a potent inducer of HR and dexamethasone (10^{-8}M). Control cells were exposed to dexamethasone alone and the cell viability in dexamethasone was measured weekly. No live cells were detected in cultures exposed to dexamethasone alone while >95% cells exposed to both nickel chloride and dexamethasone were alive following 2 weeks culture. These findings were confirmed by 3 independent experiments. The development of drug resistance was further confirmed by demonstrating no significant effects of dexamethasone at 10^{-6}M concentration for 1 week while 100% cell death in control cells by day 3. We propose that continued accumulation of new genetic changes mediated by HR, as demonstrated here, provides the genetic and molecular events required to develop drug resistance and its inhibition may allow us to successfully treat MM cells without the currently observed development of resistance. HR may be a potential therapeutic target to maintain chemo sensitivity of the tumors.

PO.1022

PRIMARY MYELOMA CELL CYTOTOXICITY *IN VITRO*: AN APPROACH TO SELECTION OF SALVAGE THERAPIES

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Multiple myeloma (MM) is characterised by bone marrow infiltration with clonal plasma cells. Most patients will respond to intensive chemotherapy followed by stem cell transplantation, however, relapse is almost inevitable, due to persistence of chemoresistant cells. Many novel therapies are emerging for the treatment of resistant myeloma, but response rates are difficult to predict in an individual. We optimised techniques for the isolation and culture of myeloma cells from bone marrow aspirates, and *in vitro* cytotoxicity testing. Myeloma cells were purified from marrow mononuclear cells using anti-CD138 coated magnetic beads and cultured in the presence or absence of purified IL-6. *In vitro* therapies tested included steroids (dexamethasone and methylprednisolone (MP)), standard cytotoxic agents (doxorubicin, cyclophosphamide) and novel agents (arsenic trioxide (ATO) plus ascorbic acid (AA) and rapamycin plus dexamethasone). Myeloma cell apoptosis in response to agents was quantitated by flow cytometric uptake of 7-AAD and Annexin V-FITC binding. Primary myeloma cells from 16 patients have been tested *in vitro* with variable patterns of response. Doxorubicin (0.1 μg/mL) was found to be the most efficient drug with >95% cell death observed after 24 hours for all patient samples. Cyclophosphamide did not show

cytotoxicity *in vitro* due to the absence of metabolites in this system. Dexamethasone was active against the myeloma cell line RPMI-8226, but showed minimal cytotoxicity versus primary myeloma cells. Intermediate response rates were observed in the presence of MP (5/7 tested) or ATO (7/9 tested). Enhanced myeloma cell apoptosis was noted when 0.5 mM AA was added to ATO (final concentration 20 μg/mL). Primary myeloma cells showed variable patterns of cytotoxicity and were generally less sensitive than myeloma cell lines. Primary plasma cells from patients with chemorefractory disease were less sensitive than primary cells from patients at diagnosis. Modifications of the system, including myeloma cell co-culture with heterologous stromal cells and cell proliferation assays using CFSE (5,6 carboxylfluorescein diacetate succinimidyl ester) will be discussed. We hypothesise that this assay system can be used to test for the development of drug resistance in a single patient over time, and may aid in the selection of an optimal second-line therapy in patients who have relapsed post-chemotherapy.

POSTER SESSION 11
IMMUNE BIOLOGY AND IMMUNE THERAPY

P0.1101

INDUCTION OF CYTOTOXIC T-LYMPHOCYTE RESPONSES AGAINST MULTIPLE MYELOMA USING DENDRITIC CELLS TRANSFECTED WITH RNA CODING TUMOR-ASSOCIATED ANTIGENS

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In Multiple myeloma (MM) intensive chemotherapy in combination with stem cell transplantation can lead to clinical remission. Adjuvant immunotherapy by vaccination with tumor antigen-loaded dendritic cells (DC) can be used to reduce minimal residual disease and prolong remission. Mage-3 and BCMA are possible target antigens for vaccination. Mage-3 is aberrantly expressed in malignant plasma cells in a significant proportion of MM patients and anti-Mage-3 CTL responses can be elicited in melanoma patients. BCMA is a B cell differentiation antigen with antibody responses found in some MM patients responding to donor lymphocyte infusions after allogeneic stem cell transplantation. Real-time RT-PCR analysis revealed that BCMA is highly expressed in malignant plasma cells of MM patients, whereas expression in more immature B-cell malignancies and normal B cells is at least 10-fold lower. No significant BCMA mRNA is detectable in non-lymphoid normal tissues, and expression in primary and secondary lymphoid tissues is 10 to 100-fold lower compared to MM tumor cells. The low expression level of BCMA in lymphoid tissue is most probably due to the presence of B cells. To get Mage-3 and BCMA expression in DC, we explored RNA electroporation as a whole antigen approach resulting in presentation of multiple peptides in different HLA molecules. We compared RNA electroporation of immature versus mature monocyte-derived DC. Immature DC (iDC) were electroporated at day 6 of culture, whereas mature DC (mDC) were electroporated 24 h after maturation with TNF-alpha and PGE2. After electroporation, both iDC and mDC were cultured for 24 h in medium containing TNF-alpha and PGE2, and tested for cell yield, viability and transfection efficiency. We found that RNA electroporation of iDC resulted in a lower yield of viable cells, and a diminished expression of CD80 and CD83. Only a slight difference in transfection efficiency between iDC and mDC was observed when electroporated with eGFP-RNA. In contrast, electroporation with Mage-3-RNA or BCMA-RNA resulted in more protein expression in mDC than in iDC as determined by western blot and flowcytometric analysis. In vitro induction of specific T-cell responses in healthy donors using Mage-3 electroporated DC resulted in Mage-3 pentamer positive cells after 4 rounds of stimulation. In conclusion, using DNA electroporation in DC we can induce protein expression of Mage-3 and BCMA. These DC can induce specific T-cell responses *in vitro*. The next step is to determine the capacity of electroporated mDC to induce CTL reactivity using PBMC from MM patients in clinical remission after chemotherapy.

P0.1102

HLA-CW6 ALLOREACTIVE CITOTOXIC T LYMPHOCITES RECOGNIZING HLA-CW6+ MYELOMA CELLS BUT NOT NON-MYELOMA HLA-CW6+ CELLS ARE PEPTIDE-DEPENDENT

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We investigated the generation of myeloma-specific cytotoxic T lymphocytes (CTL) from unrelated normal donors non-HLA matched with the myeloma cell line SBN. The aim was to obtain alloreactive CTL specific of peptides specifically presented by or restricted to myeloma cells. After coculture of SBN with PBL of unrelated donor, the T-cell line obtained was cloned and each CTL was assessed against SBN and SB-EBV (B-EBV cell line obtained by infecting B cells of SBN patient with EBV) simultaneously. Only the CTL recognizing SBN but not SB-EBV were kept for further study. Among more than 200 clones screened, we isolated two different CTL CD8+ recognizing SBN only (60% of specific lysis at effector: target ratio 5). Their cytotoxicity was blocked by antibodies against HLA-I molecules and more precisely by mAb against HLA-B/Cw molecules. Both CTL recognized also other MM cell lines that were matched with SBN for HLA-Cw*0602 (XG6, BCN). Moreover, antiserum directed against HLA-Cw6 molecules agrogated recognition of SBN, XG6 and BCN. Both CTL were not cytotoxic against BC-EBV (derived from BCN MM patient), nor against 3 other B-EBV cell lines derived from HLA-Cw*0602 donors, nor against PBL from HLA-Cw*0602 normal donors. We measured cell surface HLA-Cw6 expression of both myeloma and B-EBV cells with a scFv directed against HLA-Cw6 molecules. We showed that HLA-Cw6 was more expressed by B-EBV cells as compared to MM cells, indicating that the lack of B-EBV recognition was unrelated to HLA-Cw6 expression level. We did not find any killing inhibitor nor activator receptor expressed by the CTL suggesting that MM specific reactivity was not related to such mechanism. However, acid elution abrogated myeloma recognition and cold target competition showed that B-EBV cells did not compete with myelome cells. Both experiments suggested that CTL recognized a peptide or a set of peptides restricted to myeloma cells. Such alloreactive CTL could be helpful for GVM reaction in allotransplanted MM patients.

P0.1103

CD8+ T CELLS SPECIFIC FOR CANCER-TESTIS ANTIGENS ARE FOUND IN MANY PATIENTS WITH MULTIPLE MYELOMA AND CORRELATE WITH DISEASE BURDEN

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Proteins from the family known as *cancer-testis antigens* (CTAg) are expressed in some cases of multiple myeloma and subsets of acute myeloid leukaemia. CTAg can stimulate CD8+ T cell responses in patients with melanoma but there are no reports of CTAg-specific immune response in patients with haematological malignancy. Such information is critical to assess whether or not these antigens act as targets for tumour-specific immunity or if they could be used as targets for immunotherapy. We have used twelve peptide epitopes from a range of cancer-testis antigens which have been previously defined as epitopes for CD8+ T cells. These were used to screen for tumour-specific T-cells in blood of patients with multiple myeloma at various stages of their disease. The IFNγ cytokine secretion assay was used to detect functional responses and magnetic selection was employed to increase the sensitivity of detection. FACS analysis was used to quantitate the frequency of responding cells. Thirty-seven patients were screened with an age range of between 45 and 88 years. Blood samples were taken at monthly intervals and the percentage of CD8+ T cells responding to each peptide was calculated. 13 patients responded to 1 or more of the peptides with a range between 0.01% and 0.7% of the total CD8+ T cell pool. The frequency of the tumour-specific response fluctuated during treatment in individual patients. Analysis of the CTAg-specific immune response in relation to disease course revealed that the immune response was generally correlated with tumour burden as revealed by the paraprotein level. CTAg HLA-peptide tetramers incorporating peptides from LAGE-1 and MAGE-2 were able to directly visualize CTAg-reactive T cells in PBMC. CTAg-specific CD8+ T cells may have been primed and expanded by expression of CTAg on tumour cells or following 'cross presentation' through dendritic cells. In conclusion, T cells specific for cancer-testis antigens are present in the blood of a subset of patients with multiple myeloma. The clinical significance of this observation is currently being addressed.

P0.1104

THE EFFECT OF A THALIDOMIDE ANALOG (ACTIMID) ON T-CELL ACTIVATION IN PATIENTS WITH RELAPSED REFRACTORY MYELOMA

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Background. The discovery of the potent anti-myeloma effect of Thalidomide (Singhal et al NEJM 1999) heralded the development of a new class of agents (Thalidomide analogues) such as CC-4047 (Actimid, Celgene). This agent has been shown to have efficacy in treatment of relapsed myeloma but its exact mode of action remains unclear (Streetly M. et al, ASH 2003). Preliminary work (S.Schey, JCO 2004) suggests that thalidomide analogues can activate T cells which may in part explain their mode of action. We have previously demonstrated that measurement of serum free light chains at day 7 of treatment with Actimid can indicate likelihood of response (Patten PE, *et al.,* ASH 2003). Interleukin 2 (IL-2) is produced primarily by activated T cells. Interleukin 15 (IL-15) shares many properties with IL-2, but also stimulates the growth of natural killer cells.

Aim. The aim of this study was to see if Actimid modulated serum levels of IL-2 and IL-15 and to see if these correlated with clinical response as assessed by use of the serum free light chain (SFL) assay.

Patients and Methods. 14 patients with relapsed multiple myeloma participating in a phase I/II dose escalation study of Actimid were included in the study. All patients had serum IL-2 and IL-15 analysis performed on samples taken pre dose, and day 28. Analysis was performed using an enzyme immunoassay kit (R & D systems, USA). SFL kappa and lambda analysis was performed on samples taken pre Actimid and day 28 post Actimid. Analysis was performed using a latex enhanced immunoassay (Freelite TM kit, The Binding site, UK). The percentage change in Kappa/ Lambda ratio from baseline was calculated.

Results: 14 Patients (age range 47-83 years, median 67 years) were analysed for changes in IL-2 and IL-15 serum levels. There was a significant increase in IL-2 levels on treatment ($p<0.01$) and a non-significant reduction in IL-15 levels (p=NS.). 10 patients with complete SFL data were analysed for changes in SFL ratio on treatment and a linear regression analysis performed between changes in IL-2 levels and changes in SFL ratios with no significant correlation found (R= 0.2).

Conclusions: This study shows that serum IL-2 levels are increased in patients on Actimid with a non-significant decrease in levels of IL-15. There did not appear to be a correlation between a greater increase in IL-2 level and likelihood of response as assessed by the SFL assay. We are currently looking at cellular activation markers to confirm whether activated T-cell/ NK cell numbers are increased by Actimid.

P0.1105

INCREASED NUMBER OF T-CELL RECEPTOR Vβ, EXPANSIONS IN PATIENTS ON THALIDOMIDE MAINTENANCE THERAPY PROVIDES FURTHER EVIDENCE OF THE IMMUNOMODULATORY ACTION OF THALIDOMIDE

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Recent studies have demonstrated that following treatment with thalidomide there is increased T cell activity and NK cell cytotoxicity in the blood of patients with myeloma. The Australian ALLG MM6 trial is a multicentre randomised phase III study of low-dose thalidomide, prednisolone and Zometa versus prednisolone and Zometa for post-autologous stem cell transplant (ASCT) maintenance therapy in patients with myeloma. Laboratory studies were aimed to investigate the immunodulatory effects of thalidomide by studying the TCR Vβ repertoire of patients in MM6. TCR Vβ expansions have previously been shown to correlate with a good prognosis for patients with myeloma and are due to clonal expansions of CD3+CD8+CD57+CD28-CD27- cells. Flow cytometric analysis of TCR Vβ expansions was performed on blood samples collected prior to and post transplant (both 8 and 12 months). We assayed the

peripheral blood for TCR Vβ expansions in 35 patients enrolled in the MM6 trial. Expanded clones were seen in 54% (19/35) of patients prior to transplant. Of the patients in the thalidomide arm prior to transplant 47% had T cell expansions and in the no thalidomide arm 62% of the patients had T cell expansions. After thalidomide, 89% (17/19) of the patients had T cell expansions compared with 56% in the control, no thalidomide group. The increase in T cell expansions after thalidomide compared with no thalidomide was statistically significant ($\chi^2 = 8.34$; $p<0.005$). In the thalidomide group, 59% of the patients with clones had an expansion of more than one T cell clone compared with 12% in the control group. Further patients will be studied but these preliminary studies provide further evidence that thalidomide has an immunomodulatory action in patients with myeloma and that this is due, at least in part, to clonal expansion of cytotoxic effector cells.

P0.1106

A STUDY OF *IN VIVO* TRACKING OF MUC-1 PULSED DENDRITIC CELLS IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Dendritic cell (DC) immunotherapy is being actively studied in patients (pts) with multiple myeloma (MM) and other cancers. However, the best route of injection (inj) of these cells is unknown. The aim of this study was to determine the *in vivo* distribution of DC when given to pts with MM via varying routes of inj by using nuclear medicine imaging techniques.

Methods. Eligible pts had stable or slowly progressive MM not requiring systemic treatment, with elevated serum MUC-1 or positive marrow plasma cell MUC-1 staining. DC were produced by *in vitro* culture of autologous monocytes collected from patients via apheresis. Cells were differentiated in culture in serum-free media containing GM-CSF + IL-13, and then, after purification, cells were pulsed with Mannan-MUC1 fusion protein. Immature DC (ImDC) were matured by culture in the presence of FMKp and IFN-γ to produce mature DC (mDC). Prior to injection, ImDC/mDC were labelled with either F-18 flurodeoxyglucose (FDG) or Indium-111 (In). Patients underwent serial PET (up to 4 hours post injection) and SPECT scans (up to 72 hours post injection), respectively, to track the destination of inj cells which were given by concurrent inj of subcutaneous (sc), intradermal (id), and intranodal (in), or by the intravenous (iv) route.

Results. A total 6 pts received DC (ImDC=3, mDC=3) inj by all sc, id, in and iv routes (Table 1). PET was not useful for tracking, as the FDG-labelling efficiency of DC was low with little retention of tracer *in vivo*. In all pts after iv inj of In-labelled DC, cells were initially imaged within the lungs on SPECT. 24 hours post inj, most DC had cleared the lungs and were seen within the liver, spleen and axial and proximal appendicular skeleton. DC were still visible until 72 hours. No subsequent nodal uptake was seen after iv inj. Following sc/in/id injection, tracking of a fraction of the DC to the draining lymph node was imaged: mDC tracked to lymph node (LN) in all cases following sc inj (3/3), in 2/3 for id and 2/3 for in. ImDC tracked to lymph node (LN) in 1/3 cases following sc inj, in 1/3 for id and 1/3 for in. Tracking was seen by at least one inj route in all patients. Studies of immune responses to DC vaccination are underway.

Conclusion. We have demonstrated that it is possible to label DC with In and track their distribution *in vivo*, although FDG-labelling of DC was not feasible. Mature DC demonstrated greater ability to migrate to lymph nodes although some migratory function is maintained by ImDC. Novel PET-tracers are currently being evaluated.

Median no. of DC per apheresis (n=6)	Median % viability	Median no. of DC given per iv inj	Median no. of DCM given per sc, id or in inj	Mean % cells labelled with F-18 FDG (n=3)	Mean % cells labelled with In-111 (n=11)
3.86 x 108 (1.43-4.23)	82.1 (67.9-93.2)	1.5 x 108 (0.33-2.5)	2.0 x 107 (1.3-3.6)	29 (17-46)	86 (54-95)

P0.1107

DENDRITIC CELL NUMBERS AND THEIR SUBSETS DURING TREATMENT OF MULTIPLE MYELOMA

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Objective. In this study, the proportion of dendritic cell (DC) subsets (myeloid DC1 and plasmatocytoid DC2), T cells, B cells and NK cells was evaluated in peripheral blood of patients with multiple myeloma (MM) before and during treatment. Also control group of healthy volunteers was evaluated.

