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ABSTRACT BOOK

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European Hematology Association

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

Ancient Greek

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

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

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

Modern English

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Paris, France, May 3-6, 2011

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MAIN PROGRAM

MOLECULAR PATHWAYS/GENETICS

INTRODUCTION

H. AVET-LOISEAU

During the past decade, important progresses have been observed in our comprehension of myeloma, especially regarding molecular characteristics. Dr Croce will show our the analysis of miRNA can improve our understanding of myeloma oncogenesis. In the following talk, Dr Fonseca will analyze the most recent genomic data in order to see if it is time to identify several subclasses of myeloma, that could lead to specific therapeutic approaches. Finally, Dr Minvielle will present data on the molecular events observed during progression, that could also have implications for treatment.

MICRORNAS IN MYELOMA

C.M. CROCE

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In multiple myeloma (MM), an incurable B cell neoplasm, mutation or deletion of p53 is rarely detected at diagnosis. Using small-molecule inhibitors of MDM2, we provide evidence that miR-192, 194, and 215, which are downregulated in a subset of newly diagnosed MMs, can be transcriptionally activated by p53 and then modulate MDM2 expression. Furthermore, ectopic re-expression of these miRNAs in MM cells increases the therapeutic action of MDM2 inhibitors in vitro and in vivo by enhancing their p53-activating effects. In addition, miR-192 and 215 target the IGF pathway, preventing enhanced migration of plasma cells into bone marrow. The results suggest that these miRNAs are positive regulators of p53 and that their downregulation plays a key role in MM development.

The tumor suppressor p53 is frequently inactivated by mutations or deletions in cancer. p53 acts as a potent transcription factor and can be activated in response to diverse stresses, leading to induction of cell-cycle arrest, apoptosis, or senescence.[8,17] Although regulation of the p53 pathway is not fully understood at the molecular level, it has been well established that activated p53 suppresses cancer progression, underlining why cancer cells have developed multiple mechanisms to disable p53 function4,16. In human tumors that retain wild type (WT) p53,[8,10] p53 can be antagonized by murine double minute 2 (MDM2), a negative regulator of p53 that is also overpriced in many human tumors, offering a therapeutic strategy.[5] It has been reported that inhibiting MDM2 expression can reactivate p53 in cancer cells, leading to their demise.[5,13] TP53 mutation is rarely detected at diagnosis in many hematological cancers such as multiple myeloma (MM), acute myeloid leukemia, chronic lymphocytic leukemia, and Hodgkin's disease (HD). Thus, numerous reports have shown that therapeutic induction of p53 might be particularly suitable for the treatment of hematological malignancies.[18] Among them, multiple myeloma (MM) is a currently incurable plasma cell proliferative disorder that results in considerable morbidity and mortality.[6,9] MM develops from a benign condition called monoclonal gammopathy of undetermined significance (MGUS).[15] Individuals with MGUS often remain stable for years and do not require treatment. However, for unknown reasons, this benign condition can evolve into MM at a rate of ~1% per year, with some MMs developing after many years.[6,9] In MGUS and in the majority of newly diagnosed MM cases TP53 is WT[2,9] and the protein is rarely detectable.[14] Interestingly, in MM cells, expression of p53 protein levels can be rescued by antagonizing MDM2. Several reports have focused on the p53-mediated apoptotic pathway, upon endogenous p53 protein re-expression by the small-molecule MDM2 antagonists (Nutlins) and target genes which may be involved in p53-dependent apoptosis in MM cells have been identified.[14] MicroRNAs are an abundant class of short, non-proteincoding RNAs that mediate the regulation of target genes posttranscriptionally and that have emerged as master regulators in diverse physiologic and pathologic processes1, and oncogenesis.[3] Recently, microRNAs (miRNAs) have been reported to be directly transactivated by p53.[7] miRNAs have also been shown to target p53 and/or components of p53 regulatory pathways, thereby directly and/or indirectly affecting its activities.[11,18] We previously published the global miRNA-expression profiles of MM and MGUS and contrasted these profiles with those of normal plasma cells (PCs).[12] The findings defined a miRNA signature related to expression and regulation of proteins associated with malignant transformation of PCs, such as p53.[12] We have now examined the regulation and functional roles of miRNAs in MM development using small-molecule inhibitors of MDM2.

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THE CLASSIFICATION OF MYELOMA; SUBTYPES OF THE DISEASE

R. FONSECA

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Introduction. Multiple myeloma (MM) has also been called "Many Multiple Myelomas", alluding to the existence of well-defined subtypes of the disease, most of which carry important implications in the clinic(1-3). The subtypes of the disease are driven primarily by the founder genetic lesions that give rise to the clonal expansion of cells. It is then that any and all classifications must, directly or indirectly be guided the segregation dictated by the presence of specific DNA changes associated with disease pathogenesis (3). These founder lesions are all present since dis-

ease initiation (MGUS or "pre-MGUS") and remain constant throughout the disease course, usually present in all clonal cells. The pathogenesis of MM, while potentially contributed by external factors such as micro-environment, epigenetic factor or protein-protein interactions, has always and will always be dictated and sustained by the presence of these specific genetic factors. It is then that the biologic classification of the disease is best performed by cataloguing these DNA based aberrations and building upon that basic framework as series of secondary events that lead to disease progression and ultimately clonal evolution. Detection can be done by several methods. *Classifiers.* There are clearly two major subtypes of the disease; hyperdiploid MM and non-hyperdiploid MM (4, 5). These categories seem to be unique and define subsets of the disease at the top hierarchical level. The two disease subtypes are not mutually exclusive in all cases, and in fact up to 25% of t(4;14) cases will harbor hyperdiploidy, while a small subset of hyperdiploid cases also have translocations (5). Most of these translocations are secondary and not the primary ones. It remains puzzling why there is this minor overlap in what otherwise appear to be well-defined categories. It may mean that trisomies per se may provide a clonal advantage, and be tolerated in some translocated myelomas, but dispensable, while essential for hyperdiploid myeloma. The clonal plasma cells arise from post germinal center B cells and thus the malignancy is clearly one that possesses unique differences from B cells (6). It is incumbent that any proposals showing a possible "stem cell" for MM prove this beyond any reasonable doubt. The anatomy of translocations (at the time of isotype switching) makes the existence of B cells (pre-switch) unlikely to impossible. *Tools for classifying.* Classifying the disease can be done by one of multiple methods including those that directly detect DNA changes (e.g. FISH, chromosome studies, aCGH or mutational status) or the consequences of these aberrations (gene expression profiling)(3, 7-9). DNA based tools remain useful tools in the clinic and can be used widely, although the information they provide is limited by technical issues or depth of information provided. RNA based classifications are more attractive in that they provide comprehensive answers for classifying MM with one test. The TC classification is the one that showed that most if not all MM are characterized by the presence of cyclin D upregulation (D1, 2 or 3) (10, 11). Its subclasses clearly correlate with the genetic subtypes dictated by chromosome translocations or hyperdiploidy. This classification has two groups added that have not been well defined at the DNA level; D2 and D1+D2 subgroups. The reliability and reproducibility of the classification suggests that these two groups are likely driven by unifying genetic events yet to be identified. One limitation of this classification in the diagnostic realm is the difficulty in distinguishing boundaries for some of the neighboring groups (e.g. t(11:14) vs. D1) but overall seems to be performing well in predicting FISH category. The UAMS MM classification is very similar and results in largely overlapping groups of patients (12). The classification includes subgroups that likely represent entities that show clonal progression such as the proliferation group. This is clearly both an asset and a limitation of this classification. Other unique subtypes are also identified that correlate with clinical features such as bone disease. All other classifications are variations of the common genetic pathways dictated at the DNA level (13, 14). *Variations of classifications.* Up to this point all discussion has been based on the classification of the disease at the level of biologic founder lesions. Other classifications can be driven by the identification of features or signatures that identify subsets of the disease with a more adverse outcome. The classifications can mirror clonal initiation events (such as the t(4;14) but can also be enriched for progression events (such as -17p13 and high risk RNA signatures)(15). These classifications are more focused on clinical utility and less on the pathophysiology of the disease. Likewise there is minimal information regarding predictive factors for MM such as Her-2-neu in breast cancer. Availability of these factors would be crucial in selecting the right treatments for patients, particularly avoidance of agents of no value and with potential toxicity for specific patients. While MM is one disease the evolution of knowledge regarding disease biology has allowed for a beter understanding of the disease with implications for prognosis and potentially therapeutic selection.

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THE GENETIC PROGRESSION OF MYELOMA

S. MINVIELLE (1,2)

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Multiple myeloma (MM) is a malignant plasma cell disorder, it is estimated that 5000 new diagnoses and 3000 deaths due to myeloma occurred in 2010 in France. MM a haematopoietic cancer emblematic of gradual evolution model. MM is almost always preceded by premalignant plasma cell disorder benign termed monoclonal gammopathy of undetermined significance (MGUS) or more advanced premalignant stage called smoldering MM (SMM), then progression of intramedullary MM is associated with severe clinical features and in a fraction of patients, the tumors acquire the ability to proliferate in extramedullary sites such as blood in this case it is called plasma cell leukemia (PCL) a more aggressive disease. Considerable progress has been made in the treatment of MM in the past decade. Survival improvement in newly diagnosed MM patients is largely due to the introduction of novel effective drugs such as immunomodulatory compound thalidomide and its analog, lenalidomide and the proteasome inhibitor, bortezomib. However, almost all patients relapse, emphasizing a need of exploration of the genomic abnormalities contributing to the mechanism of progression. Previous studies with conventional karyotype and fluorescence in situ hybridization (FISH) analyses have provided a framework of the recurrent chromosomal abnormalities associated with the stages MM and highlighted chromosomal changes which impact clinical outcome. However, until recently comprehensive genome-wide analysis of DNA copy number alterations (CNAs) was hampered by the complexity of the myeloma cells karyotype coupled with the presence of genetically distinct malignant plasma cell subpopulations within the patient's bone marrow. The advent of single nucleotide polymorphism (SNP) arrays that allow genome-wide detailed exploration of copy CNAs and loss of heterozygosity (LOH) in copy-neutral LOH (CN-LOH), or uniparental disomy in cancer cells coupled with the availability of samples collected in the same individual when diagnosed with MGUS/SMM and at time of progression to active MM or diagnosed with MM and at relapse are providing important insights into the pathogenesis and progression of MM. Genomic analysis of paired samples from patients with SMM progressing to active MM revealed that progression is associated with chromosomal changes highly variable from one case to another; these changes affected the number, extent, nature and clonality of the lesions. For example, one patient acquired 4 lesions including partial 20p loss, 20q gain, and 21q loss (Fig. 1A); another patient acquired at least 8 lesions including partial 6q and 14q losses (Fig. 1B). Integrated analysis of allele ratio (AR), copy number (CN), allele specific copy number (AsCN) revealed a clonal heterogeneity in a SMM case, and clonal selection during progression to active MM in the absence of any treatment (Fig. 2). Genomic analysis shows that the chromosomal instability observed in overt MM is present in the early stage of the disease.

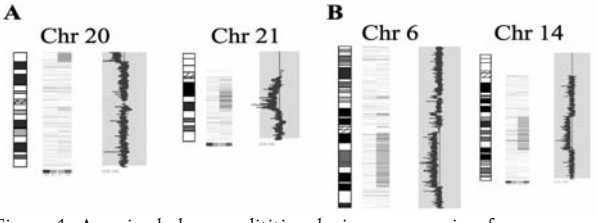

Figure 1: Acquired abnormalitities during progression from pre-malignant stage to MM. dChipSNP median-smoothed log ratio copy number heratmap showing two paired samples. Blue is deletion and red is gain. CNAs spectrum of MM sample is shown on the right.

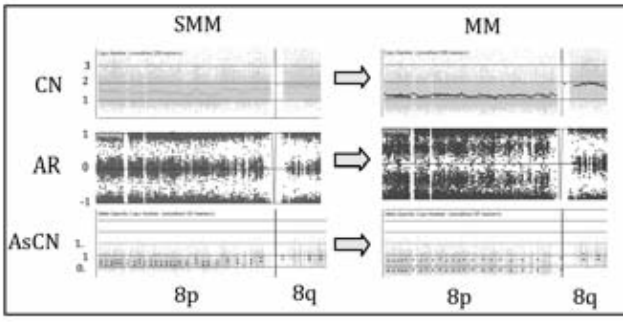

Figure 2: Assessment of mosaicism evolution during progression of SMM to MM. Left, 8p arm: CN is <2, AR is predominantly a three allele track combination (AA,AB,BB) where homozygote AA is assigned by the computer software at position 1, heterozygote AB at position 0 and homozygote BB at position –1, AsCN showed both alleles (red and blue) are between 0.5 and 1. There results suggest that del(8p) is present in minor populations. FISH analysis confirms del(8p) in minor populations (30%). Right, 8p arm CN is ~1, AR changes to a monosomic pattern with a characteristic five allele track combination (AA,AB, BB/A,B), AsCN reveals a drop of the blue allele. These results suggest expansion of the 8p minor population during progression to MM. FISH analysis confirms increase of del(8p) in MM cells (60%). 8q arm is shown as normal diploid.

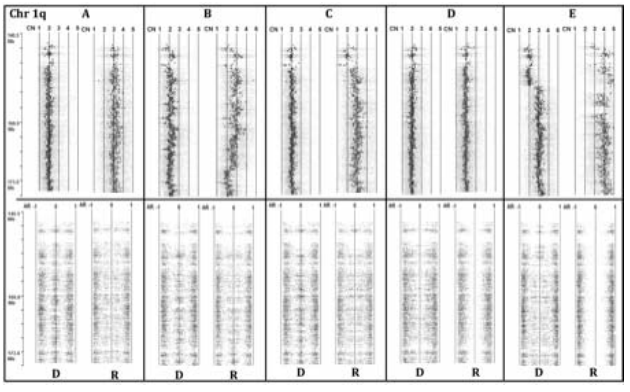

Figure 3: Chr.1q lesions acquired at relapse. Upper panel displays CN and lower panel displays AR.

Genomic analysis on matched diagnosis and relapse purified malignant plasma cell samples from patients with MM indicated that patients studied retained the majority of the CNAs or CN-LOH present at the matched diagnosis samples. The vast majority of MM cases acquired additional CNAs or CN-LOH at relapse.

Five patients acquired various chromosome 1q lesions; entire arm gain (Figure 3A), sub-arm gain (Figure 3B, C), CN-LOH (Figure 3D) and complex gain with LOH (Figure 3E). Two patients acquired del(17p) encompassing TP53. These results support the fact that relapse mechanism in MM is complex, this is not a massive genomic instability or a single genetic lesion that are responsible for relapse.

Comprehensive analysis of genomic changes in myeloma progression is promising, we can speculate that the new treatment paradigm would combine targeted therapy and subpopulations control.

PLASMA CELL BIOLOGY

THE UNFOLDED PROTEIN REPONSE: AN OVERVIEW

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Differentiation of mature B cells to plasma cells involves dramatic expansion of the endoplasmic reticulum (ER) and the up-regulation of ER chaperones that ensure synthesis, folding and secretion of large amount of immunoglobulin molecules. The transcription factor XBP-1 is induced during plasma cell differentiation, and promotes the ER expansion and induces a variety of genes in protein secretory pathway (1-4). Hence, XBP-1 deficient B cells fail to differentiate into plasma cells, and the mutant mice lacking XBP-1 in B cells produce minimal amount of antibody both at baseline and in response to antigen challenge (5-7). XBP-1 is also essential for the development of other professional secretory cells, such as acinar cells in exocrine pancreas and intestinal Paneth cells (8, 9). It is conceivable that XBP1 deficiency impairs the management of large amount of secretory proteins, causing cell death (10). XBP1 is also known as a key component of a signaling pathway called the unfolded protein response (UPR), which is instigated by three ER transmembrane proteins, IRE1 PERK and ATF6 (11). IRE1 is an endoribonuclease that cleaves XBP1 mRNA at two sites, inducing an unconventional mRNA splicing to produce THE mRNA encoding XBP-1s (12-14). It has been postulated that the UPR is activated upon increase in the concentration of the unfolded protein species in the ER, the condition called ER stress. Indeed, agents such as tunicamycin, thapsigargin and dithiotheitol that perturb ER protein folding homeostasis strongly activate the UPR. It is conceivable that large amount of secretory proteins may cause the ER stress in professional secretory cells leading to the activation of the UPR, although the precise mechanism by which the UPR is activated in plasma cells and other secretory cells and the ultimate molecular triggers in normal cell physiology remain to be clarified (15, 16). Given that multiple myeloma originates from plasma cells, it has been speculated that myeloma cells rely on robust UPR for their survival and propagation. Similar to plasma cells, multiple myeloma cells also express high levels of XBP-1 and UPR genes (17-19). The importance of IRE1/XBP-1 in the pathogenesis of multiple myeloma needs to be determined. Compounds that inhibit IRE1/XBP1 pathway may be useful to treat multiple myeloma (20).

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INSULIN GROWTH FACTOR TYPE 1 AND INSULIN IN MULTIPLE MYELOMA

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Despite multiple genetic abnormalities, particularly targeting cell cycle machinery, the growth of primary multiple myeloma cells (MMCs) of the vast majority of patients is dependent on a close interaction with the tumor environment. The identification of the cell communication signals produced by the tumor environment and making it possible survival and cell cycling of MMCs could offer promising therapeutic strategies.

Several MMC growth factors (MGFs) have been identified so far including IL6, IGF1, HGF, BAFF/APRIL, EGF family, IL10, IL21, IL15, Notch family, Wnt family. We have shown that IGF1 is a major MGF, the MGF effect of IL6, HGF, EGF family being dependent on an autocrine IGF1/IGF1 receptor loop occurring in MMCs. IGF1 receptor (IGF1R) is not expressed by normal plasma cells and is aberrantly expressed by MMCs of about 50% of newly diagnosed patients. IGF1R gene expression is a powerful factor of poor prognosis, is increased in patients with t(4;14) translocation or with a cell cycle signature. IGF1 is produced by MMCs themselves, and also by osteoclasts. High IGF1 concentration does circulate in plasma in vivo, bound to IGFBP and acid-labile subunit proteins, and syndecan-1 largely expressed by MMCs binds IGFBP3, releasing IGF1 at MMC membrane.

The aberrant IGF1R expression confers on MMCs a growth activity of insulin. Insulin, IGF-1 and their receptors are closely related molecules but both factors bind to the receptor of the other one with a weak affinity. Insulin receptor (INSR) is increased throughout normal plasma cell differentiation. INSR gene is also expressed by MMC of 203/206 newly-diagnosed patients. Insulin is a MGF as potent as IGF-1 at low concentrations, below physiological ones and requires the presence of INSR-IGF-1R hybrid receptors, stimulating INSR+IGF-1R+ MMCs, unlike INSR+IGF-1R- or INSR-IGF-1R- MMCs. Immunoprecipitation experiments indicate that INSR is linked with IGF-1R in MMCs and that insulin induces both IGF-1R and INSR phosphorylation and vice versa. Further therapeutic strategies targeting the IGF-IGF-1R pathway have to take into account neutralizing the IGF-1R-mediated insulin MGF activity.

TORC2 AS A THERAPEUTIC TARGET IN MULTIPLE MYELOMA

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In the last 5 years, studies demonstrate the PI3K/AKT/mTOR pathway is one of the most frequently hyperactivated cascades in cancer. It regulates a host of pro-tumoral functions such as proliferation, anti-apoptosis, angiogenesis, hyperactive protein synthesis and glucose metabolism. It has, thus, become a prominent target for therapy. The mTOR kinase functions within 2 separate multiprotein complexes, TORC1 and TORC2, each with distinct substrates. TORC1, composed of mTOR complexed to Raptor and mLST8, phosphorylates p70S6kinase and 4E-BP1 and, in so doing, stimulates protein translation. Cell cycle protein translation drives proliferation. TORC2, containing mTOR complexed to mLST8, SIN1, Protor and Rictor, phosphorylates and activates AKT, SGK and PKC (Figure 1).

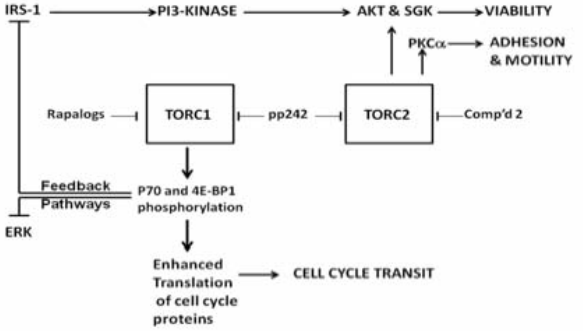

Figure 1: Feedback activation pathways after mTOR inhibition. Arrows show activation signals. TORC1/p70/4E-BP1 activity induces cell cycle transit in tumor cells but also results in feedback inhibition of PI3K and ERK. Thus, by inhibiting TORC1 in MM, rapalogs inhibit cell cycle transit but also activate AKT, SGK and ERK. Active site TOR kinase inhibitors (like pp242) overcome feedback AKT/SGK activation since they inhibit TORC2 and prevent TORC2 activation of these kinases. However, due to their additional TORC1 inhibition, ERK is still feedback activated, serving as a mechanism of resistance.

Although a fair amount of pre-clinical investigations, in vitro as well as in murine xenograft models, have demonstrated the therapeutic potential of mTOR inhibitors in multiple myeloma (MM), 1st generation rapalog mTOR inhibitors, such as temsirolimus, have been relatively ineffective in the clinic. One possible reason for lack of efficacy is the fact that rapalogs only induce G1 arrest in MM cells without induction of apoptosis. We have previously identified a possible explanation for this lack of apoptosis: Rapalogs primarily inhibit MM mTOR activity within TORC1 and TORC1 inhibition, by repressing the feedback inhibition of IRS-1, results in potent feedback activation of the IRS-1/PI3-K/AKT pathway (fig 1). This should protect against apoptosis. Feedback activation of PI3-K/AKT is one of the rationales for development of novel mTOR inhibitors that act at the ATP-binding site of mTOR (so called active-site inhibitors) and, thus, inhibit both TORC1 and TORC2. Inhibition of TORC2-induced phosphorylation & activation of AKT should overcome the feedback PI3-K/AKT pathway. Our group and the Dana Farber group has demonstrated the increased anti-MM activity of such 2nd generation TOR inhibitors like pp242 (fig 1) when compared to rapalogs.

The inhibition of TORC2 prevents AKT S473 phosphorylation and full activation of AKT and, thus, overcomes feedback activation of IRS-1/PI3-K/AKT. However, feedback activation of ERK still occurs (Fig 1). ERK activation is even more intense in MM cells treated with active site TOR inhibitors vs rapalogs. Our work shows that the ERK activation is mediated by inhibition of TORC1. Most important, ERK activation was a mechanism of resistance to 2nd generation active site mTOR inhibitors. As our previous work had demonstrated that much of the anti-MM activity of pp242 was due to inhibition of TORC2, we, thus, attempted to develop an inhibitor specific for TORC2 which would have no effect on TORC1 and, thus, not induce ERK activation. We, thus, designed a high-throughput screen of inhibitor libraries against a yeast-two-hybrid interaction between mTOR and the TORC2 protein Rictor. Compounds were identified that prevented binding of mTOR to Rictor in yeast and further tested against MM cells. One of these, a quinalone derivative termed compound 2, was effective against MM cell lines in survival assays with ED50's of 0.6, 0.8 and 1.2 uM for

MM1.S, 8226 and OPM-2 cell lines. Compound 2 also significantly induced MM cell apoptosis. Molecularly, compound 2 inhibited AKT S473 phosphorylation, the TORC2 event, while p70 phosphorylation (TORC1 event) was unaffected or even increased. In OPM-2 cells, we were able to demonstrate that compound 2 prevented association of mTOR to Rictor while having no effect on the mTOR-Raptor association. Structure-activity relationships are currently being investigated with the goal of chemical modification for enriching anti-MM activity. Most importantly, compound 2 did not induce activation of the ERK resistance pathway.

An additional player in mTOR function in MM is the mTOR-binding protein DEPTOR. DEPTOR is over-expressed in about 30% of myeloma specimens and is inhibitory within TORC1 and TORC2 complexes, downregulating their kinase activity. However, the significantly inhibited TORC1 function results in marked feedback activation of PI3-K/AKT. Knockdown of DEPTOR in MM cell lines results in cytostasis and apoptosis. We have, thus, also initiated efforts to identify compounds that inhibit DEPTOR-mTOR binding in the yeast-hybrid screen with the hope that such agents would be deleterious to high-DEPTOR-expressing MM cells.

HEPARANASE AND SYNDECAN-1: PROMOTERS OF AGGRESSIVE MYELOMA BEHAVIOR AND TARGETS FOR THERAPY

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The bone marrow microenvironment plays a key role in driving myeloma progression. Central to microenvironmental regulation of this cancer is the crosstalk that occurs between myeloma tumor cells and host cells (e.g., endothelia, stromal cells, osteoclasts). Much of this crosstalk is mediated by soluble protein effectors including growth factors, chemokines and cytokines that activate signaling pathways. Syndecan-1 (CD138), via binding of its heparan sulfate chains to these soluble effectors, is ideally suited to mediate tumor-host crosstalk. Syndecan-1 is expressed abundantly by myeloma cells and when proteolytically shed from the cell surface it accumulates within the tumor microenvironment and enhances tumor growth and metastasis in animal models of myeloma.[1] Moreover, an important regulatory role for shed syndecan-1 in myeloma is consistent with the finding that high levels of shed syndecan-1 in patient serum are an indicator of poor prognosis.[2] While investigating the mechanisms of syndecan-1 action in myeloma, we discovered that a dynamic association exists between syndecan-1 and the heparan sulfate degrading enzyme heparanase. Heparanase is expressed by both myeloma cells and by cells within the myeloma microenvironment. Like syndecan-1, elevation of heparanase expression is associated with poor prognosis in many cancers, including myeloma.[3,4] We now know that heparanase influences syndecan-1 in multiple ways. It enhances syndecan-1 shedding by elevating myeloma cell expression of MMP-9 thus contributing to high levels of syndecan-1 in the myeloma microenvironment.[5] Expression of heparanase also upregulates transcription of uPA/uPAR, HGF, VEGF and RANKL by the tumor cells. Interestingly, enhanced expression of these genes can be triggered by either upregulation of heparanase expression in the tumor cells, or by addition of exogenous heparanase to the tumor cells. This suggests that soluble heparanase within the tumor microenvironment can impact myeloma cells even if those cells are not making the enzyme. The downstream products of enhanced heparanase expression – shed syndecan-1 and growth factors – form complexes to potentiate their signaling activity in host cells. For example, the HGF/syndecan-1 complex activates the cMET receptor on osteoblasts.[6] This stimulates IL-11 signaling leading to an increase in RANKL production and a resulting increase in osteolysis. The VEGF/syndecan-1 complex enhances endothelial migration in vitro and stimulates angiogenesis in rat aortic organ cultures.[7] This is consistent with a role for heparanase in promoting myeloma angiogenesis as suggested by our previous finding that myeloma patients having high levels of heparanase enzyme activity in their bone marrow plasma exhibit high levels of microvessel density.[8] In addition to increased HGF and VEGF mediated signaling, we also discovered that heparanase expression in myeloma cells dramatically enhances osteolysis both locally and systemically in animal models. This effect was traced to the ability of heparanase to upregulate RANKL expression and secretion and is consistent with our finding that in myeloma patient biopsies, high levels of heparanase correlate with high levels of RANKL expression.[9] How is heparanase upregulating expression of genes that drive myeloma progression? Interestingly, when heparanase expression is enhanced in myeloma cells, levels of syndecan-1 in the nucleus drop dramatically.[10] Because heparan sulfate is known to inhibit activity of histone acetyltransferases (HATs), a family of enzymes linked to transcriptional activation, we speculated that loss of syndecan-1 from the nucleus would result in enhanced gene transcription. This was confirmed experimentally by showing that cells expressing high levels of heparanase have high levels of acetylated histone H3 and H4 and that addition of exogenous syndecan-1 to nuclear extracts from these cells inhibits the activity of HAT. Together these data point to the critical importance of heparanase in regulating both the location of syndecan-1 and the expression and activity of factors that control multiple pathways driving myeloma progression. Furthermore, they establish heparanase as a viable target for therapy. Heparanase is an attractive therapeutic target because: i) it is not expressed in significant levels in normal tissues, ii) there is only a single active heparanase enzyme in humans and iii) heparanase knockout mice appear normal indicating that therapeutic inhibition of heparanase will have minimal side effects. We targeted heparanase in animals models of myeloma using chemically modified heparins that bind to the heparanase enzyme via two heparin-binding domains that flank the enzyme's active site. These modified heparins are non-anticoagulant, are not cleaved by the heparanase, but are potent inhibitors of the enzymes activity. When delivered in vivo, they effectively inhibit growth of myeloma tumors growing either subcutaneously in mice or within human bones implanted in SCID mice (SCID-hu mice).[11] In summary, this work establishes the importance of syndecan-1 and heparanase working together in driving the aggressive phenotype of myeloma tumors and demonstrates the potential of heparanase inhibitors for myeloma therapy.

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BONE DISEASE AND ANIMAL MODELS

BONE DISEASE: FROM PHYSIOPATHOLOGY TO TREATMENT

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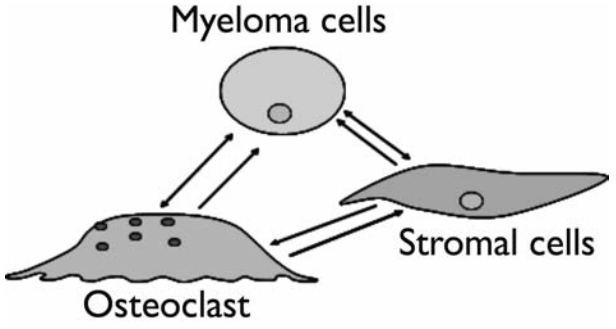
Multiple myeloma (MM) is the most frequent cancer to involve the skeleton and induces osteolytic lesions that rarely heal. Myeloma bone disease is responsible for some of the most devastating complications of MM. MM patients experience two or more skeletal-related events per year and more than 1/3 of patients sustain a pathologic fracture within 21 months. These skeletal-related events include fracture, surgery to bone, radiation to bone, and hypercalcemia. Even with the best current treatment, MM patients still experience approximately one skeletal-related event every 12-18 months. These skeletal-related events can be devastating to the patients. Seventy percent of patients present with bone pain, approximately 20% will present with a pathologic fracture at diagnosis, and over the course of their disease 60% of patients sustain a pathologic fracture. Fractures in MM patients results in a 20% increase in mortality compared to patients without fracture and an incremental cost of $63,455 for care of MM patients with bone disease. Importantly, MM bone disease can continue to progress even when patients are in complete remission from their tumor. The reason myeloma bone disease is so catastrophic is that both osteoclast (OCL) formation and activity are markedly increased while osteoblast (OB) bone forming activity is severely suppressed. Suppression of OB activity persists even when patients are in long-term complete remission and MM tumor cells are no longer detectable. The MM bone microenvironment is highly complex and is composed of multiple cell types, matrix proteins and endothelial cells that contribute to tumor growth, immune dysregulation, chemoresistance and bone destruction. How this microenvironment becomes so supportive of MM, and the contribution and interaction of the various components of the microenvironment to enhancing MM growth is only beginning to be understood (Figure 1).



Bone cells and immune cells in particular contribute to these processes through production of cytokines and expression of adhesive molecules that increase MM cell growth, enhance the chemoresistance of MM to treatment, increase OCL formation and suppress OB differentiation, polarize T cell subsets from predominantly Th-1 to Th-17 and drive dendritic cell differentiation toward the OCL lineage. Th17+ T cells produce IL-17 which enhances the effects of RANKL to further drive osteoclastogenesis in MM. Tumor associated macrophages also play an important role in MM both through immunosuppression of host responses to tumor cells and serving as OCL precursors. Preclinical models have clearly supported these findings that bone cells (OCL and OB precursors) play critical roles in myeloma bone disease in addition to their intrinsic capacity to resorb bone or form bone respectively. Marrow stromal cells are early OB progenitors. These early OB progenitors express high levels of RANKL and low levels of OPG, and thereby drive osteoclastic bone destruction. Further, they produce high levels of IL-6 and express increased levels of VCAM-1, allowing docking of MM cells to marrow stromal cells that result in increased OCL activity and tumor growth. These adhesive interactions upregulate cytokine production by both the MM cells and the stromal cells. Stromal cells from patients with MM also appear to be hyper-responsive to TNF and produce RANKL at much lower levels of TNF than normal marrow stromal cells. Finally, stromal cells support the growth of 1o MM cells, and MM cells can be passaged over stromal cells or OCL while mature OB inhibit MM cell growth. These results suggest that the blockade of OB differentiation in MM by DKK1, a Wnt antagonist, and activin A results in the induction of a conducive marrow microenvironment for growth of the tumor and osteoclastic bone destruction. Further, blocking OCL formation/activity with bisphosphonates or osteoprotegerin (OPG) decreases both tumor growth and bone destruction in preclinical models, and zoledronate has been reported to have both anti-osteoclast and anti-myeloma effects in patients in the MRC IX trial. In addition, the demonstration of the importance of RANKL in the bone destruction process of MM has led to the testing of Denosumab, a human monoclonal antibody against RANKL, in clinical trials of MM bone disease. Similarly, enhancing stromal cell differentiation to OB with anti-DKK1 or an activin A receptor antagonist decreases tumor growth and bone destruction as well as increasing bone formation. These important contributions of the marrow microenvironment to MM explain why treatments that target both the bone microenvironment as well as the tumor, such as bortezomib, which can both block tumor growth and increase OB differentiation, and the immunomodulatory drugs (IMiDs), which targets both MM cells and OCL, have been more effective than prior therapies for MM and have dramatically increased progression-free and overall survival of patients. Unfortunately, MM is still incurable for the overwhelming majority of patients, and the bone disease remains a major contributor to the morbidity and mortality of MM patients. Thus, further dissecting the underlying pathophysiology of MM bone disease, as a means to develop new treatments to prevent and/or reverse MM bone disease, is critically important to improve both the quality of life and enhance survival of MM patients. In this presentation the pathophysiology of MM bone disease will be reviewed and agents that target these processes will be discussed.

BONE ANABOLISM AND TUMOR GROWTH IN MYELOMA

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Mounting experimental data demonstrate strong association between activation of osteoblasts and control of myeloma progression.[1] Clinical data indicate that bortezomib, which directly acts to stimulate osteoblast differentiation, increases circulating levels of osteoblastic markers and stimulate bone formation in myeloma patients.[2] These studies support the notion that although mesenchymal stem cells (MSCs) from patients with myeloma are reportedly abnormal these cells can differentiate into osteoblasts and form bone upon stimulation with osteoblast activating agents. The clinical drug bortezomib[3] or experimental agents such as anti-DKK[1,4] Wnt activators,[5,6] and inhibitors of TGFβ7 and activin A signaling[8] have all been shown to activate osteoblasts in myelomatous bone. These agents also suppressed myeloma growth in bone, particularly when increased bone formation coincided with a reduction in osteoclast activity. A contrasting scenario has been reported by us demonstrating that the chimeric molecules EhpB4-Fc and ephrinB2-Fc similarly promoted bone formation in myelomatous bone but EphB4-Fc treatment only was associated with reduced tumor growth.9 In contrast to EphB4-Fc, ephrinB2-Fc had no effect on osteoclastogenesis and promoted angiogenesis,[9] suggesting that increased bone formation by itself is insufficient to create an inhospitable environment for myeloma. This study also indicates that restraining myeloma by certain bone anabolic agents is not a consequence of a decrease in marrow space due to increased bone volume; rather, it resulted from alteration of multiple cellular and molecular components in the hematopoietic marrow. The molecular mechanisms by which osteoblast activating agents restrain myeloma may involve increased production of anti-myeloma, anti-osteoclastogenic, anti-inflammatory and anti-oxidants factors by microenvironmental cells in the bone marrow, and reconstruction of the normal hematopoietic stem cell niche. We have shown that the osteogenic matrix factor, decorin, which is highly secreted during bone formation, exerts anti-myeloma, anti-osteoclastogenesis and anti-angiogenesis properties in myelomatous bone.[10] To further explore the association between bone anabolism and myeloma growth we determined therapeutic efficacy and shed light on molecular mechanisms of daily treatment with parathyroid hormone (PTH)[11] or MSC cytotherapy.[12,13] These two therapeutic approaches have been successfully used in clinical osteoporosis or bone regeneration and for improving hematopoiesis recovery. In the SCID-hu and the SCID-rab models, intra-bone injection of MSCs effectively prevented myeloma-induced bone resorption and promoted bone formation in myelomatous bone, and these effects were associated with reduced myeloma growth. The majority of the injected MSCs disappeared within 2-4 weeks, indicating that MSCs exert their effects on bone remodeling as a bystander cells (trophic effect). Using

bioluminescence imaging and immunohistochemistry we found that few intravenously injected luciferase/GFP-expressing MSCs were capable of homing to myelomatous bone and that four weekly intravenous injections of MSCs prevented myeloma-induced bone resorption, supporting the clinical relevancy of MSC cytotherapy. To shed light on molecular mechanisms of MSC cytotherapy, global gene expression profiling was performed on whole myelomatous human bone of myeloma-bearing SCID-hu hosts 0 and 24 hours following intra-bone MSCs cytotherapy. MSC cytotherapy resulted in overexpression of extracellular or soluble factors known to mediate wound healing, bone remodeling, oxidative stress and inflammation. Daily subcutaneous injections of PTH resulted in marked increased in bone mineral density (BMD) of myelomatous bones and attenuated growth of primary myeloma and the Hg myeloma cell line, capable of passaging in experimental animals.[11] Myeloma cells do not express PTH receptors indicating that reduced myeloma growth resulted from alteration of the bone marrow microenvironment. Global gene expression profiling on whole myelomatous human bone of myeloma-bearing SCID-hu hosts treated with daily PTH identified changes in molecular pathways regulating osteoblastogenesis, osteoclastogenesis and myeloma growth inhibition. PTH activated multiple bone remodeling pathways of which Wnt signaling was prominent.[11] Certain relevant microenvironmental genes, particularly unique anti-inflammation mediators, were upregulated by PTH or MSC cytotherapy indicating that these two treatment approaches mediate their effects, in part, through certain common molecular pathways. Bone anabolism that creates an effective inhospitable bone marrow environment for tumor cells may also negatively affect myeloma progression.

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EFFECT OF NEW ANTI-MYELOMA DRUGS ON BONE MICROENVIRONMENT CELLS

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The presence of osteolytic bone lesions is the hallmark of multiple myeloma (MM) and results in bone fragility and fractures in MM patients. Bone lesions are characterized by a severely unbalanced and uncoupled bone remodeling process in the area of plasma cell infiltration due to an increase in the osteoclast (OC) formation and activity and osteoblast (OB) suppression. In turn, bone microenvironment seems to have a critical role in the regulation of MM cells growth and survival. Blocking OC formation and activity through RANKL inhibition induces MM cell apoptosis in vitro and caused a marked reduction of tumor burden in vivo. Consistently it has been reported that OCs produce several MM cell growth factors as IL-6, osteopontin, BAFF and APRIL. On the other hand it has been shown that OB activation attenuates the stimulatory effect of OC cells on MM cell survival in vitro. Moreover, in the SCID-hu MM model it has been reported that the stimulation of bone formation either by the injection of mesenchymal stem cells or through the stimulation of Wnt signaling resulted in an inhibition of MM growth. Overall these results suggest that either blocking OC formation and activation or increasing bone formation in MM patients could result in a reduction in tumor burden and that bone microenvironment cells could be potential therapeutic targets in MM. In line with these observations recent data suggest that the new anti-myeloma drugs recently introduced in the therapeutic armamentarium of MM patients may affect both OC and OB cell function and activity. It has been shown that the proteaosome inhibitor Bortezomib stimulates OB differentiation and bone formation both in vitro and in vivo in mice. This evidence is strongly supported by the in vivo observations obtained in MM patients treated with Bortezomib, An increase of total alkaline phosphatase and bone specific alkaline phosphatase has been also reported in MM patients that respond to the treatment with Bortezomib. Moreover, our data indicate that Bortezomib induce osteoblast phenotype in OB progenitors increasing Runx2 activity and consequently stimulates the expression of OB markers. Consistently bortezomib stimulates bone regeneration in mice and it is able to rescue bone loss in a mouse model of osteoporosis. Interestingly it has been also reported that proteasome inhibitors including Bortezomib also inhibit OC formation and differentiation through the block of NK-kB activity in osteoclast progenitors. All these data suggest that either the anti-MM effect of Bortezomib could be de due, at least in part, to its effect on OBs and OCs or this drugs may have a positive effect on MM bone disease. Thalidomide and its derivative immunomodulatory drug (IMiD®) lenalidomide may also affect bone microenvironment. It has been shown that thalidomide and IMiD® directly inhibit OC formation and maturation. In addition we have recently investigated the potential effects of lenalidomide and the more potent IMiD® pomalidomide on MM-induced OC formation. First in a cell-to-cell contact co-culture system we found that both IMiDs® significantly blunted the production of the critical osteoclastogenic factor RANKL by human osteoprogenitor cells induced by MM cells decreasing the RANKL/OPG ratio level. Consistently the pro-osteoclastogenic property of the conditioned medium of the co-cultures was reduced in the presence of IMiDs®. Interestingly, by microarray analysis (Affymetrix®), among the genes significantly modulated by lenalidomide and pomalidomide in MM cells we identified the downregulation of the adhesion molecules ITGA4 (CD49d), ITGA8 and ICAM2 (CD102). By flow cytometry we confirmed the capacity of IMiDs® to inhibit the expression of adhesion molecules by MM cells showing that this effect was involved in the inhibition of RANKL/OPG ratio by IMiDs in co-culture. These data strongly suggest that IMiDs® affect MM-induced OC formation through the inhibition of RANKL/OPG ratio targeting the expression of adhesion molecules by MM cells.

MATHEMATICAL DESCRIPTIONS OF BONE REMODELING DYNAMICS IN MYELOMA BONE DISEASE

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We motivate the role mathematics can play in understanding and treating multiple myeloma and review some of our specific mathematical models for the dysregulated bone remodeling that occurs in myeloma bone disease.

The first of these models[1] examines the critical signaling between osteoclasts and osteoblasts. The interactions of osteoclasts and osteoblasts are modeled as a system of differential equations for these cell populations, which exhibit stable oscillations in the normal case (developed by Komarova et al.[2]) and unstable oscillations in the myeloma case. In the case of untreated myeloma, osteoclasts increase and osteoblasts decrease, with net bone loss as the tumor grows. The therapeutic effects of targeting both myeloma cells and cells of the bone marrow microenvironment on these dynamics are examined.

We extend the discussion to a new spatial representation of bone remodeling in which the dynamics of the bone/marrow interface is governed by the local interactions of cell types.[3] The sharp interface between bone and marrow regions moves in and out, i.e. in the normal direction,

due to remodeling. Based on these observations we employ the level set method to represent the spatial behavior of remodeling. We use our previous models to determine the change in bone mass that influences how the interface between bone and marrow changes.

Eventually by including in this formalism further details, such as more complex cytokine interactions and accurate parameter values, it may be possible to obtain simulations of phenomena related to bone remodeling with spatial behavior much as in vitro and in vivo. This would make it possible to perform in silica experiments more closely resembling experimental observations.

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DISSECTING THE MULTIPLE MYELOMA TUMOR MICROENVIRONMENT: A NOVEL IN VIVO PLATFORM

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The recognition of the crucial role of the bone marrow (BM) microenvironment as a potential target for novel therapeutical approaches in Multiple Myeloma (MM) has recently provided several promising strategies and a number of novel therapeutics are presently in advanced preclinical or early clinical evaluation. According to the modern view of MM pathophysiology, the interaction with heterogeneous normal populations of the human BM microenvironment (huBMM) supports the growth, the survival and induce a drug resistant phenotype of MM cells. In the last decades, however, the lack of preclinical models recapitulating the natural BM milieu, where the disease takes place, has been the major limitation for the study of the molecular interactions of MM cells with the huBMM and for the rational design and validation of novel therapeutics. Most of the early in vivo models of murine or human MM do not indeed reproduce the huBMM and therefore are not suitable systems for the study of the antitumor activity of novel agents targeting not only MM cells but also the specific huBMM. Essentially, these models involve transplantation and engraftment of established murine or human cell lines respectively in syngeneic or immunocompromised mice. These models are useful tools as first drug-screening and serve as useful filter for defining the anti-tumor activity and the pharmacokinetic properties of innovative drugs but should not be viewed as ideal models for development of anti-MM drugs. On the other hand, engineered models of human MM allow the definition of biological steps in the pathogenesis of MM and provide optimal models to test molecules that specifically target the underlying genetic lesions, but are not suited for large-scale screening of investigational drugs and do not completely recapitulate the human disease. Innovative models based on the engraftment of human primary MM cells within a huBMM obviously retain advantages over other models for the study of the pathophysiology of the disease and for preclinical evaluation of investigational drugs targeting not only the tumor compartment but also the huBMM. The development of the SCID-hu model [1-3], which is based on the implantation of a human fetal bone chip in immunocompromised (SCID) mice, is still one of the most important experimental resources and has represented a significant advancement since it reproduces an in vivo huBMM suitable for engraftment of freshly-explanted primary MM cells. Even if this model represents a powerful system for the screening of investigational drugs, there are some weaknesses associated with a limited availability of human fetal bone chips, the allogeneic nature of the fetal BM milieu versus patient-derived MM cells and the obvious differences of the fetal bone versus the elderly bone, where in the most of cases the disease takes place. In the aim to overcome the limitations of the SCID-hu system, a new model, called SCID-synth-hu, recently developed by us [4]. This model is based on the implantation into a SCID mouse of a three-dimensional (3D) bone-like poly-ε-caprolactone polymeric scaffold (PCLS). This synthetic recipient, which finely reproduces the micro architecture of a normal human femur adult bone, allows efficient coating of the 3D scaffold internal surface with human bone marrow stromal cells (BMSCs) and the successful engraftment of human primary explanted MM cells within an allogeneic huBMM in vivo. Most importantly, the injection within the biosynthetic scaffold of the whole unselected BM population from patient BM aspirate, containing both primary CD138+ MM cells and their autologous BMSCs, demonstrated a successful engraftment of all patient samples. Therefore this latter approach indeed allows the recapitulation of the human disease in its natural BM milieu. Moreover, vasculogenetic events occur within the reconstituted huBMM and we have demonstrated that this SCID-synth-hu model is suitable for investigational drug screening simply by monitoring the human paraprotein in the mouse serum. Using a sequential addition of normal or genetic engineered huBMM cell components, this system allows the dissection of biological events occurring within the huBMM, therefore providing a finely tunable innovative tool for more appropriate studies on the role of the BM milieu in the MM. In addition, preliminary results demonstrated that the polymeric scaffold can be improved by addition of hydroxyapatite, providing an optimal instrument for the study of biological events involved in the MM-related bone disease in a well defined system. An additional important point is the plasticity of this platform that can allow investigation of molecular variants of human disease by the use of personalized models. This may be relevant in the light that MM drug development cannot rely on the "one-fits-all" concept, taking in account the molecular heterogeneity of the disease. The rising interest for novel therapeutics based on the interference with molecular microenviromental networks which also involves immunological cells, strongly underscore the need of preclinical models able to recapitulate the MM cell/autologous huBMM interaction. In conclusion, more efforts are needed to further improve these preclinical platforms and allow routinely use of these models for the definition of new anti-MM therapeutic agents.

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EPIGENETIC CHANGES OF MYELOMA CELLS WITHIN THE BONE MARROW MICROENVIRONMENT

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Epigenetics refer to variability in gene expression, heritable through cell division, but without any underlying modification of the actual genetic sequence. These alterations play a fundamental role in normal physiological phenomena such as embryogenesis, genomic stability, X chromosome inactivation and imprinting, but also in cancer. Cancer cells have been shown to have genome-wide aberrations at epigenetic level, including global hypomethylation, promoter-specific hypermethylation, histone deacetylation, global down-regulation of miRNAs and upregulation of certain actors of the epigenetic machinery such as EZH2. Unlike genetic alterations, epigenetic alterations are potentially reversible and are therefore good candidates for new therapies. In multiple myeloma (MM) several genes are hypermethylated and upon treatment with a DNA methylation inhibitor they become re-expressed demonstrating the reversible nature of epigenetic modifications (1-3). Inhibition of DNA methylation using 5' azacytidine has been demonstrated to promote MM cell apoptosis, inducing double stranded DNA breaks and inhibiting IL-6 and NF-κB signaling pathways (4-5). 5-aza-2'deoxycytidine, also known as decitabine (DAC), has furthermore been shown to induce p21 and p38 mediated cell cycle arrest in the G1 and G2/M phases respectively in MM and leukemia (6-7). Besides DNA methylation, covalent histone modifications are also involved in gene transcription. The N-terminal tails of the histones are susceptible to a wide range of modifications of which acetylation, methylation and phosphorylation are the most studied so far. The degree of histone acetylation is determined by net balance of the activity of histone acetyl transferases (HATs) and histone deacetylases (HDACs) (8). Although

there is no direct evidence of altered HDAC activity or expression in MM, preclinical studies have demonstrated that HDAC inhibitors (HDACi) exhibit anti-MM activity (8,9). Our group has previously demonstrated that JNJ-26481585, a hydroxamate based HDACi, induces G1-phase cell cycle arrest and apoptosis in the murine 5T33MM cells. When used in an in vivo setting, JNJ-26481585 decreased serum M component and tumor burden in the 5T33MM model (10). Moreover, when combined with bortezomib, JNJ-26481585, clearly showed synergistic effects in the 5T2MM model on bone parameters such as reduction of osteoclasts and increase of osteoblasts, trabecular bone volume, and trabecular number compared with bortezomib as single agent, suggesting that the bone remodeling properties of bortezomib can be improved with a HDAC inhibitor (11). We here also demonstrate that Vorinostat, a HDAC inhibitor currently in clinical trial, when used as single drug directly affects the osteogenic capacity of MM patient derived mesenchymal stem cells (MSCs), as demonstrated by the expression of specific osteogeneic markers and matrix mineralization. We recently identified a novel repressed gene signature in MM cells, that was enriched for previously defined H3K27-tri-methylated genes, targets of the Polycomb group. The pan-HDAC inhibitor LBH589 activated gene expression of Polycomb target genes which were under expressed in MM and reduced the survival of the tumor clone in vitro and in vivo, using the 5T33MM model (3). In addition we also demonstrated that IGF-1 repressed expression of the pro-apoptotic gene Bim by simultaneously increasing H3K9 dimethylation (H3K9me2) and decreasing H3K4 trimethylation and H3K9 acetylation (H3K9Ac) of both the Bim and FoxO3a promoter. Interestingly, combinatorial treatment with LBH589 and DAC was demonstrated to result in a drastic and significant reduction of these H3K9me2 marks accompanied by an increase in H3K9Ac (12). When the IGF-1R was targeted (with the RTK inhibitor picropodophyllin, PPP) simultaneously with LBH589, in vitro synergistic effects on apoptosis and proliferation were observed, using both human and murine MM cells, while in vivo, using the 5T33MM model a significant prolonged survival was observed compared to single drug treatment. We here further demonstrate that besides the direct anti-MM activity of the DNA methylation inhibitor DAC and/or the histone deacetylase inhibitor JNJ-26481585; combinatorial treatment of the human and murine MM cells with both agents synergistically decreased viability and increased the number of apoptotic cells as compared to drugs used alone. When this combination strategy was used in vivo (using the 5T33MM model), synergistic effects were also observed on tumor burden and microvessel density. In conclusion, the epigenome in MM is clearly altered. Epigenetic drugs are promising not only as single agents but also in combination with other epigenetically targeted drugs or conventional used chemotherapy, targeting both the MM cells and the microenvironment. The understanding of the epigenome and the actors involved in the modulation of its interaction with genomic sequences is fundamental in the development of these new classes of therapies.

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HIGH-RISK ENTITIES OF MYELOMA: FROM BIOLOGY TO TREATMENT

T(4;14) AND GENOMIC INSTABILITY IN HIGH-RISK MULTIPLE MYELOMA

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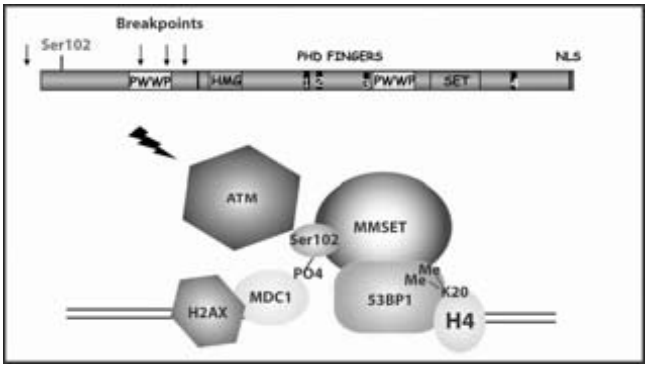
The t(4;14)(p16;q32) chromosome translocation is unique among MM IgH translocations in that the IgH breakpoint is always within a switch region, and light chain variant translocations have not been described. This results in dissociation of the intronic Eu enhancer on der(4) that dysregulates MMSET from the 3' enhancer on der(14) that dysregulates FGFR3. This suggests that it may be important in the pathogenesis of t(4;14) MM to dysregulate both of these oncogenes. Activating mutations of FGFR3 that occur in about 10% of patients with t(4;14) indicate that FGFR3 can play an important role in the progression of t(4;14) MM. However the absence of FGFR3 expression in about 25% of patients with t(4;14) MM also indicates that the continued expression of FGFR3 is dispensable, at least in some patients. The role of these two proteins in the pathogenesis of MM has remained elusive. Clinically patients with t(4;14) MM have a poor prognosis when treated with melphalan or IMiDs, whereas early mortality appears to be abrogated by the use of bortezomib, even if the poor prognosis is not completely overcome.



Recently we have identified a role for MMSET in DNA repair (Pei H, Zhang L, Luo K, Qin Y, Chesi M, Fei F, Bergsagel PL, Wang L, You Z, Lou Z. MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. Nature. 2011 470(7332):124-8). Following DNA damage MMSET is phosphorylated on Ser102 by ATM and is recruited to double strand breaks (DSB) by MDC1. MMSET is a histone methyltransferase that methylates H4K20 on the histones at sites of DSBs. Di-methylation of H4K20 recruits p53 binding protein (53BP1) a key transducer of the DNA damage checkpoint signal. 53BP1 is required for p53 accumulation, G2/M checkpoint arrest, and the intra-S-phase checkpoint in response to ionizing radiation. Approximately half of the translocation breakpoints in t(4;14) MM result in a truncated MMSET that lacks Ser102 and cannot be recruited to DSBs. These results indicate that MMSET belongs to a class of cancer causing genes such as ATM that contribute to oncogenesis by impairing the DNA damage response. Although further study is required, based on these data it may not be a useful therapeutic strategy to inhibit MMSET histone methyltransferase activity (and in fact provide caution that this may cause problems with systemic inhibition of the DNA damage response). Furthermore, they also suggest that DNA damaging agents should be used with caution in t(4;14) MM, and it they are to be used, it is probably best done when the tumor burden is lowest, and the fewest possible cells deficient in DNA repair are exposed to genotoxic agents

THE MMSET HISTONE METHYL TRANSFERASE ALTERS CHROMATIN STRUCTURE, GENE EXPRESSION AND DNA DAMAGE RESPONSE IN T(4;14) MULTIPLE MYELOMA CELLS

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The multiple myeloma SET domain (MMSET) gene is fused to the immunoglobulin locus in t(4;14)-associated multiple myeloma, and MMSET levels are elevated in these patients relative to other myeloma cases and normal cells. MMSET contains several domains commonly found in chromatin regulators including the PHD domain, PWWP domain and SET domain; responsible for histone methyl transferase (HMT) activity. What histone residues are methylated by MMSET in vivo has been uncertain. A well-folded, highly active form of the MMSET SET domain made in bacteria was promiscuous, methylating the H3K36, H3K27 and H4K20 residues of native histone as well as itself. To determine how MMSET affects chromatin in vivo and to identify genes regulated by MMSET, we engineered t(4;14)+ KMS11 cells with a tetracycline-inducible shRNA, leading to a >90% decrease in MMSET expression. Upon loss of MMSET expression, there was a striking decrease of dimethylated histone 3, lysine 36 (H3K36me3), and a strong increase in H3K27me3, a chromatin mark associated with gene repression. High MMSET levels and H3K36 methylation was associated with a more open configuration of chromatin within the myeloma cells. To ascertain the importance of the HMT activity of MMSE malignant cell growth, MMSET was re-expressed in KMS11 cells in which the rearranged and overexpressed allele of MMSET was disrupted by homologous recombination (KMS11-TKO). KMS11-TKO cells, stably infected with a retrovirus carrying MMSET, displayed high levels of H3K36me2 and loss of H3K27me3 and increased cell growth. By contrast a form of MMSET with a mutation (Y1118A) in the SET domain of MMSET that abrogated HMT activity of MMSET failed to change the chromatin state of the cells and failed to stimulate cell growth. To determine the genes regulated by MMSET and the importance of histone methylation in MMSET action, we profiled gene expression in both gain and loss-of-function systems using Illumina expression arrays. We compared these gene lists with the top 2000 genes bound by MMSET as determined in a ChIP-on-chip assay using NimbleGen 2.7kb promoter arrays. MMSET knockdown affected expression of 1845 genes (FC>1.5, $p<0.05$); 931 were upregulated and 914 had reduced expression levels. Among these, 192 genes were also bound by MMSET. Re-expression of MMSET in KMS11-TKO cells led to increased expression of 749 genes while 788 genes were downregulated; 176 of these genes were also bound by MMSET. There were 38 genes that were bound by MMSET and regulated in both systems, including BMF, BTG2 and TP53INP1. These genes implicated in apoptosis represent potential direct transcriptional targets of MMSET. Furthermore, functional annotation of genes bound and regulated by MMSET in either the knockdown or repletion system, using Ingenuity Pathway Analysis, showed enrichment of genes implicated in the regulation of cell death and the p53 pathway (e.g. BAX, BCL2, CASP6), the cell cycle (CCNE2, E2F2, TP53INP1, CDC25A) and integrin-mediated signaling (ACTB, CDC42, ITGAL). The effect of MMSET on integrin signaling is of interest given that loss of MMSET expression or repletion of KMS11-TKO cells with MMSET altered the adhesive and growth properties of KMS11 cells. Finally, gene expression changes were contrasted between re-expression of wild-type MMSET and catalytically inactive MMSET Y1118A. Strikingly, the Y1118A mutant, which was deficient in altering cell adhesion and growth and which did not change bulk histone methylation, altered expression of 1209 genes, 50% overlapping with those regulated by wild-type MMSET. Genes regulated by MMSET and the SET domain mutant were enriched mostly in cellular metabolism pathways (FDPS, IDI1, MVK) suggesting that effects on the cell cycle, adhesion and p53 pathways required the HMT activity of MMSET. These data indicate that MMSET can regulate genes in a HMT dependent and independent manner. Furthermore, MMSET target genes may be both activated and repressed upon changes in MMSET levels, indicating a complex interplay with the transcriptional machinery, likely through interactions with other transcriptional co-factors.

MMSET was recently implicated as a factor in DNA damage repair. In this regard we found that MMSET overexpressing myeloma cells demonstrated increased evidence of DNA damage at baseline and increased DNA damage after treatment of cells with mephelan or gamma irradiation as measured by comet formation and g-H2AX foci. Repletion of KMS11 knockout cells with HMT active but not HMT inactive

MMSET led to increased evidence of DNA damage. However, MMSET overexpressing cells showed increased viability after DNA damage and cells failed to undergo G1 arrest after DNA damage. These data suggest that MMSET through alteration of chromatin structure and DNA repair pathways may play a role in the chemotherapy resistance of t(4;14)-associated multiple myeloma.

WHOLE GENOME ANALYSIS OF HIGH RISK MULTIPLE MYELOMA (MM) REVEALS TEMPORAL INSTABILITY, BASELINE DIVERSITY, CLONAL TIDES, SELECTIVE PRESSURE AND LONGITUDINAL EVOLUTION

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The genetic mechanisms which underpin high risk MM have never been well defined. Indeed it is a mystery why an initiating chromosome translocation present even in MGUS should confer risk of early relapse and shortened survival. We therefore conducted comparative genomic analysis on 11 patients with known genetic risk factors and two temporally distinct samples. This analysis demonstrated a high degree of genomic instability over time in high risk MM when compared with FISH defined lower risk disease. This analysis also demonstrated baseline clonal diversity and clonal tides subject to selective pressure of therapeutics as well as the expected longitudinal evolution. Potetial clinical implications from this analysis were that:

1) Multiple drugs would be required to eradicate disease,
2) That the tumor is genomically unstable (and thus genotoxic drugs may actually worsen an already volatile situation),
3) That drug sensitive clones may re-emerge (thus explaining why seemingly drug resistant patients may later re-respond to the same drug: a phenomenon some have credited to new drugs but which seems more likely to represent clonal recrudescence)

To better understand these genetic events associated with disease progression and development of drug resistance we studied longitudinally collected samples from a single, high risk, t(4;14) MM patient at four time points. Samples included diagnosis, first progression on lenalidomide, second progression on bortezomib and plasma cell leukemia. WGS of 50bp fragment libraries provided >100x10^9 bases/sample and ~30X aligned coverage. From this analysis we identified 215 novel SNP variants (SNV) in 207 genes of which 125 were potentially damaging with 7 introducing nonsense mutation SNVs. Only 17 novel SNVs are common to all tumor time points and would be assumed to be driver events. Diagnostic and second progression shared 7 SNVs not found in the first relapse sample a finding which may lead to identification of true drug resistance (or sensitivity genes) and which supports the presence of multiple clones at diagnosis which may emerge or regress in clonal tides under treatment selection pressure. This data needs replicated in other patient samples but if true supports the use of drug therapies targeting multiple clones and challenges assumptions regarding refractory disease. Evaluation in a larger population is needed to confirm these findings.

ALLOGENEIC STEM CELL TRANSPLANTATION FOR HIGH-RISK MYELOMA

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Background. Allogeneic stem cell transplantation (allo SCT), a treatment modality based on transfer of immunocompetent donor lymphocytes offers curative potential to subjects with a variety of hematological cancers. In multiple myeloma (MM), high-dose melphalan followed by autologous stem cell transplantation (auto SCT) is adopted as a standard of care. However, it remains mostly palliative since the vast majority of patients (pts) relapse and renders allo SCT an option of interest. Deletion of chromosome 13q14 (13q-) especially when associated with additional cytogenetic abnormalities in MM has been shown to negatively impact prognosis. Therefore, improvement of therapy for these patients is highly desirable. *Patients and methods.* A prospective two-arm multi-center trial (DSMM V) was set up by our group to compare tandem high-dose melphalan 200 mg/m² (HD Mel) with a reduced intensity conditioning allo-SCT after one cycle of HD Mel for 13q- MM. Eligibility criteria were 13q- +/- additional cytogenetic abnormalies on bone marrow FISH analysis; age up to 60 years; newly diagnosed MM in Salmon and Durie stages II and III; and measurable disease. Allocation to either treatment arm was by availability of an HLA-matched (one mismatch allowed) volunteer related (VRD) or unrelated donor (VUD). Initially, all pts received four cycles of anthracycline/dexamethasone-based induction followed by chemomobilization of peripheral blood stem cells (PBSCT) and one cycle of HD Mel. Allogeneic SCT was performed after preparation with fludarabine (30 mg/m² for 3 consecutive days) and melphalan 140 mg/m². ATG was administered for VUD transplants. *Results.* 199 pts with a median age of 53 (range, 30 – 60) years were enrolled between October 2002 and March 2007 and included in this interim analysis. Sixty-seven percent had stage III disease. Allo SCT was performed in 126 of 199 pts (63%), 76 of whom (60%) received VUD allografts. The remaining 73 subjects uniformely received tandem HD Mel. Pts following allo SCT were more likely to achieve CR (59%) when compared to tandem HD Mel (32%; p=.003) within one year after end of therapy. Similarly, overall response rate was significantly higher with allo SCT (91% versus 86%; p=.003). Of note, depth of response to allo SCT was not associated with presence of acute graft-versus-host disease (GVHD): 62% CR with grades II to IV GVHD vs 58% CR with grades 0 and I (p=.75). Treatment-related mortality (TRM) at 2 years from allo SCT was 15/126 (11,9 %). At a median follow up of 41 months for tandem HD Mel and 34 months for allo SCT, projected 2-year PFS is 47,7 % for the auto-auto and 61,1 % for the auto-allo-arm. Especially in patients with an increated LDH-level and 17p deletion in addition to the 13q deletion OS is superior for the auto-allo when compared to the auto-auto-arm, respectively. *Conclusions.* This is the largest trial on first-line allogeneic stem cell transplant in MM so far. Our interim results show a higher CR rate in FISH 13q- subjects undergoing allo SCT when compared to tandem HD Mel. Despite a majority of allografts in our study being delivered from unrelated donors, TRM was comparable to trials confined to sibling transplants as in addition, TRM was identical for patients undergoing an allo SCT from a matched related or unrelated donor. With a still short follow-up, OS for very high risk patients is superior with an allo SCT when compared to tandem auto SCT.

PRACTICAL ASPECTS ON THE MANAGEMENT OF MYELOMA BONE DISEASE

BONE IMAGING IN MYELOMA: OLD SCAFFOLDS AND NEW AVENUES

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Lytic bone disease is a major feature of multiple myeloma (MM): 70-80% of patients have osteolytic lesions at diagnosis, up to 90% develop lytic lesions during the course of their disease and 10%-15% present with diffuse osteopenia or osteoporosis at diagnosis.

Conventional Radiography: Conventional radiography remains the gold standard for the evaluation of bone disease in MM patients. Lytic lesions on plain X-rays are typically punched-out lesions with absent reactive sclerosis of the surrounding bone in the flat bones of the skull and pelvis. In the long bones, there is a range of appearances from endosteal scalloping, to discrete small (<1 cm) lytic lesions, to mottled areas of multiple small lesions, to large destructive lesions. These lesions correspond to nodular replacement of marrow by plasma cells with entire bone destruction. A "complete skeletal survey", according to the IMWG guidelines, should include a posteroanterior view of the chest, anteroposterior and lateral views of the cervical spine (including an open mouth view), thoracic spine, lumbar spine, humeri and femora, anteroposterior and lateral views of the skull and antero-posterior view of the pelvis. The presence of lytic lesions in the skeletal survey is included in the definition of symptomatic myeloma, which needs therapy. However, plain X-ray has several limitations: i) it reveals lytic disease when over 30% of the trabecular bone has been lost; thus approximately 20% of patients at diagnosis have normal skeletal survey; ii) it cannot be used for the assessment of response to therapy as the lytic bone lesions seldom show evidence of healing; iii) new compression vertebral fractures do not always indicate disease progression and may occur due to ongoing bone loss or reduction of tumor mass that supports the bony cortex; iv) lack of accurate visualization of some areas; v) reduced specificity versus benign causes of osteopenia; vi) observer dependency; vii) lengthy period on the examination table; and viii) poor tolerance by patients with severe pain and extended lytic disease.