Methods. Flow cytometric determination of relative cells number in peripheral blood was based on positive expression of the surface antigen: T cells (CD3/CD4/CD8), B cells (CD19/CD20), NK cells (CD3/CD16/CD56) and DC (CD83/HLA-DR/CD11c and CD83/HLA-DR/CD123).

Results. Significant difference ($p<0.002$) was found in initial values between the group of healthy volunteers (n = 15; mean count of $CD83^+$ cells 0,26±0,15%; ratio DC1/DC2 = 0,15) and the group of patients before treatment (n = 15; 0,15±0,03% CD83+; DC1/DC2 = 4,25). In a group of patients after induction treatment with VAD regimen (vincristine, adriamycin, dexamethasone), the mean percentage of DC was higher (0,18±0,04% $CD83^+$ cells; DC1/DC2 = 4,77) than initial values. Administration of G-CSF increased the total DC numbers (0,34±0,11%; DC1/DC2 = 1,99) and intermediate levels of DC counts were found in the apheresis products (0,22±0,05%; DC1/DC2 = 0,88). Administration of GM-CSF most increased DC numbers (0,50±0,21%; DC1/DC2 = 1,63). Pretreatment DC values comparable with DC values of healthy volunteers (p<0,98) were achieved within six months after transplantation (0,24±0,08%; DC1/DC2 = 1,57). Total numbers of T cells didn't significant differ during treatment but the reverse CD4/CD8 ratio was found in majority of patients within six month after the transplantation.

Conclusions. Patients with MM have significant lower relative numbers of peripheral blood DC before treatment in comparison with healthy volunteers. The highest number of total DC was found after GM-CSF stimulation. The ratio DC1/DC2 showed relative majority of DC1 subtype and its the lowest value was found in the apheresis products. Normal DC values comparable with DC values of healthy volunteers were found in patients within six months after transplantation together with the reverse CD4/CD8 ratio.

Supported by CMG foundation and partially by grant NR 8081-3.

P0.1108

A RANDOMIZED PHASE II STUDY OF *XCELLERATED* T CELLS WITH OR WITHOUT PRIOR FLUDARABINE. THERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background. T cells from myeloma subjects can be activated and expanded *ex vivo* using the Xcellerate Process, in which peripheral blood mononuclear cells are incubated with anti-CD3 and anti-CD28 antibody-coated magnetic beads (Xcyte™-Dynabeads®). In a previous study (Borrello *et al.,* ASCO 2004), Xcellerated T Cells administered to myeloma subjects following high dose chemotherapy and autologous stem cell transplantation led to accelerated lymphocyte recovery and restoration of the T-cell receptor repertoire. In the current study, subjects with relapsed or refractory myeloma were randomized to Xcellerated T Cells with or without one cycle of fludarabine prior to Xcellerated T Cells. Fludarabine is being used to assess the influence of lymphoablation on the anti-tumor and immune reconstitution effects of T-cell therapy; it has previously been reported to have no significant activity in myeloma (Kraut *et al.,* Invest. New Drugs, 1990).

Methods. Approximately 30 subjects are planned to receive treatment. Each receives a single dose of 60-100 x 10^9 Xcellerated T Cells. Subjects on the fludarabine arm receive a single cycle (5 days at 25 mg/m^2), completed 4 days prior to the Xcellerated T-Cell infusion.

Results. 17 subjects have been enrolled and 13 treated to date, with median last f/u visit of 28 days (range 0-140). Xcellerated T Cells were successfully manufactured in all subjects, with T-cell expansion 136±61 fold (mean±SD), with 79.2±13.8 x 10^9 cells infused, and final product 98.0±2.0% T cells (n=13). There have been no reported serious adverse events related to Xcellerated T Cells. In the fludarabine arm, lymphocytes decreased from 1,228±290/mm^3 (mean ±SEM) to 402±164 following fludarabine, and then increased to 1,772±278 on Day 14 following T cell infusion (n=7). In the non-fludarabine arm, lymphocyte counts increased from 1,186±252 to 3,204±545 on Day 14 (n=4). Lymphocytes were comprised of both CD4+ and CD8+ T cells. Increases were observed in NK cells from 77±26 to 121±25, monocytes from 166±44 to 220±30 and platelets from 218±16 to 235±24 by Day 14 (n=11). In the non-fludarabine arm, neutrophils increased from 3.6±0.9 to 4.8±0.6 on Day 1. On the fludarabine arm, 3 of 6 subjects developed Grade 4 neutropenia and one developed Grade 3 thrombocytopenia. Seven subjects were evaluable for serum M-protein measurements to Day 28. One of three fludarabine treated subjects had an M-protein decrease of 38%.

Conclusions. Xcellerated T Cells were well-tolerated and led to increased lymphocytes, including T cells and NK cells. Increases in other hematologic parameters, including neutrophils and platelets were also observed. In this patient population, fludarabine is lymphoablative and also can cause neutropenia and thrombocytopenia. The fludarabine schedule has been decreased from 5 to 3 days. A decrease in M-protein has been observed in one of three fludarabine-treated subjects; data on additional subjects will be presented.

P0.1109

CELL IMMUNOTHERAPY OF MULTIPLE MYELOMA: IDENTIFICATION AND *IN VITRO* EXPANSION OF MYELOMA-SPECIFIC T CELLS

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Multiple myeloma has been considered as low immunogenic incurable disease. The attempts has been made to invert the immune status to recognize myeloma cells by T cells or other cells of the immune system. Here we studied the possibility of identification of myeloma-specific T cells *in vitro*, their clinical-grade expansion, and specific cytotoxicity of expanded T cells to myeloma cells. Irradiated myeloma cell line ARH 77 has been used as tumor antigen to stimulate peripheral blood mononuclear cells (PBMC) of 8 healthy volunteers. Activated responder T cells has been immunomagnetically separated based on surface expression of interferon gamma (Miltenyi Biotech) and expanded in 5 cases by phytohemaglutinin and repeated high-doses of interleukin 2. Cytotoxicity against the original myeloma cell line has been tested after the T cell expansion using propidium iodide. Tested expanded T cells were labeled by CFSE to distinguish them from myeloma cells. Third-party PBMC and non-expanded interferon gamma negative fraction served as controls. The percentage of interferon gamma positive cells has been enriched from 2.8±0.9 and 2.6±0.8 to 48.6±23.4 and 73.2±25.9 of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells, respectively, by immunomagnetic separation. Interferon gamma positive Tcells has been further expanded *in vitro* from $0.54x10^6±0.05x10^6$ to $214.00x10^6±103.46x10^6$ within 4 weeks. The cytotoxicity has been tested after expansion. The killing of myeloma cells reached 68.1±14.2%. Interferon gamma negative fraction killed only 0.8±0.3% of myeloma cells. As a control, killing of third-party PBMC by expanded interferon gamma positive T cells was 6.9±2.5%. These data demonstrate a specific cytotoxicity effect of expanded interferon gamma positive T cells against myeloma cell line ARH 77 and open the possibility for clinical use of tumor-specific T cells in cancer immunotherapy.

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P0.1110

IMMUNOSELECTION OF FUNCTIONAL CMRF-56+ BLOOD DENDRITIC CELLS FROM MULTIPLE MYELOMA PATIENTS FOR IMMUNOTHERAPY

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Dendritic cells (DC) loaded with tumor-associated antigens (TAA) are a promising new treatment to prevent disease relapse in multiple myeloma (MM) patients. Early phase clinical trials have demonstrated safety, efficacy and immunological responses in MM but a key issue now is the isolation of a functional, clinically relevant DC preparation.

We described a unique blood DC (BDC) isolation platform based on positive immunoselection with the CMRF-

56 antibody. To validate this as a feasible source of BDC for immunotherapy, we undertook a quantitative and functional analysis of BDC in MM patients and healthy donors. Our data demonstrate that MM patients have similar numbers of $CD11c^+CD16^+$ and $CD11c^+CD16^-$ BDC but about half the number of $CD11c^-CD123^+$ BDC in whole blood compared to healthy donors. BDC could be isolated by $CMRF\text{-}56^+$ immunoselection from all MM patients tested with similar yields and purity to healthy donors. These BDC could be activated *ex vivo* with poly I:C or LPS. Furthermore, $CMRF\text{-}56^+$ preparations could induce potent $CD4^+$ and $CD8^+$ T lymphocyte responses in both MM patients and healthy donors. Our data suggest that BDC with *in vitro* functional integrity can be isolated from MM patients in sufficient numbers to justify a clinical trial.

P0.1111

CD40, TLR-7 AND IDIOTYPE-BASED IMMUNOTHERAPEUTIC APPROACHES TO THE TREATMENT OF B-CELL MALIGNANCIES

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Idiotype–based tumor immunotherapy offers unparalleled tumor antigen specificity in myeloma. The antigen-experienced, isotype switched myeloma precursor cell eludes standard chemotherapeutic treatment modalities. We are currently exploring the therapeutic potential of CD40/TLR engagement synergy for the induction of cell-mediated immunity targeted at the idiotype of the A20 murine lymphoma model. In preliminary experiments, a systemic idiotype peptide pulse delivered along with low-dose agonistic CD40 or systemic peptide with TLR-7 agonist were separately unable to prime therapeutic cytotoxic T-lymphocyte (CTL, $CD8^+$) effectors *in vivo*. In combination, however, anti-CD40, TLR-7 agonist + idiotype peptide generated waves of effector CTLs that contributed to significantly enhanced survival kinetics compared with monotherapy- or sham-treated control mice. Follow-on experiments are underway in the 5T33MM model of murine myeloma. Combination therapy involving low-dose CD40 engagement, TLR-7 engagement and idiotype protein immunization has tremendous potential as a powerful immunotherapeutic approach to the treatment of multiple myeloma.

P0.1112

IMMUNOMONITORING AFTER IDIOTYPE PROTEIN-KLH VACCINATION IN PATIENTS WITH MULTIPLE MYELOMA RELAPSE

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Objective. Malignant cells in multiple myeloma (MM) produce a monoclonal immunoglobulin which is tumor-specific and can be used for the induction of specific T cells. The idiotype (Id) is expressed at the cell surface of malignant plasma cells and allow the recognition and targeting of these cells by Id-specific T lymphocytes. A phase II clinical study has started in our center, investigating the efficacy and toxicity of Id conjugated with keyhole limpet hemocyanin (KLH) given as a vaccine to patients with MM. The aim of this therapy was to induce specific immune response directed against the tumor cells. Twelve patients with stable disease or with slow progression not requiring systemic therapy were immunized six times with or without interleukin-2.

Methods. The specific immune response was monitored by proliferation test of mononuclear cells in presence of antigen. The production of interferon gamma (IFNγ) from activated T lymphocytes was evaluated by elispot reader. Non-specific effect of Id vaccination was controlled by flow cytometry. The expression of following antigens in peripheral blood was evaluated: T lymphocytes (CD3, CD4, CD5, CD8), B lymphocytes (CD19, CD20, CD45), NK cells (CD16, CD56, CD3), dendritic cells subtypes (CD11c, CD123, HLA-DR, CD83), monocytes (CD14), activation markers (CD25, CD69) and others (CD28, CD45RA, CD45RO).

Results. No significant toxicity was seen during vaccination. Four patients were excluded from study because of relapses or exitus. One patient from 12 patients has a positive IFNγ response before vaccination and another patient from 8 patients has a positive response after three months of vaccination. Neither specific immune response nor changes in expression of activation markers were detected. Significant changes in relative number of lymphocytes $CD3^+$ T lymphocytes and $CD83^+$ dendritic cells were found when compared with numbers before vaccination.

Conclusion. Id-protein is not strong immunogen therefore Id-KLH vaccine boostered by IL-2 can not evocate significant immune response.

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P0.1113

PROPOSAL FOR AN EARLY TAILORED COMPLEMENT TO CHEMOTHERAPY WITH ANTI-INTERLEUKIN-6 ANTIBODIES BASED ON *IN VITRO* STUDIES IN MYELOMA PATIENTS RESPONDING POORLY TO VAD REGIMENS

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In multiple myeloma (MM) patients (pts) the achievement of a complete remission (CR) is considered as a potential prelude to long-term disease control. Standard melphalan-prednisone or combination chemotherapy yield CR in no more than 5% of the pts with a median survival of less than 3 years. Dose escalation with high dose melphalan followed by stem cell rescue has improved treatment results with a response rate of more than 75%, CRs observed in up to 40-50% of cases after tandem transplants, and a longer time to progression. It has been demonstrated that CR duration is significantly longer with early onset of CR, and the sooner partial response status (PR: ≥50% reduction of the level of serum monoclonal paraprotein) is attained, the higher the CR rate is after tandem transplants, suggesting that sensitivity to high-dose MEL is greater when sensitivity to standard VAD is preserved and/or when residual tumour burden is reduced before intensification. To study the kinetics of response to induction chemotherapy we retrospectively analysed the monthly measurements of monoclonal compounds (MC) [IgG or IgA] in 60 consecutive pts enrolled in a study comparing VAD (29 pts) to Dex combined with a pegylated liposomal doxorubicin (31 pts). Response rates were: 0% CR, 9% VGPR, 57% PR, 25% minimal response (MR), 9% no change. 83% of the evaluable pts could be allocated to final response subgroups as early as the completion of the second course (Figure 1: mean decrease of MC over times) and in 91% of the case the level of MC after the sec-

ond course of chemotherapy was predictive of a final response ≥50% or <50%. Biological indicators are needed to predict induction treatment results and to propose early therapeutic alternatives in poorly responding pts. The mechanisms mediating Dex-resistance in MM cells are multifactorial. Because interleukin 6 (IL-6), among other cytokines, confers such a resistance we performed *in vitro* proliferation studies of MM cells from 5 pts who demonstrated no change (2 pts) or MR (3 pts) after 4 VAD. After a 7-day culture of bone marrow samples (i.e. MM cells together with stromal cells) in presence of Dex or BE-8 (an anti-IL6 antibody) alone, or in combination, we observed that while tested alone Dex and BE-8 induced a CD138+ cell proliferation decrease of respectively 38% (range:0-45) and 30% (0-57), the association of Dex+BE-8 reduced CD138% cells of 63% (0-99) as compared to control wells. For 4 out of 5 samples, clinically achievable concentration of anti-IL6 MoAb sensitized MM cells to Dex (Figure 2: proliferation of MM cells from 1 pt refractory to VAD). In conclusion: *in vitro* testing of MM patient cells sensitivity to Dex/anti-IL6 could allow to propose addition of anti-IL6 in poorly responding patients as early as the third course of induction.

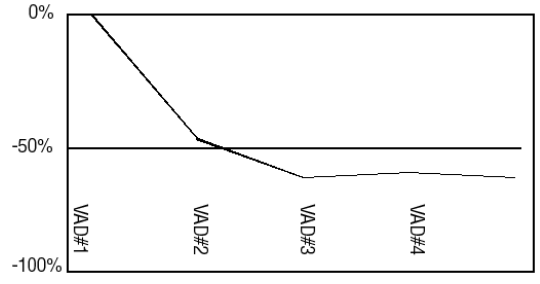

Figure 1

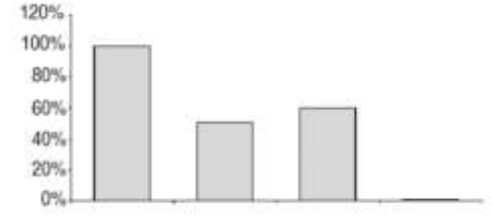

Figure 2

PO.1114

GENERATION AND PROFILING OF FULLY HUMAN THERAPEUTIC ANTIBODIES AGAINST CD38 FOR THE TREATMENT OF MULTIPLE MYELOMA

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CD38 appears on lineage-committed progenitors of lymphoid, erythroid and myeloid cells, while the most primitive pluripotent stem cells of the hematopoietic system are CD38-negative. CD38-expression is also found on plasma cells and activated B- and T cells. CD38-upregulation was detected on various cell-lines derived from B, T, and myeloid/monocytic tumors. Especially for the indication of multiple myeloma (MM), which remains an incurable malignancy with a median survival of 3-4 years, a strong expression has been reported in the majority of patient samples. Hence, over-expressed CD38 on malignant cells may provide an attractive therapeutic target for immunotherapy. CD38-specific human antibodies were selected from MorphoSys' proprietary HuCAL GOLD® phage display library by cell panning strategies. Three antibodies recognizing different epitopes on CD38 were characterized *in vitro* in detail as follows: All affinities for CD38 were in the low nanomolar range with dissociation constants between 0.5 and 6.3 nM for bivalent IgG1 and between 2.4 and 56.0 nM for monovalent Fab fragments. IHC profiles with healthy and malignant tissue from MMpatients show that the antibodies were highly specific for human CD38 and correlated well with the tumor infiltration rates. Additionally, one candidate cross-reacted with non-human primate's CD38. The human IgGs were able to kill efficiently CD38-expressing cell-lines and primary MM cells from patients by ADCC and CDC in a concentration dependent manner. EC_{50}-values of the different antibodies in ADCC and CDC range from 40 pM to 280 pM and from 410 pM to 13.6 nM, respectively, demonstrating a similar or superior activity over the reference antibody OKT10, which is currently in phase I as immunotoxin-conjugate. Most importantly, early progenitor cells were not affected in ADCC as demonstrated by a clonogenic assay. Finally, one candidate was selected for *in vivo* efficacy profiling using the MM cell-line RPMI8226 in a SCID-mouse xenograft model. After the tumor became visible, the human IgG1 antibodies were given every other day over a period of 32 days at two different concentrations resulting in a significantly reduced tumor growth at 1 mg/kg and an even stronger effect at 5 mg/kg. In conclusion, CD38 appears to be a *bona-fide* target for immunotherapy of multiple myeloma with HuCAL® anti-CD38 antibodies.

POSTER SESSION 12
BONE DISEASE - BIOLOGY AND THERAPY

P0.1201

MYELOMA CELLS PRODUCE SOLUBLE WNT ANTAGONISTS: A MECHANISM OF IMPAIRED BONE FORMATION IN MYELOMA

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Multiple myeloma (MM), a malignancy of plasma cells, develops in the bone marrow, and generates devastating bone destruction. Along with enhanced bone resorption, clinical evidence has also suggested suppression of bone formation as a contributing factor to the bone loss in MM. In contrast to recent understanding on mechanisms of osteolysis enahnced in MM, little is known about factors responsible for impaired bone formation. A canonical Wingless-type (Wnt) signaling pathway has recently been shown to play a critical role in osteoblast differentiation. Therefore, in the present study, we aimed to clarify mechanisms of suppression of osteoblast differentiation by MM cells with a particular focus on a canonical Wnt signaling pathway. Because several secreted Frizzled related protein (sFRP) and DKK family members are known as soluble Wnt antagonists, we first examined the expression of sFRP-1, 2 and 3 and DKK-1 in MM cell lines including U266, RPMI8226 and ARH77. All cell lines expressed sFRP-2 and sFRP-3 mRNA observed by RT-PCR. However, sFRP-1 was not expressed in any cell line, and DKK-1 was expressed only in U266 cells at mRNA levels. We next conducted Western blot analyses for these factors and detected only sFRP-2 in immunoprecipitants of conditioned media as well as cell lysates of all these cell lines. However, no other factors were found at protein levels. In addition to DKK-1 reported to be preferentially expressed in a mature type of MM cells, sFRP-2 mRNA and protein expression was also detected in most MM cells from patients with advanced or terminal stages of MM with bone destruction including plasma cell leukemia (3/4 and 8/10, respectively). In order to examine a biological role for sFRP-2, we added recombinant sFRP-2 to MC3T3-E1 cell culture together with BMP-2. Exogenous sFRP-2 partially suppressed alkaline phosphatase activity but almost completely mineralized nodule formation enhanced by BMP-2. Furthermore, sFRP-2 immunodepletion significantly restored mineralized nodule formation in MC3T3-E1 cells suppressed by RPMI8226 and ARH77 CM. These results suggest that sFRP-2 alone is able to suppress osteoblast differentiation induced by BMP-2 and that MM cell-derived sFRP-2 is among predominant factors responsible for defective bone formation in MM. Because MM cell-derived DKK-1 has been implicated as an inhibitor of osteoblast differentiation, soluble Wnt antagonists such as sFRP-2 and DKK-1 may potently suppress bone formation in MM. Taken together, MM cells may cause an imbalance of bone turnover with enhanced osteoclastic bone resorption and concomitantly suppressed bone formation, which leads to devastating destruction and a rapid loss of bone.

P0.1202

OSTEOPONTIN IS INCREASED AND CORRELATED WITH BONE RESORPTION IN PATIENTS WITH REFRACTORY/RELAPSED MULTIPLE MYELOMA

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Osteopontin (OPN) is a non-collagenous matrix protein produced by various cell types including osteoblasts, osteoclasts and tumour cells. Osteoclasts bind to OPN deposited in the bone matrix, through cell surface CD44 and $\alpha_v\beta_3$-integrins. These interactions are essential for the migration, attachment, and resorptive activity of osteoclasts. Serum levels of OPN are elevated in newly diagnosed patients with multiple myeloma (MM) and correlate with disease stage and extent of bone destruction. The aim of this study was the evaluation of OPN serum levels in patients with refractory/relapsed MM, the correlation with biochemical markers of bone remodelling and their alteration after anti-myeloma treatment. OPN levels were measured using ELISA method (Assay Designs, Inc., Ann Arbor, MI, USA) in the serum of 38 patients (24M/14F, median age: 64 years) with relapsed/refractory MM, before, 3 and 6 months post thalidomide/dexamethasone (Thal/Dex) combined therapy. Thirty-five patients had received intermediate dose of thalidomide (200 mg/daily), while 3 patients had received 400-600 mg of thalidomide daily. Dexamethasone was given at a dose of 40 mg/daily for 4 days every 15 days until response to treatment and then at the same dosage every month. All patients were also received zoledronic acid, monthly, both pre- and post-Thal/Dex administration. In this cohort of patients, we also measured markers of osteoclast activation [soluble receptor activator of nuclear factor κB ligand, osteoprotegerin, and 5b-isoform of tartrate resistant acid phosphatase (TRACP-5b)], markers of bone resorption [C-telopeptide of collagen type-I (CTX)], markers of bone formation (bone-specific alkaline phosphatase, osteocalcin and C-terminal propeptide of collagen type I) and markers of disease activity [β_2-microglobulin, CRP and interleukin-6 (IL-6)]. The above biochemical parameters were also evaluated in 30 healthy, gender- and age-matched, controls. Patients with refractory/relapsed MM had elevated levels of OPN compared with controls (mean values± S.D. for patients and controls, respectively: 33.48 pg/ml±17.89 pg/ml vs. 25.01 pg/ml±6.04 pg/ml, p=0.023). OPN levels showed a positive correlation with both TRACP-5b (p=0.02) and CTX (p=0.01) levels and a weak correlation with IL-6 (p=0.09). Before Thal/Dex administration, 14 patients had 1-3 lytic lesions in the skeletal survey (group A), while 24 patients had >3 lesions and/or a pathological fracture (group B). Group B had elevated OPN values than group A that showed only borderline significance (36.12 pg/mL vs. 30.35 pg/mL, p=0.086). The administration of the combination Thal/Dex did not alter significantly OPN serum levels. However, OPN serum levels post-treatment were not different between patients and controls. We found no correlation between serum OPN levels and response to Thal/Dex or disease-free survival. These results suggest that OPN is increased in the serum of patients with refractory/relapsed MM and correlates with bone resorption. Further studies with greater number of patients are needed to clarify the exact role of OPN in this cohort of patients.

P0.1203

ENDOTHELIN-1 DIRECTLY SUPPRESSES BONE FORMATION BY INHIBITING CELLULAR DIFFERENTIATION OF HUMAN OSTEOBLAST-LIKE CELLS

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Endothelin-1 (ET-1), a 21 amino acid peptide, and its G-protein coupled receptors, ETAR and ETBR are expressed by many cell types, including osteocytes, osteoblasts (OBs), osteoclasts (OCs) and vascular endothelial cells. In a cohort of 65 individuals, we have demonstrated that peripheral blood plasma levels of ET-1 are elevated in patients with multiple myeloma (MM) when compared with patients with MGUS or age-matched normal donors. Although controversial, a number of studies have shown that ET-1 stimulates bone formation in animal models and may be the major factor responsible for initiating sclerotic bone lesions in prostate and breast cancer with bone metastases. To clarify the biological significance of the elevated levels of ET-1 in MM patients with respect to bone disease, we investigated the direct effect of ET-1 on cultured human bone-derived OB-like cells by using recombinant human ET-1 and enforced expression of ET-1 in OBs with the use of a retrovirus vector containing the human ET-1 cDNA. The effect of ET-1 on the cell phenotype and function of OB-like cells was assessed using cell proliferation assays, immunofluorescence, flow cytometric analysis and *in vitro* and *in vivo* bone formation assays. Recombinant human ET-1 was found to have no effect on cell proliferation, but was found to inhibit cellular differentiation, as measured by a decrease in OB-like cell surface ALP expression. Consistent with these results, OB-like cultures in which ET-1 was constitutively expressed at high levels were found to express dramatically lower levels of cell surface ALP protein, compared with empty vector control cultures. Importantly, ET-1 over-expressing OBs, formed significantly less mineral *in vitro* and less ectopic bone in vivo compared with cells infected with empty vector. Therefore, ET-1 appears to directly suppress bone formation by inhibiting cellular differentiation of human OB-like cells. On the basis of these findings, these data also suggest that the osteoblastic bone lesions seen in other animal models are more likely to be due to the indirect effects of ET-1 on the vasculature.

P0.1204

CHARACTERIZATION OF BISPHOSPHONATE RESISTANCE BY GENE ARRAY ANALYSIS

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Amino-bisphosphonates (N-BP) constitute the standard palliative treatment for myeloma bone disease. In addition to the bone stabilising effect, several *in vitro* and some animal studies raised the hope for a direct anti-myeloma potential of these compounds. Unfortunately the anti-myeloma effect has not been demonstrated in any human *in vivo* study. We have earlier provided an obvious explanation for this discrepancy by demonstrating a rapid development of specific bisphosphonate resistance in myeloma cell lines upon continuous exposure to the drug (M Salomo *et al.,* Br J Haematol. 2003 Jul;122(2):202-10). Here we present data from gene array analysis, which provide further insight into the changes associated with development of N-BP resistance and will provide tools for the detection of N-BP resistance *in vivo.* The gene expression analysis of our myeloma cell line model of N-BP resistance was performed using the Affymetrix U133 human genome chip. Triple analysis of the original JJN-3 myeloma cell line and the N-BP resistant clone J-AR revealed > 2-fold differences in expression levels of 67 genes, including protein tyrosine phosphatase, receptor type, C (CD45), syndecan 2 and aquaporin 3. Of note, the gene expression of FPPS and related enzymes remain unaltered by the development of N-BP resistance. In conclusion, the gene expression data from the JJN-3 myeloma cell line model support our previous finding (using quantitative PCR) that N-BP resistance does not derive from transcriptional regulation of isoprenyl-pathway related enzymes. We do however identify interesting differences in gene expression in other areas known to influence myeloma pathogenesis. Moreover, these resistance-associated differences provide a potential tool to detect N-BP resistance in freshly isolated myeloma cells without the use of cellular *in vitro* assays.

P0.1205

EFFECT OF RESVERATROL ON CELLS INVOLVED IN MULTIPLE MYELOMA DISORDER

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In multiple myeloma (MM), the accumulation of malignant plasma cells in the bone marrow causes profound changes in the bone microenvironment: Bone resorption is increased due to a recruitment of more osteoclasts whereas osteoblast differentiation and bone formation are inhibited. Furthermore, the bone resorption promotes the survival and expansion of myeloma cells. Therefore, a challenging task for treating MM is to find new drugs that have multiple targets. We report here that Resveratrol (3,4',5-trihydroxy-*trans*-stilbene; RSV), a natural compound with antitumor activities in various cancer cell lines, can beneficially affect the behaviour of cells involved in MM disorder. First, the effect of RSV on the growth of 2 human myeloma cell lines, RPMI 8226 and OPM-2 was tested. After a 3-day culture, cell-numbers showed a 4- to 5-fold increase, which was dose-dependently inhibited in the presence of RSV. Full inhibition was obtained at 100microM. Second, RSV inhibits RANKL-induced osteoclast differentiation in cultures of human CD14-positive monocytes. Indeed, RSV reduced the number of Tartrate Resistant Acid Phosphatase (TRAP)-positive multinucleated cells in a dose dependent manner reaching a plateau at 25microM. This effect was associated with a fall of TRAP activity in the medium and cathepsin K gene expression was not up regulated by RANKL. RSV was effective both on early and RANKL-committed osteoclast precursors. When these cultures were performed on dentine slices, RSV abrogated the formation of resorption pits. In addition, the transcription factor NFATc1 reported to be the master switch between monocytes and osteoclasts was not induced by RANKL in presence of RSV and RANK, the receptor for RANKL was down regulated both at the mRNA and protein levels. These results suggest that RSV impairs RANKL-signalling and likely cell events occurring later during osteoclast differentiation sequence. Finally, we examined the effect of RSV on osteoblast (OB) differentiation by studying expression of OB markers in the telomerized bone marrow stromal cell line TERT20 treated with RSV for 3

days. We found that RSV significantly up-regulated the expression of osteocalcin (OC) and osteopontin (OPN) in a dose dependent manner. Moreover, RSV showed a synergistic effect with the 1.25(OH)2 vitamin D3 (VitD3), an osteogenic hormone that stimulates directly transcription of OC and OPN genes. Interestingly, the expression of VDR, the nuclear receptor that mediates VitD3 stimulation, was up-regulated in a dose dependent manner by RSV. In conclusion, our study shows that a single compound, RSV can inhibit bone resorption, promote osteoblast differentiation, and prevent myeloma cell growth. Therefore, RSV merits further investigations to determine whether it could be a useful drug for treatment of MM.

P0.1206

IMATINIB AS A POTENTIAL ANTI-OSTEOLYTIC AGENT IN MULTIPLE MYELOMA

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Imatinib is a tyrosine kinase inhibitor that specifically inhibits the growth of bcr-abl expressing CML progenitor cells by blockade of the ATP-binding site of the kinase domain of bcr-abl. Imatinib has also been shown to inhibit the c-abl, PDGF receptor, ARG and SCF receptor tyrosine kinases and has been used clinically to inhibit the growth of malignant cells in GIST and CML patients. Recent studies from our laboratory have demonstrated that imatinib also inhibits the growth of some non-malignant haematopoietic cells including monocyte/ macrophages, an effect that was not attributable to the known activity profile of imatinib. We now show that the inhibition of monocyte/macrophage development and function is due to imatinib blockade of the macrophage colony stimulating factor receptor, c-fms. Phosphorylation of c-fms was inhibited by therapeutic concentrations of imatinib, and this was not due to a downregulation in c-fms expression. Importantly, these results identify an additional biological target to those already defined for imatinib and suggest that imatinib may also be useful in treating diseases involving cells of the monocyte/macrophage lineage. As heightened bone osteolysis in patients with multiple myeloma (MM) is attributable to an increase in osteoclast number and activity, this suggests that imatinib may be useful in the treatment of the osteolytic component of MM. The effect of imatinib on the activity of osteoclasts derived from normal human monocytes was therefore examined in this study. We observed that therapeutic concentrations of imatinib inhibited the formation of TRAP positive bone-resorbing monocytes, consistent with inhibition of monocyte/macrophage differentiation.

P0.1207

MESENCHYMAL STEM CELL DEFICIENCIES IN MYELOMA PATIENTS

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Progression of Multiple Myeloma (MM) is associated with disrupted bone remodelling resulting from increased osteoclast activity and reduced osteoblast number in involved bones. The aim of this study was to investigate Mesenchymal Stem Cells (MSCs) properties in MM patients to find out whether disorders exist within the Bone Marrow (BM) microenvironment and during disease progression. We examined MSCs derived from 6 MM patients at diagnosis, 3 patients with Bone Lesions (BL-MSCs) and 3 patients Without Lesions (WL-MSCs), and 3 Normal Donors (ND-MSCs). We focused on mesenchymal phenotype, clonogenic and proliferative capacities, Growth Factor Receptors (GF-R) expression, as well as osteogenic differentiation. BM adherent mononuclear cells were used to initiate MSC cultures and 2 passages were performed when confluency was reached. Adherent MSCs were numbered after each passage and CFU-F was counted (at 11 day culture). Analysis of MSC immunophenotype and GF-R expression were performed by flow cytometry. Osteoblastic differentiation assay was performed in inducible specific medium by analysing alkaline phosphatase activity and matrix mineralisation deposition (Von Kossa method). MSCs represented 0.1 to 1% of BM nuclear cells from all sources. The characteristic MSC phenotype ($CD45^-/CD90^+/CD73^+/CD105^+$) was observed in all sources of cells (MM- and ND-MSCs). The clonogenic capacity of BL-MSCs was 2 fold lower than that of WL-MSCs (44±21 *vs* 8 ±23, $p = 0.05$) and their proliferative capacity was 7.5 fold decreased (16,5 x 10^6±15 *vs* 124 x 10^6±*68*, $p = 0.02$, total cells after 2 passages). Expression level of PDGFα & β, IGF-1, EGF and NGF Receptors was lower in MM-MSCs than in ND-MSCs (185± 39*vs* 413±*50* UA, $p = 0.0004$, 212±63 *vs* 383±*143* UA, $p = 0.01$, 227±50 *vs* 534±*67* UA, $p = 0.0007$, 229±34 *vs* 535±*84* UA, $p = 0.0001$ and 179±26 *vs* 441±*98* UA, p = 0.001, respectively), while the percentage of FGF receptor-expressing cells was dramatically decreased in MM as compared to ND (59±31.5 *vs* 90±6%, $p = 0.01$). Interestingly, the osteogenic differentiation capacity of MSCs was maintained in MM-MSCs whatever the progression status of the disease. This study demonstrates that impaired bone formation in MM could be related to a deficient proliferation capacity of MSCs associated with a low growth factor receptors expression. The lack of expansion capacity increases with disease progression. Therapeutic approaches using normal expanded MSCs grafts may improve bone reconstruction in myeloma patients.

P0.1208

RESPONSE TO VELCADE AND BONE METABOLISM IN MUTIPLE MYELOMA PATIENTS

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Myeloma bone disease is characterized by bone destruction not adequately compensated by adequate bone formation. Velcade is a potent proteasome inhibitor with a molecular target, which may vary among tumor types. Clinical evidence of osteoblastic activation was first observed at our Institution in a 63-year old female with kappa light chain MM, who relapsed after tandem autotransplant. After being treated with Velcade 1.0 mg/m^2, she promptly achieved complete remission, associated with a rapid increase in alkaline phosphatase (ALP) without changes in other liver function tests. After observing a similar increases in ALP associated with response in other patients treated with Velcade, alone or in combination to thalidomide and dexamethasone, ALP variation was analyzed in patients treated with single agent Velcade on Millenium trial 025. ALP change observed in responsive (≥25% paraprotein reduction) was compared that of no responders (Figure 1).

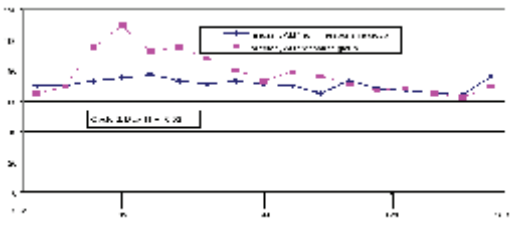

Figure 1.