Computed Tomography (CT): CT allows the detection of small osteolytic lesions that are not revealed by plain radiography. The advantages of CT vs. conventional X-rays includes: i) the superior diagnostic sensitivity of CT in revealing more osteolytic lesions, mainly in areas that cannot be accurately visualized by plain radiography, e.g. scapulae, rib or sternum; ii) the superiority in estimating fracture risk and instability; iii) the duration of the examination, which is practically three times less than that of standard radiography; iv) the complete diagnostic evaluation in a single examination without having to reposition the patients, which is important when examining patients in pain; v) the demonstration of other unsuspected pathological processes; and vi) the superiority in planning the radiation therapy or the surgical intervention. One of the negative points against CT is the radiation dose delivered to patient, which is up to 400 times higher than that of conventional radiography. To overcome this problem, whole-body low-dose CT was introduced and was found to be superior to whole-body MRI in detecting residual osteolytic abnormalities; however, this is not widely available. Another disadvantage of CT is that it is non-specific for the evaluation of osteopenia/osteoporosis. Furthermore, caution is needed regarding the use of iv contrast as this could result in significant renal dysfunction, which is common among myeloma patients.

Magnetic Resonance Imaging (MRI). MRI has been widely available for the evaluation of myeloma bone disease during the last two decades. MRI is more sensitive compared to conventional radiography in the detection of osteolytic lesions. In general, the advantages of MRI over conventional radiography and CT scan include: i) the excellent imaging of the axial skeleton due to the greater sensitivity of the method; ii) the discrimination of myeloma from normal marrow; iii) the accurate illustration of spinal cord and/or nerve root compression, soft tissue extension, head and neck plasmacytomas, avascular necrosis of the femoral head and iv) better evaluation of cardiac amyloidosis and/or soft tissue amyloid deposits. Furthermore, the presence of focal lesions on MRI correlates with shorter overall survival (OS) in several studies, in patients who received both conventional chemotherapy and novel agent-based regimens. One of the advantages of MRI is the depiction of marrow infiltration. Five MRI patterns of marrow involvement in myeloma have been recognised: (1) normal appearance of bone marrow despite minor microscopic plasma cell infiltration; (2) focal involvement; (3) homogeneous diffuse infiltration; (4) combined diffuse and focal infiltration; and (5) variegated or "salt-and-pepper" pattern with inhomogeneous bone marrow with interposition of fat islands. Low tumor burden is usually associated with a normal MRI pattern, but a high tumor burden is usually suspected when there is diffuse hypointense change on T1-weighted images, diffuse hyperintensity on T2-weighted images and enhancement with gadolinium injection. Diffuse MRI marrow pattern correlates also with poor prognosis in patients who are treated with conventional chemotherapy or novel agent-based therapies and changes in MRI pattern correlate with response to therapy. MRI may also contribute in the definition of symptomatic disease in asymptomatic myeloma patients with normal skeletal survey. However, further studies will reveal if abnormal MRI is going to be incorporated in the definition of symptomatic myeloma.

The Role of PET/CT in Multiple Myeloma. F18-fluorodeoxyglucose PET/CT (FDG-PET/CT) is reliable for most bone lesions that are at least 1 cm in diameter using a standard SUV cut-off of 2.5 to indicate the presence of disease. For lesions smaller than 5 mm in diameter, it has been suggested that any amount of FDG uptake should be considered positive regardless of SUV. Lesions between 5–10 mm are considered indeterminate if the SUV is less than 2.5. The sensitivity of FDG PET in detecting myelomatous involvement is approximately 85% and its specificity is approximately 90%. By combining MRI of the spine/pelvis and FDG-PET/CT, the ability to detect sites of active MM is >90%. PET/CT provides additional information for the assessment of MM bone disease in areas not covered by MRI, but further studies are needed before its broader use in MM.

Other Imaging Techniques. Traditional technetium bone scintigraphy scanning is not recommended for MM patients as its specificity and sensitivity at diagnosis and follow-up is lower compared to conventional radiography. Dual-energy X-ray absorptiometry (DXA) is a valuable method for the definition of osteoporosis and in MM it may influence the decision to begin bisphosphonate treatment. Limitations of the method include its influence by spondylosis, spinal osteophytes and vertebral collapse, its difficulty to recognize myeloma osteoporosis from other forms of osteoporosis. DXA fails to predict disease progression.

In conclusion, conventional radiography remains the cornerstone for the evaluation of MM bone disease. Whole body MRI can give complementary information to skeletal survey and is recommended in patients with normal conventional radiography. MRI of the whole spine should be performed in addition to the skeletal survey as part of staging in all patients with a solitary plasmacytoma of bone. Urgent MRI is also the diagnostic procedure of choice to assess suspected cord compression in myeloma patients even in the absence of vertebral collapse. CT of the spine or other areas of the skeleton may be considered to clarify cases of clinical concern in the absence of MRI and to guide tissue biopsy. PET/CT can give complementary information to MRI but its use in MM has to be clarified by further studies. The incorporation of abnormal MRI to the definition of symptomatic myeloma also needs to be clarified.

WHOLE BODY MAGNETIC RESONANCE IMAGING IN MONOCLONAL PLASMA CELL DISEASES

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Whole body magnetic resonance imaging (wb-MRI) is an emerging technique in the assessment of malignant hemato-oncologic and especially in monoclonal plasma cell diseases. First investigations with MRI in multiple myeloma have already been published in 1987 by Ludwig et al (Ludwig Lancet 1987). While until the early years of this century there was no practicable way to examine the whole body of patients by MRI, the introduction of the so called rolling table device and whole body array curls it has become possible to examine more and more parts of the body in one examination within reasonable time and with adequate spatial resolution (Figure 1). Therefore several studies on the application of wb-MRI in patients with monoclonal plasma cell diseases have been published. Baeuerle et al. compared the findings of MRI of the spine and the sacral bone with wb-MRI and were able to demonstrate that it is worth to perform the latter if available because mere axial MRI misses about 10% of patients who present with exclusively extra-axial lesions (Baeuerle Radiology 2009).

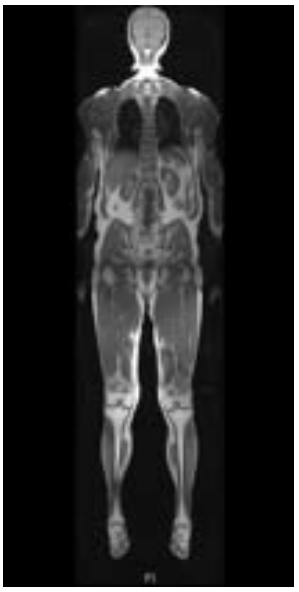

Figure 1 Composed T1-weighted Wb-MRI in coronal orientation

Comparison of wb-MRI derived classification according to the Durie/Salmon PLUS staging system with the conventional Durie/Salmon stage revealed a rather weak concordance of 45% of both systems. However, the meaning of these findings for the routine diagnostic work-up of patients with monoclonal plasma cell disease is still unclear (Fechtner Radiology 2010) Fig. 2. MRI detects diffuse involvement as well as focal lesions representing accumulations of myeloma cells w/o effects on the concerning mineralized bone. Both manifestations have been demonstrated to be of adverse prognostic significance. In a multivariate analysis of 149 patients with smoldering or indolent myeloma a number of more than one focal lesion detected by wb-MRI was the strongest adverse prognostic factor for progression free survival defined as transformation into symptomatic disease (Hillengass JCO 2010. Figure 3). The current guidelines of the international myeloma working group still recommend radiological skeletal survey as most common imaging method for routine work-up of patients with monoclonal plasma cell diseases mainly because it is widely available and most clinical trials are based on conventional x-ray findings (Dimopoulos Blood 2011; Durie Leukemia 2009). Comparison of different imaging methods revealed that wb-MRI is superior to radiological skeletal survey (Ghanem Eur Radiology 2006, Gleeson Skeletal Radiol 2008) and whole body computed tomography (wb-CT) (Baur-Melnyk AJR 2008) and equivalent or superior to positron emission tomography (PET) w/o CT (Shortt AJR 2009; Zamagni Haematologica 2007) for the detection of lesions caused by the accumulation myeloma cells not only in bone marrow but also in soft tissue.

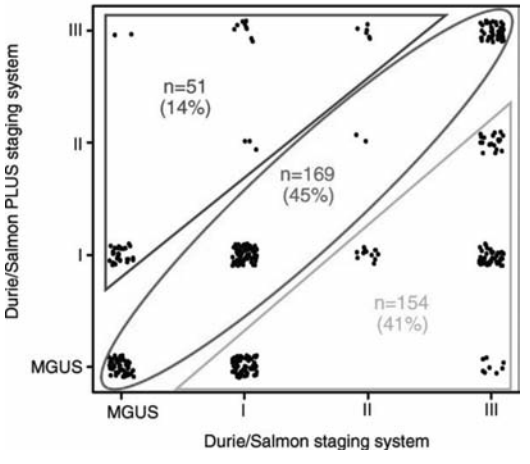

Figure 2. Comparison of Durie/Salmon and Durie/Salmon PLUS staging system in 404 patients. Fechtner K. et al. Radiology 2010.

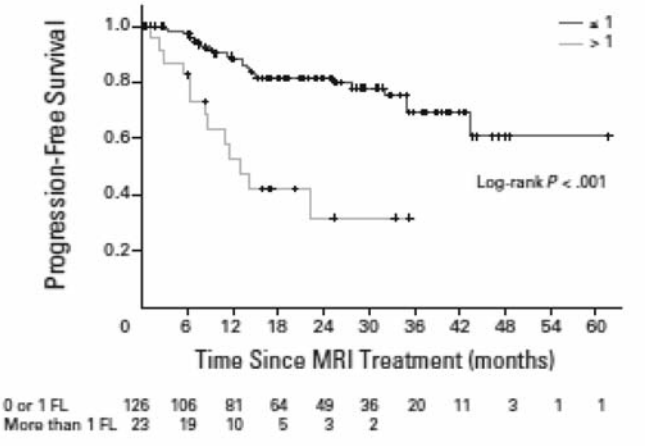

Figure 3. Prognostic significance of focal lesions in wb-MRI in patients with smoldering/ indolent multiple myeloma Hillengass J. et al. JCO 2010.

However, other studies demonstrated a superiority of CT and PET/CT over spinal MRI and in the assessment of response to treatment (Nanni Eur J Nucl Med Mol Imaging. 2006). Furthermore, the correlation of serological and MRI-derived response to systemic treatment was rather weak in 100 patients examined before and after therapy in our department (Hillengass Abstract 2977 ASH 2010). This may be at least in part be caused by the fact that focal lesions detected by MRI in many cases do not disappear completely but leave cystic spots which can not be differentiated from vital plasma cell tumors. In this case PET/CT delivers complementary information because the vitality of lesions can be detected (Zamagni Abstract 369 ASH 2010).

In conclusion, if available wb-MRI is a valuable method to assess tumor mass in patients with monoclonal plasma cell disease independent of the secretory activity. Plasma cell tumors in bone marrow can be detected before mineralized bone is affected. If the detection of changes in MRI should lead to initiation of systemic treatment is still a question to be addressed by further prospective studies. Especially in patients with asecretory myeloma or with soft tissue masses wb-MRI is a valuable tool superior to all other available imaging tools.

Accompanying morphologic wb-MRI the development of functional imaging sequences as dynamic contrast-enhanced MRI and diffusion-weigthed imaging is under way. These techniques allow to measure microcirculation and cellularity of bone marrow and may in future help to differentiate between vital myeloma lesions and cystic residua.

PAMIDRONATE: CAN THE DOSE BE LOWERED?

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Introduction. Prophylactic bisphposphonates have been standard therapy in multiple myeloma for the last three decades. Both clodronate and pamidronate were proved to decrease the number of skeletal related events (SRE) significantly compared to placebo.(1-3) Also a better survival was suggested in subgroups of patients.(4;5) When the more potent zoledronic acid was developed, it was proved to be as effective as the standard treatment with pamidronate 90 mg both given as monthly infusions in patients with multiple myeloma.(6;7) Zoledronic acid could be administered as 15 min infusion and was soon used worldwide instead of a 2-4 hour infusion of pamidronate. It was known, almost from the beginning of the treatment, that both pamidronate and zoledronic acid were nephrotoxic depending on the administered dose and the infusion rate and therefore the treatment had to be modified in many myeloma patients depending on their renal impairment. Eight years ago came the first reports on the bisphosphonate induced osteonecrosis of the jaw (ONJ)(8) and it became obvious that the risk increased with the more potent zoledronic acid and with the cumulative dose of both N-containing bisphosphonates (9). This lead to modification of the guidelines for the use of bisphosphonates in multiple myelom limiting the treatment to 2 years in newly diagnosed multiple myeloma (10;11). Therefore the question of 'what is the optimal dose of the bisphosphonates?' became an issue. Only few studies have addressed this question either by com-

paring different doses of a certain bisphosphonates (12;13) or by comparing bisphosphonates with different potency (7;12;14). In the Nordic group (NMSG) we conducted a study of PAM 30 mg versus 90 mg. *Dose-efficacy.* Berenson *et al.* compared monthly treatment of three dosis of zoledronic acid (0.4, 2, and 4 mg)(ZOL0.4, ZOL2, and ZOL4) with pamidronate 90 mg (PAM90) and showed no significant clinical difference on SRE or skeletal pain score between ZOL2 and ZOL4 and PAM90, but lower efficacy of ZOL 0.4 mg. Bone metabolic markers showed dose depending relation with the three ZOL doses. However, the number of patients in each group was relatively low (12). Rosen at al compared PAM90 with ZOL4 and zoledonic acid 8 mg, but had to modify the dose in the last group (ZOL4/8) in a large study of myeloma and breast cancer patients. They found no significant difference between the three groups for myeloma patients with respect to any SRE, pain score, ECOG performance status or survival (6;7). In the study of the Nordic myeloma study group Gimsing et al compared PAM90 with PAM30 (monthly infusions of pamidronate 30 mg) and found no significant difference in performance status (EORTC QLQ-C30), time to first SRE, progression free survival (PFS) or overall survival (OS) (13). In MRC Myeloma IX trial Morgan et al compared clodronate (1600 mg daily) with ZOL4 and showed recently significantly higher PFS and OS for the ZOL4 group (14) and for SRE though still not finally published. *Dose-toxicity.* More patients had increasing creatinine level in the ZOL4 and PAM90 compared to ZOL2 and ZOL0.4 but there was no significant difference in the renal serious adverse events (SAE) (12). In the study of Rosen *et al.* the zoledronic 8 mg treatment was to be stopped and the infusion time of zoledronic acid had to be prolonged from 5 to 15 min. due to concerns of the renal safety. After these modifications only ZOL4/8 had significantly increased nephrotoxicity compared to ZOL4 and PAM90 (6). Neither of these studies reported data on ONJ since this was not an issue at that time. In the Nordic study we found a trend towards more cases of ONJ in the PAM90 compared to PAM30 (8 vs. 2 cases) and more patients stopping treatment due to nephrotoxicity (15 vs. 7 cases) (13). In the MRC myeloma IX trial significantly more patients of ZOL4 had ONJ compared to clodronate treated patients (35 vs. 3 cases) while there was no significant difference in nephrotoxicity (14).

Conclusions. Monthly infusions of pamidronate 30 mg can be recommended as the effective and safe treatment dose of prophylactic pamidronate. The higher efficacy of monthly zoledronic acid 4 mg compared to daily oral clodronate also for survival raises the question whether this is due to the higher potency of zoledronic acid or the fact that zoldronic acid like pamidronate is a N-containing bisphosphonate that is known to have higher anti-myeloma effect in vitro than e.g. clodronate due to another mechanism of action. The final answer to this question demands a new study comparing PAM30 with ZOL4.

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EFFECT OF ZOLEDRONIC ACID (ZOL) VERSUS CLODRONATE (CLO) ON SKELETAL-RELATED EVENTS (SRES) IN PATIENTS (PTS) WITH MULTIPLE MYELOMA (MM) DURING INTENSIVE (INT), NON-INTENSIVE (NON-INT), AND THALIDOMIDE MAINTENANCE (TM) THERAPIES: MRC MYELOMA IX STUDY RESULTS

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The Medical Research Council (MRC) Myeloma IX Study examined the effects of ZOL vs CLO in pts with newly diagnosed MM assigned to antimyeloma therapy (Int or Non-Int, both followed by TM or no maintenance [NM] in eligible pts) and randomized to ZOL (n = 981; 4 mg intravenously every 21 to 28 days) or CLO (n = 979; 1600 mg orally every day). ZOL and CLO were continued and SRE data collected at least until disease progression. SREs included fractures, spinal cord compression, new osteolytic lesions, and radiation or surgery to bone. Time to first SRE was evaluated using a Cox model, and multiple event analyses used Andersen-Gill methodology. Among 1960 evaluable pts with 3.7-years' median follow-up, ZOL significantly prolonged survival (P = .012) and reduced the ongoing risk of SREs by 28% vs CLO (hazard ratio = 0.72; P < .0001). In both Int and Non-Int pathways, ZOL reduced the mean SREs/pt-year by 50% (0.4 vs 0.8 for CLO) and provided profound reductions in vertebral fracture rates. ZOL significantly increased time to first SRE vs CLO overall (P < .001), in Int (P = .003) and Non-Int pathways (P = .008), and with TM (P = .044) or NM (P = .003). SRE kinetics were similar when new osteolytic lesions were excluded as an SRE. Both bisphosphonates were generally well tolerated and had similar renal safety. Osteonecrosis of the jaw rates (ZOL, 3.6%; CLO, 0.3%) compare favorably with previous reports in MM. These analyses suggest that ZOL provides SRE benefits vs CLO regardless of myeloma treatment regimen.

ZOLEDRONIC ACID IN THE MANAGEMENT OF MULTIPLE MYELOMA: RESULTS FROM THE MRC MYELOMA IX STUDY

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Bisphosphonates (BPs) have proven efficacy in reducing the risk of skeletal-related events (SREs) and treatment of hypercalcaemia in multiple myeloma (MM). The nitrogen-containing bisphosphonates (N-BPs) in particular are potent inhibitors of osteoclastic bone resorption. There is increasing pre-clinical evidence that these agents have anti-cancer cell activity, decreasing proliferation, adhesion and angiogenesis and promoting apoptosis; one mechanism, peculiar to the N-BPs, being the blocking of prenylation of small signaling proteins. There has, as yet, been little good evidence of improvement in outcomes, particularly overall survival (OS) in MM. In a previous UK Medical Research Council (MRC) study, clodronate (CLO) was shown to slow progression of skeletal disease suggesting benefit from long-term treatment[1] and, in subgroup analysis, OS was significantly improved by CLO vs placebo in those patients who had not had fractures before study entry (N=153; p=0.006).[2] MRC Myeloma IX incorporated a comparison of oral CLO (the standard BP) with a N-BP with respect to occurrence of SREs, response and survival outcomes, selecting zoledronic acid (ZOL) because of its potent anti-resorptive activity and short infusion time.

Patients with newly diagnosed MM (NDMM) were allocated to one of two pathways – intensive which included HDM and autograft (ASCT) and non-intensive, determined by physical status and informed discussion rather than rigid age cut-off. In addition to induction chemotherapy randomizations (CTD v CVAD/CTDa v MP) there was randomization to maintenance thalidomide or not. All patients were eligible for upfront randomization to either 4 mg ZOL (15-min infusion) every 3-4 wks (4wkly post-induction) or 1600 mg CLO orally daily, to be continued at least until progression. This was an open-label study. Analysis was by intention to treat and between group differences were assessed using Cox proportional hazards and logistic regression models. Overall, ZOL significantly reduced the occurrence of SREs by 26% versus CLO (HR=0.74; p=0.0004). SRE reduction was shown to occur whether or not there were bone lesions at baseline (Fig 1). Furthermore, there appeared to be consistent reduction in the cumulative incidence of SREs in years 1 to 5 in patients receiving thalidomide, though numbers at risk were small beyond 2 years, supporting ongoing use to prevent SREs.

Across the study as a whole OS was significantly improved with ZOL compared with CLO during the first 4 months and during the full follow up period, reducing the mortality by 16% (HR=0.84; CI 0.74-0.96; p=0.0118) extending median survival by 5.5 months (50 vs 44.5 months; p=0.04) (Fig 2).[3] Further analysis has shown the OS benefit emerging during the first 4 months of therapy and apparently restricted to patients with bone disease at outset. For patients in the intensive and non-intensive pathways combined, more early deaths (disease or treatment-related) occurred in CLO patients than ZOL patients (p=0.0008). ZOL treatment was associated with significant improvement in PFS of 12% vs CLO (HR=0.88, 95% CI 0.80-0.98; p=0.0179 and increased median PFS of 2 months (19.5 vs 17.5 months; p-0.07). There were no significant interactions between the various regimens of BP plus induction chemotherapy for the differences in OS and PFS with ZOL vs CLO. The CR+VGPR rates were significantly higher for ZOL vs CLO in the non-intensive pathway (p=.018). The incidence of acute renal failure was low and similar in the ZOL and CLO groups in both pathways. Overall, thromboembolic events were more common in patients on ZOL than those on CLO, but rates were not significantly different in each pathway separately. Confirmed osteonecrosis of the jaw (ONJ) was uncommon, but higher with ZOL than CLO (4% vs <1%). In MRC Myeloma IX, ZOL has been shown to be superior to CLO across several endpoints. The improvement in survival, independent from the prevention of SREs, is entirely consistent with an important anti-myeloma cell effect. The very early emergence of OS benefit with ZOL vs CLO, supporting its use in the treatment of NDMM at the outset, also suggests possible synergy with induction chemotherapy. Generally, patients who received thalidomide and ZOL had better outcomes. If anything, there is a greater reduction in relative risk of SREs with ZOL vs CLO in the absence of bone lesions at presentation (HR = .53) than when bone lesions are present (HR=.77), supporting early initiation of ZOL in all patients with symptomatic NDMM for whom chemotherapy is being started. The continuing reduction in SRE risk in follow-up, including the maintenance phase, indicates that long term therapy with ZOL may be appropriate (perhaps with increased intervals between pulses of treatment). The detailed analysis of Myeloma IX data to coincide with the 6 year median follow-up may shed further light on this aspect. In additional studies and current analyses we are examining whether the effects of ZOL differ in delineated biological risk groups.

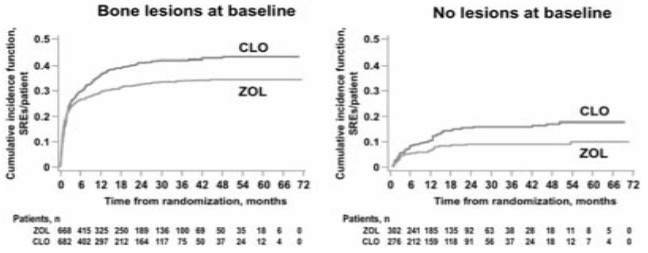

Fig. 1. Reductions in SRE incidence over time for ZOL versus CLO by presence or absence of bone lesions at baseline.

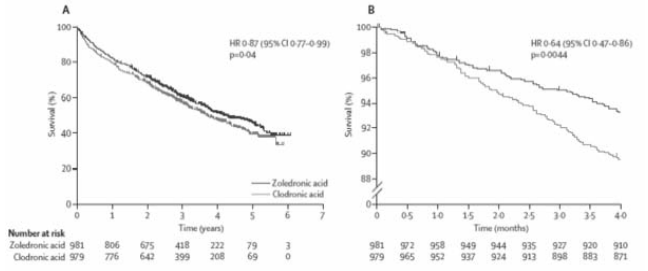

Fig. 2. Kaplan-Meier curves for patients randomized to ZOL and CLO for OS during the full follow-up period (A) and during the first 4 months of treatment (B). HR=hazard ratio.

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OSTEONECROSIS OF THE JAW

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Osteonecrosis of the jaw (ONJ) was first identified in 2003, a few years after the introduction of Pamidronate and Zoledronic acid for treatment of cancer associated bone disease [1]. It has been defined by the American Society for Bone and Mineral Research (ASBMR) [2] and the American Association of Oral and Maxillofacial Surgeons (AAOMS) [3] as a lesion of exposed bone in the maxilla or mandible that persists for 8 weeks, in patients treated with bisphosphonates not receiving radiotherapy to the craniofacial area. This definition excludes the recently defined stage 0 where there is clinical suspicion of ONJ, without clinical evidence of necrotic bone, with no specific clinical findings and symptoms. The lesion can be preceded or accompanied by pain, swelling of the mucosa, ulcer and loose teeth or can manifest as a non-healing ulcer after tooth extraction. Although the association between ONJ and bisphosphonates has been reported by several authors, the causality and the pathophisiology are still a matter of investigation. Several hypotheses have been proposed. Profound inhibition of bone remodelling induced by the potent nitrogen containing aminobisphosphonates, favoring the accumulation of microdamage in an area particularly prone to microtrauma secondary to mastication may be one such hypothesis. This hypothesis is supported by the observation that gene expression profiling of MM patients treated with BP with and without ONJ, showed downregulation of genes involved in both osteoclastogenesis and osteoblastogenesis and osteoblast function [4]. Alternative hypothesis consider the antiangiogenic properties of aminobisphosphonates [5] supported by

the recent description of cases of ONJ in patients receiving anti-antiangiogenic compounds. In vitro demonstration of toxic effects on epithelial cells by aminobisphosphonates advances the hypothesis that delayed healing of the mucosa after tooth extraction may play a role in the pathogenesis of this complication [6]. Others have looked at SNP arrays and polymorphisms to explain propensity to develop ONJ. Given that there is emerging data on patients developing ONJ with the use of other antiresorptives such as denosumab, the theory that this is a consequence of altered bone remodelling which is specific to the entire class of antiresorptive agents seems more likely. Importantly, recent reports of stress fractures in patients with prolonged amino-bisphosphonate exposure are likely another manifestation of the same pathophysiologic process. This has been demonstrated by us in our in vivo models studying bone mechanical strength [7]. The frequency of ONJ in the cancer population is estimated approximately between 0.8% to 12% [8,9] in retrospective studies, while the recent MRC IX prospective trial noted an incidence of 3.8% in the zoledronic acid treated patients [10]. The adverse event of ONJ needs further studying given improvements in myeloma patient survival and the potential to remain exposed to anti-resorptives for prolonged period. Here we will discuss staging, clinical presentation, risk factors, treatment and preventive strategies to minimize the adverse event of ONJ without compromising bone health.

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SHOULD WE USE MARKERS OF BONE REMODELING IN MYELOMA? WHICH ONE AND WHEN?

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Lytic bone disease is a frequent complication of multiple myeloma (MM). Lytic lesions rarely heal and X-rays are of limited value in monitoring bone destruction during anti-myeloma or anti-resorptive treatment. Biochemical markers of bone resorption [N- and C-terminal cross-linking telopeptide of type I collagen (NTX and CTX or ICTP, respectively] and bone formation [bone specific-alkaline phosphatase, osteocalcin and procollagen type I N- and C-propeptide (PINP, and PICP, respectively)] have been investigated as tools for evaluating the extent of bone disease, risk of skeletal morbidity, and response to anti-resorptive treatment in MM. *Bone Turnover Markers and Extent of Myeloma Bone Disease:* Comparison between bone resorption markers revealed that serum ICTP and urinary NTX better reflected the extent of myeloma bone disease and could better predict early progression of the bone disease after conventional chemotherapy (CC). However, serum ICTP remained more sensitive than the urinary assays when patients with impaired renal function are excluded from the analyses. Furthermore, serum ICTP was elevated in MM patients who did not have detectable osteolytic lesions by plain radiograph but had abnormal bone MRI scans, while high or intermediate urinary NTX correlated with an increased risk for skeletal-related events (SRESs) development compared with low NTX values. High NTX values also correlated with an increased risk for developing a first SRE. A recent study in 282 myeloma patients who participated in a randomized phase III study comparing zoledronic acid and pamidronate showed that high urinary NTX was independently associated with elevated risk for the development of first SRE (68% increase in risk for SRE development per 100-unit increase of NTX; p=0.005). Serum CTX has been also correlated with the extent of bone disease but larger studies will reveal its value in comparison to urinary NTX or serum ICTP. Markers of bone formation have produced variable results in the different studies and thus at present, their clinical utility is doubtful. *Correlations of Bone Turnover Markers with Myeloma Activity and Survival:* In several studies, biochemical markers of bone resorption strongly correlated with stage of MM. Serum ICTP and TRACP-5b and urinary NTX were higher in myeloma stage II/III than in stage I disease. Markers of bone remodeling have also correlated with well-characterized markers of disease activity, such as beta2-microglobulin and interleukin-6 and also with overall survival (OS). ICTP was an independent prognostic factor for OS in MM patients treated with CC, while incorporation of ICTP in the ISS separated four risk groups with a 5-year OS rate of 95, 65, 46 and 22%, respectively. Increased baseline levels of urinary NTX (≥50 nM BCE/mM creatinine) also correlated with an 88% increased risk of death in a randomized study with 282 patients. *Bone Markers during Anti-resorptive Therapy:* Biochemical markers of bone turnover have been used in MM both to monitor bisphosphonate treatment, and to determine which subjects will benefit most from bisphosphonate therapy. In a large, randomized study comparing 4 mg zoledronic acid with 90 mg pamidronate, given IV every 3 to 4 weeks, in patients with bone metastases from breast cancer or with MM osteolytic disease, urinary NTX was strongly suppressed (up to 64% below baseline in both treatment groups) for the duration of the study. Bone marker data from this and other bisphosphonate studies clearly demonstrate that there is a subset of myeloma patients who do not respond to, or who become refractory to bisphosphonate therapy. Patients with persistently elevated bone marker levels are at higher risk for SREs and disease progression compared with patients who respond to bisphosphonate therapy and have normalized bone resorption. In an important study, patients who had high baseline NTX levels (≥ 64 nM BCE/mM creatinine) and continued to have elevated NTX levels after 3 months of zoledronic acid therapy (n=26, 15%) had a significantly increased risk of developing a first SRE (RR=1.71; p=0.035) and shorter SRE-free survival (RR=1.65; p=0.039) compared to subjects who normalized NTX in response to bisphosphonate treatment (n=137, 81%). In this study, among patients with high NTX at baseline, 15% treated with zoledronic acid and 30% treated with pamidronate did not normalize NTX levels after 3 months of bisphosphonate therapy. Although unknown, one might speculate that patients who did not have biochemical improvement in their NTX levels may have an osteoclast-independent mechanism of bone resorption and might therefore benefit from additional therapies. Denosumab is a fully human monoclonal antibody against receptor activator of nuclear factor-kappaB ligand (RANKL), the most potent osteoclast activator to-date. In a recent study, 1776 adult patients with solid tumors or MM (n=10% of the total) who were naive to intravenous bisphosphonates were randomized to receive either subcutaneous denosumab 120 mg or intravenous zoledronic acid every 4 weeks. Denosumab produced similar results regarding the delay in time to first on-study SRE or subsequent SREs compared to zoledronic acid, while it also rapidly and potently reduced (by more than 80% within the first month) urinary NTX levels. *Novel Markers Regulating Osteoclast/Osteoblast Function:* Novel molecules that regulate osteoclast function (RANKL, osteoprotegerin, osteopontin, CCL-3), osteoblast function (dickkopf-1, sclerostin) or both (activin-A) have been measured in myeloma studies but their results are preliminary and their value has not been confirmed. *Effect of Novel Anti-Myeloma Agents on Bone Remodeling Markers:* The available data suggests that immunomodulatory drugs (thalidomide and lenalidomide) reduce osteoclast function but have little or no effect on osteoblast activity. Bortezomib reduces markers of bone resorption and increases markers of bone formation. Furthermore, bortezomib showed beneficial effects on bone formation in the clinical setting, increasing bone volume and bone mineral density at least in a subset of responding myeloma patients. *Conclusions & Future Directions:* Serum ICTP and urinary NTX seem to be more accurate than other bone resorption markers in reflecting both the severity

of bone destruction and the efficacy of response to bisphosphonate treatment. To date, data on CTX remains sparse, but studies are ongoing. There is also a strong correlation between serum ICTP and urinary NTX with increased risk for progressive bone disease, development of SREs and OS. Symptomatic patients who continue to have increased levels of NTX after 3 months of anti-myeloma and anti-resorptive therapy remain at high risk for both SREs and shortened OS; accordingly such patients may require more aggressive therapy. The value of bone markers in the setting of asymptomatic myeloma has also to be evaluated as it may reveal patients at high risk for progression. In the current era of concern about bisphosphonate-associated adverse side-effects (i.e. renal impairment, osteonecrosis of the jaw, subtrochanteric femoral fractures), bone turnover markers may be of particular use: i.e. low levels of serum ICTP/CTX or urinary NTX may provide impetus for deciding to lengthen bisphosphonate dosing regimens (i.e. changing from monthly to 3-month intervals). Such trials are urgently needed (and indeed should be highly encouraged) before final conclusions are made about the introduction of biochemical markers of bone remodeling into the routine clinical care of MM patients.

MYELOMA CAST NEPHROPATHY AND AL AMYLOIDOSIS

THERAPEUTIC ELIMINATION OF AMYLOID DEPOSITS: IS IT POSSIBLE?