A statisically significant difference was observed p=0.002. The source of the ALP spikes was then prospectively evaluated in three MM patients by serial measurements of ALP, bone specific alkaline phosphatase (BALP) and intact parathyroid hormone (PTH) (figure II). Rise in ALP after treatment with Velcade was associated with myeloma response and with a parallel increase of BALP and PTH. Our work is the first clinical evidence suggesting that Velcade activates osteoblasts, which in turn is associated with an anti-myeloma effect.

P0.1209

THE COMBINATION OF INTERMEDIATE DOES OF THALIDOMIDE PLUS DEXAMETHASONE IMPROVES ABNORMAL BONE REMODELLING THROUGH THE REDUCTION OF SRANKL/ OSTEOPROTEGERIN RATIO IN PATIENTS WITH REFRACTORY/RELAPSED MYELOMA

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The combination of thalidomide and dexamethasone (Thal/Dex) is very effective in refractory/relapsed multiple myeloma (MM). The pathway of receptor activator of nuclear factor κB (RANK)/osteoprotegerin (OPG) is crucial for the activation and differentiation of osteoclasts. The aim of this study was the evaluation of the effect of Thal/Dex combination on bone remodelling in patients with refractory/relapsed MM. We studied 35 patients (23M/12F, median age: 63 years), who received thalidomide at a dose of 200 mg/daily. Dexamethasone was given at a dose of 40 mg/daily for 4 days every 15 days till response and then at 40 mg/daily for 4 days monthly. All patients were also given zoledronic acid, monthly, both pre- and post-Thal/Dex therapy. We measured, pre-, 3 and 6 moths post-treatment, the following parameters in the serum of patients: markers of osteoclast activation [soluble RANK ligand (sRANKL), OPG, tartrate resistant acid phosphatase isoform-5b (TRACP-5b)], markers of bone resorption [C-telopeptide of collagen type I (CTX)], and markers of bone formation [bone alkaline phosphatase (bALP), osteocalcin (OC), and C-terminal propeptide of collagen type I (CICP)]. The above markers were also measured in 30 healthy, gender- and age-matched, controls. Before the administration of Thal/Dex, patients had increased levels of sRANKL (p=0.008), OPG (<0.001), sRANKL/OPG ratio (p=0.01), TRACP-5b (<0.0001), CTX (p=0.001) and CICP (p=0.01), while they had reduced levels of bALP (p<0.0001) and OC (p=0.001) compared with controls. However, after *correction* of OPG values with patients' renal function, OPG had no differences between patients and controls (median values of OPG/creatinine ratio: 6.713 vs. 6.507, for patients and controls, respectively). The ratio of sRANKL/OPG correlated with the extent of bone disease pre-treatment (p=0.04). Thal/Dex administration resulted in a significant reduction of sRANKL (p=0.056 and p<0.0001, at 3 and 6 months post-treatment, respectively), sRANKL/OPG ratio (p<0.0001 at 6 months post-treatment), TRACP-5b (<0.0001 at 3 and 6 months post-treatment) and CTX (p=0.005, and p=0.001, at 3 and 6 months post-treatment, respectively). Markers of bone formation and OPG did not show any significant alteration during 6 months post Thal/Dex therapy, although OC values had an increase of borderline significance at 6 months (p=0.056). Changes of sRANKL/OPG ratio correlated with changes of both CTX and TRACP-5b. The combination of Thal/Dex was given for a median time of 10 months. Response rate was 65.7%. Median survival was 18 months. Beta2-microglobulin before treatment and type of response predicted for survival. Patients who had a sRANKL/OPG ratio value of $>15x10^{-2}$ had median survival of 13 months, while patients who had lower values had a median survival of 20 months. However, this difference was not significant due to the low number of patients of this study. These results suggest that the combination of intermediate dose of Thal with Dex is very effective in patients with refractory/relapsed MM and improves bone remodelling through the reduction of sRANKL/OPG ratio.

P0.1210

OSTEONECROSIS OF THE JAWS IN MYELOMA: ANALYSIS OF RISK FACTORS INCLUDING TIME DEPENDENCY OF AREDIA® AND ZOMETA® USE, STEROID USE AND UNDERLYING DENTAL PROBLEMS

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Osteonecrosis of the jaws was evaluated in 812 myeloma patients responding to a web-based survey. Data items collected included age, sex, diagnosis, type and duration of bisphosphonate treatment, dental problems such as pain, bone spurs, tooth decay, poor healing, infection, gum disease, and other details, as well as treatment for dental problems. Information about other treatments was also recorded, including chemotherapies, biologic therapies and transplantation. Details of radiation therapy were gathered, with special reference to head and neck irradiation. Of the 812 myeloma patients, 46 (5.7%) indicated a diagnosis of osteonecrosis of the jaws and an additional 46 patients had findings suspicious for early osteonecrosis, giving a total of 92 patients or 11.4% of respondents affected. The strongest correlation was with use of Aredia® and/or Zometa® in both univariate and multivariate analyses (p<.0001). The only other therapy with a significant correlation was prednisone as part of a melphalan/prednisone or alone, but not other steroid use. In an analysis of time dependency, only Aredia® and/or Zometa® and prednisone use were separate factors in the multivariate analyses. The odds ratio is > 1 even at 3 months. The p-value becomes significant at 12 months; more so at 24 and 36 months. For these and other correlations, there

was an identical trend with osteonecrosis and/or suspicious findings. Additional analysis assessed the interaction between steroid use, prednisone in particular, and increased risk combined with Aredia® and/or Zometa®. There was no indication of interaction. Both Aredia® and/or Zometa® and prednisone use were separate factors in the multivariate analyses. The full clinical details and the impact of dental treatment and preventative measures are being further evaluated. 78% of patients with osteonecrosis of the jaws gave a history of prior dental problems. Specific increased risk was noted in patients undergoing tooth extraction, root canal and other surgical procedures, as well as a decreased incidence in patients with recent dental prophylaxis. These issues require further analysis and investigation.

Conclusions. The new entity of osteonecrosis of the jaws in myeloma patients is most strongly associated with use of Aredia and/or Zometa. This risk is time-dependent and becomes significant at 12 months, increasing thereafter to 36 months. Prednisone is an additional and separate risk factor that is not time-dependent. Risk is increased by major dental procedures and poor dental hygiene. These analyses can help form the basis for new recommendations for bisphosphonate use, as well as dental treatment and prevention strategies.

Table 1.

	≥3 mos.	≥6 mos.	≥9 mos.	≥12 mos.	≥24 mos.	≥36 mos.
Odds Ratio	1.33	1.40	1.97	2.20	2.44	2.56
p-Value	0.46	0.32	0.06	0.03	0.004	0.004

P0.1211

PAMIDRONATE AND ZOLEDRONATE - ASSOCIATED OSTEONECROSIS IN MYELOMA IS AN INCREASING AND UNDER-RECOGNIZED PROBLEM

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Pamidronate and zoledronate are bisphosphonate drugs that reduce the risk of skeletal events in myeloma patients with bone lesions. The drugs are widely used off-label in patients without bone lesions and often in smoldering disease. Safety data are available only for short-term use (2 years) but they are usually administered for longer periods of time. Jaw osteonecrosis has recently been identified as complication in bisphosphonate-treated patients. 11 cases of confirmed osteonecrosis have been seen in ~650 myeloma patients seen from March 2001 to August 2004; 2 more cases are undergoing radiological evaluation for confirmation currently. All patients were on pamidronate or zoledronate at the time of diagnosis, and the duration of bisphosphonate therapy was 4-75 months (median 50). All had received corticosteroids previously, and 9 had received thalidomide. 3 had received local radiation to the jaw previously, and 5 had a history of dental extraction within the 1-3 years preceding the diagnosis. Amongst confirmed cases, the mandible was involved in 9, the maxilla in 1, and both in 1. The outstanding symptom was local pain and discomfort, and protruding bone within the oral cavity. Therapy was variable, and the multiple approaches used included cessation of bisphosphonates (n=11), systemic analgesics (n=10), surgical debridement (n=7; repeatedly in 3), gingivoplasty (n=1), teeth extraction (n=5), prolonged antibiotic therapy for persistent anerobic infections (n=4), and hyperbaric oxygen therapy (n=2). Some symptom control was achieved in all patients, but all continued to have persistent problems at the time of last contact (n=10) or death from progressive myeloma (n=1). Amongst patients undergoing surgical intervention, symptoms were controlled for a short while but surgery was invariably followed by worse symptoms with necrosis of more bone. Investigations by the NIH-funded RADAR (Research on Adverse Events and Reports) Project disclosed no reported cases in the FDA MedWatch database, and information submitted by the manufacturer to the FDA in support of a package insert revision includes an osteonecrosis estimate of <1 in 10,000. Our findings suggest that pamidronate- and zoledronate-associated osteonecrosis is a severe, difficult to treat, and under-reported, adverse event. We suggest the following approach to bisphosphonate-treated myeloma patients: (1) careful assessment of the need for continued bisphosphonate therapy beyond 2 years, (2) querying patients about jaw/tooth symptoms, (3) jaw imaging if there are any local symptoms, (4) discontinuation of bisphosphonates in patients developing osteonecrosis, (4) reporting cases of osteonecrosis to FDA MedWatch program, and (5) avoidance of bisphosphonates in plasma cell dyscrasias where their utility has not been established (e.g. smoldering myeloma). Moreover prospective studies are essential such as careful clinical assessment and detailed jaw bone imaging in all myeloma patients who have received bisphosphonates for 3 years or more.

P0.1212

BISPHOSPHONATE THERAPY AND INCREASED INCIDENCE OF MANDIBULAR/MAXILLARY OSTEOMYELITIS

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Bisphosphonates provide skeletal support for patients with multiple myeloma by inhibiting the resorbtion of bone and reducing the incidence of pathologic fractures and hypercalcemia. Recently, bisphosphonates have been linked to osteonecrosis of the jaw in multiple myeloma patients. We report 16 cases of multiple myeloma patients being treated with bisphosphonates who developed periodontitis/osteomyelitis of the jaw. Sixteen patients were evaluated. All were being treated with bisphosphonates for an average of 8 months (range 4-17 months) prior to the onset of new jaw symptoms. Fifteen of the 16 patients are 51 years or older. None of the patients had been irradiated in the jaw nor had obvious osseous manifestation of multiple myeloma in the jaw as evidenced radiologically or pathologically. Six patients were receiving zoledronic acid and 10 pamidronate. Jaw biopsy was preformed in all 16 patients. Actinomycosis was cultured in 4 specimens, two biopsies had multiple positive cultures and the other 10 biopsies did not grow any bacteria. All patients are currently controlled on suppressive antibiotic therapy. The mandible and maxilla are generally bisphosphonate seeking bones as evidenced by scintigraphy revealing increased bone turnover with repetitive chewing motion. Therapeutic levels of bisphosphonates will inhibit osteoclastic activity. The role bisphosphonates play in perpetuating an infection is likely multi-factorial. Angiogenesis inhibition may be involved as well as its effect on preventing the clearing of debris by osteoclasts. The immune system in myeloma is also compromised resulting in a fertile medium for infection and further destruction. Additionally, in an acidic environment, such as one potentially resulting from an infection, bisphosphonates are released more rapidly from hydroxyapatite of bone into the surrounding

area and are cytotoxic to the local stromal cells. As the nitrogen containing bisphosphonates are rapidly released, it may up regulate the host inflammatory response with stimulation of IL-1 and IL-6. As more inflammation ensues there is more release of bisphosphonates. This continuous cycle perpetuates an unstable local environment. We recommend, therefore, that patients have a full dental examination at the time of diagnosis of the plasma cell dyscrasia especially if bisphosphonates are to be considered as part of the therapy. In addition, we suggest to hold bisphosphonates for a period of 3 months prior to invasive dental procedures to allow for the osteoclastic activity to recover, allowing for debris removal and lessening the chance of creating a fertile bacterial medium. Patients with multiple myeloma on bisphosphonate therapy whom experience dental/jaw discomfort should have an aggressive evaluation to rule out any infectious etiology.

PO.1213

RENAL SAFETY OF IBANDRONATE IN MULTIPLE MYELOMA PATIENTS WITH RENAL DETERIORATION

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Background. Bisphosphonates are the standard of care for the treatment of skeletal complications due to bone metastases and multiple myeloma. However, clinical studies have shown that some intravenous bisphosphonates are associated with an increased risk of renal safety issues. The aminobisphosphonate, ibandronate, is indicated for use in patients with bone metastases from breast cancer. In phase III trials, intravenous ibandronate was shown to have a renal safety profile comparable to placebo. Here we present the results of a study that assessed the safety of intravenous ibandronate 6mg in patients with multiple myeloma and pre-existing renal insufficiency.

Methods. In an open-label study of patients with multiple myeloma (n=21, creatinine clearance 8–120 ml/min), intravenous ibandronate 6mg was administered over 30 minutes. Ibandronate excretion and serum levels were measured over 24 hours. AUC of serum ibandronate levels were calculated. Renal function deterioration was graded depending on creatinine clearance (grade 0:≥80, 1:50–79, 2:30–49, 3:<30 ml/min). Markers of tubular damage, αglutathione-S-transferase [αGST] and β-N-acetyl-glucosaminidase [βNAG], were measured at baseline and at 24 and 72 hours after ibandronate infusion.

Results: At baseline, 4 patients had normal renal function and the rest had varying degrees of renal insufficiency. Mean proteinuria was 1483±1588mg/24 hours. There was a statistically significant positive correlation between ibandronate elimination and creatinine clearance (r=0.81; p<0.001). The AUC for ibandronate was not significantly different over the four grades of creatinine clearance. Serum creatinine and urinary enzymes did not change significantly within 72 hours of ibandronate infusion. βNAG showed a significant positive correlation to proteinuria (r=0.49, p=0.01)

Conclusions: In this study, the elimination of ibandronate correlated with renal function; however, the AUC of ibandronate serum levels did not significantly increase. This may indicate that the amount of ibandronate bound to the bone increases with renal insufficiency. In patients with varying degrees of renal insufficiency, intravenous ibandronate was well tolerated. There was no evidence of acute renal toxicity with ibandronate in these high-risk patients. These data suggest that ibandronate may be suitable for use in multiple myeloma patients with pre-existing renal impairment.

PO.1214

CLODRONATE AND DEXAMETASONE AS MAINTENANCE THERAPY IN RESPONDING MULTIPLE MYELOMA PATIENTS

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Background. Osteolytic lesions and pathological fractures are peculiar features in Multiple Myeloma (MM). It is now well established that clodronate decreases the progression of osteolytic lesions, bone pain and fractures also in MM patients. *In vitro* studies demonstrated that biphosphonate can inhibit myeloma cell proliferation inducing apoptosis and may have a direct effect on the tumor cells increasing patient's survival (Berenson *et al.,* 1998).

Aims. Based on these considerations and on the debated issue concerning the best approach for MM responding patients after induction therapy, we evaluated the efficacy and safety of the association clodronate and Dex as maintenance therapy.

Methods. 22 responding patients with MM were enrolled in this study: 14 M/ 8 F, median age 69 years (range 52-88 years), 3 patients had stage IIA, 17 IIIA and 2 IIIB, 13 IgG, 6 IgA and 5 light chain. All patients were considered responders (with > 50% decrease of monoclonal component, Hb level >10 g/dL, reduction in bone pain, improvement in Performance Status): 10 of them to a first-line therapy (8 patients received Melphalan + Prednisone while 2 received VAD regimen) and other 10 to different lines of treatment. Our maintenance therapy consists of clodronate (1600 mg/day) combined with dexamethasone (Dex) (20 mg for 4 days.) given every 28 days. All patients were treated on outpatient basis and received at least 6 courses of maintenance therapy. The biochemical and clinical controls, during the treatment, were performed monthly.

Results. After 38 months of study, the median follow-up is 15 months (range 6-38 months) and the median number of maintenance courses given is 13 (range 7-31). To date 18 patients are alive and 13 patients persist in continuous response. Among the 8 patients relapsed after 6, 8, 9, 13, 15, 18, 19 and 26 months, 5 died in relapse of disease. Median duration of response from starting maintenance is 12 months (range 6-38).

Conclusions. In our experience this combined treatment demonstrated to be effective in maintaining hematological and clinical response. Therapy was well tolerated also in elderly and improves the quality of life.

PO.1215

BIOMARKERS OF MYELOMA-ASSOCIATED LYTIC BONE DISEASE USING SELDI-TOF MASS SPECTROSCOPY

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MGUS is a pre-malignant state, with a 1% annual risk of progression to overt myeloma (Kyle, NEJM 346:564, 2004).

Progression of MGUS to myeloma is preceded and can be predicted by increased rates of bone turnover (Betaille, JCI 88:62, 1991). Progress to overt myeloma is associated with lytic bone disease in about 80% of patients. A simple, reliable test that identifies MGUS patients at risk of imminent progression to symptomatic myeloma would elucidate biological mechanisms that govern the process and identify targets for early intervention. The intimate association of progression with changes in bone biology suggested that early recognition of changes in bone turnover would serve such a purpose. Therefore, we sought to identify protein biomarkers of bone disease in the sera of patients with myeloma that can be used as early predictors of progression. Given the limited reliability of individual markers of bone turnover to identify changes in bone metabolism, we elected to adopt a global proteomics approach. Sera from 62 untreated myeloma patients were collected and stored at -80°C for future analysis. 35 serum samples were from myeloma patients with 1-26 lytic bone lesions as identified on X-ray skeletal surveys and 27 serum samples were from patients without lytic lesions. The sera were analyzed by surface-enhanced laser desorption and ionization-time of flight mass spectroscopy (SELDI-TOF MS) using Ciphergen's ProteinChip Biology System II (PBS II), to identify protein patterns associated with lytic bone disease. Each sample was applied in 4 replicates to randomly assigned spots on 12 IMAC30 ProteinChips placed in a bioprocessor and activated with Cu^{++} ions using a Biomek2000 robot. The chips were read on a PBS II reader. The mass spectra of proteins, generated using an average of 66 laser shots, were calibrated using peptide and protein standards and normalized to total ion current using CiphergenExpress 2.0 software. To develop a classification model that separates non-treated patients with focal lesions from those without focal lesions, we identified among the low molecular weight (1500-25000 kDa) proteins a set of 17 protein peaks that were differentially expressed between the two groups at a significance level of $p<0.005$. We then randomly selected 80% of patients for developing a model based on these peaks using a stepwise logistic regression method; 198 calibrated and normalized spectra from 50 randomly selected patients,28 with lytic bone lesions and 22 without lytic lesions (2 samples had only 3 replicates) were used for model development. The model used 10 protein peaks to classify the patients, with a receiver operating characteristic (ROC) area of 0.897. Finally the model was challenged by the set-aside test set of 50 spectra from 12 randomly selected patients (one patient had 6 replicates). The model exhibits high sensitivity and specificity, and its overall prediction accuracy is around 90%. Studies are underway to identify the biomarkers and to validate the utility of this model to predicting progression of MGUS to overt myeloma.

P0.1216

ELEVATED SERUM LEVELS OF SDF-1· ARE ASSOCIATED WITH INCREASED OSTEOCLAST ACTIVITY AND OSTEOLYTIC BONE DISEASE IN MULTIPLE MYELOMA PATIENTS

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Multiple myeloma (MM) is an incurable plasma cell (PC) malignancy able to mediate massive destruction of the axial and craniofacial skeleton. The aim of this study was to investigate the role of the potent chemokine, stromal derived factor-1 alpha (SDF-1α) in the recruitment of osteoclast (OC) precursors to the BM. Our studies show that MM PC produce significant levels of SDF-1α protein and exhibit elevated plasma levels of SDF-1α when compared with normal, age-matched subjects. The level of SDF-1α positively correlated with the presence of multiple radiological bone lesions in individuals with MM, suggesting a potential role for SDF-1α in OC precursor recruitment and activation. To examine this further, PB-derived $CD14^+$ OC precursors were cultured in an *in vitro* OC-potentiating culture system in the presence of recombinant human SDF-1α. Whilst failing to stimulate an increase in $TRAP^+$, multinucleated OC formation, our studies show that SDF-1α mediated a dramatic increase in both the number and the size of the resorption lacunae formed. The increased OC motility and activation in response to SDF-1α was associated with an increase in the expression of a number of OC activation-related genes including, RANKL, RANK, TRAP, matrix metalloprotease-9 (MMP-9), Carbonic Anhydrase II (CA-II), and Cathepsin K. Importantly, the small-molecule CXCR4-specific inhibitor, 4F-Benzoyl-TE14011 (T140), effectively blocked OC-formation stimulated by the myeloma cell line, RPMI-8226. On the basis of these findings, we believe that the synthesis of high levels of SDF-1α by MM PC may serve to recruit OC precursors to local sites within the BM and enhance their motility and bone resorbing activity. Therefore, we propose that inhibition of the CXCR4-SDF-1α axis may provide an effective means of treatment for MM-induced osteolysis.

P0.1217

CORRELATION OF OSTEOPROTEGERIN AND SRANKL CONCENTRATIONS IN SERUM AND BONE MARROW OF MULTIPLE MYELOMA PATIENTS

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Background. Receptor activator of NF-κB (RANK) is a TNF receptor superfamily member expressed on the surface of osteoclasts and their precursors that mediates their differentiation, survival, and activation upon interaction with its ligand, RANKL, expressed by osteoblasts, stromal and myeloma cells. RANKL is produced as a membrane bound protein and cleaved into a soluble form by a metalloprotease. The primary secreted form is produced by activated T-lymphocytes. Osteoprotegerin (OPG) is a secreted TNFR, it acts as a decoy receptor for RANKL and inhibitor of RANK-RANKL interaction. Recent *in vitro* studies have shown that an imbalance of OPG/RANKL system is crucial in the myeloma induced bone disease. It has been reported recently that the serum ratio RANKL/OPG is increased and correlates with the extent of bone disease, bone resorption, and survival in MM patients (Terpos et al. Blood 2003; 102, 1064-1069). Therefore in this study we analyzed the concentrations of BM and serum OPG and soluble RANKL in MM patients.

Materials and methods. Determinations of bone marrow (BM) and serum OPG and sRANKL concentrations were performed in 45 MM patients (19M 26F, median age 66 range 38-86; 11 at stage I, 13-II, 16-IIIA, 5-IIIB acc. to DS.; 29 had lytic lesions at skeletal X-ray survey; 4 had hypercalcemia; monoclonal protein IgG was in 29 patients, IgA – 10, IgM-

1, Bence - Jones – 4, NS – 1) and 42 age and sex matched healthy controls by means of ELISA method using Osteoprotegerin ELISA and sRANKL ELISA kits (Biomedica GmbH, Vienna, Austria).

Results. In MM patients, serum OPG concentration in particular patients ranged from 42 to 445 pg/ml with a mean level of 128±76, median 106 pg/ml while in healthy persons OPG level ranged from 43 to 159 pg/ml, mean 82±26, median 81 pg/ml (p=0,000109). In MM patients serum sRANKL concentration ranged from 0 to 293 pg/mL, mean 28±55, median 8 pg/mL while in healthy persons sRANKL level ranged from 0 to 43, mean 4,4±8,0, median 1pg/ml (p=0,000367). In MM patients, BM OPG concentrations ranged from 40 to 397 pg/mL with a mean concentration of 122±71, median 103 pg/mL while sRANKL concentration ranged from 0 to 130 pg/mL, mean 24±26, median 21 pg/mL. There was found correlation between BM and serum OPG concentrations (r=0,838; p=0,000..13) and between BM and serum sRANKL concentrations (r=0,702; p=0,000..15). No correlation was between BM OPG and sRANKL concentrations (r=0,1338; p= 0,38) and between serum OPG and sRANKL concentrations (r=0,15; p=0,32). Median values of the OPG/sRANKL ratio for BM and serum of MM patients were 4,9 (range 0-79) and 4,0 (range 0-176) (p=0,64), respectively. The differences in OPG/sRANKL level ratio depending on occurrence of osteolysis and stage of disease were not statistically significant: in BM - of patients with osteolysis median ratio was 7,7 range 1-480 and of patients without osteolysis median 5,4 range 1-66 (p=0,50); in serum - of patients with osteolysis median 9,2 range 0,7-406 and of patients without osteolysis median 3,4 range 0,4-168 (p=0,09); in BM – in stage I- median 4,9 range 1-66, in stage II+III - median 6,4 range 1-480 (p=0,5532); in serum - in stage I- median 8,5 range 0,4-168, in stage II+III - median 7,3 range 1,3-406 (p=0,96). Median value of the OPG/sRANKL ratio for serum of healthy persons was 0,93, range 0-159.

Conclusion. OPG/sRANKL ratio is increased in BM and serum of MM patients. Biological and clinical significance of BM and serum OPG and sRANKL concentration correlation is not clear.

POSTER SESSION 13: MGUS AND EVOLVING MYELOMA

P0.1301

SERUM FREE LIGHT CHAIN RATIO IS AN INDEPENDENT RISK FACTOR FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background. We hypothesized that an abnormal serum free light chain (FLC) ratio, indicates clonal evolution in the neoplastic plasma cell and increases the risk of progression to malignancy.

Patients and Methods. Of 1,384 Southeastern Minnesota MGUS patients diagnosed between January 1, 1960 and December 31, 1994, 1148 patients who had cryopreserved serum samples collected within 30 days of MGUS diagnosis were studied with the serum FLC assay. The FLC levels were determined using the serum FLC assay (Freelite™, The Binding Site Limited, Birmingham, U.K.). The FLC assay allows the assessment of clonality based on the ratio of kappa/lambda light chain levels (normal reference range, 0.26 to 1.65). Patients with a kappa/lambda FLC ratio <0.26 are typically defined as having monoclonal lambda free light chain and those with ratios >1.65 are defined as having a monoclonal kappa free light chain.

Results. An abnormal FLC ratio (kappa/lambda ratio <0.26 or >1.65) indicating presence of monoclonal FLC was detected in 379 (33%) patients. In a Cox proportional hazards model, the risk of progression in patients with an abnormal FLC ratio was significantly higher compared to patients with a normal ratio (hazard ratio, 3.5; 95% CI, 2.3-5.5; p<0.001). The increased risk was independent of both the size and type of the serum monoclonal (M) protein by multivariate analysis. There was a good correlation between increasingly abnormal FLC ratio and the relative risk of progression. The annual rate of progression was 0.8% when the serum kappa/lambda FLC ratio was 1/4-4.0, 2% for FLC ratio 1/4-1/8 or 4.0-8.0, and 3% when the ratio was <1/8 or >8.0. We constructed a model for predicting the risk of progression of MGUS based on the size of the serum M protein and presence of an abnormal FLC ratio (<0.26 or >1.65). The use of these two risk factors identified 3 cohorts of MGUS patients with significantly different rates of progression: A high-risk subset (15% of MGUS patients) with a 46% risk of progression to myeloma or related disorder at 20 years; an intermediate-risk subset (32% of MGUS patients) with a 26% risk of progression; and a low-risk subset (53% of MGUS patients) with only a 7% risk of progression.

Conclusions. The FLC ratio is a clinically and statistically significant predictor of progression in MGUS, and is independent of the size and type of the serum M protein. We present a new risk-stratification model for progression of MGUS that identifies a low-risk subset of MGUS patients. Since this subset accounts for more than half of all patients with MGUS, this is a finding of significant importance for the management of MGUS.

P0.1302

MONOCLONAL GAMMOPATHY SCREENING: IMPROVED SENSITIVITY USING THE SERUM FREE LIGHT CHAIN ASSAY

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Introduction. Screening for monoclonal gammopathy (MG) in the UK commonly consists of serum protein electrophoresis (SPE), serum immunoglobulin (Ig) levels and detection of Bence Jones proteinuria (BJP). With this approach the reported sensitivity in detection of clinically significant plasma cell dyscrasia is 96%. Important conditions such as nonsecretory myeloma, light chain myeloma where the renal threshold for light chain excretion has not been reached; amyloidosis and light chain deposition disease may be missed. We aimed to compare the ability of serum free light chain (sFLC) assays, in combination with routine screening methods, to detect patients with monoclonal gammopathies (MG).

Methods. We assessed 217 consecutive samples referred to a district general hospital for investigation of possible monoclonal gammopathy and 24 known cases of multiple myeloma. The 217 samples received routine SPE (Sebia) and total Ig measurement (Beckman Immage). Immunofixation electrophoresis (IFE - Sebia) was performed if an abnormality was detected in SPE, immunoglobulin levels or if BJP was detected. All serum samples were then assessed by FreeliteTM for the presence of abnormal serum free kappa and lambda levels and kappa/lambda ratios.

Results. The combination of sFLC ratios and SPE detected 24/24 of the known monoclonal gammopathy cases. The remaining 217 samples were shown to be normal by SPE, suggesting no new cases of monoclonal gammopathy. However, 8 of these cases (3.3%) had an abnormal free light chain ratio. Where available, urine protein electrophoresis/urinalysis did not reveal Bence Jones protein (5/8). IFE was performed in all 8 cases and shown to be normal. Review of the case notes confirmed 3 cases of multiple myeloma; 2 requiring treatment. 2 cases had hypogammaglobulinaemia. The remaining five cases are under investigation.

Discussion. For proven cases of MG, this series shows that the combination of sFLC assays with SPE has higher sensitivity {27/27 cases (100%)} than SPE with BJP {24/27 cases (89%)}. The improved sensitivity has important implications for the detection of clinically important plasma cell dyscrasia. This combination has the advantage of ease of single sample collection for patients and suggests that urinalysis is unnecessary. For the laboratory this approach may reduce staff workload both for preparation of samples and for their interpretation. The space required for storage of samples is also substantially reduced. Further work is in progress to confirm these findings.

P0.1303

SERUM FREE LIGHT CHAIN LEVELS IN ASYMPTOMATIC MYELOMA

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Patients with asymptomatic myeloma fulfil two of the diagnostic criteria for myeloma having more than 10% bone marrow plasma cells and an M protein of greater than 30 g/L, but they are asymptomatic with no evidence of end organ or tissue damage. The median time to disease progression is 12-32 months. These patients do not require treatment but do require monitoring for progression to symptomatic myeloma. Predicting progression of asymptomatic myeloma would be of clinical benefit to optimise monitoring and initiate treatment prior to substantial end organ damage. However monoclonal spike, plasma cell labelling index, bone marrow plasmacytosis, immunoparesis and the presence of Bence Jones protein have limited value in predicting progression. Abnormal levels of serum free light chains are present in 95% of all multiple myeloma patients and have clinical benefit in diagnosis and monitoring of disease. In monoclonal gammopathy of undetermined significance (MGUS) 60% of patients have abnormal serum free light chain ratios and are an independent risk factor for progression to myeloma. The aim of this study was to examine the serum of asymptomatic patients for serum free light chains at diagnosis and to determine if they are predictive of disease progression. Archived presentation sera were studied from forty three asymptomatic myeloma patients who had been registered into United Kingdom Medical Research Council trials (1980-2002). Archived presentation sera were assayed for serum free light chains using the serum free light chain assay on an Olympus AU400 analyzer. Times to progression for those with abnormal versus normal serum free light chain ratios were compared. Times to progression were examined by Kaplan-Meier survival curves and log-rank sum statistical analysis. Abnormal serum free light chains were present in 36/43 (84%) of asymptomatic myeloma patients at the time of diagnosis and the remaining 7 patients had normal ratios. The median follow-up time for all 43 patients was 2807 days. Six patients with a normal kappa/lambda ratio had a median time to progression of 1323 days. In contrast, 26 patients with abnormal serum free kappa/lambda ratios had a median time to progression of 713 days. Ten patients who had an abnormal kappa/lambda ratio had not progressed at the time of follow-up. Although the median time to progression of patients with normal serum free light chain ratios was greater than those with abnormal ratios, this did not reach statistical significance ($p<0.13$). In summary, 84% of asymptomatic myeloma patients have an abnormal kappa/lambda ratio at diagnosis, in comparison with 95% of multiple myeloma and 60 % of (MGUS) patients. Furthermore, our data suggest that those with normal serum free light chain ratio may progress more slowly than those with abnormal ratios. Due to the small number of patients in this study, this did not reach statistical significance. In the spectrum of malignancy from MGUS to asymptomatic and symptomatic myeloma serum free light chain levels have an increasing frequency of abnormality and are associated with increased risk of progression to symptomatic myeloma.

P0.1304

SERUM LEVELS OF CD138, BONE DISEASE OF MULTIPLE MYELOMA AND DIFFERENTIATION BETWEEN MM AND MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE

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Syndecan-1 (CD138) is a glycoprotein trans-membrane present in plasma cells. This factor has an important role in the regulation of several relevant cell functions (proliferation, cell adhesion and apoptosis). It has also been recognized importance has been described as an essential factor for the internalitation of Osteoprotegerin (OPG) by plasma cells, and therefore it could be involved in the pathogenesis of bone disease in multiple myeloma (MM).

Aims. To investigate the possible significance of the serum levels of CD138 in the differentiation between MM patients with distinct degrees of bone disease, as well as for distinction between MM patients and monoclonal gammopathy of unknown significance (MGUS) patients. Also to evaluate the possible relationship between serum levels of CD138 and biochemical markers of bone remodelation (BMBR) or others cytokines implied in MM bone disease.

Materials and methods. 61 patients were included: 45 MM (median age 71, M/F: 1.37), 16 MGUS (median age 68, M/F: 0.37) and 8 healthy individuals (HI) (median age 59, M/F 0.17). The serum levels of CD138 were measured by ELISA (Euroclone®). We measured 6 BMBR (Pyrt, Dpyrt, β-crosslaps, Trap5b, bone alkaline phosphatase and Osteocalcin) and 10 serum cytokines (IL-6, sIL-6R, TNF-α, IL-1β, HGF, VEGF, OPG, sRANKL, MIP-1 α, and IGF-I). All parameters were measured in serum by ELISA, except Pyrt and Dpyrt measured in urine by HPLC. Statistical methods: Non-parametric tests (U de Mann-Whitney, Kruskal-Wallis and Spearman's correlation).

Results. The serum levels of CD138 were significantly higher in MM patients (102.0 ng/mL) than MGUS patients (40 ng/mL) (p<0,05), and HI (37, 1 ng/mL) (p<0,01). CD138s levels do not differentiate between MM patients with different degrees of bone affectation. The serum levels of CD138 significantly correlated with some BMBR (Pyrt) and with the serum measure of sIL6R and MIP-1α. No correlation was found with serum levels of RANKL or OPG.

Conclusions: In our study, the serum levels of CD138 were useful to differentiate MM patients from MGUS patients and HI. Although it didn't show any value in discriminating between MM patients with different degree of bone disease, it did show correlation with some BMBR and MIP-1α.