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Although some monoclonal immunoglobulins, including free light chains, may sometimes have direct toxic effects on cell and organ functions, the important pathological changes responsible for clinical disease in systemic AL amyloidosis, and entirely responsible for disease is all other forms of systemic amyloidosis, are caused by the extracellular deposition of amyloid[1]. The protean clinical manifestations of amyloidosis, the need to think of the diagnosis and the requirement to conduct the appropriate diagnostic investigations, all result in the diagnosis usually being made late in the course of the disease. Substantial amyloid deposits which have already caused significant and often irreversible organ damage are generally present. The only therapeutic approach which is clinically effective comprises replacement of damaged organ function, by dialysis and/or transplantation, to sustain life, and measures to sharply reduce the abundance of the respective amyloid fibril precursor protein. If this can be achieved amyloid deposition can be arrested and in some cases the deposits then regress with clinical benefit. However the necessary chemotherapy, powerful anti-inflammatory treatment or organ transplantation may be slow to act and/or of limited effectiveness as well as being difficult, dangerous and expensive. There is thus an urgent need for new treatments which directly target amyloid deposits for safe elimination. Amyloid deposits are predominantly composed of amyloid fibrils which are very stable structures with a common cross core fold regardless of the type of fibril protein. Deposits are always rich in proteoglycans and glycosaminoglycans of the heparan and dermatan types, some of which are tightly associated with the fibrils and further stabilise them against proteolytic degradation by phagocytic cells. Macrophage action is almost certainly the only mechanism by which amyloid deposits can be cleared in vivo. All amyloid deposits of all types always contain the non-fibrillar normal plasma protein, serum amyloid P component (SAP), a member of the pentraxin family of proteins[2]. This trace plasma protein circulates in all subjects, including those with systemic amyloidosis, in the range of about 15-50 mg/l, and undergoes reversible but very avid calcium dependent specific binding to all types of amyloid fibrils. As a result it becomes highly concentrated in amyloid deposits so that there can be as much as 20,000 mg of SAP in the amyloid deposits of a patient with extensive systemic amyloidosis compared to the total of 50-100 mg which is normally present in the blood and extracellular fluid[3]. We have demonstrated that human SAP in amyloid deposits is identical to SAP in the circulation and is thus completely intact and not degraded[4], even though the half life of SAP in amyloid deposits, for example in the liver, is about 30 days compared to its half life in the plasma of about 24 h. Furthermore the binding of SAP to amyloid fibrils powerfully stabilises both components of the complex against proteolytic degradation by proteases or phagocytic cells in vitro, and presumably has the same effect in vivo[5]. In vitro amyloid fibrillogenesis is strongly enhanced by seeding with preformed fibrils or pre-fibrillar aggregates and SAP promotes this process. SAP is thus very likely to contribute to formation and persistence of amyloid in vivo. Indeed induction of systemic AA amyloidosis is retarded and reduced in mice with targeted deletion of the SAP gene[6]. Having validated SAP as a therapeutic target we developed a new chemical entity, the palindromic bis-D-proline compound, (R)-1-[6-[(R)-2-Carboxy-Pyrrolidin-1-yl]-6-oxo-Hexanoyl]Pyrrolidine-2-Carboxylic acid (CPHPC), intended to both block the binding of SAP to amyloid fibrils and to remove bound SAP from amyloid deposits in vivo[7]. We hoped that complete removal of all SAP from amyloid deposits would expose the fibrils to accelerated elimination by normal macrophage clearance activity. Surprisingly and unexpectedly CPHPC triggers rapid and almost complete depletion of all SAP from the circulation and extracellular fluid, which persists for as long as the drug is given[7]. SAP binds CPHPC in stable complexes containing two SAP molecules cross linked by 5 CPHPC molecules and these assemblies are instantly cleared and catabolised by the liver[7,8]. The depletion of plasma SAP also clears most of the SAP from amyloid deposits but the affinity of SAP for CPHPC is insufficient to produce complete dissociation of all SAP from amyloid deposits in the face of the continuous production of 50-100 mg of new SAP per day and the avid binding of SAP to the solid phase ligands provided by amyloid fibrils[9]. CPHPC is well

tolerated by amyloidosis patients and has been administered to more than 60 subjects for a total of more than 50 patient years without any adverse effects[8,9] (and unpublished). But up to about 10% of the amyloid associated SAP remains in major visceral amyloid deposits even after months of continuous CPHPC treatment[9]. Nevertheless we have not observed any new amyloid accumulation in patients on CPHPC, even those in whom there was progressive deposition before and after CPHPC exposure[9]. Furthermore there were encouraging signs of prolonged renal and possibly patient survival in subjects receiving CPHPC[9]. However we have not detected any amyloid regression[9]. Thus while CPHPC may be a useful adjunct to other therapy for amyloidosis it does not itself promote elimination of the deposits. The capacity of CPHPC to clear essentially all SAP from the circulation while leaving significant amounts of SAP specifically bound in the amyloid deposits, suggested the possibility of using antibodies to SAP to target amyloid for destruction via the residual SAP in the tissues. We explored this avenue in the mouse model of systemic AA amyloidosis which very closely resembles the corresponding human disease. The mouse amyloid deposits were loaded with human SAP either by using human SAP transgenic mice and clearing their circulating SAP with CPHPC, or by simply injecting human SAP into wild type AA amyloidotic animals. Administration of IgG anti-human SAP antibodies, once the circulating human SAP had cleared, was tolerated with no clinical or other adverse effects[10]. Within one day after antibody treatment the deposits were massively invaded by macrophages which proceeded to surround, ingest and destroy the amyloid deposits, in the process fusing to form multinucleate giant cells[10]. Almost all the amyloid in liver and spleen was destroyed by 10-14 days and at 28 days after a single antibody does virtually no amyloid or inflammatory cells were detectable and histological appearances were normal[10]. The process does not require the IgG Fc region but is complement dependent and absolutely macrophage dependent[10].

This approach should be applicable to all forms of human amyloidosis although its safety and efficacy, especially for amyloid in different organs and tissues will have to be carefully evaluated. GlaxoSmithKline have licensed the invention and have fully humanised one of our optimal mouse monoclonal anti-human SAP antibodies. We are currently working together towards early clinical testing.

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MANAGEMENT OF AL AMYLOIDOSIS IN 2011

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Systemic immunoglobulin light chain (AL) amyloidosis is caused by misfolded monoclonal light chains which deposit in target organs as fibrillar aggregates causing progressive organ dysfunction. Differently from multiple myeloma, in AL amyloidosis the clinical picture and prognosis are determined by the organ dysfunction caused by the amyloidogenic light chain, posing unique challenges to the treatment and follow up of patients with this disease. On the one hand, multiorgan failure makes these patients particularly susceptible to treatment toxicity. Yet, on the other hand, reductions in the concentration of the circulating free light chain (FLC) can rapidly result in marked clinical improvement and prolonged survival. The therapy of AL amyloidosis is directed to recovering the function of the target organs through the fastest and deepest reduction of the concentration of the misfolded amyloidogenic light chains, minimizing treatment toxicity and supporting the function of the damaged organs. A timely and correct diagnosis is the key to effective therapy. Early diagnosis is essential since it allows a broader range of therapeutic options and the potential recovery of the damaged target organs. Any patient who presents with a clinical syndrome consistent with AL amyloidosis should undergo a biopsy to detect amyloid deposits. If the patient has one of the clinical amyloidosis syndromes with a monoclonal gammopathy, it is important to exclude the possibility of senile systemic amyloidosis, particularly in older men with isolated cardiac involvement, and of reactive or familial amyloidosis with an incidental MGUS. It is essential to unequivocally identify the protein responsible for the disease before embarking on therapy. Mass spectrometry can confirm the amyloid protein composition and will likely become the gold standard for identifying the protein forming amyloid deposits. The major determinant of outcome in amyloidosis is the extent of cardiac involvement. Cardiac imaging techniques, echocardiography and magnetic resonance, have been successfully used for the diagnosis and prognosis of amyloid cardiomyopathy. Cardiac biomarkers provide a quantitative assessment of cardiac damage (troponin I or T) and cardiomyocyte stress (BNP, NT-proBNP), and are the most important predictors of outcome in amyloidosis. These two biomarkers are at the basis of a staging system that is now used to stratify patients who are registering for clinical trials. The use of cardiac biomarkers has been validated, and many other prognostic factors reflecting burden of disease and organ dysfunction have been recently proposed. The consensus criteria for hematologic and organ response have been recently updated at the 12th International Symposium on Amyloidosis.[1] Achieving a hematologic response translates into improved overall survival. Although partial responses can be beneficial, it appears that significant reductions in free light chain levels are associated with the best clinical responses. Several active regimens are now available for the treatment of AL amyloidosis, including high-dose dexamethasone-based regimens combined with melphalan (MDex), thalidomide (ThalDex), and cyclophosphamide-thalidomide (CTD), high-dose melphalan followed by rescue with autologous stem cell transplantation (SCT), and the new agents, lenalidomide (Len), bortezomib (Bor) and pomalidomide. More recently, the combination of new agents with melphalan- or cyclophosphamide-based regimens (LenMDex, LenCDex, BorMDex, CyBorD) are being tested in several trials. A multinational phase III study comparing MDex to BorMDex has just started. MDex and SCT are the two most widely used regimens. The French Myeloma Collaborative Group compared these two regimens in a randomized trial and found no significant differences for hematologic or organ responses. In a recent update, with a longer follow-up, the authors did not find any superiority in the intensive (SCT) arm in survival or remission duration even in the landmark analysis eliminating treatment related mortality.[2] Treatment for AL amyloidosis is highly individualized and is based on age, organ dysfunction, and regimen toxicities. The choice of novel agent depends on organ function and pace of disease. Close monitoring of clonal response, evaluated by FLC assay, and of cardiac response, evaluated by NT-proBNP or BNP, should guide regimen changes and duration of therapy. Whenever possible, patients should be treated within controlled clinical trials. Although important therapeutic advances have been made in recent years, a significant proportion of patients die within six months from diagnosis (Figure 1) and this has remained unchanged over the past 25 years. Most of these patients present with advanced cardiac disease and their treatment is an unsolved challenge. These patients may not tolerate high-dose corticosteroids or multidrug regimens. If they have isolated cardiac disease, orthotopic heart transplantation should be considered, followed by chemotherapy to prevent amyloid deposition in the transplanted heart. If the patient is not a transplant candidate, a low-dose regimen (for instance, low-dose BorMDex), should be considered. Better understanding of the biology of the amyloidogenic plasma cell clone and of the molecular mechanisms underlying the light chain misfolding, tissue targeting and toxicity will define disease-related prognostic criteria and lead to a true risk-adapted therapeutic strategy. Furthermore, advances in the understanding of the molecular events involved in amyloid formation and tissue damage have revealed several new drug targets and therapeutic approaches including innovative options to enhance clearance of amyloid deposits. These novel therapeutic opportunities should raise the clinician's awareness of AL amyloidosis, in order to make a diagnosis in the early stages, when full recovery of vital organ function can still be achieved through a concerted therapeutic approach.

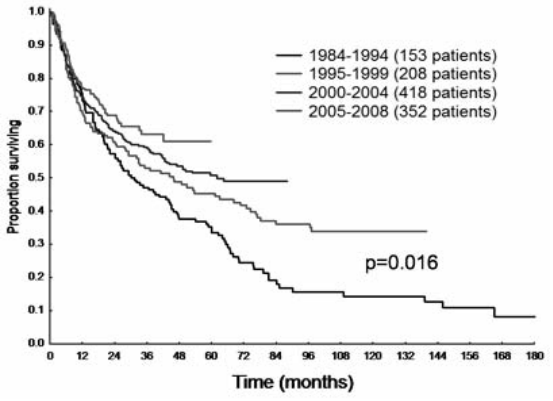

Figure 1. Survival of patients with AL amyloidosis according to the year of diagnosis.

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MYELOMA CAST NEPHROPATHY

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Renal insufficiency (RI) is a severe and frequent complication in multiple myeloma (MM), that occurs in nearly half of the patients during the course of the disease. At diagnosis, 20 to 40% of patients have evidence of RI, 10% of whom requiring dialysis. In most cases, RI results from myeloma cast nephropathy (MCN), characterized by cast formation in the renal distal tubule lumen, secondary to the interaction of monoclonal immunoglobulin (Ig) light chains (LCs) with Tamm-Horsfall protein. Ig LCs, which are freely filtered through the glomerulus, are physiologically reabsorbed in the proximal tubular (PT) cells, through a mechanism of endocytosis mediated by the tandem receptors cubilin and megalin, and then degraded in the lysosomal compartment of the PT cell. Therefore, MCN is nearly always observed in the context of high-grade MM secreting large amounts of free LCs, exceeding the capacity of PT reabsorption and catabolism. RI in MCN results from both tubular obstruction by LC casts and severe tubulo-interstitial inflammation, characterized by infiltrates of mononuclear cells and giant cell reaction around casts. Tubulo-interstitial inflammation is triggered by massive PT reabsorption of LCs, leading to activation of redox pathways, mitogen activated protein kinases and NF-κB, leading to production of pro-inflammatory cytokines such as TNF-α, interleukin 6 and 8, and MCP-1. Cellular injury is accompanied by morphologic and functional alterations of PT epithelium, including epithelial-mesenchymal transition. Tubulo-interstitial fibrosis rapidly develops in the absence of rapid reduction of circulating free LCs. If the amount of urine LC excretion influences the risk of RI, which is high when LCs proteinuria is above 2 g/day, MCN is usually triggered by factors that modify renal perfusion, including dehydration, hypercalcemia, infections, contrast media, or nephrotoxic drugs (non-steroidal anti-inflammatory agents, diuretics, angiotensin conversing-enzyme inhibitors). MCN usually presents as isolated acute RI, that often reveals the underlying MM. Diagnosis relies primarily on urine electrophoretic analysis, typically showing predominant LC proteinuria. In some cases, kidney biopsy is required to confirm diagnosis or exclude another LC-related renal disease. Renal prognosis of MCN is poor, as recovery of renal function occurs in 50 to 60% of patients, and in only 20 to 30% of those who require dialysis support. Moreover, persistence of RI in MM significantly reduces patient survival. Avoiding delays in the diagnostic assessment and initiation of disease specific treatment is therefore essential to improve outcomes. RI imparts a significant negative effect on the overall survival of patients with MM. Even small changes in renal function can have large effects on survival. Patients who are dialysis dependent have the worst fate. While RI is generally viewed as a marker of disease severity, several studies have suggested that reversal of RI can restore the normal life expectancy of these patients. Therefore, it is important that patients with RI be appropriate treated to insure the best outcome. In an effort to standardize the renal responses for clinical studies, the International Myeloma Working Group recently published its Consensus Statement which includes response criteria differentiated in complete response (CRenal), partial response (PRenal) and minimal response (MRenal). In the past, the secreted monoclonal protein (M-protein) has been the target of therapy for MM-related renal failure. However, since whole Igs are not involved in the pathogenesis of MCN, M-protein may not be the best target. A more appropriate target is the serum free LC level (sFLC) since filtered LCs are the quintessential component of MCN. Recent studies suggest a relationship exist between sFLC and risk of MCN. In addition, 2 separate studies have now shown that a minimum of 50% reduction in sFLC is necessary for recovery of renal function in patients with biopsy proven MCN. Finally, it is important that this reduction is achieved early in the patient's management, those patients with only moderate sFLC reduction at day 12 were dialysis dependent for significantly longer than those with a significant early reduction. Overall, to enable renal recovery in 80% of the population a reduction of 60% in sFLC is required by day 21. Aside from supportive treatment, chemotherapy should be initiated as soon as possible. The lack of nephrotoxicity or need for dose adjustment even with dialysis patients makes bortezomib a good front line agent in these patients. Data from CREST and SUMMIT showed bortezomib was effective in patients with advanced RI and had similar rates of adverse events as patients with normal renal function. Bortezomib also has quick time to response. In VISTA, patients with advanced RI (<30 ml/min) who received bortezomib melphalan and prednisone (VMP) were much more likely to have a hematologic (37% vs 13%) and renal response (37% vs 7%) than patients treated with MP. Bortezomib may have an advantage over other agents because of its ability to directly inhibit MCP-1 which is responsible for the inflammatory reaction that leads to the irreversible chronic tubulointerstitial fibrosis. Corticosteroids have similar properties and a retrospective review from a single institution demonstrated reversal of RI in 73% of their patients treated with high dose dexamethasone with novel agents. A subgroup analysis of 2 phase 3 trials (MM-009 and MM010) using lenalidomide and dexamethasone found 72% of the patients with RI had at least 1 level of improvement in renal function according to chronic kidney disease stages. However, the serum creatinine cutoff for these two studies was 2.5 mg/dl and patients with RI will require dose reduction with lenalidomide. To enable a rapid sustained reduction in sFLC, we must think beyond simple switching off the high rate of production by the plasma cell clone and consider how free LCs are removed from the serum. In the setting of normal renal function or moderate RI the Ig LCs will be rapidly cleared from the circulation by glomerular filtration. However, in the context of severe RI the serum half-lives of LCs are increased substantially, from 3-6 hours to 2-3 days. When this reduced renal clearance is combined with a slow tumour response to some chemotherapy regimens, high sFLC can result for considerable periods after treatment has commenced. This in turn causes ongoing tubular injury and a progressive fibrosis. To help break this cycle, Ig LCs can be directly removed from the circulation by either plasma exchange (Plex) or high cut-off haemodialysis (HCO-HD). Plasma exchange has been used in this setting for 30 years and is very effective at clearing the intravascular compartment. But the distribution of Ig LCs is predominately extravascular and time for redistribution of LCs from extra- to intra-vascular compartments is required. Therefore with a short duration treatment such as Plex the total body clearance of LCs may actually be quite small. This may explain why the 3 randomised controlled trials of Plex in this setting have failed to show a consistent benefit for the procedure. To safely allow extended removal of LCs from the serum, HCO-HD can be considered. These new dialysis membranes allow effective removal of all middle molecules including LCs by haemodialysis and hemofiltration. Early pilot studies have shown that in a population of patients with biopsy proven MCN, when LC removal by HCO-HD is combined with effective chemotherapy there is a sustained reduction in sFLC. This was associated with improved renal recovery and patient survival. Two randomised controlled trials are now further evaluating this new treatment option: EuLITE (UK + Germany) and MYRE (France).

NEW DRUGS AND THERAPEUTIC APPROACHES

PRE-CLINICAL EVALUATION AND NOVEL THERAPEUTICS

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The introduction of novel therapeutics has seen an improvement in the median duration of survival for those multiple myeloma (MM) patients with access to these agents. Furthermore, the achievement of complete remission (CR) with first line therapy now represents a not unexpected goal in newly presenting patients, however, no evidence yet exists that MM is curable with presently available chemotherapeutics. The goal of our work is to try to improve the rationality of clinical therapeutic evaluation via 1. improved pre-clinical testing of potential therapeutics, 2. the identification of new therapeutic targets, and 3. the optimisation of clinical testing of potential therapeutics. Examples of these strategies are described. ***Improved pre-clinical testing of potential therapeutics.*** Unfortunately, pre-clinical compound evaluation strategies have frequently demonstrated disappointing translation into the clinical setting. The mainstay of pre-clinical therapeutic testing has involved the use of immortalized human myeloma cell lines (HMCL). While gene expression profiling (GEP) studies have suggested that HMCL are representative of primary MM and that the predominantly CD45 positive sub-set of HMCL that retain some degree of IL-6 dependency may be more so, the extra-medullary growth capacity of HMCL clearly demonstrates their limited relevance to primary disease. With clear recognition of this limitation we and other investigators have explored the use of HMCL co-culture systems in an attempt to recapitulate the primary disease and have incorporated the use of reproducible primary MM cell assays for drug testing. Using co-culture of HMCL and the stromal cell line HS-5 we can reproducibly induce MM drug resistance and clearly demonstrate activation of critical proliferation and survival signaling pathways. In this context we have evaluated the JAK inhibitor CYT387. CYT387 significantly abrogates co-culture induced STAT3 phosphorylation inducing both apoptosis and cell cycle arrest of HMCL. Importantly CYT387 consistently synergized with melphalan in inducing MM cell death when tested at low micro-molar concentrations against both HMCL and primary MM tumour cells. ***The identification of potential therapeutic targets.*** The recognition of new potentially targetable cellular proteins remains an ongoing challenge. Several investigators have evaluated pre-clinically and in early phase clinical trials a variety of small molecule inhibitors of Heat Shock Protein 90 (HSP90). Our investigations with a potent orally bioavailable HSP90 inhibitor have demonstrated the putatively beneficial anti-tumour effects of HSP90 inhibition, including significant reduction in AKT activation, subsequent reduced AKT protein expression and cellular mislocalistion of MEK. As would be expected coincident compensatory overexpression of the HSP90 chaperones HSP70 and HSP27 was observed. HSP27, a member of the family of small HSPs is ATP-independent, has anti-apoptotic capacity and has been implicated in oncogenesis. Evaluation of primary MM cells demonstrated an elevated level of expression of HSP27 when compared to normal plasma cells and a greater than 5-fold increased level of expression in CD45 negative/IL-6 independent HMCL when compared to CD45 positive/IL-6 dependent HMCL. Moreover, IL-6 withdrawal resulted in a rapid increase in HSP27 expression in CD45 negative HMCL that returned to baseline upon IL-6 addition. Based on these preliminary data we hypothesise that HSP27 may function in a pro-survival role in MM and thus represent a potential novel therapeutic target. ***Optimisation of clinical testing of potential therapeutics.*** De-acetylase inhibitors (DACi) represent a novel class of anti-cancer agents presently being evaluated in MM. The rationale for their use in MM is largely extrapolated from other malignancies but this fact notwithstanding pre-clinical data has demonstrated significant activity against HMCL. Putative mechanisms of action include the re-expression of previously silenced genes via histone de-acetylation and the functional modulation of oncogenic cytoplasmic proteins. DACi may be active against nuclear [Class 1] and/or cytoplasmic [Class IIB] de-acetylases. We hypothesized that a sub-set of patients may be more responsive to particular DACi and strategies facilitating the identification of these patients would enable more rational evaluation of DACi in clinical trials. Our preliminary investigations have revealed over-expression of Class I but not Class IIB DAC in primary MM cells when compared to normal plasma cells. Consistent with this, evaluation of a panel of DACi with Class I and/or Class IIB inhibitory activity against a range of primary MM cells while demonstrating marked inter-patient variation in cell killing also revealed that in all cases maximal killing occurred with Class I and not Class IIB inhibition. Finally, GEP of HMCL resistant or sensitive to DACi has revealed only a limited set of differentially expressed genes. Validation of these data in vitro and in the context of a soon to be commenced Phase II clinical trial are pending.

THE EMERGING ROLE OF SECOND GENERATION PROTEASOME INHIBITORS, INCLUDING CARFILZOMIB AND OTHER AGENTS: CLINICAL DATA

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Proteasome inhibition has been validated as a highly effective strategy in the treatment of multiple myeloma (MM). Bortezomib (Btz), a small molecule boronate peptide and the first-in-class, reversible proteasome inhibitor (PI) to have been approved, has shown substantial clinical activity both as a single agent and in combination, with dramatic improvements seen in survival for MM patients with its use both as initial therapy and in relapse. However, the development of peripheral neuropathy (PN) and other dose-limiting toxicities (DLTs) can limit its extended use at full dose and thus curtail its potential effectiveness. Moreover, resistance to Btz-based therapy can emerge over time. To further improve treatment outcomes in MM, a second generation of proteasome inhibitors, including carfilzomib (PR-171), salinosporamide (NPI-0052), MLN9708, CEP18770, and ONX 0912 have entered clinical trials with the intent of reducing side effects and overcoming resistance. Studies with carfilzomib (Cfz), a selective and irreversible PI, exhibiting activity via the epoxyketone moiety, are the most advanced among the new PIs currently under development. Initial phase I evaluation established a dosing regimen of Cfz at 20 mg/m^2 for 2 consecutive days weekly for 3 weeks in 4 week cycles. Based on favorable tolerability and activity at this dose, the phase II 003 trial in relapsed and refractory MM was expanded to a 003-A1 pivotal trial1 with dose-escalation of Cfz from 20 mg/m^2 in the first cycle to 27 mg/m^2 in the second and subsequent cycles. The study enrolled 266 patients with advanced, progressive MM, which was refractory to last therapy. Patients had at least 2 prior lines of treatment, which must have included Btz and either thalidomide or lenalidomide (Len). Median time since diagnosis was 5.4 years, and all but 1 patient received prior Btz. Nearly half (44%) of all patients were refractory to Btz as part of the last line of therapy, and overall 88% were either refractory or intolerant to Btz. In this heavily pre-treated patient population, Cfz showed promising activity with 24% of patients achieving PR or better, 34% MR or better, and 69% at least SD. Response rates in patients refractory to Btz in the last prior therapy versus any prior therapy were comparable (≥MR 31% vs 28%, respectively). The DOR was 8.3 months for all patients which was identical for patients with ≥PR or ≥MR, and comparable for patients with disease refractory to Btz as part of last therapy (8.4 months). PFS for all patients was 3.7 months, but was more favorable for patients with MR (8.1 months), PR (8.8 months), and VGPR (11.6 months). Median OS was also encouraging at 15.5 months. Overall, Cfz was well-tolerated. Of importance, Grade 3/4 neutropenia was observed in only 10% of patients and the incidence of PN was low (overall 12%, ≥ Grade 3 1 %). Fatigue and thrombocytopenia were seen, as was transient elevation in creatinine, which proved manageable with the introduction of low dose dexamethasone as premedication and hydration as part of supportive care. The results from phase II 004 trial, which enrolled less heavily pre-treated patients with relapsed and refractory MM after 1–3 prior lines of therapy, provide additional evidence of activity.[2] Of particular interest, a cohort of 66 Btz-naïve patients achieved an overall response rate (≥PR) of 54%, including 29% of patients with responses ≥VGPR. The median DOR for this cohort had not been reached at the time of preparation of this abstract. Interestingly, there was a trend towards increased depth and duration of responses with the higher doses of Cfz. As in the 003 study, Cfz was generally well-tolerated and PN was limited. Importantly, Cfz, like Btz, appears to overcome the adverse prognosis of unfavorable cytogenetics, defined as the presence of del13, del17p13, t(4;14) or t(14;16).[2,3]

Carfilzomib combinations are now in the early phase of clinical development. At the time of this review, initial results are available for the CRd combination (Cfz + Len + Dex). In the phase I portion of the CRd study in patients with relapsed and refractory MM following 1–3 prior lines of prior therapy, the overall response rate (>PR) was 78% including a VGPR rate of 40%.[4] The regimen was well tolerated, with no DLTs seen up to the maximum planned dose levels of Cfz 20/27 mg/m^2, Len 25 mg per dose, and Dex 40 mg, allowing prolonged administration for up to 2 years. A more recent phase 1/2 study of CRd in the treatment of frontline MM enrolled 31 patients, including both transplant and non-trans-

plant candidates, requiring initial treatment.[5] Transplant candidates underwent stem cell collection after 4 cycles of CRd but stem cell transplant could be deferred with treatment continuing for a total of 8 cycles followed by CRd as maintenance therapy. Toxicities to date have been manageable, with the MTD not yet reached and patients now at the highest planned dose level of Cfz 36 mg/m^2, Len 25 mg, and Dex 40 mg. After a median of 6 cycles (range 1–13), the best response rates include >PR 96%, >VGPR 70%, CR/nCR 55%. Responses occurred rapidly, with all but 1 patient achieving PR after 2 cycles. Depth of response also appears rapid and further improves with the increasing duration of treatment. CRd maintenance also appears feasible, without emergence of significant PN or myelosuppression reported. Encouragingly, all patients are alive and none have shown evidence of disease progression. Although still early, these results compare favorably to the most effective regimens for newly diagnosed MM, such as RVD. The results of both CRd studies provide additional support to the recently initiated phase III ASPIRE trial of CRd vs Rd in relapsed MM. Clinical studies with other proteasome inhibitors are in early phase. NPI-0052 is a non-peptide-based natural product targeting all 3 active sites of the proteasome. Initial evaluations adopted a convenient weekly dosing schedule (D1, 8 and 15 every 4 weeks). Preliminary results from an ongoing phase I study using this schedule in relapsed and refractory MM, including a significant proportion of Btz-refractory patients, show paraprotein response and prolonged SD, even at low doses.[6] Also, inhibition of proteasome activity equals or exceeds that obtained with efficacious doses of Btz, without producing significant AEs including PN, although renal toxicity is a potential concern. Enrollment for a twice weekly dosing schedule is currently underway, with paraprotein responses described. Similarly, studies with MLN9708, an orally bioavailable, reversible boronic acid-based PI and CEP-18770, another boronic acid-based inhibitor, are actively enrolling, with encouraging initial results reported. In single-agent studies of MLN9708 in patients with relapsed and refractory MM, paraprotein responses have been seen and toxicities have proven manageable, with no significant PN reported to date. Combination studies with Len and Dex in the upfront setting are now underway. With the same goal of oral therapy, ONX 0912 is a potent, irreversible, orally bioavailable, peptide epoxyketone PI and a structural analog of Cfz. It is currently undergoing clinical evaluation in a phase I trial in patients with advanced solid tumors with plans for studies in MM. In summary, the results from these early studies with novel, second generation PIs, are encouraging and build on the success seen with Btz. It appears that particular PIs may have unique features, distinguishing them from other agents in this same broad class, including overcoming resistance to Btz and having different toxicity profiles. Second generation PIs therefore have the promise of providing important additional therapeutic options for the treatment of MM.

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CURRENT STATUS OF POMALIDOMIDE IN MYELOMA

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Pomalidomide is the newest immunomodulatory drug. Phase I trials in relapsed myeloma established pomalidomide as being well tolerated in doses ranging from 1-5 mg/day with response rates (>PR) ranging from 25- 50% (1-3) depending on how heavily pre-treated was the population of patients. The first phase II study using pomalidomide and dexamethasone (Pom-Dex) for myeloma was conducted in patients with relapsed disease after 1-3 prior regimens4. Pomalidomide was given orally at 2 mg/day, continuously, along with dexamethasone (40 mg) given weekly. The study included 60 patients and showed an overall response rate (≥PR) of 63%, including 33% who achieved VGPR or CR (Table 1). Additionally 82% had at least a 25% decrease in their M-spike. Importantly, the study showed responses among 40% of patients who were lenalidomide refractory, suggesting non-cross resistance with other IMiDs. Responses were seen in 74% of patients with high risk cytogenetic or molecular markers. The median progression free survival was 11.6 months and was not significantly different in the patients with high risk disease compared to those with standard risk disease. Long-term follow-up of this cohort indicates the median duration of response was 21.3 months. Two year survival was 76%. Follow up trials have focused on developing pomalidomide as salvage therapy for patients with relapsed myeloma that is refractory to novel agents. A cohort of lenalidomide-refractory patients5 was treated with responses of ≥PR in 31% of patients and response duration was 9.1 months. The median overall survival was 13.9 months. The MM-002 phase I/II study3 included patients who had previously been treated with both bortezomib and lenalidomide and were refractory to their most recent regimen. Responses of PR or better were seen in 25%. The IFM 2009-02 trial6 included myeloma patients who were symptomatic and progressing following at least two cycles of lenalidomide and bortezomib (either separately or in combination). Pomalidomide was given orally either at 4 mg/day on days 1–21 of each 28-days (arm A) or continuously on days 1–28 of each 28-day cycle (arm B). Dexamethasone was given orally at 40 mg daily on days 1, 8, 15 and 22 of each cycle. Among 92 patients enrolled, responses of PR or better were seen in 42% (Arm A) and 39% (Arm B).

Table 1. Pomalidomide use in myeloma.

	Regimen	#prior regimens, median	Schema	Doses	≥ PR	PFS/DOR/OS, months
Phase I trials						
Sehey et al. JCO 2004	Pom	3	28/28	MTD 2 mg	54%	9.7/-/22.5
Streetly et al. BJH 2008	Pom	4	28/28	MTD 5 mg QOD	50%	10.5/-/33
Richardson et al. ASH 2010	Pom±Dex	6	21/28	MTD 4 mg	25%	5/5/20
Phase II trials						
Lacy et al. JCO 2009	Pom/dex	2	28/28	2 mg	63%	11.6/21.3/76% at 2 yrs
Lacy et al. Leuk 2010*	Pom/Dex	4	28/28	2 mg	32%	9.1/4.8/13.9
Leleu et al. ASH 2010	Pom/Dex	4	21/28	4 mg	42%	7.3/4/88% at 4 m
Leleu et al. ASH 2010	Pom/Dex	4	28/28	4 mg	39%	5/4/85% at 5 m
Richardson et al. ASH 2010	Pom±Dex	5	21/28	4 mg	25%	NA
Lacy et al. ASH 2010**	Pom/dex	6	28/28	2 mg	26%	6.5/12/78% at 6 m
Lacy et al. ASH 2010**	Pom/dex	6	28/28	4 mg	26%	3.3/NA/69% at 6 m

*Lenalidomide; **Lenalidomide and bortezomib refractory.

Mayo Clinic investigators recently reported results of pomalidomide therapy comparing two different dosing strategies in sequential Phase II trials for patients with relapsed myeloma that was refractory to both lenalidomide and bortezomib7. Pomalidomide was given orally 2 mg/day or 4 mg/day, on days 1–28 of a 28-day cycle, with dexamethasone given 40 mg daily on days 1, 8, 15 and 22. Responses of PR or better were seen in 26% in both cohorts. The 2 mg cohort showed responses of minor response (MR) or better in 49% versus 40% in the 4 mg cohort. The median duration of response in the 2 mg cohort was 12 months. Toxicity consisted primarily of neutropenia with grade 3 or 4 neutropenia seen in 49% of the patients treated with 2 mg daily and 66% of those treated with 4 mg daily. This data suggests that there is not a dose response for pomalidomide and that there is no distinct advantage for 4 mg over the 2 mg per day dose. The data presented here again confirms remarkable activity of the Pom/dex regimen. In myeloma, pomalidomide appears to overcome resistance to both lenalidomide and bortezomib.