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P0.1305

ACCURATE PREDICTION OF OUTCOME FOR WITH IgC AND AND IgA MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE PATIENTS USING FLOW CYTOMETRY

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Only a small proportion of patients with monoclonal gammopathy progress to myeloma, but long term follow up is required. Utlising six-parameter flow cytometry, mRNA microarray expression analysis, FISH karyotyping, assessment of immunoglobulin sequence heterogeneity and in vivo kinetic analysis, we have identified and characterised human short-lived and long-lived bone marrow plasma cells. Myeloma is derived from the latter plasma cell subset, whilst the majority of normal bone marrow plasma cells are short-lived. In at least a proportion classified as having monoclonal gammopathy of undetermined significance (MGUS), the paraprotein is produced by an expansion of reactive long-lived plasma cells. More accurate characterisation of the plasma cells present in the bone marrow of MGUS patients is likely to predict outcome. Extensive analysis of phenotype and genotype is not feasible for routine diagnosis, but we have demonstrated that simple flow cytometric analysis is potentially sufficient to discriminate patient groups with distinct outcomes in a series of selected 88 patients from a single institution. The aim of this study was to perform a more extensive analysis of outcome on a series of patients from a number of centres. Bone marrow aspirate and trephine biopsy samples were assessed in 396 consecutive patients (M:F = 1.2:1; median age 75 years; age range 38-103 years) with an eventual diagnosis of MGUS by standard criteria made between 1995 and 2000. Plasma cells represented a median 0.8% of bone marrow leucocytes (range 0.1-9.2%). Five-parameter flow cytometric analysis was performed in all cases. Patients with no normal short-lived (CD19+56-) plasma cells or an excess of long-lived plasma cells (CD19- or CD19+56+ plasma cells representing >1% of bone marrow leucocytes) were classified as having a neoplastic profile (n=161, 41%). Patients with short-lived plasma cells representing at least 70% of the total plasma cell pool were classified as having a normal profile (n=92, 23%). The remainder were classified as having an intermediate profile (n=143, 36%). Progression to myeloma occurred in 18/161 (11.2%) of patients with a neoplastic profile at a median of 3.3 years from presentation (range 1.3-7.2 years). In contrast, none of the patients with a normal profile developed myeloma. Disease progression occurred in only 1/143 (0.7%) of patients with an intermediate profile (p<0.0001). Three of eight intermediate patients undergoing later bone marrow analysis had developed a neoplastic profile after a median 3 years of follow-up, confirming that continued monitoring is necessary for this group. The data confirms that basic flow cytometric analysis is extremely powerful at predicting progression to myeloma in IgG and IgA MGUS. It is possible to identify patients with a negligible risk of disease progression over a five year follow-up period. These patients may be reassured and spared the worry and uncertainty of long term follow up while the remaining patients may be monitored in a more detailed and systematic way. Trials of therapeutic intervention may be feasible in patients with a high risk of progression.

P0.1306

DISSECTING THE LYMPHOCYTE AND PLASMA CELL COMPONENTS IN MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE

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Monoclonal gammopathy is a biochemical finding that is often unattributed to a specific disease entity, but which may represent the earliest stages of a heterogeneous group of disorders including myeloma, Waldenstroms macroglobulinaemia, CLL and other B-cell neoplasias. In some cases,

the paraprotein may be the product of a reactive process. Our hypothesis is that patients with a reactive paraproteinaemia are unlikely to develop a malignant disorder; whilst progression in those with a neoplastic basis for their paraprotein will be dependent on the type of neoplastic cell responsible. The aim of this study was to determine whether it is possible to identify a neoplastic cellular population responsible for paraprotein production, and if so to determine the clinical disorder most closely associated with the abnormal cellular population. Bone marrow aspirate and trephine biopsies were analysed from 96 patients (male:female ratio = 1.6:1; median age 73 years, range 39 – 105 years) with MGUS/MG[u]. The paraprotein was IgM in 30/102 (29%) and IgG or IgA in 72/102 (71%). Flow cytometric analysis was used in all cases to determine B-cell light chain expression and CD19/CD56 expression in plasma cells. More extensive phenotypic and genotypic analysis was performed as necessary. Patients were classified as having an abnormal B-cell profile if the kappa:lambda ratio was below 0.5:1 or above 3:1 and an abnormal plasma cell profile if fewer than 10% of marrow plasma cells were short-lived (CD19+CD56-). Patients with an IgG or IgA paraprotein showed a neoplastic plasma cell profile in 24/72 (33.3%) of cases, whilst 29/72 (40.3%) showed a plasma cell profile that we have previously demonstrated to be associated with a low risk of disease progression. Both plasma cells and B-cells were normal in 10/72 (13.9%) of cases, whilst 9/72 (12.5%) showed a monoclonal B-cell abnormality (5/9 typical CLL immunophenotype, 4/9 unclassifiable CD5- B-cell abnormalities). Patients with an IgM paraprotein showed a monoclonal B-cell abnormality in 17/30 (56.7%) of cases (3/17 typical CLL immunophenotype, 14/17 unclassifiable CD5- B-cell abnormalities). A neoplastic plasma cell profile was not detected in any patients with an IgM paraprotein, although 5/30 (16.7%) showed a reactive plasma cell profile. Both plasma cells and B-cells were normal in 8/30 (26.7%) of IgM MGUS cases. These results demonstrate that it is possible to characterise the abnormal B-cell or plasma cell population in many patients with MGUS by utilising routinely applicable flow cytometric analysis. IgM paraproteins are predominantly derived from neoplastic non-CLL B-cell expansions, but many patients have reactive plasma cell expansions. IgG or IgA paraproteins are usually plasma cell derived, but B-cell disorders and particularly CLL should be excluded. It is probable that the outcome of patients with MGUS correlates with the cellular fraction generating the monoclonal immunoglobulin, and bone marrow assessment with flow cytometry should be routinely applied at presentation.

P0.1307

MALIGNANT TRANSFORMATION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: PROPOSAL OF A PREDICTIVE SCORE

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The risk of malignant transformation of MGUS is estimated about 1% per year.Two predictive factors of malignant transformation (level and isotype of monoclonal component) were previously described. In order to identify predictive factors we performed a retrospective study on 134 MGUS.

Patients and methods. From 1980 to 1995, 134 MGUS (118 Ig G, 15 Ig A, 1 Biclonal) were diagnosed. Mean age was 67.3 years (Range 37 to 89 years). Sex ratio was 1. The following parameters were evaluated at diagnosis: hemoglobin, creatininemia, calcemia, level of monoclonal component, level of normal immunoglobulins, bone marrow plasmocytosis. Medium follow-up was 84 months (12 to 240 months). Malignant transformation and survival were evaluated in all patients.

Results. In 90 patients (72,9%) MGUS was stable. In 25 cases (18,65%) a malignant transformation was observed: 21 multiple myeloma, 3 non Hodgkin's lymphoma and 1 AL amyloidosis. The rate of malignant transformation was estimated at 11.27% at 5 years and 22.0% at 10 years. Two prognostic factors were identified : a level of monoclonal component ≥15g/l and a bone marrow plasmocytosis ≥5%. When combining these 2 factors 3 risk groups were identified:

	Risk of malignant transformation	
	5 years	10 years
Group 1: low risk < 15g/L and < 5% 36 patients	0% *	10.5% *
Group 2: intermediate risk ≥ 15g/L or ≥ 5% 63 patients	11.0%*	20.6%*
Group 3: high risk ≥ 15 g/L and ≥ 5% 35 patients	33.6%*	48.3 %*

**Log rank: 0.0002.*

Conclusion. In patients with MGUS Ig G and Ig A, we proposed a predictive score of malignant transformation based on level of monoclonal component and bone marrow plasmocytosis. This model allows to identify 3 risk groups.It requires validation on a prospective study.

P0.1308

DIFFERENCE IN OVEREXPRESSION OF PLASMA CELL FOLATE RECEPTOR CAN DISTINGUISH MULTIPLE MYELOMA FROM MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING MYELOMA

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Background. Folate receptor (FR) is a recently recognized tumor marker that is being investigated for targeted delivery of drugs, imaging of cancer cells, and tumor immunotherapy. FR is an attractive candidate for targeted therapy of tumors overexpressing the FR because of the small size of the molecule, simple conjugation chemistry and lack of immunogenicity (*Lu Y, Low PS. Folate-mediated delivery of macromolecular anticancer therapeutic agents. Adv Drug Deliv Rev. 2002 Sep 13;54(5):675-93).* FR is overexpressed in 90% of ovarian cancers and targeted therapy and imaging utilizing this approach is being explored. A number of other human tumors including endometrial, colorectal, breast, and neuroendocrine carcinomas also overexpress FR and are also being studied. FR is negatively or only weakly expressed in most normal tissues including some myeloid cells. In view of the above we wished to learn if overexpression of FR by marrow plasma cells (PC) might differentiate MM from MGUS and SMM.

Methods. We obtained marrow biopsy sections from 20 patients each with MM, MGUS, or SMM, and 11 normals and stained them by immunohistochemistry using a FR-beta antibody. Expression was graded 0 (negative), 1 (equivocal), 2 (positive) or 3 (strong positive) based on immunohistochemical staining of marrow PC. We recorded the percent

PC, percent positive PC, and the staining of other cell types including myeloid, erythroid, lymphocytes, macrophages, megakaryocytes and control tissues.

Results. PC of patients with MM positively expressed FR compared to MGUS, SMM and normal PC ($p<0.0001$). Of interest, 6 of 20 SMM patients showed positive expression. Maturing myeloid cells were also positive, acting as internal positive controls. Macrophages were often positive; some endothelial cells were positive; early erythroid precursors were rarely positive; megakaryocytes, maturing erythroid precursors and lymphocytes did not express FR. The chromosomal location of the FOLR gene cluster is 11q13. Gene expression arrays of purified PC failed to show evidence of overexpression of FOLR1, FOLR2, or FOLR3. We did confirm expression of the FR protein product in myeloma and ovarian cell lines via Western blot. We are presently establishing flow cytometric assays to detect FR and have utilized confocal microscopy to demonstrate the presence of FR in myeloma and ovarian cancer cell lines.

Conclusion. Immunohistochemical studies show differential expression of FR in MM PC compared to absent or equivocal expression in MGUS and normal PC and variable expression in SMM. Western Blot confirms the presence of FR in myeloma cells. The data suggest that FR could be utilized as a diagnostic tumor marker and there are implications for development of folate-based tumor imaging, drug delivery, and immunotherapy in myeloma.

P0.1309

IgM MONOCLONAL GRAMMOPATHY OF UNDETERMINED SIGNIFICANCE – LONG TEAM FOLLOW UP OF 56 PATIENTS

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Among MGUS few studies have focused specifically on Ig M isotype. We performed a retrospective study on 56 patients in order to define the rate of malignant transformation and to identify predictive factors.

Patients and methods. From 1980 to 1995, fifty six patients were diagnosed as Ig M MGUS. Mean age was 67.5±10.4 years with 34 men and 22 women. Mean level of monoclonal component was 12.4g/l. Median follow up was 72 months (Range 12-180 months).

Results. In 31 patients the diagnosis of MGUS was maintained. Nine patients died for other reasons. Malignant transformation was observed in sixteen patients (28.6%):9 Waldenstroem's Macroglobulinemia and 7 Non Hodgkin Lymphoma. The estimated rate of malignant transformation was 12.3% at 5 years and 32.8% at 10 years. According to the level of monoclonal component, the estimated rate of malignant transformation at 5 years was 10.3% in 43 patients with a level < 20 g/L and 42.3% in 13 patients with a level ≥20 g/L (log rank: 0.0057).

Conclusion. In our experience the estimated rate of malignant transformation of Ig M MGUS seems to be higher than in Ig G or Ig A MGUS. The level of monoclonal component is a significant predictive factor of malignant transformation.

POSTER SESSION 14: WALDENSTRÖM'S MACROGLOBULINEMIA, AMYLOIDOSIS AND POEMS SYNDROME

P0.1401

INCIDENCE AND SURVIVAL OF WALDENSTRÖM'S MACROGLOBULINEMIA: A POPULATION STUDY

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Background: Waldenström's Macroglobulinemia (WM) is an uncommon B-cell lymphoproliferative disorder defined by the International Waldenström's Macroglobulinemia Working Party and the World Health Organization as a predominately inter-trabecular bone marrow infiltration of small lymphocytes with an IgM monoclonal gammopathy. Data from several studies suggests that the concentration of monoclonal protein does not correlate with the extent of bone marrow infiltration and has little or no prognostic relevance. There is little reliable incidence data in the U.K. as clear diagnostic criteria have only recently been defined and the disease is often grouped with other plasma cell dyscrasias in epidemiological studies. Furthermore, no prospective randomized study exists to inform the clinician which treatment is the most effective and management is therefore highly variable. This study identifies incidence rates and survival in an unselected population of patients presenting with WM.

Materials and methods: The study was conducted in the South Thames area, covering a population of 7.0 million people managed by 12 Health Authorities and 70 haematologists covering 27 NHS Trusts. A registration form was designed to define cases of WM prospectively between 1999 and 2000. Trained haematology data officers from the Thames Cancer Registry (TCR) collected data on haematological and biochemical laboratory results. All deaths in the U.K. are registered with the Office of National Statistics (ONS) and if the cause of death is from a cancer this information is forwarded to the TCR where they are correlated with registered cases. Conversely, all live cases of WM registered by the TCR are linked three monthly with the ONS to obtain death details. All patients diagnosed with WM during the period 1999-2000 were followed up until December 2002. Statistical analyses: the age standardised rate (ASR) was evaluated using the method of Jensen *et al.,* (1991). The survival curves were generated using the Kaplan-Meier method and were compared using the log-rank test.

Results: 113 *de novo* cases of WM were identified. The crude incidence rate was 1.03 per 100,000 inhabitants corresponding to ASR of 0.62 and 0.41 per 100,000 (European and World Standard Population respectively). 81% cases were IgM kappa and 19% IgM Lambda. The male to female ratio was 1:1.26 with 63 (55%) males and 50 (45%) females. The median age was 74 years (range 42-98 years): 24 (21%) were less than 65 years and 89 (79%) 65 years or greater; 43 (38%) were less than 70 years. The age-specific incidence rates rise steadily with increasing age from 1.41 at age 55-64 years to 5.00 per 100, 000 by age 75-84 years. The overall age-specific incidence rate was 1.26 per 100,000. The median follow up is 29 months (range 0.2 to 47 months). The median overall survival for the whole group was not reached at 45 months. One year survival was 78% (95% CI

68% to 85%) and three year survival was 61% (95% CI 50% to 72%). There was no significant difference between patients aged less than 65 years and those aged 65 years or greater, (p=0.414). Haemoglobin was <10 gms/L in 34% of patients and there was a significant difference in survival between the 2 groups [p=0.043]. 9% of patients presented with a platelet <100,000 x 10^9/l but survival difference was observed. 93% of patients had a WHO PS score of 0-2 at presentation.

Conclusions: This is the first population-based study in the U.K. to evaluate the incidence and survival of patients with WM. This study has shown WM to be a rare disease, which increases with age and has an incidence comparable to that reported from multi-centre studies. It has shown that it is a disease with a median age of 74 years, which is higher than previously reported from multi-centre studies. We did not see any survival advantage according to age but haemoglobin of <10 gms/L appears to be a prognostic factor for survival.

PO.1402

WALDENSTROM'S MACROGLOBILINEMIA: A RETROSPECTIVE ANALYSIS OF 20 PATIENTS FROM 1993 TO 2004 AT SAINT ANTONIO'S GENERAL HOSPITAL (OPORTO, PORTUGAL)

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Introduction. Waldenström's macroglobulinemia (WM) is a rare lymphopoliferative disorder characterized by lymphoplasmocytic infiltration, with intertrabecular pattern, of the bone marrow and/or ocassionally other tissues, and by the presence of a serum monocloral IgM.

Objective. The current study was conducted to analyse the prognostic value of presenting features of a series of patients with WM, who were homogeneously treated.

Materials and Methods. We did a retrospective study, from 1993 to 2004 at Sto Antonio's Hospital, of 20 newly diagnosis WM patients, by collecting data from the clinical processes of the patients. In the analysis it was used the statistical package SPSS, v. 12.0.

Results. We evaluated 20 patients with WM, the mean age at diagnosis was 70 years (range, 53 to 83); the ratio male/female was 1.5; with a median overall follow-up was 32.5 months (range, 1 to 101), 37.5 (range, 6 to 74) for living patients and 38.0 (range, 1 to 101) for those who died. A 3-years overall survival 71,4% and 5-years overall survival 60%. Six patients (30 %) died with the median interval from diagnosis being 8 months (range, 1 to 101). The 20 untreated WM patients received either chorambucil (n=13) or no treatment (n=7); of these, 6 patients were asymptomatic and the other one was symptomatic but died before sarted treatment. The analysis showed that the mean value of serum β_2-microglobulin at presentation of the patients who died was superior to the living patients (p=0,002). The patients who died had the variable age at presentation superior to those who didn't died (p=0,013).

Conclusions. This retrospective analysis confirms that clorambucil is an effective first-line agent in WM, and that β_2-microglobulin levels and age at diagnosis are important prognostic markers in WM that may influence the survival of the patients. However, the precise levels of β_2-microglobulin with prognostic significance remain to be determined.

PO.1403

EXPANDED CLONES OF CYTOTOXIC SUPPRESSOR T CELLS IN THE BLOOD OF PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINEMIA

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Previous studies have suggested that expanded T-cell clones are found in the blood of 59% of patients with multiple myeloma. These expanded T-cell clones are associated with prolonged overall survival and thus it has been suggested that they may have anti-tumor activity. The aim of these studies was to search for similar T-cell clones in the peripheral blood of patients with Waldenström's Macroglobulinemia by using flow cytometry to determine the T cell receptor (TCR) Vbeta repertoire. Expanded T-cell clones were detected in 9 of 15 (60%) patient samples. Of the nine patients with TCR Vbeta clones, for patients had multiple clones. The TCR Vbeta clones were not identical, consisting of a variety of clones across the TCR Vbeta repertoire. The clones in myeloma are cytotoxic T cells that are CD3+CD8+ CD57+CD45RA-CD28- and perforin+. We found that TCR Vbeta clones in Waldenstroms Macroglobulinemia are also CD3+CD8+ cytotoxic T cells unlike those found in elderly normals which are predominantly CD4+. To determine if the expanded clones are CMV specific we used an HLA-A0201 pp65 tetramer. Of the 15 patients tested only 5 were HLA-A0201 positive and of the 9 patients with TCR V beta clones only 2 were HLA-A0201 positive. Preliminary data suggests that the expanded clones are not CMV specific, although this will require further investigation. Further work is necessary to determine whether the presence of expanded TCR V beta clones in Waldenström's Macroglobulinemia patients is associated with increased survival or any anti-tumor activity.