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DEACETYLASE INHIBITORS IN MULTIPLE MYELOMA

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Despite the improvement observed in the patients' outcome in the last years, MM remains incurable and novel therapeutic options are still necessary for relapsed or refractory patients. In this regard, several drugs, such as DAC inhibitors, that target specific mechanisms of the tumor cells are currently being explored. Deacetylases (DACs) are enzymes specialized in removing acetyl groups from their client proteins, which can be both, histone- and non-histone proteins such as α-tubulin, p53, p73, retinoblastome, several steroid receptors, E2F family members, Bcl-6, Hsp90, or HIF-1α among others. The rationale for using these drugs in MM relies in the fact that there is a general pattern of deacetylation in neoplastic cells that could be reverted with the use of DAC inhibitors. Moreover, several of these non-histone proteins play an important role in the pathogenesis of MM. This is the case of p53, an important tumor suppressor gene for all malignancies, but specifically for multiple myeloma. Another mechanism potentially targeted by DAC inhibitors is the unfolded protein response: DACs have an important role in this pathway at least through two different mechanisms: the deacetylation of HSP-90 that allows its correct functioning and the formation of aggresomes by DAC6. The inhibition if both mechanisms with DAC inhibitors abrogates this pathway of survival of the MM tumor cell. Several groups of DAC inhibitors are currently available: Aliphatic acids (valproic acid); cyclic peptides (romidepsin (FK-228)); benzamides (entinostat (MS-275) or MGCD0103) and hydroxamates (vorinostat (SAHA), panobinostat (LBH589), TSA (Trichostatin A), belinostat (PDX-101), resminostat (RAS2410), LAQ824 or ITF2357). A different small molecule that does not fit into any of the previous classes is tubacin, which was recently described as a specific inhibitor of DAC6 and induces acetylation of tubulin and Hsp-90, without affecting histone acetylation. Preclinical studies have demonstrated interesting activity of most of these drugs mediated through various mechanisms: the induction of apoptosis and cell cycle arrest mainly by the upregulation of p21; the interference with the interaction between plasma cells and the microenvironment or the inhibition of angiogenesis. Moreover they also have a role in protecting murine models from myeloma bone disease. Regarding the clinical efficacy, four trials have explored the activity of panobinostat, ITF2357, vorinostat and romidepsin in monotherapy (table 1) with modest results in all of them but with an acceptable safety profile, being the most frequent adverse events general symptoms (fatigue, anorexia), haematological and GI toxicity (dehydration, diarrhea, and nausea). The observation of disease stabilization and some responses, together with the in vitro data showing marked synergism in combination with other antimyeloma agents, prompted the investigation of the activity of DACi in combination with conventional (melphalan, doxorubicine or dexamethasone) or novel (bortezomib or lenalidomide) antimyeloma agents (Table 2). These combinations have resulted in general in good response rates even in patients previously refractory to the drugs used in the combination. Regarding the combination with bortezomib there is a good preclinical rationale for its use as it simultaneously targets three steps in the unfolded protein response pathway: the proteasome, the chaperone system (Hsp-90) and the aggressome. The main toxicity for this combination apart from the specific toxicity of this class of drugs has been the thrombocytopenia. A further step has been to add Pegylated liposomal doxorubicine (PLD) to this combination with some good quality responses. Both vorinostat and panobinostat have also been combined with lenalidomide and dexamethasone in two phase I trials, with general symptoms and myelosuppresion being the most significant side effects. In conclusion, the solid preclinical rationale for the use of DACi in MM as well as the positive clinical results, particularly in combination with other agents such as bortezomib or lenalidomide, suggest that this class of drugs may reach approval as antimyeloma agents. At this moment, phase III randomized trials are underway in order to prove if the combination of DACi with either bortezomib or lenalidomide plus dexamethasone are superior to the standards used at present for relapsed/refractory patients.

Table 1. Most relevant results of clinical trials with DACi in monotherapy in MM.

DACi	Reference	Phas	n	ORR
Panobinostat	Wolf ASH 2008	II	38	3%
Vorinostat	Richardson Cl Lymph 2008	I	13	10%
ITF2357	Gally Ann Hemat 2010	II	19	7%
Romidepsin	Niesvizky ASH 2005	II	12	0%

Table 2. Most relevant results of clinical trials with DACi in combination in MM.

DACi	Combination	Reference	Phas	n	ORR
Vorinostat	Bortezomib	Badros CCRes 2009	I	23	42%
Vorinostat	Bortezomib	Weber IMW 2008	I	34	43%
Vorinostat	Bortezomib	Weber IMW 2008	I	13*	38%
Panobinosta	Bortezomib	San Miguel ASCO 2010	Ib	47	55%
Romidepsin	Bortezomib	Harrison ASH 2008	I	25	67%
Vorinostat	PLD + Bortezomib	Voorhees ASH 2010	I	32	72%
Panobinosta	Lenalidomide + Dex	Mateos ASCO 2010	Ib	46	57%
Vorinostat	Lenalidomide + Dex	Richardson ASH 2010	I	30	53%
Panobinosta	Melphalan	Berenson ASH 2009	I	15	27%
Panobinosta	Melphalan + Thalidomide + Dex	Offidani ASH 2010	I/II	24	50%

* All patients were previously exposed to Bortezomib

MONOCLONAL ANTIBODIES IN THE TREATMENT OF MULTIPLE MYELOMA

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Monoclonal antibodies offer the possibility of targeted therapy to eliminate the tumor cells selectively with minimal side effects. The initial antibodies produced using hybridoma technology established in 1975, were mouse monoclonal antibodies that were highly immunogenic. Subsequent improvement in antibody engineering resulted in the development of chimeric, humanized and fully humanized monoclonal antibodies that are better tolerated and perform well in clinical setting.

Multiple myeloma is an excellent disease for treatment with monoclonal antibodies. The tumor cells express a wide range of surface antigens that can be targeted. The myeloma tumor growth is dependent on its interaction with the microenvironment, especially osteoclasts, osteoblasts and endothelial cells. Therefore the antibody can be targeted against growth factors or its receptors or surface antigens expressed by stromal cells upon interaction with tumor cells. Currently, more than 10 monoclonal antibody candidates have entered clinical development. Monoclonal antibodies directed against myeloma cells surface antigens (CD 40, HM 1.24, IGF-1R, CD56, CS1, CD138, CD74, IL-6R, CD 38, TRAIL-R1) or growth factors and microenvironment targets (IL-6, RANKL, DKK1, VEGF, BAFF). Monoclonal antibodies targeting the CD 20 antigen expressed by the mature B cell has been highly successful in the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia and Waldenstrom's macroglobulinemia. Clinical studies with Rituximab have been generally ineffective in the treatment of multiple myeloma due to poor expression of this antigen in plasma cells. CD40 is a member of the tumor necrosis factor receptor super family and is highly expressed on multiple myeloma cells and on bone marrow stromal cells. Two anti-CD 40 antibodies, dacetuzumab (SGN-40) and lucatumumab (CHIR-12.12, HCD122) had shown promise in the preclinical model, but failed to show significant clinical activity. CD 74 is a transmembrane protein that forms the invariant portion of HLA-DR and is expressed on myeloma cells. A humanized anti-CD74 monoclonal antibody coupled

with doxorubicin (IMMU-110) is being evaluated in a phase 1/2 study. CD56 is expressed aberrantly in myeloma tumor cells. A humanized monoclonal antibody to CD56 conjugated to spindle poison maytansinoid has shown some clinical activity. Humanized antibody to CD38 (HuMax-CD38) has shown preclinical activity and is currently in phase 1/2 safety study in patients with relapsed or refractory myeloma. Syndecan-1 or CD138 is a cell surface heparan sulfate proteoglycan that is highly expressed by plasma cells, myeloma cells and rarely in epithelial cells. BT062 is a chimeric monoclonal antibody conjugated to maytansinoid and is currently in phase 1 clinical trial. Preliminary results on 27 evaluable patients indicated clinical benefit in half of the patients treated with stabilization of disease for >9 weeks. Elotuzumab is a fully humanized monoclonal antibody against CS1 which is universally expressed at high levels on plasma cells and myeloma cells. Phase 1 clinical trial showed no dose limiting toxicity but also no efficacy. In phase 1 study of elotuzumab plus bortezomib objective response was noted in 48% of 27 evaluable patients with a median time to progression of 9.5 months. When elotuzumab was combined with lenalidomide and dexamethasone in phase 1B trial of 28 patients the overall response rate was 82%. Among 22 patients with treated until progression, the median time to progression is not reached at one year. The results were confirmed in a subsequent phase 2 study of 26 patient with the same combination resulting in an overall response rate of 85%. Based on the positive phase 1 and phase 2 results, a phase 3 trial of lenalidomide and weekly dexamethasone with or without elotuzumab is currently underway. A chimeric IgG1κ Anti-IL-6 monoclonal antibody, siltuximab (CNTO 328) has been evaluated either as a single agent or in combination regimens for multiple myeloma. Suppression of serum C-reactive protein (CRP) following treatment with siltuximab is a pharmacodynamic marker of IL-6 bioactivity. In a phase 1 study among 15 patients with relapsed multiple myeloma partial response was noted in 3 patients and long-lasting stable disease in 2 patients (224 days and 533 days). In a phase 2 study of siltuximab and bortezomib in relapsed/refractory multiple myeloma the overall response rate was 57% among 21 patients treated with a median time to progression of 8.7 months. Similarly, in another phase 2 trial in relapsed and refractory multiple myeloma siltuximab and dexamethasone showed a response rate of 20% among 44 evaluable patients. Currently a phase III trial of MPV with and without siltuximab in underway. Denosumab (Prolia) is a fully human IgG2 monoclonal antibody to receptor activator of nuclear factor-B ligand (RANKL), an osteoblast-derived glycoprotein. Myeloma tumor cells induce over expression of RANKL by marrow stromal cells resulting in osteoclast activation and lytic bone lesions. In a phase II trial 95 patients with relapsed myeloma (53 patients) or in plateau phase (43 patients) were treated with denosumab. Denosumab substantially suppressed bone resorption in both relapsed and plateau-phase MM subjects, as measured by serum C-terminal telopeptide of type 1 collagen (sCTx). There was minimal antitumor response. Denosumab is currenty approved for the prevention of skeletal-related events in solid tumors but not in myeloma. In multiple myeloma overexpression of Dickkopf-related protein 1 (DKK1) by plasma cells with correlated with the lytic bone disease. DKK 1 primarily inhibits cell osteoblasts while RANKL activates osteoclasts resulting in pure lytic bone disease seen in multiple myeloma. A fully humanized anti-DKK 1 monoclonal antibody, BHQ880, is currently in clinical trial both in the relapsed myeloma as well as in high-risk asymptomatic multiple myeloma. A number of monoclonal antibodies are currently in clinical trial for the treatment of myeloma. Most of the antibodies tested to date have not been efficacious as a single agent. The combination of lenalidomide and dexamethasone with elotuzumab appears promising and is currently in a phase 3 pivotal trial. Thus monoclonal antibody therapy will be most effective when combined with existing treatments for multiple myeloma.

SECONDARY MALIGNANCY IN MYELOMA: AN EMERGING ISSUE?

MAINTENANCE TREATMENT WITH LENALIDOMIDE AFTER TRANSPLANTATION FOR MYELOMA: ANALYSIS OF SECONDARY MALIGNANCIES WITHIN THE IFM 2005-02 TRIAL

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High dose therapy (HDT) is a standard treatment for young patients with myeloma. Residual disease is always present after HDT and an effective maintenance treatment is required to prevent relapse. This phase 3 trial investigated the efficacy of lenalidomide (LEN) for maintenance after transplantation. Patients, under 65, with non-progressive disease after a first line HDT were randomized to receive consolidation with LEN (25 mg/d, 21 days/month, for 2 months) followed by maintenance with either placebo (Arm A) or LEN (10 to 15 mg/d) until relapse (Arm B). From July 2006 to August 2008, 614 patients were randomized. Patient's characteristics of each group were similar. On July 2010, the trial was unblinded and the final analysis was performed with a median follow up of 34 months from randomization and 44 months from diagnosis. Consolidation with LEN improved the very good partial response rate (VGPR) ($p<0.0001$). Maintenance with LEN improved the progression-free survival (PFS): median 24 months from randomization in arm A, versus 42 months from randomization in arm B (HR=0.5, $p<10-8$). This benefit was observed across all stratified subgroups of patients. In multivariate analysis, PFS was related to maintenance with LEN ($p<0.0001$), and to response (CR/VGPR) after consolidation ($p<0.01$). Although maintenance treatment with LEN was well tolerated, several patients developed malignancies including AML, MDS, ALL, HD and solid tumors. All IFM centers were asked to report every secondary malignancies for patients within this trial. Updated data will be presented at the meeting.

PHASE III INTERGROUP STUDY OF LENALIDOMIDE VERSUS PLACEBO MAINTENANCE THERAPY FOLLOWING SINGLE AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) FOR MULTIPLE MYELOMA (MM): CALGB ECOG BMT-CTN 100104

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Disease relapse/progression is the primary cause of treatment failure after ASCT for MM. The primary objective of this study was to investigate if maintenance lenalidomide (Len) would prolong time to progression (TTP) following single ASCT with melphalan 200 mg/m2. Eligibility included Stage I-III MM, ≤1 year from diagnosis, ≥2 months of induction with stable disease or better (≥SD), age <70 years. Patients (pts) with ≥SD were randomized at day 100 post-ASCT to Len or placebo until disease progression, after stratification by β-2 microglobulin level and prior thalidomide or Len therapy. Starting Len dose was 10 mg/day, increased to 15 mg/day or decreased for toxicity; 568 pts were enrolled (04/2005-07/2009). The fifth interim analysis based on 460 randomized pts at a median follow-up of 17.6 months at study unblinding (12/2009) demonstrated a significantly longer TTP with Len. 46/231 Len and 97/229 placebo pts experienced an event (one-sided unadjusted $P<0.0001$); estimated HR of 0.37. The median TTP was 43.6 months for Len and 21.5 months for placebo. Using all follow-up events as of 02/2011, 65/231 Len and 114/229 placebo pts have experienced an event (one-sided unadjusted $P<0.0001$); HR of 0.43. Deaths in the Len and placebo arms were 13 and 24 respectively (unadjusted $P<0.049$) up to 12/2009 and as of 2/2011 are 21 and 37 respectively (unadjusted $P<0.019$). Significant delay in TTP in the Len arm was observed, regardless of stratification. Len initiated at day 100 post-ASCT in MM patients significantly improves TTP and appears to improve overall survival.

SECONDARY MALIGNANCIES IN ELDERLY MYELOMA PATIENTS

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Advances in cancer detection and treatment have tripled cancer survivorship since 1971, and the population of cancer survivors has grown by 2% each year (Travis et al, 2006). However, longer survival increases the risk of second primary malignancy (SPM) development in these patients, possibly through late sequelae of treatment, aging, and/or genetics (National Cancer Institute; Fraumeni et al, 2006). Among these, age is a well-characterized risk factor for primary malignancy development, and data from the Italian region demonstrate increased incidence rates per year of life for each 10-year age group studied: 0.40% (ages 45-54), 0.97% (ages 55-64), 1.82% (ages 65-74), 2.54% (ages 75-84), and 3.86% (ages 85+). The risk of SPM development should be reported as SPM incidence per year of follow-up, and age should be considered a risk factor. Multiple myeloma (MM) is an incurable malignancy characterized by multiple relapses, and eventually, refractory disease and death. Therapy advances have resulted in significant survival benefits, with 5-year relative survival rates among patients with MM of all age groups having increased from 26% to 38% for patients first diagnosed in 1975-1977 and 1999-2006, respectively (Travis et al, 2006). In these patients, prolonged survival may increase the risk of developing an SPM. Registry studies conducted in the United States and Sweden have shown that MM patients are at a higher risk for the development of non-Hodgkin's lymphoma (NHL; 1.7-fold), acute myeloid leukemia (AML; 8-fold), and chronic myeloid leukemia (CML; 2.5-fold) compared with all cancer patients (Dores et al, 2006; Dong et al, 2001). The goal of this presentation is to discuss the issue of SPM emergence in recent clinical trials evaluating the use of lenalidomide in autologous stem cell transplant (ASCT)-eligible and -ineligible patients with newly diagnosed MM (NDMM). The current report describes SPM incidence in patients enrolled in MM-015 as well as spontaneous SPM reports from investigators for additional investigator sponsored trials. MM-015 is a phase 3 trial designed to evaluate the efficacy and safety of continuous lenalidomide treatment (melphalan, prednisone, and lenalidomide induction followed by lenalidomide maintenance [MPR-R]) vs fixed-duration regimens of melphalan and prednisone (MP) or melphalan, prednisone, and lenalidomide (MPR) in transplant-ineligible patients aged ≥ 65 years. A post hoc analysis evaluated incidence rates (IRs) per 100 person-years for SPMs and risks of SPM relative to disease progression risk. As of the May 2010 data cut-off prior to site-unblinding (median follow-up: 25 months), 12 total cases of SPMs were reported (4/150 in MPR-R [IR = 1.40], 6/152 in MPR [IR = 2.05], and 2/153 in MP [IR = 0.67]), including 2 myelodysplastic syndromes (MDS) cases in MPR-R and 2 AML cases in each of MPR-R and MPR arms. In the MPR/MPR-R vs MP group, the incidence of AML/MDS was 3.6% vs 0.7%, respectively. A longer follow-up is needed to draw definite conclusions. Solid tumors were reported in 6 patients (1/150 in MPR-R, 3/152 in MPR, and 2/153 in MP) and were of heterogeneous tumor types. No B-cell malignancies were reported. Of note, the early detection of some solid tumors may end up in their cure.

The progression-free survival (PFS) benefit afforded by lenalidomide maintenance outweighs the increased SPM risk, as indicated by increased progression risk for MP and MPR vs MPR-R. The median PFS for MPR-R vs MP was 31 vs 13 months ($P < .001$), resulting in a 60% reduction in the risk of disease progression (hazard ratio [HR] = 0.395) (Palumbo et al, ASH 2010). Similarly, 2-year PFS rates were higher for patients receiving MPR-R vs MP (55% vs 16%) (Palumbo et al, ASH 2010). The impact of relapse and the occurrence of a second cancer on patient outcomes has been evaluated. For patients receiving lenalidomide maintenance (MPR-R), the risk of death or progression at 2 years is 45% and the risk of SPM at 2 years is 3%. In patient receiving MP alone the risk of death or progression at 2 years is 84% and the risk of developing an SPM for these patients at 2 years is <1%. Similarly, when SPMs were added as additional events for PFS, the hazard ratio for disease progression and SPM development for MPR-R vs MP (HR = 0.408; $P < .001$) was similar to the PFS analysis that did not include SPMs (HR = 0.395; $P < .001$). Although longer follow-up is needed, these preliminary data suggest that the risk-benefit profile remains in favor of lenalidomide maintenance.

In the Dutch MPR-R vs melphalan-prednisone-thalidomide followed by thalidomide maintenance (MPT-T) study, 200 patients were evaluated; so far 3 cases of SPM were observed. In an EMNTG study, 319 patients ≥ 65 years were randomly assigned to receive treatment with either lenalidomide plus low-dose dexamethasone (Rd), MPR, or cyclophosphamide-lenalidomide plus low-dose dexamethasone (CRd). To date, after a median follow-up of 8 months, no SPMs were reported. In a previously published study evaluating lenalidomide as consolidation-maintenance following bortezomib, adriamycin, and dexamethasone (PAD) induction and ASCT in NDMM patients (Palumbo et al, JCO 2010), 102 patients (median age 67) received lenalidomide maintenance and 2 cases of SPMs were reported. In another EMNTG study comparing MPR with tandem melphalan 200 mg/m2 (MEL200) and ASCT (Palumbo et al, ASH 2010), 402 patients < 65 years of age were included, and after 2 years of follow-up 2 cases of SPM were reported. In a phase 3 study (343 patients) comparing cyclophosphamide-lenalidomide-dexamethasone (CRD) with MEL200 followed by ASCT, no SPMs were reported. On the contrary, one case of SPM was detected in a GIMEMA phase 2 study evaluating the role of MPR-R as induction in elderly NDMM (54 patients; Palumbo et al, JCO 2007). A total of 46 NDMM patients were enrolled in a study assessing the role of lenalidomide-prednisone (RP) as induction followed by MPR and no SPM cases were reported. The rates of SPM reported here are those currently available in our database; they may be underestimated due to the lack of a specific query on SPM to the participating centers. Efforts are currently ongoing and specific queries are requested to update the incidence of SPM in these studies. Results will be presented at the meeting.

RETROSPECTIVE ANALYSIS OF THE LONG TERM SAFETY OF LENALIDOMIDE (LEN) ± DEXAMETHASONE (DEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) PATIENTS (PTS): ANALYSIS OF POOLED DATA AND INCIDENCE RATES (IR) OF SECOND PRIMARY MALIGNANCY (SPM)

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Background. The occurrence of SPM in MM pts has been well recognized based on disease natural history and associated treatments. Given the association of aging with development of malignancies and recent concerns over the potential of increased SPM risk with long term Len exposure, SPM incidence from Len studies was compared with expected background invasive cancer incidence from the US SEER Cancer Registries, 2003-2007. *Methods*. Data from Len-based arms of 11 Celgene-sponsored studies in RRMM pts who received ≥24 months (mos) of Len were analyzed for SPM incidence. By SEER definition, non-melanoma skin cancers and in situ malignancies were excluded. *Results.* Median age at enrollment was 64 (range 29-92). Median Len duration for all studies was 5 mos (range 0.03-58.27); 313 (8.2%) pts received ≥24 mos (median duration 34 mos [range 24-58.3]). In all pts, 57 SPMs were identified in 56 pts (8 MDS, 1 AML, 2 B-cell malignancies [low grade B-cell lymphoma, Epstein-Barr virus associated lymphoproliferative disorder], 46 solid tumors [ST]) corresponding to an IR of 2.15 and a standardized incidence ratio (SIR) of 0.77 (95% CI 0.43-1.28). No B-cell malignancies were reported in pts with ≥24 mos of Len. SPM IRs compare favorably with IR from SEER (range 1.3-2.2/100 patient-yrs [PY] for persons aged 60-85+). *Conclusion.* Len-based therapy for RRMM, including durations ≥24 mos, did not significantly increase the SPM IR compared to IR for invasive cancers reported by SEER. Rigorous efforts are underway to further characterize SPM and identify risk factors.

Characteristic (N=3839)	# SPM Cases (IR per 100 PY)
Age, yrs	
<65 (n=2060)	19 (1.27)
65-75 (n=1335)	32 (3.71)
>75 (n=444)	6 (2.46)
Cumulative Len dose, mg (n=3812)[a]	
<2400	15 (3.50)
≥2400	41 (1.89)
Treatment duration, mos	
≥12	31 (2.12)
≥18	25 (2.13)
≥24	22 (2.35)
Regimen	
Len (n=731)	19 (2.42)
Len+Dex (n=3108)	38 (2.09)

[a]Cumulative dose incalculable for 27 pts due to missing data.

RISK OF SECOND PRIMARY MALIGNANCIES (SPM) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) PATIENTS (PTS) TREATED WITH LENALIDOMIDE AND DEXAMETHASONE (LEN+DEX): ANALYSIS OF MM-009/010 SPM INCIDENCE RATE (IR)

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Background: The phase 3 MM-009/010 trials demonstrated a significant survival benefit for Len+Dex compared with placebo (PBO)+Dex (Dimopoulos 2007; Weber 2007). However, pts who live longer have an increased SPM risk. Among persons aged 65+ the incidence of invasive cancer is 2.1 per 100 person-years. Thus, we examined SPM risk in MM-009/010. *Methods:* This post hoc analysis was based on pooled MM-009/010 data. SPM IRs per 100 person-years were evaluated during active treatment. Incidence rates of SPM among 23,838 MM pts in the US SEER Cancer Registries (1973-2000) were used to calculate Standardized Incidence Ratios (SIRs) for solid tumor (ST) malignancies (MDS data not available in SEER). *Results:* SPMs were low, with 6 ST and 2 MDS cases in the Len+Dex arm and 2 ST cases in the PBO+Dex arm. No AML or B-cell malignancies were observed. IRs for ST malignancies were equivalent by arm (1.06 per 100 person-years in Len+Dex vs. 0.90 per 100 person-years in PBO+Dex). SIRs were similar for Len+Dex and PBO+Dex active treatment (0.76 [95% CI: 0.28-1.68] vs 0.64 [0.11-2.12]). With an additional median 1.5 years of follow-up only one new SPM was identified in the Len+Dex arm. *Conclusions:* ST IRs were low during active treatment, did not differ between arms, and were consistent with background incidence. SIRs were not consistent with increased ST risk. During long-term follow-up, detection of new malignancies decreased greatly as only survival data were collected. SPM risk is not increased, resulting in no change to the benefit-risk profile for Len in RRMM.

RISK OF SUBSEQUENT PRIMARY MALIGNANCIES IN PATIENTS WITH MULTIPLE MYELOMA BEFORE AND AFTER THE INTRODUCTION OF NOVEL THERAPIES: A POPULATION-BASED STUDY IN SWEDEN

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Background. The risk of developing acute myeloid leukemia (AML) and/or myelodysplastic syndromes (MDS) following the use of alkylating agents to treat multiple myeloma (MM) has been recognized for many years. We aimed to characterize the risk of subsequent primary malignancies in MM pts before and after the introduction of novel therapies. *Methods.* Using high-quality population-based data from Sweden, we assessed the risk of secondary malignancies in all MM pts (n=9,926) diagnosed 1986-2005 (follow-up until 2006). We estimated standardized incidence rates (SIRs) for all subsequent primary hematologic and solid tumors overall and separately for pts diagnosed before/after 1995 (introduction of highdose melphalan/ASCT) and 2000 (introduction of IMiDs), respectively. *Results.* We found an overall excess risk of AML (SIR=7.6; 95% CI 4.6-11.7), non-specified myeloid leukemias (including MDS) (SIR=21.7; 11.6-37.1) and non-melanoma skin cancer (SIR=2.2; 1.7-2.7). There was no significantly increased risk for other hematologic or solid tumors. The results were the same when we stratified by calendar period (before/after 1995 and 2000). *Conclusions.* Based on all MM pts diagnosed in Sweden 1986-2005, we confirm the excess risk of developing AML/MDS. With the exception of non-melanoma skin cancer, no other malignancies were significantly increased. Results were very similar in analyses restricted to pts diagnosed before vs. after 1995, suggesting that highdose melphalan/ASCT may not alter short term risk of second cancers. Longer follow-up is needed to better define risks in the IMiD-era.

MONOCLONAL IMMUNOGLOBULIN AND THE NERVE, WALDENSTROM'S MACROGLOBULINEMIA

NEUROPATHY IN MONOCLONAL GAMMOPATHY

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Introduction. The association of neuropathy with monoclonal gammopathy has been known for several years, even if the clinical and pathogenetic relevance of this association is not completely defined. This is not a marginal problem as: a) monoclonal gammopathy is present in 1-3% of the population above 50 year in whom it is often asymptomatic, and, b) in at least 8% of patients is associated with a symptomatic neuropathy with a prevalence in the population above 50 years of at least 1 per 1,000, representing one of the leading causes of neuropathy in aged people. Monoclonal gammopathy may result from malignant lymphoproliferative diseases including multiple myeloma or solitary plasmocytoma, Waldenström's macroglobulinemia (WM), other IgM secreting lymphoma or chronic lymphocytic leukaemia, as well as from primary amyloidosis (AL) and cryoglobulinemia. In most instances it is not associated with any of these disorders and is defined monoclonal gammopathy of undetermined significance (MGUS) for its possible, though infrequent, evolution into malignant forms. Several data support the pathogenetic role of the monoclonal gammopathy in the neuropathy particularly when of IgM isotype. There is however not yet defined therapies for these neuropathies, as their efficacy have not been confirmed in randomized trials. *Neuropathy and IgM monoclonal gammopathy.* A symptomatic neuropathy has been reported in up to 50% of patients with IgM monoclonal gammopathy. Some of these patients have WM or other forms IgM secreting lymphoprolipherative disease. The majority has however an IgM MGUS whose only clinical manifestation is the neuropathy leading to its inclusion in the group of IgM-related disorders. Different forms of neuropathies have been associated with IgM monoclonal gammopathy including cranial nerve palsies, mononeuropathies or mononeuritis multiplex often associated with WM and lymphoma and related to lymphoplasmacytic infiltration of nerves, amyloid deposition, cryoglobulinemic vasculitis or microangiopathy of endoneurial vessels. The vast majority of patients have however a chronic progressive, symmetric and predominantly distal neuropathy which is often attributed to a reactivity of the M-protein with neural antigens including the myelin-associated glycoprotein (MAG), sulfatide and several gangliosides. These reactivities are found in approximately two thirds of the patients, and are particularly frequent in those with MGUS (84%). *Neuropathy associated with anti-MAG IgM.* In almost 50% of the patients with IgM-related neuropathy the M-protein reacts with MAG and other cross-reactive glycoconjugates (Nobile-Orazio et al. 1998). Almost 80% of these patients have and IgM MGUS while most remaining patients have an otherwise asymptomatic WM. This neuropathy is quite homogeneous being characterized by distal and symmetric, predominantly sensory involvement, gait ataxia and postural tremor in the upper limbs. Motor impairment is usually less prominent and often appears later. The neuropathy mostly affects men in their sixties or seventies and usually runs a slowly progressive course with approximately 50% of the patients requiring a support to walk after 15 to 20 years (Niermeijer et al. 2010). Electrophysiological and morphological studies are consistent with a demyelinating neuropathy. The possible role of anti-MAG antibodies in the neuropathy is supported by their almost invariable association with the neuropathy and by the fact that their presence often predicts the development of neuropathy In addition pathological studies on nerve biopsies often disclose the presence these antibodies and complement on myelin and complement mediated demyelination of nerve was experimentally induced in animals by intraneural or systemic injection of these antibodies. These data led to the use of several therapies directed at reducing monoclonal IgM antibodies in these patients. Even if almost 50% of patients were reported to improve after one of more of these therapies their efficacy was not confirmed in randomized trials (Lunn and Nobile-Orazio 2006). More recently approximately 30% of the patients were reported to improve after therapy with the humanised anti-CD20 monoclonal antibody (Rituximab). The results did not however achieve statistical significance in two randomized trials (Dalakas et al. 2009; Leger et al. 2010). It remains unclear the long-term benefit on the neuropathy of these therapies as the follow-up seldom exceeded two years (Benedetti et al. 2008). This data would be particularly important considering the slow progression of the neuropathy and the adverse effects of these therapies. *Neuropathy associated with anti-sulfatide and antiglycolipid antibodies.* Several other anti-neural reactivities of IgM M-proteins have been reported in patients with IgM related neuropathies but their role in the neuropathy is still debated (Joint task force EFNS/PNS 2010). High titers of anti-sulfatide IgM antibodies can be found in 4% of the patients with a demyelinating sensorimotor neuropathy associated with IgM monoclonal gammopathy (Nobile-Orazio et al. 2008) while lower titers can be found in patients with other neuropathies. The possible pathogenetic relevance of this association is supported by morphological studies on nerve biopsy showing deposits of the M-protein and of complement. Few data are however available on the clinical response to treatment in these patients. IgM antibodies to the ganglioside GM1 were originally reported in patients with IgM monoclonal gammopathy and multifocal motor neuropathy, even if most subsequently reported patients did not to have IgM monoclonal gammopathy. A few patients with neuropathy and IgM monoclonal gammopathy reacting to the disialosyl containing gangliosides GQ1b, GD1b, GT1b, GD3 and GD2 have been reported (Willison et al. 2001). Most patients have a chronic sensory demyelinating ataxic neuropathy associated with mild or no weakness, recurrent ophthalmoplegia and cold agglutinin activity of the M-protein. Willison proposed for this syndrome the acronym CANOMAD (Chronic Ataxic Neuropathy with Ophthalmoplegia, M-protein, cold Agglutinins and anti-Disyalosil antibodies). Most of these patients improve after therapy with IVIg (Attarian et al 2010) which are often ineffective in other IgM-related neuropathies confirming that the search for these reactivities has practical implication for the treatment of this neuropathy. *Neuropathy and IgG monoclonal gammopathy.* Less clear is the relationship between neuropathy and IgG monoclonal gammopathy. Some patients have multiple myeloma where the neuropathy is occasionally the presenting symptom but more frequently occurs in patients with established disease. In these patients the neuropathy is clinically heterogeneous reflecting the presence of different pathogenetic mechanisms. More typical are the features of the neuropathy associated with osteosclerotic myeloma which is often associated with other non-neurological manifestations typical of the POEMS (Polyneuropathy, Organomegaly, Endocrinopathy, M-protein and Skin changes) syndrome (Dispenzieri et al. 2004). The majority of patients have however an IgG MGUS, which is often found during the work-up or follow-up of the neuropathy (Nobile-Orazio et al. 2002). Almost 50% of the patients have a chronic demyelinating neuropathy clinically and therapeutically indistinguishable from chronic inflammatory demyelinating polyradiculoneuropathy, while most of the remaining has a predominantly sensory axonal or mixed neuropathy. The possible role of IgG M-proteins in the neuropathy remains unclear as no consistent reactivity of IgG M-proteins with nerve or endoneurial deposits of IgG have been reported and in over 50% of patients the M-protein become manifest after the neuropathy. *Neuropathy and IgA monoclonal gammopathy.* Only few patients with neuropathy and IgA monoclonal gammopathy have been reported representing in most series a small proportion of the patients with neuropathy and monoclonal gammopathy. Some patients have myeloma or a POEMS syndrome (see above) while others have IgA MGUS. The clinical and electrophysiological features of the neuropathy in these patients are quite heterogeneous (Nobile-Orazio et al 2002) making it difficult to identify a prevailing type of presentation except that the neuropathy is almost invariably chronic progressive. Anti-neural reactivity or endoneurial deposits of IgA M-proteins have been rarely reported in these patients and few patients have been reported to improve with immune therapies so that the pathogenetic role of the monoclonal gammopathy in the neuropathy remains unclear.

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POEMS SYNDROME: DIAGNOSIS AND TREATMENT

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Recognition of the complex of a combination of peripheral neuropathy, organomegaly, endocrinopathy, monoclonal plasmaproliferative disorder (typically λ), skin changes, papilledema, extravascular volume overload (peripheral edema, pleural effusions, ascites), sclerotic bone lesions, thrombocytosis, Castleman disease, increased levels of circulating vascular endothelial growth factor, and abnormal pulmonary function tests is the first step in diagnosing POEMS syndrome, a rare paraneoplastic syndrome due to an underlying plasma cell dyscrasia. Peripheral neuropathy dominates the clinical picture, with an ascending, symmetric, sensorimotor, predominantly demyelinating neuropathy. Table 1 includes criteria for a diagnosis of POEMS syndrome. -existing Casleman's disease or lymph nodes with Castleman-like pathology is not unusual. Over the past two decades, restrictive lung disease, pulmonary hypertension, and arterial events, including stroke, have become increasingly recognized. Although the majority of patients have osteosclerotic myeloma, these same patients usually have only 5% bone marrow plasma cells or less (almost always monoclonal λ), and rarely have anemia, hypercalcemia or renal insufficiency. Serum M-spikes are small, and although free light chain elevations are common, the κ/λ ratio is more often normal than not. Fewer than 10% of patients have proteinuria exceeding 0.5 g/24 hours and/or serum creatinine greater than or equal to 1.5 mg/dL. The renal histology is diverse with membranoproliferative features and evidence of endothelial injury being most common. These characteristics and the superior median survival differentiate POEMS syndrome from multiple myeloma The pathogenesis of this multisystem disease is complex. Elevations of vascular endothelial growth factor and pro-inflammatory cytokines are the hallmark of this disorder. Little is known about the plasma cells except that more than 95% of the time they are λ light chain restricted with restricted Vλ germline gene usage. POEMS syndrome is not an immunoglobulin deposition disease. The dominant feature of this syndrome is the peripheral neuropathy, and not infrequently patients are initially diagnosed with chronic inflammatory demyelinating polyneuropathy (CIDP) or, less frequently, Guillain-Barré. The two best ways to distinguish POEMS from monoclonal gammopathy associated peripheral neuropathy and AL amyloidosis is to measure a plasma or serum VEGF level. The course of POEMS syndrome is usually chronic with reported median survivals ranges from 33 months to nearly 14 years. The number of POEMS features does not affect survival. Fingernail clubbing and extravascular volume overload, i.e. effusions, edema, and ascites, and respiratory symptoms are all associated with a significantly shorter overall survival.

For patients with a dominant sclerotic plasmacytoma, first line therapy is irradiation. Systemic therapy is appropriate for patients with diffuse sclerotic lesions or absence of any bone lesion and those who have not demonstrated stabilization of their disease 3 to 6 months after completing radiation. Useful approaches include therapy with corticosteroids, low dose alkylator therapy, and high dose chemotherapy with peripheral blood stem cell transplant. Intensive supportive care measures must also be instituted including physical therapy, orthotics, diuretics, analgesics, and CPAP. Plasmapheresis and/or intravenous gammaglobulin are not effective. The role of anti-VEGF therapies, immune modulatory drugs, and proteasome inhibitors has not yet been well defined, but drugs with known high rates of treatment related neuropathy should not be considered as first line therapy. Despite the theoretic rational for using bevacizumib in patients with POEMS syndrome, this therapy may result in as much harm as good and should not be considered a standard. Thalidomide and lenalidomide both have activity, but potential side-effects of neuropathy and thrombosis should not be ignored.

When therapy is effective, response of systemic symptoms and skin changes typically precede those of the neuropathy, with the former beginning to respond within a month, and the latter within 3-6 months with maximum benefit frequently not seen before 2 to 3 years. Clinical response to therapy correlates better with VEGF level than M-protein level, and complete hematological response is not required to derive substantial clinical benefit.

*Table 1. Criteria for the Diagnosis of POEMS Syndrome**

Mandatory or major criteria	1. Polyneuropathy (typically demyelinating)
	2. Monoclonal plasma cell-proliferative disorder (almost always λ)
Other major criteria	3. Castleman disease
(one required)	4. Sclerotic bone lesions
	5. Vascular endothelial growth factor elevation
Minor criteria	6. Organomegaly (splenomegaly, hepatomegaly, or lymphadenopathy)
	7. Extravascular volume overload (edema, pleural effusion, or ascites)
	8. Endocrinopathy (adrenal, thyroid,* pituitary, gonadal, parathyroid, pancreatic*)
	9. Skin changes (hyperpigmentation, hypertrichosis, glomeruloid hemangiomata, plethora, acrocyanosis, flushing, white nails)
	10. Papilledema
	11. Thrombocytosis / polycythemia†
Other symptoms and signs	Clubbing, weight loss, hyperhidrosis, pulmonary hypertension/ restrictive lung disease, thrombotic diatheses, diarrhea, low vitamin B12 values
Possible associations	Arthralgias, cardiomyopathy (systolic dysfunction), and fever

*POEMS, polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes. The diagnosis of POEMS syndrome is confirmed when both of the mandatory major criteria, one of the three other major criteria, and one of the six minor criteria are present. *Because of the high prevalence of diabetes mellitus and thyroid abnormalities, this diagnosis alone is not sufficient to meet this minor criterion. †Anemia and/or thrombocytopenia are distinctively unusual in this syndrome unless Castleman disease is present.*

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THE ROLE OF PURINE ANALOGS IN THE FRONT LINE TREATMENT OF WALDENSTROM'S MACROGLOBULINEMIA

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Waldenstrom's macroglobulinemia (WM), a rare B-cell malignancy, is incurable. Conventional treatment consists of alkylating agents (especially chlorambucil), purine analogs and Rituximab. Purine analogues such as fludarabine and cladribine are active. Response rates to first-line therapy range from 38 to 95%. Discrepancies in response rates between different studies could be due to the small patient populations and to differences in patient characteristics and response criteria. Trials of purine analogs combination therapy with drugs such as cyclophosphamide with or without Rituximab give higher response rates in small series. To better determine the efficacy of purine analogs as first line therapy, we have conducted a randomized trial in France and UK, comparing the efficacy of chlorambucil to fludarabine in untreated patients. The WM1 study was a prospective international randomized open-label study that included patients with previously untreated WM MZL and LPL. At registration, patients were stratified as having WM, SLVL, or LPL, and were randomized in the two arms. The aim of the study was to compare the efficacy of oral CBL at a dose of 8 mg/m^2 for 10 days every 28 days to a maximum of 12 cycles with oral F at a dose of 40 mg/m^2 orally for 5 days every 28 days to a maximum of 6 cycles. 418 patients were enrolled into the study from 07/01 to12/09. 414 patients received at least one course of chemotherapy.

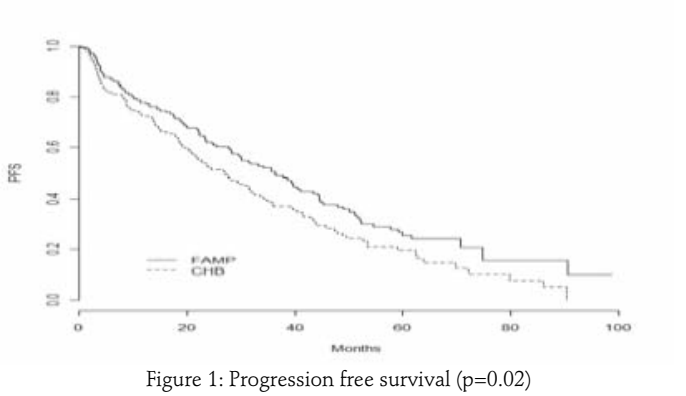

Figure 1: Progression free survival (p=0.02)

There were 339 WM, 37 MZL and 38 LPL with a median age of 68 years (40-89). 207 patients were randomized in the F arm and 207 patients in the CBL arm. At inclusion, the median of haemoglobin (g/L), platelets (Giga/L), albumin (g/L) and beta 2 microglobulin (mg/l) were 10, 220, 37.1 and 3.7 respectively. The overall response rate (CR+PR) was 47.8 in the F arm versus 38.6 in the CBL arm (p=0.06). With a median follow-up time of 36 months, the median of progression free survival time (PFS) and disease free survival (DFS) were statistically longer in the F arm: PFS 36.3 m vs 27.1 m (p=0.02, Figure 1) and DFS 38.3m vs 19.9 m (p= 0.001, Figure 2). In WM group, factors influencing negatively PFS were CBL arm, albumin< 40 g/L and Beta2 microglobulin >3 mg/L. Main toxicity was haematological with 13.7% vs 3.4% of grade IV neutropenia and 12% vs 7% of grade IV anemia in F and CBL arms respectively. F by oral route is a safe and effective ambulatory treatment in WM patients, even the elderly and more effective than CBL with a duration of response over 3 years.

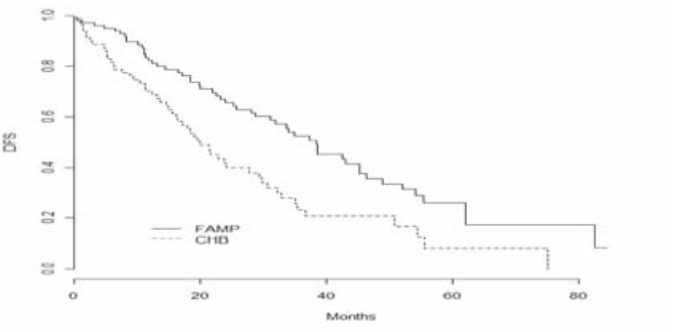

Figure 2: Disease free survival (p=0.001)

ORAL COMMUNICATIONS

0-01

IMMUNOTHERAPY IN MYELOMA, MYTH OR REALITY

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There is considerable evidence for immune mediated control of disease in myeloma. Stable levels of paraprotein during plateau phase disease and the impact of GvM in the allogeneic setting demonstrate the ability of the host's immune system to control disease. Why then has immunotherapy proven to be only marginally effective in myeloma? Possible explanations include the inability to select an appropriate antigen, exhaustion and anergy of cytotoxic T cell clones, and the complex immunosuppressive interactions between the tumour and its host causing dysfunctional dendritic cells, an inbalance in the control by Treg/Th17 cells and an increased number of T-cells with acquired regulatory functions. Our studies demonstrate the number of Tregs in the blood is increased, Th17 cells reduced, and Treg function is impaired compared with aged matched controls, but increased in patients on lenalidomide, compared with untreated patients. New data on Trogocytosis demonstrates that myeloma cell membrane antigens can be passed to T-cells. Acquired expression of antigens such as HLA-G on T-cells induces Tregs in patients with myeloma. HLA-G expression is quite heterogenenous on malignant plasma cells ranging from 0-96% and carries adverse prognostic significance. Therefore these complex interactions make induction of successful immunotherapy problematic, not only related to the inability to choose the appropriate antigen but predominately because of the complex immunomodulatory interactions between the tumour and its host.

0-02

DEFINITE ANALYSIS OF DONOR VERSUS NO DONOR COMPARISON OF NEWLY DIAGNOSED MYELOMA PATIENTES INCLUDED IN THE HOVON 50/54 STUDY

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Allogeneic Stem Cell Transplantation (Allo-SCT) as a treatment option for multiple myeloma (MM) patients is controversial. We have presented two earlier interim analysis (donor versus no donor: ASH 2008 and EHA 2010) of the Hovon 50/54 study in which we found no benefit for Auto/Allo-SCT as part of first line therapy as compared to Auto-SCT followed by maintenance with thalidomide or Interferon. Comparable studies performed by the Italian study group led by B. Bruno and the EBMT however have shown that prolonged follow-up may be necessary to find a favourable Graft Versus Myeloma effect. We will perform a new analysis to present the definite donor versus no donor comparison after a median follow-up of 72 months. Only patients are included in the analysis that have received the full induction therapy, the HDM200, had all sibs HLA typed and were treated in a centre with an Allo-SCT policy. 122 patients had a sibling donor of which 100 patients underwent the Allo-SCT between 2 and 6 months following HDM after conditioning with low dose TBI only (2Gy). 139 patients had no sibling donor of which 122 patients started with maintenance therapy. As shown by earlier analysis - median follow-up of 60 months-, PFS and OS were comparable between the two groups. For patients that did receive their allocated treatment the curves began to diverge in favour of the Allo-transplanted group. The final analysis will be performed in the beginning of 2011 and the results presented during the workshop may show if a delayed Graft versus Myeloma effect becomes apparent after prolonged follow-up.

0-03

MULTIEPITOPE VACCINE TO TREAT PATIENTS WITH MYELOMA-RELATED DISEASE: POTENTIAL CLINICAL APPLICATION

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The efficacy of peptide vaccine can be enhanced by using multiple immunogenic epitopes derived from different tumor-associated antigens (TAAs) and inducing broad cytotoxic T lymphocyte (CTLs) responses against tumor cells. The goal of our study was to examine the effectiveness of combination of four immunogenic HLA-A2+ peptides specific to XBP1 unspliced, XBP1 spliced, CD138 and CS1 antigens to induce CTLs response targeting multiple myeloma (MM) cells. The peptide-specific CTLs were generated ex vivo by repeated stimulation of T lymphocytes from HLA-A2+ normal donors with antigen-presenting cells pulsed with each individual peptide or all peptides combined together. The CTLs were evaluated for their specific subtypes and immune function using MM cell lines or primary cells. We observe that CTLs generated by the combination peptides induced tumor-specific immune responses including IFN-γ production, cell proliferation, and cytotoxicity in response to HLA-A2+ MM cells. In addition, stimulation with the multiple peptides induced significantly increased CD8+ CTLs, as well as activated (CD69+) and effector memory (CD45RO+/CCR7-) CTLs, but decreased naïve (CD45RO-/CCR7+) CTLs compared to control. Moreover, the CTLs generated using the combination peptides demonstrated similar or greater response, compared to CTLs generated using individual peptides. Thus, targeting multiple TAAs, XBP1, CD138 and CS1, using a cocktail of specific peptides may provide an effective immune response for therapeutic application in patients with MM and related plasma cell disorders.

0-04

JUMPING TRANSLOCATIONS 1Q12 CONTRIBUTE TO COPY NUMBER (CN) VARIATIONS IN MULTIPLE MYELOMA (MM): UNEXPECTED CN GAINS INVOLVING DUPLICATIONS AND TRANSLOCATIONS OF RECEPTOR CHROMOSOMES (RC).

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MM is characterized by complex chromosome aberrations including an unbalanced rearrangement known as a jumping translocation 1q12 (JT1q12). Two types of JT1q12 can occur: One type results in the translocation of the entire 1q to the telomere of a RC, increasing the CN of genes on the 1q. A second type of JT1q12 results in a whole-arm unbalanced translocation which increases the CN of genes on 1q, while decreasing the CN on the RC. We investigated 60 cases with 1q aberrations by G-banding, FISH and SKY for CN changes relating to JT1q12. Thirty-five cases showed deletions in RCs, including 11 with 16q-, 6 with 19q-, 3 with 6q-, and two each with 5q- and 8p-. Surprisingly, four cases showed unexpected translocations of RCs, with two cases demonstrating telomeric JT1q12s resulting in the translocation of the distal portion of the RCs. One of these cases showed the duplication of distal 18q including BCL2 and its subsequent translocation to 21p. The other telomeric case showed an inverted duplication of distal 8q, including c-MYC, and the 1q12~23 amplicon, thus co-amplifying these two segments. Two cases with whole-arm 1q12 and 16q11.2 translocations showed jumping translocations of 16q11.2. The most striking case showed amplification of both 16q11.2 and the 1q12~23, resulting in multiple CN increases in 16q11.2 and 1q12~23, and CN losses of 16q- and 11p-. These findings demonstrate that JT1q12 aberrations can be involved in the duplication and translocation of non-homologous chromosome segments and result in an unexpected CN increase for genes on RCs.

O-05

CEREBLON (CRBN) IS A REQUIREMENT FOR THE ANTI-MYELOMA ACTIVITY OF THALIDOMIDE, LENALIDOMIDE AND POMALIDOMIDE

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Only 30% of MM patients respond to single agent IMiD therapy and the mechanism of action of IMiDs is unclear. Cereblon (CRBN) was identified as a primary teratogenic target of thalidomide. We investigated the role of CRBN in the anti-MM activity of IMiDs. CRBN RNAi knock-down in MM lines induced cytotoxicity post transfection. Cells which survived with stable CRBN depletion are highly resistant to both lenalidomide (Len) and pomalidomide, but not to bortezomib, dexamethasone or melphalan. Isogenic MM cell lines (sensitive or resistant to Len) carry deletions of genomic CRBN on array CGH eg. in MM1.S, a mono-allelic deletion in the sensitive line becomes bi-allelic in the resistant line. Gene expression changes induced by Len are dramatically suppressed by CRBN knockdown further demonstrating that CRBN is required for Len activity. A lower CRBN expression level was detected by RT-PCR and mRNA sequencing from 3 patients at relapse after Len treatment compared with baseline patient samples. GEP analysis indicated that MM cell lines (generally more resistant to IMiDs) have a lower expression level of CRBN compared with primary MM cells. However, only 12% of HMCL and 1.2% of MM patient have a monoallelic deletion of CRBN on array CGH. Gene expression profile (GEP) identified 123 shared changes in MM cells treated with Len or CRBN knockdown. Those genes are enriched for cell survival and immune response signaling. In summary, CRBN is an essential requirement for IMID activity and low levels correlate with lack of drug response. CRBN levels should predict therapeutic response.

O-07

IDENTIFICATION OF GSK-3 AS A PRIMARY TARGET OF IMIDS AND BIOMARKER OF CLINICAL RESPONSE USING DROSOPHILA

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Immunomodulatory drugs (IMiD®s) have proven beneficial in the treatment of multiple myeloma (MM). Pre-clinical studies demonstrate multiple direct and indirect anti-tumor activities including anti-angiogenic, proapoptotic, anti-proliferative and immunomodulatory effects. Evidence suggests a putative interaction of IMiDs with Wnt signaling, though the precise target in this pathway remains unknown. The genetic simplicity of Drosophila has proven useful in delineating Wnt signaling in mammalian systems. Accordingly, we have applied Drosophila to elucidate the mechanism of action of IMiDs on Wnt signaling. Drosophila fed IMiDs present with morphological phenotypes that precisely replicate wingless (Wg) pathway mutants suggesting that IMiDs directly inhibit Wg/Wnt signaling in Drosophila. Furthermore, using epistasis analysis, we show that thalidomide represses Dll expression in WT Drosophila however genetic mutants lacking Sgg/GSK-3 activity fail to respond suggesting that functional GSK-3 is required for biologic activity. We show that IMiDs disrupt membrane localization of Sgg indicating that the bioactivity of IMiDs is achieved through the translocation and potentiation of Sgg/GSK-3 in Drosophila. Finally, we demonstrate that IMiDs fail to induce GSK-3 translocation in pomalidomide resistant myeloma cell lines and remarkably in a Phase II clinical trial of single agent lenalidomide for CLL, GSK-3 localization predicted for clinical response. In summary, our results indicate that IMiDs target GSK-3 function and identify GSK-3 as a potential biomarker of clinical response.

O-08

A PHASE III STUDY OF ENOXAPARIN VS ASPIRIN AS THROMBOPROPHYLAXIS FOR PATIENTS WITH NEWLY DIAGNOSED OF MULTIPLE MYELOMA TREATED WITH LENALIDOMIDE-BASED REGIMENS.

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Preliminary studies on multiple myeloma (MM) patients (pts) treated with a combination of lenalidomide and dexamethasone have shown an increased risk of thrombosis (between 11% and 75%). This randomized phase III trial compared the efficacy and safety of low-molecular weight heparin (LMWH) or aspirin (ASA) as thromboprophylaxis in newly diagnosed MM pts younger than 65 yrs, treated with lenalidomide-based regimens. A total of 402 transplantation candidates received four 28-day cycles of lenalidomide (25 mg d 1-21) and low-dose dexamethasone (40 mg d 1,8,15,22) (Rd) as induction and randomized to consolidation with six 28-day cycles of melphalan (0,18 mg/Kg d 1-4), prednisone (2 mg/Kg d 1-4) and lenalidomide (10 mg d 1-21) (MPR) or tandem melphalan 200 mg/mq with stem-cell support (MEL200). Eligible pts were randomly assigned to receive LMWH (Enoxaparin 40 mg/d, N=166) or ASA (Aspirin 100 mg/d, N=176) during Rd induction and MPR consolidation. The incidence of any G3-4 thrombotic events occurred early during induction (median 1,3 months) in 2 (1,20%) and 4 (2,27%) pts in the LMWH and ASA groups, respectively (risk difference= -1.07%; 95%CI= - 3.86% to 1.72%, p=0.452). Only one thrombotic event was detected during MPR consolidation. Deep vein thrombosis were equally distributed (2 and 2 pts) while 2 pulmonary embolism were observed only in the ASA group. Only 1% of minor bleeding was seen. In conclusion our data indicate a low overall incidence of thrombosis and suggest that both LMWH and ASA have a similar thromboprophylactic effect in lenalidomide treated MM pts.

O-09

MINIMAL RESIDUAL DISEASE (MRD) ASSESSMENT USING MULTIPARAMETER FLOW CYTOMETRY (MFC) PREDICTS OUTCOME IN BOTH INTENSIVELY AND NON-INTENSIVELY TREATED PATIENTS: RESULTS FROM THE MRC MYELOMA IX TRIAL

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The purpose of this study was to evaluate the impact of MRD on outcome in both younger patients treated with autologous transplantation and older patients treated with less intensive regimens. In an analysis of 711 intensively treated patients MRD was assessed following induction with either CVAD or CTD and at day 100 post HDM. The presence of MRD at day 100 (demonstrable in 66%) was highly predictive of outcome (PFS 21 months versus 39 months for MRD- patients, p=0.0001). A particularly favourable outcome was demonstrable in the subset of patients who became MRD- at the end induction therapy (PFS 46 months, p=0.0015). When the effect of maintenance thalidomide was considered the PFS was shortest in those MRD+ patients who did not receive maintenance and longest in those who were MRD- and received thalidomide (p=0.004). Subsequent analyses demonstrated a consolidation effect in that 32% of MRD+ pts who received maintenance became MRD-. Similarly a maintenance effect was also demonstrable as 80% of MRD- patients who received maintenance remained MRD- versus 46% for no maintenance (p=0.01). MRD was also assessed following the completion of therapy (CTDa or M&P) in 510 non-transplant eligible patients. Only 8% became MRD- but a significantly

improved PFS was demonstrated (p=0.028). MRD assessment using MFC is highly predictive of outcome in the context of both intensive and non-intensive therapies. The effects of maintenance strategies can also be evaluated and our data would suggest that maintenance thalidomide can eradicate MRD in a significant proportion of patients.

O-10

PROGNOSTIC RELEVANCE OF 18F-FDG PET/CT IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS RECEIVING UP-FRONT AUTOLOGOUS STEM-CELL TRANSPLANTATION (ASCT): A PROSPECTIVE STUDY

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We prospectively analyzed the prognostic relevance of PET/CT at diagnosis, after induction and after ASCT in 192 newly diagnosed MM patients. At baseline, 44% of the patients showed ≥ 3 focal lesions (FLs), 46% had SUV values > 4.2 and in 6% extramedullary disease (EMD) could be detected. These 3 variables adversely affected 4-year estimates of PFS (≥ vs < 3 FLs: 43% vs 65%, P= 0.01; SUV > vs ≤4.2: 43% vs 64%, P= 0.008; presence vs absence of EMD: 25% vs 65%; P= 0.0008) and OS (≥ vs < 3 FLs: 77% vs 90%, P= 0.03; presence vs absence of EMD: 60% vs 89%; P= 0.0008). Persistence of severe FDG uptake after induction predicted for shorter PFS (P= 0.004). After 3 months from ASCT, PET/CT was negative in 65% of the patients whose 4-year PFS and OS estimates were superior in comparison with those of patients with positive PET/CT (PFS: 62% vs 43%, P= 0.02; OS: 88% vs 75%, P=0.02). In multivariate analysis, both severe PET/CT involvement at diagnosis (SUV > 4.2 and/or EMD) and persistence of FDG uptake after ASCT were independent predictors of worst PFS (SUV > 4.2= HR: 2.0, 95%CI: 1.13-3.72; EMD= HR: 15.0, 4.0-55.8; FDG uptake after ASCT= HR: 2.12, 1.19-3.77) and OS (EMD= HR: 6.99, 2.28-21.46; FDG uptake after ASCT= HR: 3.57, 1.03-12.39). PET/CT involvement at diagnosis and after ASCT is a reliable predictor of prognosis in autografted MM patients. In particular, post-ASCT complete FDG suppression is associated with extended PFS and OS.

O-11

IMPROVED PROGRESSION FREE SURVIVAL WITH BORTEZOMIB CONSOLIDATION AFTER HIGH DOSE MELPHALAN; RESULTS OF A RANDOMIZED PHASE III TRIAL

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A randomized phase III trial was designed to explore the effect of consolidation with single agent bortezomib, given from 3 months after high dose melphalan with autologous stem cell support (ASCT) to bortezomib naïve patients. Between November 2005 and April 2009, 370 were randomized to either no consolidation therapy or to bortezomib consolidation which was given in a dose of 1.3 mg/m^2 twice weekly in a 3 week schedule for the first 2 cycles. In the following four cycles, bortezomib was given once weekly in a 4 week schedule for a total of 20 injections over 21 weeks. All analyses were performed on an intention to treat basis. The mean total dose of bortezomib given was 82% (median 90%) of the planned dose. At the time of randomization the proportion of patients in CR/nCR was 20% in the bortezomib group and 21% in the control group and patients in ≥ VGPR were 39% in both groups. *Results.* The median progression free survival measured from the time of randomization was 27 (95% confidence interval 24-29) months for patients in the bortezomib group compared to 20 (95% confidence interval 17-23) months for patients in the control group, p = 0.02. The proportions of patients achieving ≥ VGPR were 70% in the bortezomib group versus 58% in the control group, p = 0.01. The 2-years OS is 90% in both groups. *Conclusions.* Our results indicate that consolidation with bortezomib given as a single agent after high dose melphalan is feasible and does prolong progression free survival after ASCT.

O-12

A PHASE 1/2 MULTI-CENTER, RANDOMIZED, OPEN LABEL DOSE ESCALATION STUDY TO DETERMINE THE MAXIMUM TOLERATED DOSE (MTD), SAFETY, AND EFFICACY OF POMALIDOMIDE (POM) ALONE OR IN COMBINATION WITH LOW-DOSE DEXAMETHASONE (DEX) IN PATIENTS (PTS) WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM) WHO HAVE RECEIVED PRIOR TREATMENT (TX) THAT INCLUDES LENALIDOMIDE (LEN) AND BORTEZOMIB (BORT)

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Background: POM is a distinct IMiD® immunomodulatory drug with potent anti-proliferative and pro-apoptotic activities in vitro, which is effective in pts with relapsed MM after multiple lines of tx. This Ph1/2 study evaluated MTD, safety, and efficacy of POM ± 40mg dex/wk in pts with RRMM after ≥2 prior regimens, including Bort and Len. Results from Ph1 and preliminary results from Ph2 are reported. *Methods:* In Ph1, 28-day cycles of POM (2-5mg; D1-21) with the option to add dex 40mg/wk after 4 cycles if no response or progression. All pts received thromboprophylaxis. In Ph 2, pts were randomized to receive POM at MTD (4mg) ± dex 40mg/wk. *Results:* Enrollment was completed with 38 and 221 pts in Ph1 and Ph2. Data from 28 evaluable pts in Ph1 and 120 pts in Ph2 (all pts starting tx as of April 30, 2010; median follow up 27 wks) are presented. Median number of prior tx was 6 (range 2-17) and 5 (range 2-13), respectively. Neutropenia (53% and 42%), infections (30% and 31%), thrombocytopenia (16% and 22%), and anemia (21% and 20%) were the most common Gr 3/4 AEs in Ph1 and Ph2, respectively. In Ph 1, partial response or better (≥PR) was 25%, with minimal response or better (≥MR) 50%. In Ph2, ≥PR is 25%, with ≥MR 38% reported to date. In Ph1, median duration of response, PFS, and overall survival were 20.1 wks, 20.1 wks, and 79.6 wks each. *Conclusions:* POM 4mg/day on D1-21 of each 28-day cycle with or without dex shows promising efficacy and manageable toxicity in pts who have RRMM, and have resistant disease after multiple lines of tx, including both Len and Bort.

O-13

RESULTS OF PX-171-003-A1, AN OPEN-LABEL, SINGLE-ARM, PHASE (PH) 2 STUDY OF CARFILZOMIB (CFZ) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (R/R MM)

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Introduction: CFZ is a highly selective proteasome inhibitor in development for treatment of MM. PX-171-003-A1 was an open-label, single-arm ph 2b study in pts with R/R MM. *Materials and Methods:* Pts with ≥2 prior therapies, including bortezomib (BTZ) and lenalidomide or thalidomide, received CFZ on Days 1, 2, 8, 9, 15, 16 of each 28-day cycle (C), at 20 mg/m^2 in C1, escalating to 27 mg/m^2 for C2–12. Primary endpoint was overall response rate (ORR). Secondary endpoints were clinical benefit response (CBR), duration of response (DOR), overall survival, time to progression, progression-free survival, and safety. *Results:* 257/266 enrolled pts were response-evaluable. Median prior lines of therapy was 5 (range 1–20); 99.6% received prior BTZ. Median ORR was 24.1%; median DOR was 8.3 mo (95% CI 5.6, 9.2). CFZ was effective despite baseline peripheral neuropathy (PN) (see Table).

Response category	*All pts (N=257) n (%)**	*Pts with baseline PN (N=202) n (%)**
Complete response	1 (0.4)	1 (0.5)
Very good partial response	13 (5.1)	10 (5.0)
Partial response	48 (18.)	37 (18.3)
Minimal response	26 (10.1)	20 (9.9)
Stable disease	89 (34.6)	67 (33.2)
Progressive disease	69 (26.8)	57 (28.2)
Not evaluable	11 (4.3)	10 (5.0)
Overall response rate (≥PR)	62 (24.1)	48 (23.8)
95% confidence interval	19.0, 29.8	18.1, 30.2
Clinical benefit response (≥MR)	88 (34.2)	68 (33.7)
95% confidence interval	28.5, 40.4	27.2, 40.6

**Response as assessed by Independent Review Committee.*

27 pts (10%) completed all 12C and continued onto extension study PX-171-010. The most common adverse events (AEs) Grade ≥3, regardless of relationship to CFZ, were thrombocytopenia (27%) and anemia (22%). <1% of pts had Grade 3/4 PN. *Conclusions:* Single-agent CFZ achieved durable responses in pts with R/R MM. CFZ was well tolerated; there were no unexpected toxicities, AEs were manageable, and worsening PN was uncommon. Pts entering the study with baseline PN had an ORR of 23.7% suggesting that baseline PN does not preclude CFZ treatment and response. Cumulative AEs were not observed, suggesting the potential for prolonged use of CFZ for MM.

0-14

DECLINING MYELOMA MORTALITY RATES IN THE UNITED STATES FOLLOWING INTRODUCTION OF NOVEL THERAPIES

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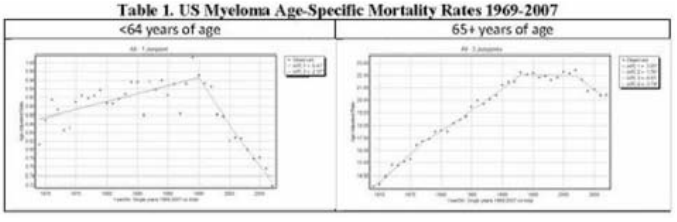
Improved survival of young patients (<65) with multiple myeloma (MM) has resulted from usage of oral melphalan and prednisone, autologous stem cell transplantation and introduction of the novel agents (thalidomide, bortezomib and lenalidomide). This success is evident in clinical research trials but any influence on the MM general population is unknown.We studied a national database to analyze the impact of the introduction of the novel agents on survival rates for MM patients of all ages. Death records from the US National Center for Health Statistics were used to characterize time trends in MM mortality rates in the United States (US) during the period 1969-2007. Temporal trends in MM mortality rates were characterized with joinpoint regression techniques. In the US population under 65 years of age, MM mortality increased from 1969-1995 (Annual Percent Change (APC) =0.4; p<0.05) and decreased rapidly thereafter (APC =-2.4; p<0.05). Among those 65 years of age and older, increasing MM mortality rates from 1969-93 were followed by a plateau during the period 1993-2002; rates among the elderly declined after 2002 (APC =-1.7; p<0.05).

Table 1. US Myeloma Age-Specific Mortality Rates 1969-2007



Declining MM mortality rates in young patients were observed during a time period after bone marrow transplantation became the preferred therapy for patients in this age group. Similarly, declining MM mortality rates for elderly patients were observed shortly after thalidomide was licensed by the FDA for treatment of this disease in 2001. Conclusion: the novel agents are contributing to improved survival for myeloma patients of all ages.