PO.1404

IMPACT OF CHROMOSOMAL ABNORMALITIES ON SURVIVAL IN PRIMARY LIGHT CHAIN AMYLOIDOSIS

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Primary light chain amyloidosis (AL) is associated with a subtle monoclonal plasma cell population. Due to the small percentage and low mitotic index of these abnormal plasma cells, cytogenetic studies in AL have been restricted to analysis by interphase fluorescence in situ hybridization (iFISH), with published reports of <100 patients. In a recent iFISH study we reported the incidence of chromosomal abnormalities involving the IgH locus and deletions of chromosome 13, del(13), in a series of 26 systemic AL patients from the National Amyloidosis Centre, UK (Harrison, *et al.,* 2002, Br. J.Haematol. 117, 427-435). In this present study, we report an updated series with a total of 45 patients including the clinical details and survival data (on 43 patients) in order to investigate the impact of specific chromosomal changes on survival in AL. Median age at presentation was 59 years (range 40-83 years); the male to female ratio was 1.7:1; 37 patients had systemic and six had localized AL (bladder n=3, lymph node n=1, lung n=1). Due to a significantly different disease course, patients with localized AL

were excluded from the survival analysis. A total of 38 (88%) patients had abnormal serum free light chains while 11 (25%) had intact paraprotein. SAP scintigraphy showed organ involvement as: liver n=15 (34%), spleen n=24 (55%), kidneys n=22 (51%) (including 7 with renal failure) and heart (by echocardiography) n= 12 (27%). Twenty-two (51%) patients had ≥5% plasma cells in the bone marrow. Chromosomal abnormalities were detected in 24 (64%) patients with systemic AL (none were found in localized AL) of which 75% occurred in patients with ≥5% plasma cells: del(13) n=13 (35%) (>5% plasma cells n=10); IgH translocations n=19 (51%): t(11;14)(q13;q32) n=17 (45%) (>5% plasma cells n=9); unknown translocation partner n=2 (5%). One patient had trisomy 9. There was no association between chromosomal abnormality and pattern of organ involvement or response to treatment. Median follow-up was 22 months. Median overall survival (OS) of patients with systemic AL was 11 months (range 2-61). There was a trend towards poorer survival in patients with any chromosomal abnormality [median OS 7 months (range 2-61) vs. 14 months (range 2-42); p=0.34]. This was particularly marked in patients with del(13) [7 months (range 2-61) vs. 15 months (range 2-42); p=0.28]. These differences were not statistically significant but that may be due to the small patient numbers. Translocation, t(11;14) [median OS 12 months (range 2-61) vs.11 months (range 2-42); p=0.43] does not appear to have an impact on survival. In conclusion, chromosomal abnormalities appear to be more frequent in AL patients with ≥5% plasma cells in their bone marrow. Presence of a chromosomal abnormality, specifically del(13), are associated with a trend towards a poorer outcome. A larger study is in progress to further investigate these observations.

P0.1405

DEFINITION OF ORGAN INVOLVEMENT AND TREATMENT RESPONSE IN PRIMARY SYSTEMIC AMYLOIDOSIS (AL): A CONSENSUS OPINION FROM THE 10TH INTERNATIONAL SYMPOSIUM ON AMYLOID AND AMYLOIDOSIS

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Introduction. Previously, specific criteria for recognizing organ involvement and defining response in amyloid (AL) had minimal utility and was primarily of academic interest. However, new therapies directed at the plasma cell, including conventional dose chemotherapy, myeloablative chemotherapy, and agents directed at disruption of fibril structure are regularly being used to treat patients. Currently criteria for response and organ involvement differ from institution to institution, making it difficult to directly compare outcomes reported on therapy from academic centers. Outcomes are directly related to the number of organs involved with amyloid and accurate definition as to what constitutes organ involvement are more than an intellectual exercise. Thirteen leaders in the field were invited to submit institutional criteria from which the current guidelines were developed.

Results. The panel drew up consensus guidelines for the following questions: 1) What is required for a diagnosis of amyloidosis?; 2) Differentiating systemic from localized amyloidosis; 3) How is amyloidosis characterized as AL type?; 4) Definitions of organ involvement; 5) Criteria for organ response (heart, kidney, liver, nerve and soft tissue); 6) Organ progression; 7) Hematologic response criteria (including utilization of the newly developed immunoglobulin nephelometric free light chain assay) 8) Definitions for progressive disease both hematologic and organ based.

Conclusion. Defining organ involvement and response criteria, both hematologic and organ-based for amyloidosis AL has always been challenging. The thirteen members of the consensus panel have defined criteria proposed to be used worldwide by physicians who treat patients with this disease and to permit uniform reporting criteria of treatment-related outcomes. Hopefully number of organs invloved, frequency of response, response duration and time to progression will now have the same meaning in all published reports of therapy and natural history. In the future integration of new imaging techniques, as well as serum biomarkers such as serum troponin and brain naturetic peptide will further define the definitions of organ involvement and response.

P0.1406

CIRCULATING SOLUBLE LIGHT CHAIN OLIGOMERS IN SERA OF PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS

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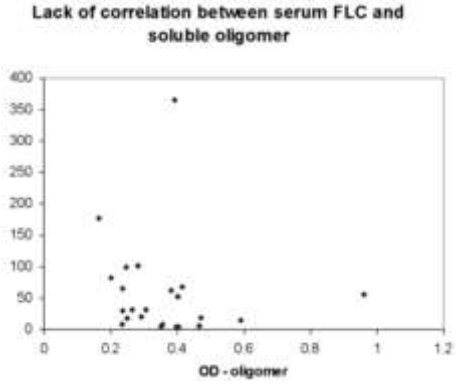
Light chain amyloidosis (AL) is a plasma cell dyscrasia characterized by amyloid formation of the monoclonal light chain. We have previously reported the presence of circulating free light chains (using FREELITE™, The Binding Site, Ltd.) in the sera of these patients and their utility in diagnosis and monitoring after therapy. More recently, there is growing evidence that multimeric complexes of soluble free light chains (oligomers) are likely to play a critical role in mediating organ dysfunction in light chain amyloidosis. There is evidence from other amyloid protein folding diseases that soluble oligomers may be the toxic species in the amyloid formation pathway. To determine if AL patients had circulating soluble oligomers, we analyzed sera from 23 AL patients using an anti-oligomer antibody (rabbit anti-human polyclonal) developed by Kayed and Glabe (University of California, Irvine) against oligomers of the Abeta protein. This antibody recognizes conformation-specific epitopes and therefore, has been shown to interact with oligomers from several amyloidogenic proteins, including light chain amyloid. All 23 AL patients tested showed detectable soluble oligomers in circulation, with levels ranging from 3% to 96% greater than the positive control (Abeta oligomer). Two patients showed greater than 100% increase in oligomer levels compared to the positive control. There did not appear to be a linear correlation between

free light chain levels and oligomer levels (See Figure), which would suggest that oligomer presence is not merely a function of the presence of free light chain. Freezing does not appear to destroy the oligomers since frozen (thawed) sera from the 23 patients showed comparable oligomer levels to fresh sera samples from another 3 AL patients. In addition, preliminary data from 1 myeloma (MM) patient (control) with a very high level of lambda free light chain (732 mg/dL) showed substantially lower oligomer levels than the AL patients. We propose to extend this analysis by analyzing a larger cohort of AL and MM (control) patients and performing clinical correlations between oligomer levels and clinical parameters of disease. This is the first report of circulating soluble amyloid-conformation-specific oligomers in the sera of AL patients and will be of significance in improving our understanding of the pathogenesis of this disease.



PO.1407

INTERIM ANALYSIS OF A PHASE II STUDY OF RISK-ADAPTED INTRAVENOUS MELPHALAN FOLLOWED BY ADJUVANT DEXAMETHASONE AND THALIDOMIDE FOR NEWLY DIAGNOSED PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS

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High-dose melphalan (M) with autologous stem cell transplant (ASCT) is effective therapy for systemic al amyloidosis (AL), but treatment-related mortality (TRM) remains high, and hematologic complete responses (CR) occur in a minority of patients. In this study, we asked whether a risk-adapted approach to IV M dosing could decrease TRM, and whether adjuvant dexamethasone (D) +/- thalidomide(T) could improve hematologic and amyloid-involved organ response rates. Low-risk patients (1 or 2 major organs involved, no advanced cardiac disease) receive M 100, 140, or 200 mg/m^2 with ASCT based on age, cardiac involvement, and renal function. High-risk patients (≥3 organs involved or advanced cardiac disease) receive 2 cycles of M 40 mg/m^2 without ASCT. Patients with persistent clonal plasma cell disease at 3 months receive 9 months of adjuvant D+T or D alone (if history of deep venous thrombosis or neuropathy). Since 9/02, 42 patients (median age=58 (range 34-73), 64% males) have enrolled a median of 1.5 months (range 0.5-7) from diagnosis. Organ involvement includes 14 (33%) cardiac, 26 (62%) renal (14 with renal only), 13 (31%) liver/GI tract, and 11 (26%) peripheral nervous system. Only 4 high-risk patients have enrolled; all had symptomatic cardiac involvement and died a median of 4 months (range 3-6) after M. Thirty-four patients in the low-risk group have been treated, and TRM is 6.4% (2/31), with an additional 3 patients alive <100 days post-treatment. Four low-risk patients have died of progressive disease (PD) a median of 11.7 months (range 7-18) after M. At 3 months, 19/30 (63%) evaluable low-risk patients had hematologic responses (5 CR, 14 PR) and 11 had stable disease. Twenty patients with persistent clonal disease began adjuvant therapy with D +/- T (see Table). At 12 months, 11/15 (73%) evaluable patients had responses (5 CR, 6 PR) and 8 (67%) had objective improvement in amyloid-related organ function. Preliminary analysis of serum free light chain data (n=31) shows an association between a persistently abnormal κ:λ ratio 3 months after treatment and an increased risk of death (RR=1.54, 95% CI=1.12-2.12, p=0.02). Analyses of the prognostic significance of serial troponin and BNP levels and plasma cell cyclin D1 expression are ongoing. In conclusion, risk-adapted dosing of IV M in newly diagnosed AL patients has a low TRM. Adjuvant D +/- T is feasible, has moderate toxicity, and has to date benefited 20% (4/20) of patients with persistent clonal plasma cell disease 3 months post ASCT.

PO.1408

CLONAL DISEASE RESPONSE AND CLINICAL OUTCOME IN 229 PATIENTS WITH AL AMYLOIDOSIS TREATED WITH VAD-LIKE CHEMOTHERAPY

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In AL amyloidosis low dose chemotherapy has poor efficacy, and high dose therapy is marred by treatment related mortality of at least 13%. We report here our experience with intermediate dose combination chemotherapy comprising VAD (vincristine-Adriamycin-dexamethasone) and related regimens in 229 pts who were evaluated in our centre and were followed-up every 6 months following treatment that was undertaken at referring hospitals. 125 pts were female, median age at treatment was 55 yrs (range 29-75), and a median 2 (1-5) organ systems were involved with amyloid (renal in 202, cardiac 98, hepatic 75, soft tissue 47, neuropathy 47, gastro-intestinal 20). 167 received VAD, 35 C-VAMP, 16 Z-Dex and 9 C-VAD; this was first-line therapy in 203 cases. Median time from diagnosis to treatment was 3 mo, and a median 4 (1-9) cycles were given. Clonal immunoglobulin production was identified at baseline in 218 patients by abnormal serum free light chain (FLC) ratio in 181pts (95% of evaluable cases), serum paraprotein (sPP) in 138 (62%), and urinary BJP in 144 (65%) cases. Monoclonal immunoglobulin production fell by more than 50% on FLC and sPP measurements in 61% and 63% of cases respectively. We confirm and extend our previous findings (Lachmann *et al.,* BJH 2003;122:78) that FLC response is a powerful predictor of survival after treatment in AL amyloidosis. Median survival for patients whose FLC normalised (24% of cases) was not reached at 110mo, was 80 mo among patients with >50% reduction of FLC (38% of pts), 42 mo in those with a 25-50% fall (6% of pts), and 42mo in non-responders (33% of pts), $p<0.0001$. Median duration of clonal remission was 60 mo in patients whose FLC were suppressed by >50%. Evidence of a partial response of clonal disease using conventional assays of sPP and BJP was obtained in 47% of cases. 61% of patients proceeded to have further therapy, although the rate was significantly lower in those who achieved >50% reduction in FLC. At median follow-up of 29 mo, 86 patients have died. Overall survival (OS) for the cohort is 80mo from the time of treat-

ment (Kaplan-Meier). Treatment toxicity necessitated early cessation in 60pts (26%) after a median of 3 cycles, and included 5 deaths (2%). Survival of patients classified using published risk factors and exclusion criteria for 'eligibility' for stem cell transplantation was a median of 96 mo versus 47 mo for ineligible cases. Function of amyloidotic organs improved in 21% (kidney 28%, heart 6%, liver 21%, soft tissue 40%), remained stable in 50%, and worsened in 29%. Whole body amyloid load on SAP scintigraphy decreased in 25%, remained stable in 56%, and increased in 19% of cases. In conclusion, clonal disease responses and clinical outcomes of patients with AL amyloidosis treated with VAD-like chemotherapy are comparable with literature reports of those who have undergone stem cell transplantation. Treatment-related mortality was low.

P0.1409

UNEXPECTED HEMATOLOGIC TOXICITY ASSOCIATED WITH THE USE OF INTRAVENOUS INTERMEDIATE DOSE MELPHALAN AND DEXAMETHASONE IN PATIENTS WITH CARDIAC AL AMYLOIDOSIS

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Background. Due to the highly variable bioavailability of orally administered melphalan, intravenous melphalan has been assessed in myeloma and AL amyloidosis. Intermediate dose melphalan and dexamethasone is an increasingly utilised regimen in patients with AL amyloidosis although little toxicity data is available regarding this combination.

Methods. We conducted a pilot study in patients with AL amyloidosis using a previously published melphalan dose (Eur J Haematol 1998;61:306). Patients received melphalan 25mg/m^2 i.v. on d1 and dexamethasone 20mg p.o. d1-4. Treatment was repeated every four weeks provided the neutrophil count was > 1 x 10^9/L and platelet count > 80x10^9/L, and treatment was continued until plateau. G-CSF was only administered in the event of delayed neutrophil recovery or neutropenic fever.

Results. Nine patients were treated between Dec02 and Jun04: median age 66 yrs (range, 41-73); 44% male. All patients had advanced disease: cardiac involvement in all, renal in 5, liver in 2 and neurologic in 2; 4 had active heart failure; median ECOG performance status 2 (range, 1-3); median number of organs involved 2 (range, 1-4). One patient had received prior oral melphalan and prednisone for 4 cycles. Median cycles received was 3 (range,1-5). Two patients were not assessable for haematologic toxicity having died within one week of therapy. All remaining patients developed Grade IV neutropenia (median nadir 0.13 x 10^9/L, range 0.01-0.46) and 3/7 developed Grade IV thrombocytopenia (median nadir 19 x 10^9/L, range <10-27). Five developed neutropenic fever. All patients receiving >2 cycles required dose delays and only one did not need dose reduction. Overall five patients have died: two early sudden cardiac deaths from advanced amyloidosis on day 3&6 of cycle 1, two of multi-organ failure associated with infection after cycle 1 & 2, and one of progressive cardiac failure 10 months post-treatment. Of the five patients who survived beyond three months, four achieved a 50% reduction in their monoclonal free light chain and one achieved CR by traditional serum electrophoresis criteria. Median survival is 10 months.

Conclusions. Intravenous melphalan at a dose of 25mg/m^2 causes significant myelosuppression and is unable to be delivered to the most patients with advanced AL. We suggest a dose of intravenous melphalan at 20mg/m^2 may be more tolerable.

P0.1410

THALIDOMIDE TREATMENT IN 99 PATIENTS WITH AL AMYLOIDOSIS: TOLERABILITY, CLONAL DISEASE RESPONSE AND CLINICAL OUTCOME

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Thirty% of patients with refractory or relapsed myeloma respond to thalidomide, and twice this number respond to thalidomide coupled with dexamethasone when it is given as first line treatment. We report here the use of thalidomide in 99 patients with systemic AL amyloidosis in whom cytotoxic therapy had been deemed either ineffective or too toxic to pursue. Forty-seven pts were female, median age was 64 yrs (range 32-83), and the patients had had a median of one type of prior chemotherapy (0-5). Thalidomide was taken for a median of 5 months (0.4-34), at a median dose of 100 mg/day (50-600). Thalidomide was prescribed alone in 56 patients, along with dexamethasone in 12, cyclophosphamide in 8, cyclophosphamide and dexamethasone in 13, melphalan in 5, melphalan and dexamethasone in 4, and on a background of maintenance anti-rejection therapy in one recipient of a solid organ transplant. Median follow-up was 11 months. Adverse events occurred in 75 patients, including fatigue or somnolence (33), neuropathy (27), significant constipation (18), mental changes (9) and/or oedema (8). Six had venous thromboses, one related to a Hickman line and another resulting in fatal pulmonary embolism, and two patients had arterial clots resulting in CVA and leg amputation respectively. Thalidomide was discontinued due to adverse effects in 41 cases, lack of effect in 9, planned cessation of therapy in 4 and death from progressive amyloid disease in 19. Clonal disease responses of >50% were recorded in 34% of patients who were followed by serum free light chain measurements, and in 36% of patients who had paraproteins that were quantified conventionally. Overall survival for the whole cohort was estimated at 26mo from the start of treatment by Kaplan-Meier analysis. Overall survival was significantly better when dexamethasone was included as part of the regimen (p<0.012). Organ function improved or remained stable in 23% and 39% of evaluable cases respectively, and SAP scintigraphy showed regression of amyloid in 18% of patients. Thalidomide alone or in combination with other agents is probably as effective in AL amyloidosis as it is myeloma, but it frequently produces adverse effects.