POSTERS ONLY

P-001

17 MYELOMA CELL SURVIVAL GENES, IDENTIFIED IN COCULTURES, ARE ASSOCIATED WITH PATIENT POST RELAPSE SURVIVAL AND PRESENT TARGETS FOR THERAPY

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Interaction with osteoclasts and mesenchymal stem cells supports survival of primary myeloma cells. We identified changes in global gene expression profiling (GEP) of freshly isolated myeloma cells after coculture with osteoclasts or with mesenchymal stem cells. Interaction with osteoclasts induced expression changes in 675 genes and with mesenchymal stem cells changed the expression of 296; 58 genes were similarly changed in both coculture systems. To determine if these genes revealed potential therapeutic targets, we analyzed whether changes in the expression of these genes at relapse were associated with post relapse survival of 71 patients with GEP at baseline and at first relapse. We also determined whether the level of expression of these genes at first relapse was associated with post relapsed survival of 127 patients. All patients were treated on TT2 protocol. While the change of expression (relapse/baseline signal) of 7 genes, dichotomized at the median, was marginally predictive of survival of the 71 patients (>40% misclassified), expression of 17 genes, dichotomized at the median, was significantly predictive of post relapse survival of the 127 patients (log-rank p=0.0001, median survival 13 vs. 61 mo, HR 4.4). We validated the model on 32 relapsed patients after TT3 therapy (p=0.003, median 8 vs. 25 mo, HR 3.9), and 98 patients who relapsed after various regimens (p=0.0054, median survival 15 vs. 57 mo, HR 2.2). We identified survival associated genes whose expression affect post relapse survival and which present potential targets for myeloma therapy.

P-002

ANALYSIS OF IGG AND IGA MONOCLONAL GAMMOPATHIES BY NEPHELOMETRIC MEASUREMENT OF INDIVIDUAL IMMUNOGLOBULIN κ/λ RATIOS

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Availability of antibodies which bind to conformational epitopes spanning the junctional regions between bound κ or λ light chains and their respective heavy chain partners has allowed the specific measurement of serum IgGκ, IgGλ, IgAκ and IgAλ concentrations. In turn, this has enabled calculation of IgGκ/IgGλ and IgAκ/IgAλ ratios (heavy/light chain or HLC ratios) for individual patients (Bradwell et al. Clin Chem 2009; 55: 1646). In this study, diagnostic value of HLC ratios was compared with serum protein immunofixation (IFE) results. Fresh and archived, frozen sera from 31 patients with monoclonal gammopathy including 25 with multiple myeloma were assayed. Serum protein electrophoresis and IFE were performed using HYDRASYS 2 apparatus (Sebia) and antisera from the same company. Igκ/λ pairs concentrations were measured on Siemens BNII nephelometer, using reagents from Binding Site. IFE and HLC ratio results were concordant for 24 of the 31 IFE – monoclonal protein positive samples. IFE detected 2 "minor" IgGκ bands (1 oligoclonal after ASCT, 1 reactive) and 1 IgAλ with polyclonal background that were not detected by HLC assay. In 5 cases with diclonal IgGκ + IgGλ gammopathy revealed in IFE, HLC ratios were normal and in 3 cases with diclonal IgG + IgA gammopathy, including 2 IgGκ + IgAλ cases and 1 IgGκ + IgAλ, HLC ratios were concordant with IFE. Conclusion. HLC assay cannot replace IFE although provides numerical results.

P-003

EVOLUTIONARY SEQUENCE OF CYTOGENETIC ABERRATIONS DURING THE ONCOGENESIS OF PLASMA CELL DISORDERS. DIRECT EVIDENCE AT SINGLE CELL LEVEL

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Only indirect evidence is available regarding the exact temporal sequence of cytogenetic aberrations during the oncogenesis of plasma cell disorders (PCD). We investigated bone marrow specimens from 185 patients with PCD by fluorescence in situ hybridization (FISH). The Δ13, p53 deletion and IGH disruption were found in 47.2, 7.5 and 58.9% of cases, respectively. Incidences of IGH/FGFR3, IGH/CCND1 and IGH/c-MAF aberrations within the IGH positive group were 22.6, 21.7 and 6.6%, respectively. In 25 cases harboring at least two specific aberrations, combined FISH analysis has been performed at single cell level. Clonal evolution was observed in 16% of cases. Recurrent IGH translocations were the earliest aberrations followed by Δ13 and p53 deletion. Our results not only support the current model of the oncogenesis of PCD but provide the first direct evidence at single cell level. In 25% of IGH positive cases examined by combined FISH analysis the recurrent IGH translocations were presented only in a subset of purified plasma cells. Subsequently, we extended our observation to all 185 patients screened in this study. In 21.8% of cases harboring specific recurrent IGH translocation (IGH/FGFR3 20.0%, IGH/CCND1 16.7% and IGH/c-MAF 28.6%) the aberration was presented in less than two-thirds of purified plasma cell population. The presence of more than one IGH translocations was excludable in these cases. Recurrent IGH translocations are early genetic events during the oncogenesis of PCD but their initiating role is questionable in a not negligible subset of cases.

P-004

THE ROLE OF TJP1 IN SENSITIZING MULTIPLE MYELOMA CHEMOTHERAPY AGENTS

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Chemotherapy agents are extremely important in the treatment of liquid malignancies, including multiple myeloma (MM). Unfortunately, chemotherapy resistance in these situations is the most significant cause of treatment failure. Thus, identification of novel genes that plays a crucial role in MM chemosensitivity may greatly help to predict, treat, or circumvent chemoresistance in MM and improve clinical outcomes. Toward this purpose, we have successfully developed a high-throughput siRNA based functional target validation approach and identified 34 potential chemosensitivity genes, in which one gene identified with the top frequency was TJP1 (Tight junction protein 1). Our further studies on TJP1 suggested that targeting TJP1 led to tumor cell resistant to several chemotherapy agents, including doxorubicin (Dox), cisplatin (Cis), methotrexate (MTX), and bortezomib. Further analysis with 264 bortezomib treated MM patients indicated that expression level of TJP1 correlated with patient response to bortezomib. Two clones and pooled RPMI 8226 MM cell line, which were developed against bortezomib treatment in our lab, showed loss of TJP1 expression, suggesting a role of TJP1 may play in bortezomib resistance development. More importantly, TJP1 targeting in myeloma cells resulted in cell resistance to bortezomib treatment. Together, these findings have led us to propose that TJP1 is important determents of myeloma chemotherapy.

P-005

CLINICAL IMPACT OF LOW PLASMA MIR-92A LEVELS IN MONOCLONAL GAMMOPATHIES

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Purpose: The aim of this study is to evaluate the clinical relevance of the miR-92a in plasma obtained from patients with monoclonal gammopathies, because miR-17-92 polycistronic miRNA plays a crucial role in neo-angiogenesis and lymphoid development and neoplastic transformation. *Methods:* We measured plasma miR-92a values (miR-92a/miR-638) using qRT-PCR in 166 patients with monoclonal gammopatyies, and compared results with those in normal controls. The association between plasma miR-92a levels and clinical features, including therapeutic response, was also evaluated. *Results:* Plasma miR-92a values were significantly low in newly-diagnosed symptomatic MM compared with normal subjects (P < .0001), irrespective of CRAB symptoms . However, no significant differences of miR-92a levels were found between smol-

dering myelomas (P = .208) or MGUS (P = .894) when compared with healthy subjects. The cut-off level of plasma miR-92a/miR-638 in all MM patients at diagnosis was 0.2331, and the sensitivity of miR-92a/miR-638 level at the time of diagnosis of MM was 89.29% (95% CI : 78.12% to 95.97%). The plasma miR-92a of MM at the complete response recovered to similar level of healthy subjects. In contrast, the plasma miR-92a was still lower even in MM with VGPR than in healthy subjects (P = .0007). *Conclusion:* This is a first report of detectable miRNAs in plasma obtained from multiple myeloma and its related diseases. The current results indicate that the plasma miR-92a value could be a novel biomarker not only for diagnosis but also for monitoring myeloma patients after chemotherapy.

P-006

GROWTH DIFFERENTIATION FACTOR 15 IN MULTIPLE MYELOMA: IN VITRO BIOACTIVITY ON MYELOMA CELLS AND PROGNOSTIC SIGNIFICANCE IN PATIENTS

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We previously reported that growth differentiation factor 15 (GDF15) is overproduced by bone marrow mesenchymal stem cells from patients with multiple myeloma (MM) as compared with those from healthy individuals, with pathophysiological consequences that remain undefined. We report here that in vitro, GDF15 increased survival of a stroma-dependent (MOLP-6) MM cell line but not that of a stroma-independent (MM1.S) line. This GDF15 bioactivity involved Akt-dependant signaling, with T308 Akt phosphorylation in MOLP-6 and primary MM cells but not in MM1.S cells. In addition, GDF15 could confer resistance to melphalan, bortezomib and to lenalidomide in both MM cell lines, with an Akt-dependant signaling for MOLP-6, but not for MM1.S. Considering these results, we then tested the significance of a plasma concentration of GDF15 (pGDF15) in 131 MM patients and found that it was correlated with the main prognostic factors of the disease : International Staging System, Durie Salmon disease stage, ß2 microglobulin level, haemoglobin, presence or absence of del13, creatinemia, calcemia, serum M protein and albumin. For the 81 patients with high pGDF15 level, the probabilities of event-free and overall survival 30 months after diagnosis were 50% and 75%, respectively. For the 50 patients with low pGDF15 level, these probabilities were 80% and 97%, respectively (P<0.0045 and P<0.013, respectively). Microenvironment-derived GDF15 is a survival and chemoprotective factor for MM cells and pathophysiologically linked to initial parameters of the disease and patient survival.

P-007

MULTIPLE MYELOMA PLASMA CELL (MM PC) CO-EXPRESSION OF HEPATOCYTE GROWTH FACTOR (HGF) ISOFORMS AND OF THE RECEPTOR CMET IS ASSOCIATED WITH OVERALL SURVIVAL (OS) IN MM PATIENTS

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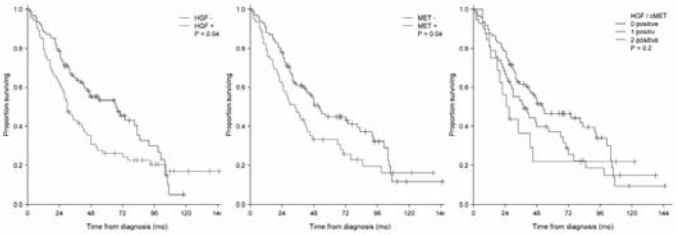
Aberrant production of HGF/cMet in MM PCs has been described. Studies on HGF and MM have focused on measuring full-length or all isoforms of HGF expression. However, naturally occurring shorter HGF isoforms are known to work as partial inhibitors. We examined the total and HGF isoforms and cMET expression levels in isolated MM PC of >150 newly diagnosed patients with MM, MGUS and healthy volunteers(HV). *Methods:* Aberrant MM PCs were sorted by fluorescence activated cell sorting, a cDNA archive was generated by global reverse transcription and amplified (Rasmussen et al, BJH 2003). Quantitative PCR was performed using β-actin as internal reference gene. Determination of positivity or negativity was made from a cut-off-value at 10E-05. The HGF primers covered full-length HGF (mRNA1 and 3), HGF2 primers mRNA 2 and 4 and HGF5 primers mRNA 5. The MM patients were treated with either HD melphalan with ASCT or melphalan-prednisone according to age. *Results:* ≥1 of the HGF isoforms were found to be expressed in PCs from 43% of MM patients, 32% of MGUS and 0% of HV. Expression of ≥1 HGF isoform was associated to an adverse OS (p=0.04). Looking at isoforms, only the quantitative expression of transcript variant 5 was associated with an adverse OS (p=0.02). Expression of cMET was observed in 38% of MM patients and associated to an adverse OS (p=0.04). Expression of total HGF was correlated with the expression of cMET (p=0.002). Thus, the co-expression of total HGF/cMet in MM patients seems to identify a group with an adverse prognosis, possibly through autocrine mechanisms.



P-008

FUNCTIONAL PROPERTIES OF CD138- AND CD138+ CELLS IN MULTIPLE MYELOMA: STUDY IN THE 5T33MM MODEL

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Multiple myeloma (MM) contains a heterogenous mix of tumor cells including CD138- and CD138+ cells. While some studies described that CD138- cells are a minor population containing MM clonogenic and therapy-resistant cells, others show that only CD138+ cells are able to proliferate and engraft. These studies clearly demonstrate that phenotypical and functional different tumor populations exist, but results are controversial. We investigated CD138- and CD138+ tumor populations in the murine syngeneic 5T33MM model. Both in vitro (clonogenic assay) and in vivo engraftment studies suggest that both populations form colonies and induce MM disease in a syngeneic environment, albeit CD138+ cells more apparent than CD138- cells. Real-time PCR for Blimp-1 and bcl-6 indicate that CD138- cells are less differentiated than CD138+ cells. We investigated in vitro the sensitivity of CD138- and CD138+ cells to drugs (bortezomib (Bz), MG132, melphalan, LBH589, 17AAG) by measuring cell viability and caspase3/7 activation. CD138- cells tend to be more resistant to drugs. Subsequently, we investigated, the phenotype of 5T33MM cells after in vivo treatment with Bz. The percentage of CD138- 5T33MM cells was significantly increased when mice were treated with Bz compared to vehicle. Preliminary results indicate that CD138- cells express more ABCG2, an ABC transporter involved in resistance. In conclusion, both CD138+ and CD138- cells are important in MM development with the CD138- cells being more therapy-resistant indicating that CD138- MM cells should be included in further investigations.

P-009

IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISMS IN GENES INVOLVED IN INFLAMASSOME AND STRUCTURAL CYTOPLASM FRAMEWORK IN SURVIVAL AND PROGRESSION IN PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Polymorphisms in proteins involved in cytosolic macrocomplex with regulatory functions in the immune system have previously shown to have prognostic impact after stem cell transplantation. Otherwise, SNPs in miRNA proteins pathway and in the target genes binding sites have been observed to miRNAs-related alterations in solid tumors with different prognostic implications. In both cases, no experience in multiple myeloma (MM) has been reported. *Methods:* 137 patients with chemosensitive MM (73M/64F, median age 55 years) intensified with autologous stem cell transplantation (SCT) have been studied. The genes evaluated were NLRP2, NLRP3, ATBF1 and EP300 for innate immune system and KRT81, AFF1 and FAM179b for miRNA target genes. *Results:* Overall survival was significantly longer in patients with SNPs in KRT81 (rs3660; p=0.029) and NLRP2 (rs1043684; p=0.053)(Figures 1 and 2). There was a trend toward a longer in progression-free survival for KRT81 (p=0.17), but not for NLRP2. A polymorphism in EP300 (rs20551) was associated with higher incidence of implant syndrome (42% vs. 12%, p=0.015). No other associations with prognosis or toxicities were observed. *Conclusion:* NLRP2 was only associated with OS, as previously reported in the allogeneic SCT setting. In contrast, EP300 and NLRP3 did not show any relation with progression and mortality. This is the first report in haematological malignancies that a polymorphism in the miRNA binding site in keratin gene (KRT81) has been associated with prognosis. Further studies on proliferation and miRNA interaction are encouraged.

Figure 1. Overall survival after autologous stem cell transplantation according to the presence of polymorphism rs3660 in KRT81. (C/C= SNPs, C/G=heterozygous SNP, G/G=wild type)

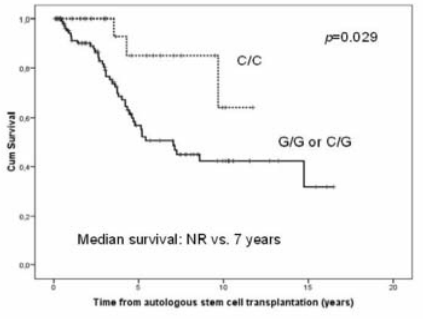
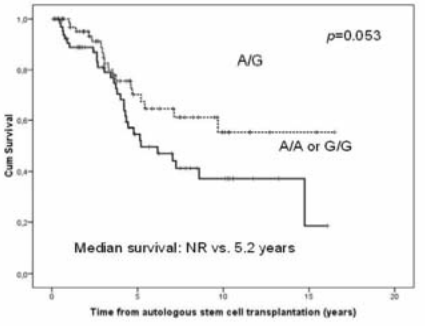


Figure 2. Overall survival after autologous stem cell transplantation according to the presence of polymorphism rs1043684 in NLRP2. (A/A= SNPs, A/G=heterozygous SNP, G/G=wild type)



P-010

DNA REPAIR SINGLE NUCLEOTIDE POLYMORPHISMS IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE: IMPACT ON RESPONSE AND SURVIVAL

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Background: Thalidomide was the first of the so-called new drugs incorporated in the treatment of multiple myeloma (MM). The analysis of polymorphisms in drug metabolism pathways could help to identify patients with possible different treatment response and outcome. *Methods:* Single nucleotide polymorphisms (SNPs) in 12 genes involving multidrug resistance, drug metabolic pathways, DNA repair systems and cytokines were examined in 28 patients (13M/15F; median age 59 years) with relapsed/refractory multiple myeloma (MM) treated with single agent thalidomide and the results were correlated with response, toxicity and overall survival (OS). *Results:* The response rate to thalidomide was higher in patients with hetero- (AC) or homozygous (CC) SNPs in ERCC1 (rs735482) than in those with wild type (AA), with the ERCC5 heterozygous SNP rs17655 (CG) than those with the homozygous SNP (GG) or wild type (CC), and with heterozygous XRCC5 (AG) polymorphism rs1051685 than those with wild type (AA). Longer OS was associated with the homo- and the heterozygous SNP in ERCC1 (AC + CC vs. AA; p=0.005) and with the heterozygous SNP in XRCC5 (rs1051685) (AG vs. AA; p=0.02) (Figures 1 and 2). The heterozygous polymorphism in GSTT1 (CT vs. TT) was associated with a lower frequency of thalidomide-induced peripheral neuropathy (p=0.04). *Conclusion:* SNPs in ERCC1 and XRCC5 were strongly associated with higher response rate and longer OS to thalidomide in patients with relapsed /refractory MM. SNPs in ERCC5 were also associated with greater response rate.

Figure 1. Overall survival of relapsed/refractory MM patients treated with thalidomide according to ERCC1 (rs735482) genotype (AA=wild-type, CC=homozygous SNP, AC=heterozygous SNP).

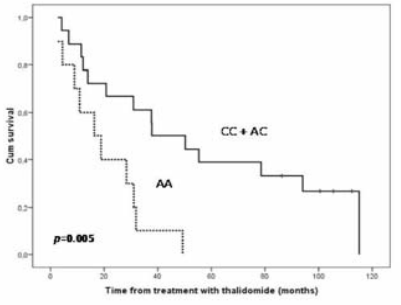
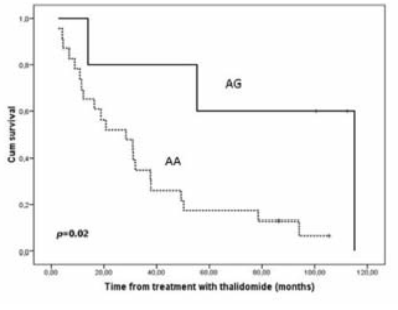


Figure 2. Overall survival of relapsed/refractory MM patients treated with thalidomide according to XRCC5 (rs1051685) genotype (AA=wild type, AG=heterozygous SNP).



P-011

PROGRESSION THROUGH MGUS TO MULTIPLE MYELOMA DRIVES DIFFERENTIAL ACCUMULATION OF T CELL SUBSETS VIA BONE MARROW EXPRESSION OF CCR4 AND CXCR3 LIGANDS

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Multiple Myeloma is a malignant disease of bone marrow plasma cells characterised by immunodeficiency and T cell abnormalities. We explored how changes in chemokine levels reflect chemokine receptor expression on T cell populations in the blood and bone marrow of normal, MGUS and myeloma patients to assess the impact of altered chemokine gradients on T cell distribution. We also investigated T cell adherence and migration using bone marrow mesenchymal stem cells (BMMSC) from these patient groups. CXCR3 ligands, CCR4 ligands, CXCL8 and CXCL12 were significantly increased in the blood and bone marrow often progressively from MGUS to myeloma. The frequency of CCR4 expressing T cells in the bone marrow was higher in myeloma patients for both CCR4+ CD4+ (p=0.0002) and CCR4+ CD8+ T cells (p=0.0053). The CCR4+ Th17 CD4+ population showed a significant reduction in the blood paralleling an increase in the bone marrow. The frequency of both CXCR3+ CD4+ and CXCR3+ CD8+ T cells were reduced in the blood but maintained in the bone marrow in both MGUS and myeloma. Functional studies showed increased T cell adherence and migration to myeloma BMMSCs compared with that towards control BMMSC. Chemokine gradients and T cell migration are factors in T cell dysfunction in the myeloma microenvironment. Here we show CXCR3 chemokines and CCR4 chemokines are important to the altered T cell distribution. Therapies targeted against the excess chemokines may have beneficial effects directly against the tumour cells expressing chemokine receptors and in reversing the distorted T cell environment.

P-012

TARGETING BTK AS A TREATMENT FOR MYELOMA STEM CELLS

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Evidence of the existence of a myeloma stem cell (MMSC) has been provided; MMSC are enriched in the CD138- and CD19+/CD27+ fraction, indicating a memory B-cells phenotype. Bruton's tyrosine kinase (BTK) is critical for B cell development, differentiation, and signaling. The loss-of-function mutations of BTK results in lack of mature B lymphocytes and immunoglobulins (Igs). In this study, MMSC (CD138- fraction) were isolated from 4 MM cell lines. Stem cell characteristics were verified by clonogenic in vitro assays and tumor formation in NOD/SCID mice. The real-time PCR analyses indicated that BTK levels were significantly higher levels in MMSC than in CD138+ bulk MM cells. Over-expressing BTK in CD138+ cells resulted in enhanced clonogenic potential, increased drug resistance and increased Wnt, Hh and Notch activity, as evidenced by higher expression levels of ?-catenin, Smo and GLI1, and Notch1, respectively. Furthermore, increased BTK modulated myeloma cell adhesion and migration. Whereas knockdown of BTK by shRNA or a BTK inhibitor LFM-A13 induced MM cell death, growth inhibition, decreased clonogenesis, and altered cell adhesion and migration. Our results indicate that BTK may play an important role in maintaining myeloma stemness and myeloma cell adhesion and migration. It represents a logical target to attack both myeloma stem cells and the bone marrow microenviroment.

P-013

MICRORNAS CHANGES OCCUR IN MULTIPLE MYELOMA CELLS IN THE CONTEXT OF BONE MARROW (BM) MILIEU

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Introduction: Primary MM cells present with a specific miRNA signature as compared to normal plasmacells. Nevertheless, miRNA changes that occur in MM cells in the context of BM milieu have not been examined yet. *Methods:* miRNA profiling has been performed on MM cell lines either alone or in co-culture with BM stromal cells, MM primary CD138+ cells, normal CD138+ cells. In vitro and in vivo functional studies were performed on miRNA-15a- and -16-1-precursors-transfected cells. In vivo MM cell growth has been evaluated by either using in vivo imaging model, bioluminescence. Angiogenesis has been studied both in vitro and in vivo. Result: Pre-miRNA-15a- and -16-1-transfected MM cells showed decreased DNA synthesis, decreased cyclinD1/cyclinD3/cdk6/pRb protein expression and cell cycle arrest, vs scramble probe-transfected MM cells. NFkB pathway inhibition was also observed. Inhibition of MM cell growth was confirmed in vivo; anti-angiogenic properties of miRNA-15a and -16-1 were validated both in vitro and in vivo. miRNA profiling of MM cells cultured with BMSCs differ from MM cells which were not grown in contact with BMSCs: predicted targeted genes of increased miRNAs included negative regulators of NFkB, PI3K/Akt/mTOR, and MAPK/ERK signaling pathways; as well as tumor suppressors, pro-apoptotic factors and cyclin-dependent kinases inhibitors. Summary: miRNAs play a key role in MM pathogenesis by regulating plasmacell tumor clone growth. BMSCs exert a modulatory effect on miRNA profiling in MM cells, which results in promoting MM cell growth and inducing MM cell survival.

P-014

APRIL (A PROLIFERATION INDUCING LIGAND) LINKS MYELOPOIESIS TO NORMAL AND MALIGNANT PLASMA CELL SURVIVAL IN BONE MARROW

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Introduction: It is not fully understood why the bone marrow (BM) is so efficient in sustaining long-term survival of plasma cells (PC), a process required to maintain humoral immune memory. In the present study we analyzed the role of APRIL, a cytokine of the TNF-family, in sustaining normal and malignant PC survival in the BM microenvironment. Methods and *Results:* Staning sections of human BM with antibodies specific for either cells producing APRIL or secreted APRIL, we showed that myeloid precursors, the main hematopoietic component of BM, were the dominant producers of APRIL. We confirmed these findings by in vitro cultures and by detailed FACS analysis on BM cell suspensions, and found that early hematopoietic cytokines, including the stem cell factor, were able to induce APRIL in myeloid precursors. APRIL mediated PC survival in in vitro co-culture experiments and in a mouse model, in which we blocked APRIL with specific antibodies.To investigate the role of APRIL in multiple myeloma (MM), we used a new mouse model of MM, the MOPC-315BM model, in which a variant of the plasmocytoma cell line MOPC-315 leads to full-blown MM after in iv injection. We found that APRIL levels stayed high despite hematopoiesis impairment occurring upon BM invasion by the MOPC-315BM cells, and that APRIL-producing myeloid precursors were selectively protected during this invasion in an IL-6 dependent manner. *Conclusion:* Our study demonstrates a link between myelopoiesis and PC/MM cell survival, and points to new treatment approaches for this still incurable disease.

P-015

DLL1/NOTCH INTERACTION INDUCES DRUG RESISTANCE TO BORTEZOMIB BY TWO DISTINCT MECHANISM IN MULTIPLE MYELOMA

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One of the greatest challenges in multiple myeloma (MM) treatment is to overcome drug resistance. Dll1 is a Notch ligand expressed in bone marrow (BM) stromal cells. We have demonstrated that the Notch pathway could be activated by coculture of MM cells with Dll1 overexpressing stromal (MS5.Dll1) cells. We found that Dll1/Notch interaction could induce drug resistance to the proteasome inhibitor bortezomib both in murine 5T33MM and in human RPMI8226 MM cells. In addition, the Notch pathway inhibitor, DAPT, could increase the sensitivity to bortezomib. We observed that CD138- MM cells are less sensitive to bortezomib, have a higher Notch activation and a higher expression of ABCG2 and Cyp1a1, genes involved in drug resistance, compared to CD138+ MM cells. The Cyp1a1 activity in CD138- MM cells is much

higher than that in CD138+ MM cells. After Dll1/Notch interaction, we detected an increased percentage of CD138- MM cells and a down-regulation of CD138 mRNA expression. This could be reverted by inhibiting the Notch pathway with DAPT. Similar results were found in human RPMI8226 cells. Furthermore, we found that the anti-apoptotic proteins Bcl-2, Mcl-1, Bcl-xl were up-regulated and the pro-apoptotic protein Bim was down-regulated after Dll1/Notch interaction in CD138+ MM cells, which also can contribute to Dll1/Notch induced drug resistance. In conclusion, our data show that Dll1/Notch interaction can induce drug resistance to bortezomib, by shifting MM cells to a more resistant CD138- phenotype and by up-regulating anti-apoptotic proteins in CD138+ MM cells.

P-016

TREG NUMBER, TREG FUNCTION AND TH17 CELLS IN PLASMA CELL DYSCRASIAS

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There is conflicting data on regulatory T (Treg) cells in myeloma (MM). This is mainly due to technical differences between antibodies and permeabilisation. We used CD3+CD4+CD25h+CD127- expression to quantitate Treg numbers and an intracellular IL-17 assay to quantitate Th17 cells. Treg function was determined using CFSE tracking of Treg negative T cells stimulated with anti-CD3CD2CD28 beads +/- 1:1 flow sorted Treg cells. Tregs were increased in MM (n=32; mean=8.9%) compared to aged-matched controls (6.5%; t=3.1; p=0.009). No difference was observed for MGUS (mean=7.5%) and WM patients (mean=6.0%). Th17 cells in the blood of MM patients were decreased compared with controls (n=22) (0.7%; 2.0% (t=2.3; p=0.03). However Th17 cells in MGUS (2.2%) and WM (1.1%) were not significantly different from normal. The Treg/Th17 ratio of MM (16.1) was significantly different to the Treg/Th17 ratio of the control group (6.6) (t=4.1; p=0.0002). There was a trend for an increase in Treg numbers after IMiD therapy. The suppressive function of Tregs from MM patients (n=15) was variable. Treg function from patients on lenalidomide (n=4) was increased (mean=65%) compared with patients on thalidomide (n=3; mean=23%), other MM patients and age-matched controls (n=11; mean=31%). rhTGFb increased the suppressive capabilities and rhIL-12 reduced the function of Tregs. In conclusion the balance in Treg/Th17 cells is dysfunctional in patients with MM. Tregs are increased whereas Th17 cells are reduced. Tumour derived cytokines and therapy, especially IMiDS have a major impact on Treg function.

P-017

HLA-G CAN BE ACQUIRED BY T CELLS VIA TROGOCYTOSIS AND IS A CLINICALLY RELEVANT IMMUNOREGULATOR IN PATIENTS WITH MYELOMA

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HLA-G is a "non-classical" HLA class I molecule responsible for immune tolerance in a fetus, allograft or tumour by inhibiting the cytotoxic lymphocyte response and decreasing NK cell function. HLA-G expression is increased in various malignancies. T cells can acquire HLA-G by trogocytosis. We have studied the significance of HLA-G on malignant plasma cells and T cells of patients with myeloma (MM). The incidence of HLA-G expression on plasma cells (CD38++) was heterogeneous (0.2 to 96%; mean = 21.3%) but clinically relevant as HLA-G+ plasma cells were associated with a significant reduction in overall survival (χ2=12.4; p<0.0004) with no difference in β2M, age or M protein isotype. Low HLA-G expression was found on both CD4 and CD8 age-matched normal T cells (n=15; mean=0.19%) but 26% of MM patients (10 of 38) demonstrated a concentration of HLA-G-expressing CD3+ cells above the mean+2SD of controls (χ2=4.9;p<0.03). HLA-G was acquired by T cells in cultures (trogocytosis) from HLA-G+ plasma cells. Flow-sorted CD3+ HLA-G+ T cells significantly reduced the proliferation of CFSE-labeled CD3+ HLA-G- T cells in 4 day cultures. HLA-G+ T cell inhibition was similar to the inhibition due to CD38++ HLA-G+ plasma cells. CD3+ HLA-G+ T cells were CD25- and thus were not Treg cells. In conclusion, HLA-G expression is clinically relevant in patients with MM. HLA-G+ T cells are potent immunoregulators. Tumor escape in patients with MM is at least partly due to the inhibition of normal T cell proliferation by other T cells which acquire ectopic antigens

P-018

INCIDENCE AND RELEVANCE OF TROGOCYTOSIS IN MULTIPLE MYELOMA AND OTHER B CELL MALIGNANCIES DETERMINED BY FLOW CYTOMETRY

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Trogocytosis is the transfer of membrane proteins between cells during contact. We studied trogocytosis in patients with multiple myeloma (MM) and other B cell malignancies as it has been suggested that acquired antigens may alter T cell function and promote immune tolerance. An in vitro model of trogocytosis was established in which plasma cell lines and flow-sorted CD38++ cells of patients (n=11) or malignant B cells from CLL (CD5+CD19+) (n=4) and WM patients were biotinylated and cultured with MM or normal mononuclear cells. Acquisition of biotinylated proteins was measured by flow cytometry and confocal microscopy. There was a greater acquisition of biotinylated proteins by CD3+ cells (mean=13.6%) than B cells (mean=2.4%; t=2.80; p<0.05) or NK cells (mean=3.1%; t=2.57; p<0.05). There was no difference between biotin transferred to normal or MM T cells nor from cell lines or primary plasma cells. However <1% T cells acquired membrane fragments when cultured with biotinylated malignant B cells from patients with CLL or WM (t =3.86; p<0.05). Upon culture with biotinylated flow-sorted normal T cells, only 2% of CD38++ plasma cells acquired membrane fragments. Approximately 71% of HLA-A2 matched and 82% HLA-A2 unmatched T cells acquired biotinylated fragments. Trogocytosis is more common in MM than other B cell malignancies, is mainly unidirectional, is HLA independent and T cells are more likely to be involved than other lymphocytes. The inappropriate juxtaposition of acquired antigens on T cells may create acquired regulatory cells.

P-019

CORRELATION OF GENE EXPRESSION WITH CENTROSOME AMPLIFICATION IN MULTIPLE MYELOMA

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Introduction Centrosome amplification (CA) may lead to genomic instability of malignant plasma cells in multiple myeloma (MM). Study objective was to evaluate association of CA with expression of genes controlling centrosome duplication. *Methods* Centrin number was used to define presence of CA in PCs (CD138+) of MM patients: more than 4 signals were considered to be positive. Samples with ≥10% of PCs with >4 fluorescence signals of centrin were considered CA positive. 18 MM patients were evaluated for CA in PCs. The patients' characteristics were: males/females 9/9, median age of 69 years, newly diagnosed and relapsed patients. qRT-PCR (6 CA positive vs 12 CA negative patients) were performed on PC focusing on chosen genes. *Results* Gene expression analysis showed significant increase in relative quantification coefficient R of following genes (data presented as median [range]): AURKA 0.11 [0.02-0.26] vs 0.34 [0.11-0.9], PCNT 0.51 [0.11-1.52] vs 1.84 [0.51-3.20] and TACC3 0.2 [0.03-0.45] vs 0.82 [0.04-2.63] for CA negative vs CA positive patients, resp. (p<0.03). Also, coefficient R of genes PCNT, AURKB, AURKA, CCNB2, PLK4, HMMR, TACC3 was strongly correlated with percentage of CA positive PC (rs>0.5, p<0.05). In this study, we showed close interaction between presence of CA and expression of genes controlling centrosome duplication (PCNT, AURKB, AURKA, CCNB2, PLK4, HMMR, TACC3). Some of these genes (AURKA, PCNT and TACC3) reflect even CA positivity of patients. This work was supported by grants NT11154, NS10207, NS10406, NS10408, LC06027, MSM0021622434, GAP304/10/1395.