P0.1411

THALIDOMIDE ALONE AND IN COMBINATION WITH OTHER AGENTS IN THE TREATMENT OF PATIENTS WITH AL AMYLOIDOSIS

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A recent report from the Mayo clinic (Dispenzieri et al 2003, Amyloid 10:256-261) showed that high dose thalidomide was poorly tolerated in 12 patients with AL amyloidosis and all patients were withdrawn from the study because of poor tolerance, disease progression or death. We report our preliminary results on 5 patients with AL amyloidosis treated with thalidomide, alone or in combination

with other drugs. The patients were diagnosed between 1997 and 2004 and are all currently alive. 3 of the patients had an underlying multiple myeloma as well as AL amyloidosis, and two had primary AL amyloidosis only. Four patients had proteinuria at presentation and this was the presenting feature in three. The fourth patient presented with bone tumours which on biopsy showed amyloidosis of bone and proteinuria was an incidental finding. One patient had kappa secreting plasma cells and 4 patients had lambda secreting plasma cells and in one of these there was also secretion of intact IgG immunoglobulin. This last patient presented with bleeding per rectum and the amyloidosis was mainly confined to the gut. Four of the patients had prior VAD chemotherapy and one had an autologous stem cell transplant. Thalidomide was given alone in 1 patient but 3 others also received monthly pulsed dexamethasone. The dose of thalidomide was 100 mg daily in 3 patients and 200 mg in one patient. The fifth patient tolerated thalidomide poorly at 100 mg and the dose was therefore reduced to 25 mg for two weeks every 4 weeks. All patients showed variable but clinically useful responses. One patient showed a complete response and has discontinued treatment after 9 months, in a second patient the SFLC ratio became normal and there was an arrest of renal function deterioration. In the other two patients the clonal serum free light chains levels were reduced by 75% and 68% of the levels at the start of treatment. These include the two patients with primary AL amyloidosis. All of the responding patients tolerated the treatment, one of these patients had fluid retention, managed with diuretics; and a tremor but is continuing with the treatment. The fifth patient has an underlying multiple myeloma, which relapsed in 2003 with plasmacytomas in the chest treated with VAD and relapsing again in 2004. He showed an initial response to a combination of thalidomide, oral melphalan and dexamethasone with a reduction of the tumour masses by 50% on CT scans, and a reduction of serum lambda free light chains from 1669 mg/L to 493 mg/L. He had two further courses of this treatment but without the dexamethasone because of massive fluid retention including a non-cardiogenic pulmonary oedema. However he has currently relapsed 8 weeks after the third course of treatment. We therefore believe that low dose thalidomide can be used successfully in patients with AL amyloidosis. The major adverse effect in some of the patients with nephrosis appears to be fluid retention, which can be marked.

P0.1412

MINI-AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA AND AMYLOIDOSIS – A SINGLE CENTER EXPERIENCE

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Mini-autologous transplantation has been used to treat multiple myeloma and amyeloidosis in patients who can not tolerate single autologous transplant in order to achieve a better response rate and a prolonged disease free survival.

Aim. To evaluate the outcome, benefits, complications and mortality of multiple myeloma and amyloidosis patients treated with mini-autologous transplantation.

Patients and methods. 12 patients (11 MM and 1 amyloidosis AM) treated up-front with mini-autologous PBSCT during the period from August 2002 to September 2004, median age 70.5 years (range, 53-74) , male-to-female ratio 1:1 , median time from diagnosis to the first transplant 7.5 months (range, 5-13). Four patients with MM had an unfavorable cytogenetic risk (13q-, p53 deletion) and five patients had high β_2 microglobulin levels. Nine of 12 patients underwent double transplant while 3 had only one transplant, due to delayed recovery in two cases and severe septicemia with subsequent renal failure in one case. All patients received three cycles of induction chemotherapy (VAD), Nine patients underwent stem cell mobilisation with high dose cyclophosphamide and G-CSF, while the remaining three patients (1 AM and 2 MM) underwent mobilisation with G-CSF alone.

Results. Complete remission achieved in 4 patient (44%) post 2nd transplant, 1 patient (8%) post 1st transplant and none of the patient achieved complete remission post conventional chemotherapy. After a median follow up period of 12 months (range, 7-26), all 12 patients were alive and 10 remained in remission (3 CR, 7 PR), while two MM patient showed disease progression.

Conclusion. Mini-autologous transplantation is well tolerated with no treatment related mortality and the responses to treatment are comparable to the responses achieved in younger patient treated with single autologous transplant (200 mg/m^2).

P0.1413

LOCALIZED AMYLOIDOSIS: CLINICAL FEATURES AND OUTCOME IN 235 CASES

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Localised amyloidosis is uncommon but causes a wide variety of recognised clinical syndromes. We report 235 patients (132 males, mean age 58 yrs) presenting with localised amyloidosis, defined by: 1) diagnostic Congo-red histology, 2) absence of hepatic, renal, cardiac and nerve involvement, and 3) negative SAP scan except for involved sites. In most cases, amyloid deposits were limited to characteristic distributions in the urinary tract (37 cases), lung and airways (40), larynx (28), nasopharynx (25), combinations of contiguous respiratory sites (7), skin (25), lymph nodes (22), eye/orbit (20) and bowel (11). There were also less common presentations with localised amyloidosis in bone/spine (9), brain (2), synovium (2), and 1 case in each of breast, nerve, salivary gland, thigh and vulva. Two patients, both followed for some years, had amyloid in more than one site; one in skin and nasopharynx, one in urethra and tonsil; neither showed progression to systemic amyloidosis. 162 of the biopsies were available for review at the NAC, and the fibril was AL in the 100 cases (73% lambda and 27% kappa) where the fibril type could be determined. AL type was further supported by appropriate negative genetic testing. Evidence of a systemic clonal plasma cell disorder (abnormal free light chains or serum paraprotein, BJP or abnormal BMBx) was identified in 61 cases (26%), significantly more often in patients with bowel (p=0.002), bone/spine (p=0.0003) or lymph node (p=0.0002) amyloidosis. Mean follow-up was 39 mo. The course of the disease was generally benign, progressing in 46%, stable in 23% and improving in 31%. Active therapies were pursued in 111 cases (surgery or excision in 77 cases, other local therapy (18), medical and/or chemotherapy (16)) ameliorating the condition in 47%. 12 of 116 pts followed conservatively improved spontaneously and were stable in another 82 (71%). Only 2 patients subsequently developed systemic AL, one of whom died. Another 11 died, 1 of localised AL (pulmonary), 2 of complications from localised AL, 2 of myeloma, 3 of unrelated cancers and 3 of unknown cause.

This large cohort of patients confirms the generally excellent prognosis associated with localised AL amyloidosis.

P0.1414

ROLE OF SERUM FREE LIGHT CHAINS IN DIAGNOSIS AND MONITORING RESPONSE TO TREATMENT IN LIGHT CHAIN DEPOSITION DISEASE

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Light chain deposition disease (LCDD) is a rare non-amyloidotic immunoglobulin deposition disease accounting for 2% of renal histological abnormalities in patients with plasma cell dyscrasias. The underlying plasma cell dyscrasia may be subtle with only light chain secretion, leading to diagnostic delay and problems with monitoring response to therapy. Circulating free immunoglobulin light chain (FLC) concentration was measured with a sensitive nephelometric immunoassay in 17 patients with biopsy proven LCDD. The presenting features were: Male – 13, female – 4; median age 55 yrs (range 27-76). All patients had renal involvement [end stage renal failure 4(23%), chronic renal failure 12 (70%), proteinuria with normal renal function one (5%)], 3(17%) had cardiac and 2 (10%) had liver involvement. The median creatinine clearance was 26ml/min (range 9.6-102) with median proteinuria of 3.4g/24hrs (range 1.7-15.5). The median time from the presenting symptom(s) to confirmation of diagnosis was 7 months (range 1-25). 6 (35%) had underlying myeloma. 13 (76%) had immune paresis. 8 (47%) had a measurable paraprotein with 2(11%) having only immunofixation positive monoclonal band. 7 (41%) had free light chains in the urine. Serum free light chains were abnormal with a clonal bias in 15 (88%). 11(64%) had a κ excess, 4(23%) had λ light chain excess while 2 (11%) had a polyclonal rise. The median λ levels were 317mg/L(range 8.5-2260) while the median λ levels were 64mg/L (range 17-10700). A total of 10 patients received systemic chemotherapy for the underlying plasma cell dyscrasias as follows: VAD – 4, C-VAMP -1, VAD followed by autologous stem cell transplant – 2, melphalan and prednisone – 1 and intermediate dose melphalan – 2. 8(80%) patients had a light chain response with a median decrease of 63% (range 31-95%) compared to the pretreatment values, 1(10%) had no change in light chain level (which did not show clonal bias pre treatment) but had a very good partial response on paraprotein criteria. Only two patients had complete normalization of free light chain levels. Renal function improved in 2, remained unchanged in 5 (including 3 patients with ESRD) and worsened in 1 patient. Both patients with abnormal liver functions and cardiac involvement showed improvement. The median overall survival was 59 months. In summary, measurement of FLC in patients with LCDD can detect 33% more cases of plasma cell dyscrasia than standard electrophoretic methods. The assay is also useful for monitoring response to treatment. Detection of abnormal FLC may shorten time to diagnosis in patients without an abnormal paraprotein. However, the presence of renal failure makes assessment of complete responses using absolute κ or λ values difficult (because of renal failure induced polyclonal rise in light chains), though using κ/λ ratio can compensate. Measurement of serum free light chains can be recommended as a useful addition to the screening tests for patients with suspected LCDD and also monitoring response to chemotherapy.

P0.1415

HIGH DOSE THERAPY AND AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION IN 15 PATIENTS WITH POEMS SYNDROME

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POEMS syndrome is a plasma cell dyscrasia with osteosclerotic bone lesions and λ light chain isotype characterized by the combination of polyneuropathy, organomegaly, endocrine abnormalities and skin changes. In patients with diffuse bone marrow infiltration or multifocal lesions who cannot be cured by radiotherapy prognosis is poor with conventional chemotherapy mainly because of progressive neuropathy. In 2002 we reported in Blood 5 patients with POEMS syndrome treated with intensive chemotherapy with stem cell support with a very good efficacy. We report here the follow-up of these patients and 10 other patients who received intensive treatment with stem cell support in 3 french centers. There were 5 women and 10 men, median age was 52 (range 44-62). A plasma cell dyscrasia with λ light chain was present in all patients with multiple osteosclerotic bone lesions in 11 and bone marrow involvement in 7. All patients had distal bilateral sensory disturbance predominating in the lower limbs associated with abolition of deep tendon reflexes. Nine patients also presented with motor deficiency, including 4 who were bedridden because of tetraparesia. Electromyographic studies provided evidence for demyelinating lesions in all patients either isolated or associated with axonal degeneration. Other manifestations of POEMS syndrome included POEMS related nephropathy in 1 patients, organomegaly in 9 patients, impotence, diabetes mellitus, adrenal insufficiency and/or hypothyroidism in 9 patients, papilloedema in 4 patients, hyperproteinorachia in 6 patients and skin changes in 11 patients. Two patients had had prior cerebrovascular accidents, one a history of myocardial infarction, and another a jugular vein thrombosis. Blood stem cell collection was performed after mobilization by chemotherapy in 9 patients and with G-csf alone in 6 patients. Tandem transplant was performed in 2 patients with total body irradiation, 13 patients received 140 (n=2) or 200 mg/ m2 (n=11) of melphalan. Local radiation was considered in patients who had a prominent focal bone lesion and was performed in 5 patients No toxic death occurred during stem cell mobilization or transplantation. Post transplant hematopoietic recovery was satisfactory in all cases with no unusual toxicity. After HDT 12 patients were in complete remission and 3 in very good partial remission. In all cases, remission of plasma cell proliferation was associated with improvement in performance and/or in neurological symptoms. In addition to neurological symptoms, other manifestations of the POEMS syndrome improved. Within a median follow-up of 31 months since HDT (range 3-94), only 1 patient experienced a relapse after 22 months and died 3 years after HDT. Serum VEGF levels before and after HDT will be reported in the meeting. In conclusion HDT for POEMS syndrome results in clinical improvement in a majority of patients and should be considered as a therapeutic option for patients with disseminated disease or when complete remisson is not achieved with irradiation.

P0.1416

LONG-TERM FOLLOW-UP OF A PATIENT WITH POEMS SYNDROME

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Introduction. The POEMS syndrome is a multisystem disorder characterized by presence of sensorimotor polyneuropathy, organomegaly or osteosclerotic bone lesions, endocrinopathy, monoclonal gammopathy and skin changes. Progressive worsening of axonal degeneration and demyelinization were usual outcomes until agressive combined treatment was introduced

Case report. A 70-years old man with a 3-year history of severe sensorimotor polyneuropathy of lower extremities leading to complete immobility. In October 1998, the diagnosis of POEMS syndrome was done, according to following features in clinical manifestation: skin hyperpigmentation and hypertrichosis, skin angiomas, bilateral axillar adenopathy (histological examination showed the Castleman disease - angiofolicular hyperplasia). Abnormal laboratory findings consisted from polyglobulia (Hb 170 g/L), thrombocytosis (578 x 10^9/L), presence of M-protein IgG-lambda (12.8 g/L) in serum and in liquor. In the bone marrow, mild increase in monoclonal IgG-lambda plasmocytes was found (13%), and also signs of partial fibrosis with osteoblastic and osteoclastic activity. The EMG examination of lower extremities showed severe neuropathy with both axon and myelin damage. Radiography of the skeleton discovered 8cm long osteosclerotic lesions in proximal parts of diaphysis in both humeri, while the bone Tc-99m scintigraphy scan was normal. DEXA revealed higher bone mineral density (BMD) in the region of humerus (1.24 g/cm^3 right, 1.21 g/cm^3 left) than in the forearm region (0.98 g/cm^3). Other pathological findings were: hypeglycaemia (7.6 mmol/L), C-peptide elevation (1265 ng/mL), FSH (41.9 IU/mL), prolactin (8 ng/mL), ACTH (113 pg/mL) as signs of endocrinopathy, and S-beta$_2$-microglobulin (2.8 mg/mL), VCAM-1 (1389 ng/mL), IL-2 (61.4 g/mL), osteocalcin (40.2 ng/mL), PICP (39 ng/mL), and DPYR (7.8). After 6 courses of chemotherapy (melphalan and prednisone), local radiotherapy of osteosclerotic lesions and axillar lymfadenopathy (30Gy) and after 6 courses of BMCP regimen, the complete recovery of the muscle strength, mobility, hemogram and normalization of B2M were achieved, while levels of prolactine, PICP, M-protein and plasmocyte number decreased. BMD of both humeri decreased to 1.1 g/cm^3. Levels of FSH, ACTH and DPYR stayed unchanged. In September 2003, increased levels of thrombocytes, M-protein and plasmocytes in bone marrow were observed again, and after 8 courses of CIDEX chemotherapy they all fall down to normal levels. The monoclonal protein is 1.9 g/L, lymph nodes are normal size.

Conclusion. In a 7-years follow-up of a patient with POEMS syndrome treated by conventional chemotherapy in combination with actinotherapy of osteosclerotic lesions and axillar lymphadenopathy we observed a very good laboratory response following by improving of the performance status, mobility and muscle strength, although the neuropathy of lower extremities is still present.

P0.1417

LONG TERM COMPLETE REMISSION OF WALDENSTROM'S MACROGLOBULINEMIA AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION: A CASE REPORT

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Man, 55 years, was referred in January 2004 for fatigue and symptoms of anemia. Blood analysis revealed a hemoglobin of 63 g/L, platelet count of 41x10^9/L,white blood cell count of 3.9x10^9/L,with 65% (atypical) lymphocytes and 35% neutrophils.

In 1997, the patient was diagnosed with Waldenstrom's macroglobulinemia, his marrow contained 84% lymphocytes and 8% plasmacytoid lymphocytes, serum globulin 80 g/L, IgM 32.5 g/L, a tall narrow spike was seen in the γregion on serum protein electrophoresis. Immunoelectrophoresis demonstrated a monoclonal IgM paraprotein of the κtype. The patient was treated with multiple courses of cyclophosophamide, vincristine, melphalan, prednisone and interferon-a, with poor effect. In 1998, autologous PBSCT was performed. The patient received a single high-dose mephalan (140 mg/m^2) as conditioning. Transplantation was followed by sustained hematologic engraftment. The patient regained his health, hemoglobin, white blood cell count and platelet count returned to normal, and the paraprotein could not be detected within 10 months after transplantation. Bone marrow biopsy revealed normal cellularity without atypical lymphocytes. The patient remains well within 6 years. At this time of admission to our department, laboratory examinations were follows as: serum globulin 83.4 g/L, IgM 81 g/L, and paraprotein of the κtype 10.2 g/L. Bone marrow contained 80.75% lymphocytes and a number of plasmacytoid lymphocytes. Flow cytometric analysis of the marrow confirmed these abnormal cells were CD20+/CD19+/ CD56+/CD79a+ , without myeloid markers or stem cell markers (CD34) expression. He was diagnosed with relapse of macroglobulinemia and was treated with COP regimen, with no effect. He was then died of bacterial infection.

The experiences we got from this case showed that high-dose chemotherapy supported by autologous PBSC was a highly effective therapy, and long term disease-free survival could be gained, no residual disease was found 72 months after transplantation.

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