P-020

VDELTA-1 GAMMA-DELTA T CELL-MEDIATED KILLING OF MYELOMA CELLS: A NEW CANDIDATE FOR IMMUNOTHERAPY

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Gamma-delta T cells are potent effector lymphocytes of innate immunity and are involved in anti-tumour immune surveillance. There are two major subsets of gamma-delta T cells in humans; Vdelta1 and Vdelta2 subsets. Each subset recognize tumours by separate mechanisms involving phosphoantigens and stress-induced ligands for the activating receptor NKG2D. The Vdelta2 subset is known to recognize Multiple Myeloma (MM) but Vdelta1+ cells targeting MM has not previously been investigated. We have examined the cytotoxic potential of Vdelta1+ cells to lyse myeloma cell lines RPMI8226 and U266, and plasma cell leukaemia ARH77. Vdelta1+ cells were purified from peripheral blood mononuclear cells from healthy donors by immunomagnetic sorting and expanded (purity of 95-97%) over 3 weeks on irradiated PBMC. Vdelta1+ cells were co-cultured with target cell lines at 5:1 and 10:1 E:T ratio in a 4-hour flow cytometric killing assay. The expanded Vdelta1+ cells showed prominent anti-myeloma reactivity by specific lysis against all three cell lines, with the highest lysis of U266 cells (mean 73.23%, SD 11.9). Vdelta1+ cells isolated from peripheral blood of myeloma patient also showed high cytotoxic reactivity of 71.6% against U266 cells. Vdelta2+ gamma-delta T cell-mediated lysis was not significantly different to lysis by Vdelta1+ cells (mean 64.13%, SD 9.3). Multiple Myeloma remains incurable and most patients relapse or become resistant to conventional treatment. Our results identify myeloma-reactive Vdelta1+ gamma-delta T cells as promising candidates for a novel tumour immunotherapy.

P-021

A PROLIFERATION-INDUCING LIGAND (APRIL) ATTENUATES DEXAMETHASONE-MEDIATED APOPTOSIS OF PRIMARY CYCLIN-D2 (CCND2) EXPRESSING MULTIPLE MYELOMA (MM) CELLS

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Introduction: the TC classification identifies subgroups of patients with differing clinical features and outcomes on the basis of distinct genetic lesions. The bone marrow (BM) environment is central to MM pathogenesis and drug resistance, but much of the evidence is from human MM cell lines, derived from extra-medullary tumours, and little is known of how TC subgroup dictates drug resistance in primary tumours. APRIL is abundant in MM BM and is critical for B-cell differentiation and plasma cell survival. *Materials & Methods:* the protective effect of APRIL, in the context of anti-MM agents, was investigated in primary CD138+ MM cells, freshly isolated from MM BM. Cells were cultured in RPMI + 20% pooled MM plasma, and viability evaluated by Annexin V/PI staining. *Results:* APRIL, in the absence of anti-myeloma drugs, had no effect on survival but reduced Dex (1uM)-induced apoptosis after 48-72 hrs. The protective effect of APRIL was confined to CCND2 MM cells with t(4;14) or t(14;16) (n=8; P=0.003), with no protection seen in CCND1 MM cells +/- t(11;14) (n=12; P=0.118). APRIL-mediated protection from apoptosis was confirmed by changes in PARP cleavage and CASP 3 activity. *Conclusions:* our work indicates that APRIL protects MM cells from Dex, particularly those expressing CCND2 with IgH translocations. This segregation by genetic subtype suggests different mechanisms of drug resistance in the different TC subgroups, offering important new targets for sub-group-specific drug therapy in MM.

P-022

ISOLATION AND CHARACTERIZATION OF CD146+ MSC SUBPOPULATIONS: COMPARATIVE STUDY FROM MM PATIENT AND HEALTHY SUBJECT

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It has been demonstrated that plasmocytes and hematopoietic stem cells (HSC) co-localized in special medullar niche. Plasmocytes interact to CXCL12-expressing reticular cells and CD146+ mesenchymal stromal cells (MSC) are critical in establishment of HSC niche. We previously reported that multiple myeloma (MM) MSC are abnormal. The goal of this project is to study the CD146+ subpopulations MSC from healthy donor (HD) and MM patient. We have selected CD146High/Low clonal MSC population from the bone marrow of HD and MM patients. We studied progenitor frequency, differentiation potential, hematopoietic support activity and support of MM cells proliferation. Whatever the status of subjects, clonogenic CD146High MSC present lower level of proliferation and higher frequency of progenitor than CD146Low MSC. However, MM clones proliferate two fold lower than HD clones independently of CD146 expression. By qualitative and quantitative analysis, first results show that CD146High MSC possess higher potential to differentiate in osteoblast than CD146Low MSC. We also observed that CD146Low MSCs seem to better support hematopoiesis in vitro, as demonstrated by a better HSC proliferation with CD146Low MSC. Finally, preliminary results demonstrated that CD146High MSC induce a higher proliferation of a stroma-dependent MM cell line (MOLP-6) compared to CD146Low counterparts observable with MM MSC only. Taken together, these results demonstrate differences between bone marrow specific subpopulations CD146+ cells from HD/MM patients and allow us to have a better understanding of MM niche.

P-023

POLYCOMB TARGET GENES ARE SILENCED IN MULTIPLE MYELOMA

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We have deciphered an ISS stage III prominent under expressed gene signature in MM patients as compared to normal counterpart plasma cells using an integrative genomics approach. This dataset was found to be enriched for previously defined H3K27 tri-methylated Polycomb group (PcG) target genes in human embryonic fibroblasts. During embryogenesis the PcG proteins constitute an important part of the epigenetic memory by repressing lineage-specific developmental genes and maintaining self-renewal. Validation for enrichment of the target genes for H3K27 tri-methylation was done by chromatin immunoprecipitation (ChIP) using CD138+ isolated MM patient cells and cell lines. As the data implied that the Polycomb-targeted gene profile would be highly relevant for pharmacological treatment, we used two compounds to chemically revert the H3K27-tri-methylation mediated gene silencing in MM. The S-adenosylhomocysteine hydrolase inhibitor 3-Deazaneplanocin (DZNep) and the histone deacetylase inhibitor LBH589 (Panobinostat) reactivated the expression of genes repressed by H3K27me3, depleted cells from the PRC2 component EZH2 and induced apoptosis in human MM cell lines. LBH589 treatment in the immunocompetent 5T33MM in vivo model resulted in gene upregulation, reduction in the tumor load and increased overall survival. Collectively, our results point to a common gene signature in MM mediated by gene silencing via the PRC2 complex. Unraveling the molecular mechanisms underlying gene silencing by PcG may be helpful in understanding tumor stemness in MM and have new therapeutic implications.

P-024

COMPARISON OF STROMAL CELLS OF ADIPOSE TISSUE FROM MULTIPLE MYELOMA PATIENTS AND HEALTHY DONORS

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Introduction: Multiple Myeloma (MM) is a B-cell neoplasia causing severe and irreversible bone disease and cytopenia. We have proposed to treat those lesions with mesenchymal stem cells (MSC) of the bone marrow using their ability to differentiate into osteoblasts and to support hematopoiesis. But MM MSC are abnormal. We thus studied the adipose derived stromal cells (ASC), cells with similar properties than MSC and isolated from adipose tissue (AT), comparing ASC from MM

and healthy donors (HD). *Patients and Methods:* 1) 15 MM and HD ASC sample from subcutaneous tissue and cultured for 3 passages (P) of 21 days; 2) flow cytometry at the end of the first P for stromal, adhesion, enzymatic and plasmocyte feature; 3)osteogenic, chondrogenic, adipogenic differentiation assay; 4) Elisa measurement of IL-6, DKK-1 and GDF-15 in culture supernatant; 5) Co-culture assays of ASC with MOLP-6 (stroma-dependant MM cell line proliferation support) and CD34 cells (hematopoiesis support). *Results:* the cell culture parameters (progenitor frequency, cell population doubling and expansion capacity) didn't show any difference betwenn MM and HD ASC. In the same way, phenotypic, differentiation and hematopoiesis support assay didn't underline any difference. Interestingly there was no difference for ELISA and MOLP-6 proliferation support assay between MM and MM ASC while MM MSC datas were higher to those obtained with HD MSC in previous laboratory works. *Conclusion:* these preliminary datas suggest that MM ASC are normal and could potentially be used in autologous stem cell transplantation.

P-025

DOMINANT RESPONSES WITH CONSERVATION OF T CELL RECEPTOR USAGE IN THE CD8+ T CELL RECOGNITION OF A CANCER TESTIS ANTIGEN PEPTIDE PRESENTED THROUGH HLA-B*702 AND CW7 IN PATIENTS WITH MULTIPLE MYELOMA

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Cancer testis antigens exhibit physiological expression within germ cells and are frequently expressed in malignant tissue. Immunological tolerance to cancer testis proteins is not established and expression of CTAg proteins within malignant cells can therefore lead to induction of cellular and humoral immunity. A considerable body of evidence now indicates that CD8-specific immunity plays an important role in the control of cancer cell growth and a number of immunotherapy studies are in progress.

We have previously identified CTAg-specific immune responses in patients with multiple myeloma and reported that recognition of the MAGE-A1289-298 peptide restricted by HLA-B*0702 is the most common specificity. Here we studied seven CD8+ T cell clones specific for this peptide which were isolated from three myeloma patients at several timepoints. The affinity of peptide recognition was high with 50% maximal interferon-gamma production observed at a peptide concentration of 10-10M and variation of only one order of magnitude between the affinities of the clones. Importantly, all the clones were able to recognise and kill multiple myeloma cell lines. Interestingly, one patient did not express HLA-B*0702 but three clones from this patient recognised the MAGE-A1289-298 peptide on a lymphoblastoid cell line (LCLs) expressing HLA-Cw7. The T cell receptor gene usage was determined in 5 clones and showed conserved features in both the alpah and beta chain genes indicating correlation between T cell receptor usage and peptide specificity of cancer testis antigen-specific T cell clones.

P-026

SERUM FREE LIGHT CHAINS ANALYSIS CAN EARLY DETECT RELAPSE/PROGRESSION IN INTACT IMMUNOGLOBULIN MULTIPLE MYELOMA

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We have analysed 174 MM patients diagnosed at our hospital between years 2002 and 2008, for which a concomitant measurement of sFLC and serum protein electrophoresis (sPE) was done during follow-up. There were 53% males, median age at diagnosis 57 years (34-72), 120(69%) patients had IgG, 52(30%) IgA and 2(1%) IgD. We were interested to monitor the behaviour of sFLC and sPE in a way to early detect relapse/progression independently of treatment type. Slopes of the increase period corresponding to each measurement were compared using the student t-bilateral test. We observed 117(67%) patients with relapse or disease progression and 57(33%) patients were still in response to treatment. Among the 117 patients, in 77(66%) cases, relapse or progression was detected by concomitant increase of both sFLC and the intact immunoglobulin level (igl) with a significant earlier increase for sFLC. In 17(15%) patients, the relapse or progression was characterised by the only increase of sFLC without any increase of the igl. Contrarily, in 5(4%) patients there was only an increase of the igl without increasing the sFLC. Finally, in 18(15%) patients, the relapse or progression was revealed by the increase of igl faster than the concerned sFLC. When comparing slopes of increasing sFLC comparing to increasing igl, we found a very high significant difference ($p<0.001$), thus showing that sFLC have a faster detection of relapse or progression. Since there are no uniform recommendations for the use of this analysis during follow-up, we recommend its concomitant use with sPE, waiting for guidelines

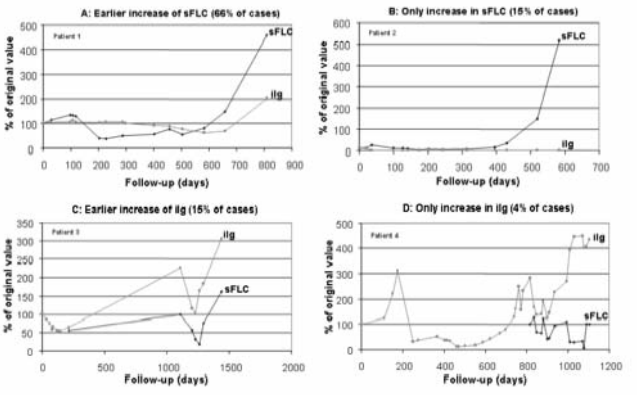

Figure1: Different relapse/progression profiles

P-027

SERUM FREE LIGHT CHAINS RATIO (SFLCR): AN INDEPENDENT PROGNOSTIC FACTOR FOR OVERALL SURVIVAL AND PROGRESSION FREE SURVIVAL IN MULTIPLE MYELOMA

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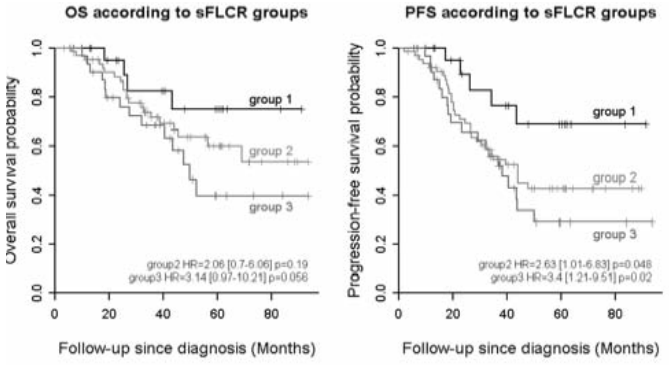
To evaluate the impact of sFLCR, measured at diagnosis in multiple myeloma (MM) patients, on the progression free survival (PFS) and overall survival (OS); a total of 118 consecutive patients diagnosed between years 2002 and 2008, and for which we have assessed the sFLCR at diagnosis were included in this study. There were 73(62%) males and 45(38%) females, median age: 57 years [34-72], 63 IgG, 35 IgA, 2IgD and 18 light chains; 11(9%) in stage I, 13(11%) in stage II and 94(80%) in stage III. Among 55(47%) FISH analysis done, 28(24%) detected a chromosome 13 deletion; no information about deletion-17 was available. According to the distribution of the different ratios, we have defined three groups: group1(n=25): patients with $0.13<sFLCR<3.3$ which represents the double of the normal range(0.26-1.65); group2(n=63): patients with $sFLCR>3.3$ and group3(n=30): patients with $sFLCR<0.13$. Kaplan Meier and cox regression analysis were performed to study the PFS and OS in different groups. After a median follow-up of 38 months [3.3-93.7], the probability of OS at 5 years for groups 1, 2 and 3 was 75%[56-100], 60%[47-76] and 40%[23-69] respectively; and the probability of PFS at 5 years was 69%[49-96], 43%[31-60] and 29%[15-54] respectively (Figure).The multivariate analysis showed that both OS and PFS are worslty affected with a more abnormal sFLCR. Our study has showed that abnormal sFLCR at diagnosis affects OS and more strongly the PFS independently of any other concomitant variable. We suggest that this factor deserves more focus for its validation as a prognostic factor in MM.



P-028

SPONTANEOUS HUMORAL RESPONSES AGAINST CANCER-TESTIS ANTIGENS OF THE MAGE FAMILY IN MYELOMA PATIENTS

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Background: Cancer-testis (CT) antigens are specifically expressed in the bone marrow (BM)-infiltrating plasma cells of patients with multiple myeloma (MM) and that among CT antigens members of the MAGE family are most commonly detected. In this study, we investigated for the first time the occurrence of spontaneous humoral responses against these promising targets for the antigen-specific immunotherapy of MM. *Methods / Results:* 1347 plasma samples from 225 MM patients were screened for antibody responses by ELISA. MAGE-A11-, MAGE-A1-, MAGE-A8-, and MAGE-A3-specific humoral responses occurred in 17 (7.6%), 5 (2.2%), 4 (1.7%), and 3 (1.3%) of MM patients, respectively. Humoral responses against MAGE-C2/CT10 were most commonly detected being present in 33 (15%) of the patients. For the first time we were able to demonstrate the presence of MAGE-C2/CT10-specific memory B cells in the blood of MM patients in an ELISPOT assay. Spontaneous MAGE-C2/CT10-specific immune responses, which consisted mainly of the IgG2 subtype, proved to recognize both natural and recombinant MAGE-C2/CT10 protein. Epitope mapping using overlapping peptides showed that antibody responses were restricted to certain MAGEC2/CT10 regions corresponding to amino acids 1-40, 80-90, and 350-360. *Conclusions:* Cancer-testis antigens of the MAGE family, especially MAGE-C2/CT10, are capable of inducing spontaneous humoral response in MM patients. These antigens represent promising targets for the antigen-specific immunotherapy of MM but might also be of use as diagnostic and/or prognostic parameters for myeloma patients.

P-029

RESTORATION OF MIR-214, MIR-196B AND MIR-375 EXPRESSION REDUCES CELL GROWTH OF MYELOMA CELLS.

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MicroRNAs have been demonstrated to be deregulated in multiple myeloma (MM). We have previously reported the downregulation of miR-214, miR-196b and miR-375 in MM compared to normal plasma cells. To gain insights into the role of these miRNAs in myeloma biology, gain-of-function experiments overexpressing pre-miRNA-214, pre-miRNA-196b and pre-miRNA-375 in JJN3 and H929 cell lines were carried out. Ectopic expression of each of these miRNAs reduced cell growth in myeloma cells inducing apoptosis. In order to identify the potential direct target genes of miR-214, miR-196b and miR-375, gene expression profiling was obtained from JJN3 cell line transfected with miRNA precursors. After crossing the list of deregulated genes with the databases of computationally predicted target genes, we identified 6 candidate targets down-regulated upon miR-196b induction, 29 after miR-214 transfection and 25 when miR-375 was re-expressed. Since the highest apoptosis effect was observed for miR-214 induction, we analyzed firstly the transcriptome effects of this miRNA. Among the 29 downregulated target genes after miR-214 induction, we focused on TRAF1 which is involved in regulation of NFkB signaling, on PSDM10 involved in p53/MDM2 interaction and ASF1B, a histone chaperone. The downregulation of the 3 target genes was confirmed at mRNA and protein level. 3' UTR reporter gene assay also confirmed the direct effect of miR-214 on PSDM10 and ASF1B. All together, these results suggest a tumor suppression function for miR-214, miR-196b and miR-375 by regulating myeloma cell proliferation.

P-030

MYELOID-DERIVED SUPPRESSOR CELLS ARE ELEVATED IN MGUS AND MM PATIENTS

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells consists myeloid progenitors and immature myeloid cells. In healthy individuals, these immature myeloid cells differentiate into granulocytes, macrophages and dendritic cells. In contrast, pathological conditions including cancers and auto-immune diseases MDSCs are expanded and impose suppressive functions on immune cells. In this study using flow cytometry we screened MDSCs in 78 peripheral blood (PB) and bone marrow (BM) samples with discrete clinical representations of monoclonal gammopathies (MGs) including: MGUS-(13/78), newly diagnosed MM-(31/78), relapsed MM-(16/78) and patients in remission-(18/78). For comparison 10 healthy volunteers (HVs) PB was also analyzed. Phenotype of MDSCs was characterized as CD33+CD11b+CD14-(HLA-DR)- and quantification was performed from all myeloid cells. A significant elevation was observed in the level of PB MDSCs for MG patients compared to HVs [0.44% (0.13%-5.38%) vs. 0.13% (0.02%-0.45%); P=0.0004]. Comparison of PB MDSCs between HVs and various clinical entities of MGs are summarized in Table 1. BM MDSCs showed an insignificant pattern of increase in MGUS cohort compared to MM cohort [0.52% (0.12%-1.72%) vs. 0.38% (0.07%-9.02%); P=0.60]. Correlation analysis showed a negative association in between ?2-microglobulin level and BM MDSCs (r=-0.34; P=0.002). In conclusion, these elevated MDSCs in MM patients might induce immune deregulation by their suppressive function. This study was supported by GACR-GAP304/10/1395, MSM0021622434 and NPVII2B06058.

Table1. Comparison of peripheral blood MDSCs between HVs and Monoclonal gammopathy patients

HVs (median % & range %)	Patients (n)	(median % & range%)	P value
0.13 (0.02-0.45)	MGUS (13)	0.31 (0.14-5.38)	0.071
	MM (31)	0.69 (0.15-3.14)	**0.0008**
	MM Relapse (16)	0.29 (0.13-3.81)	0.24
	MM Remission (18)	0.39 (0.13-3.46)	0.093

P-031

PLASMA MEMBRANE PROTEOMICS IDENTIFIES BIOMARKERS ASSOCIATED WITH T(4;14) MULTIPLE MYELOMA

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Multiple myeloma (MM) is characterized by recurrent chromosomal

translocations. Patients with t(4;14)(p16;q32) have the worst prognosis. MMSET, identified by its fusion to the IgH locus in t(4;14) MM, is universally overexpressed in t(4;14) MM. In order to identify cell surface biomarkers associated with t(4;14) MM for small molecule or antibody based therapies, we knocked down MMSET expression with shRNA and generated a cell line pair from KMS11, a t(4;14) MM cell line. Using stable isotope labelling by amino acids in cell culture (SILAC) followed by enrichment of plasma membrane proteins by cell surface biotinylation/avidin-affinity chromatography and analysis by GeLC-MS/MS, MMSET associated differences in plasma membrane proteins were analysed. By this approach, 45 cell surface proteins were identified as differentially expressed between KMS11 and KMS11/shMMSET. Three targets down regulated in KMS11/shMMSET were selected for further validation. Flow cytometry analysis indicated SLAMF7 was overexpressed and decreased by shMMSET treatment in KMS11 and other t(4;14) MM cell lines (KMS18, KMS28BM, NCI-H929 and OPM2). Quantitative RT-PCR analysis indicated shMMSET treatment resulted in significant reduction of SLAMF7 mRNA, suggesting that MMSET might regulate the transcription level of SLAMF7. Western blot analysis indicated shMMSET treatment decreased FGFR3 level in KMS11, KMS18, KMS28BM and OPM2, and reduced IL6ST level only in KMS11 and OPM2. Overall, these results illustrated that SLAMF7 might be a novel cell surface protein associated with t(4;14) MM.

P-032

BONE MARROW RESIDENT MONOCYTES ARE PRIMARY TARGET FOR VASCULAR ENDOTHELIAL GROWTH FACTOR

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It has been shown that vascular endothelial growth factor (VEGF), produced by multiple myeloma (MM), suppresses development and function of dendritic cells (DC). However, little is known about the effect of VEGF on human DC during their lifespan in bone marrow (BM) and peripheral blood (PB). We analysed expression of VEGF receptor(R)-1 and VEGFR-2 on myeloid (M)DC (CD11c+CD16+MDC and CD11c+CD16-DC) and CD14+ monocytes that reside in the BM of humanised (hu)NOD/SCID mice or in PB of adult healthy subjects, and explored their capacity to interact with rhVEGF165. The CD11c+CD16-DC and monocytes located in the femur, tibia and pelvic bones had elevated VEGFR-1 and VEGFR-2 surface expression compared to those located in the vertebrae and skull. In contrast to BM resident MDC and monocytes, surface expression of VEGFR-1 and VEGFR-2 was negligible on MDC and monocytes in PB. However, VEGFR-1 mRNA was detected in CD11c+CD16+MDC and monocytes, and VEGFR-1 and VEGFR-2 mRNA in CD11c+CD16-DC in PB. Treatment with rhVEGF165 enhanced the antigen presenting capacity of BM resident monocytes but not MDC and had no effect on maturation status of MDC and monocytes as measured by MHC-class II, CD40, CD86, CD83 expression. Treatment with rhVEGF165 also had no effect on either antigen presenting function or maturation status of MDC and monocytes in PB.

These data suggest that BM resident monocytes are the primary target for paracrine action of rhVEGF165. This raises the possibility that VEGF produced by MM cells in the BM can enhance the role of surrounding monocytes in MM immunosurveillance.

P-033

MULTIPLE MYELOMA-INDUCED ALLOREACTIVE T CELLS REGULATE ELIMINATION AND ESCAPE PHASE OF MULTIPLE MYELOMA

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The graft-versus-myeloma (GVM) effect, followed by relapse, is the clinical example donor alloreactive T cells regulating the elimination and escape phase of multiple myeloma (MM). In this study, we investigated whether MM cells induce and shape alloreactive T cell responses by adoptively transferring human T naive (TN) cells into MM-bearing mice in which human RPMI8226-TGL MM cells localized exclusively in bones. We showed that MM cells primed donor TN cells upon arrival to myeloma-infiltrated bones. T cell priming led to multiple CD8+T cell divisions and the emergence of double positive (DP) CD8αα+CD4+T cells which were degranulated, expressed INF-γ and secreted perforin. MHC class I-dependent contact with MM cells was required for CD8+T cell division and the production of DP T cells in vitro, but additional stimuli supported the development of DP T effector cells in vivo in myeloma-infiltrating bones. MM-induced alloreactive T cells were associated with an increased number of apoptotic MM cells in myeloma-infiltrated bones and were capable of lysing MM cells isolated from T-cell recipient mice and suppressing tumour burden for approximately 12 days. Thereafter, as determined by bioluminescence imaging, MM recurred despite the persistence of alloreactive T cells in the medullar space and acquired the ability to form extramedullary MM masses. The contribution of MM-induced alloreactive T cell in the elimination and escape phase of MM, demonstrated in this preclinical MM model, has direct implications for donor leukocyte infusion therapy in MM patients.

P-034

HGF AND IGF-1 POTENTIATE SDF-1 ALPHA-MEDIATED MYELOMA CELL MIGRATION THROUGH ACTIVATION OF PAK

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Accumulation and dissemination of myeloma cells in the bone marrow requires cell migration. Stromal cell-derived factor (SDF)-1α, insulin-like growth factor (IGF)-1 and hepatocyte growth factor (HGF) are potent positive mediators of myeloma cell migration in vitro, and in this study we found a synergistic effect on migration when combining SDF-1α with either HGF or IGF-1. The synergy was only seen in migration along a positive gradient of SDF-1α, whereas concentration of HGF or IGF-1 could be uniform along the migration pathway. JJN-3 cells, which secrete large amounts of HGF, migrated slower towards SDF-1α when exposed to an inhibitor of the HGF receptor c-Met, demonstrating cooperative activity between autocrine HGF and exogenous SDF-1α.The synergy could not be explained by changes in receptor expression levels as HGF and IGF-1 did not affect the SDF-1α receptor level, or vice versa. There was, however, a clear positive correlation between the degree of cytokine-induced migration and p21-activated kinase (PAK) activation both in the myeloma cell lines INA-6 and IH-1 cells, as well as in primary myeloma cells. Downregulation of PAK 1 and PAK 2 with siRNA in the INA-6 cell line resulted in lower cell migration, indicating a role for PAK in cytokine-induced cell migration. This study shows synergy between SDF-1α and HGF/IGF-1 as mediators of myeloma cell migration and points to PAK as a possible target in the treatment of multiple myeloma.

P-035

PODIA IN MULTIPLE MYELOMA CELLS PROMOTE INTERACTION WITH BORE MARROW FIBROBLASTIC STROMAL CELLS

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The physical contact between MM cells and BM microenvironment can lead to cell adhesion mediated drug resistance. Although some components of adhesive structures in MM cells have been identified very little is known about their exact morphology and dynamics. We have observed formation of CD138 and F-actin containing membrane extensions in MM cells in in vitro cultures of BM aspirates and in sections of BM trephines of MM patients. MM membrane extensions elongated on the surface of BM stromal cells or interconnected MM cells forming seemingly cellular networks. Morphologically, MM membrane extensions appear similar to haematopoietic structures such as nanotubes in T-cells involved in cell communication and podia in CD34+ and leukemia cells of unknown function. Dexamethasone increased the length and the percentage of MM cells with membrane extensions. This correlated with enhanced adhesion of MM cells on fibroblastic stromal cells and protection of MM cells against apoptosis. Blocking CXCR4 signalling with Plerixafor inhibited the rate of formation and length of MM membrane extensions and correlated with sensitisation of MM cells to Dexamethasone. Similar results were obtained using Dexamethasone in combination with the kinase inhibitor Dasatinib. Neither Dasatinib nor Plerixafor at doses achievable in patients directly induced apoptosis in MM or BM stromal cells. We conclude that MM membrane extensions/podia are involved in the interaction with BM stromal cells, require CXCR4 and Src and/or c-Abl activity and could be involved in resistance to treatment with Dexamethasone.

P-036

COMMON FRAGILE SITE GENE FHIT (FRAGILE HISTIDINE TRIAD GENE) AND WWOX (WW DOMAIN CONTAINING OXIDOREDUCTASE) EXPRESSION ARE FREQUENTLY ALTERED IN MULTIPLE MYELOMA

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The common fragile sites (CFSs) are regions of profound genomic instability, and hot-spots for deletions and other alterations in cancer cells. We have previously shown that promoter of FHIT is frequently methylated in multiple myeloma (MM) and correlated with worse prognosis. WWOX is located at a CFS region on chromosome 16q23.3, similar to FHIT, this gene was also target of alterations in multiple cancers. The objective of current study is to find out if these potential tumor suppressor genes in MM are altered. Six myeloma cell lines, bone marrow mononuclear cells of 84 MM and 12 MGUS patients were subjected to this study after obtaining informed consent. Isolated CD138 positive plasma cells (PC) of 8 MM patients were examined to see if the alteration occurred really in myeloma cells. Using methylation specific PCR, WWOX promoter methylation was detected in 15% of MM and 0% of MGUS patients, 2of 6 cell lines. Using nested RT-PCR, aberrant short transcripts of WWOX were detected in 85% of MM and 63% of MGUS patients, 4 of 6 cell lines. In isolated PC, aberrant WWOX were detected 100% of MM. Aberrant transcripts of FHIT were not often observed compared to WWOX (2.5% of MM, 16% of MGUS, none of cell lines). In isolated PC, FHITexpression was very low in 87.5% of MM. Various WWOX transcripts lacking exons coding catalytic SDR domain are expressed only in cancers. High frequency of aberrant WWOX expression of myeloa cells implies an important role of this gene in MM. Our result also indicated that two CFS genes FHIT and WWOX were frequently altered but in different way.

P-037

IMMUNOPHENOTYPIC CHARACTERISTICS OF PERIPHERAL BLOOD MOBILIZED CD34+ HEMATOPOIETIC PROGENITOR CELLS AFTER DIFFERENT INDUCTION THERAPIES IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS

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APBSCT is the gold standard for young MM pts and new drugs are increasingly used as part of the induction therapy. The impact of new drugs on hematopoietic stem cells biology is still unclear. We aimed to identify CD34+ cell subsets from apheresis collection best predictive of early and long term hematopoietic recovery after APBSCT in newly MM pts. Therty one MM pts, treated with different induction therapies, were prospectively evaluated: 12 VAD like, Bor 8 and 11 Len. Flow cytometry analysis on cryopreserved apheresis samples was used to evaluate the following CD34 subsets: CD34/CD38, CD34/CD90, CD34/CD110, CD34/CD117, CD34/CD133, CD34/HL-DR, CD34/VEGFR-2. Higher percentages of hematopoietic stem cells subsets CD34/CD133+ and CD34/CD117+ were observed in patients treated with Len versus patients that received Bor and VAD induction therapies (68.59±27.52% vs 23.5±27.0% p=0.020 and 30.94±22.94% vs 5.4±5.4% p=0.009, respectively). With regard to the prediction of platelet engraftment after PBSCT, statistical analysis showed a significant correlation between the infused CD34+/CD133+ cells and early platelet engraftment (p=0.031) and 3 or 6 months platelet count (r=0.47, p=0.026 and r=0.57, p=0.009, respectively). No correlation was observed with neutrophil engraftment. In MM pts, Lenalidomide treatment promotes mobilization of immature hematopoietic stem cell subsets (CD133+ and CD117+); hematopoietic stem cells infused expressing CD34+/CD110+ and CD34+/CD133+ best predicted early and long term platelet reconstitution during the first 6 months

P-038

LOW FCγRIIB BINDING ABILITY IN MULTIPLE MYELOMA (MM) CELLS REDUCES IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIF (ITIM) SIGNALING

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Immunoreceptor tyrosine-based activation motif (ITAM) and ITIM provide the basis for two opposing signaling modules that duel for control of plasma cell activation. FcγRIIb mediated SH2-containing inositol 5-phosphatase (SHIP) phosphorylation activates downstream ITAM or ITIM signaling. We first investigated the IgG-binding ability of 30 MM patients and 29 normal donors to FcγRIIb. Each serum sample was incubated with MHC1 cells that only express FcγRIIb but do not express FcγRI and FcγRIIa. The results showed MM patients' serum IgG have much lower FcγRIIb-binding ability than normal human IgG ($P<0.05$) by using both of flow cytometric and IFA assays. We further analyzed the FcγRIIb-SHIP signaling pathway in normal B-cells and Raji B-cells. The cells were exposed to MM patients' or normal human sera for 5, 15, 30, or 60 minutes to determine the time of maximum SHIP phosphorylation. The maximum time point of phosphorylation was 15 minutes. However, when MM tumor cells were exposed to MM patients' or normal human IgG, FcγRIIb was not activated by Fc and phosphorylation/total SHIP and Syk phosphorylation/total Syk as well as AKT phosphorylation/total AKT were not changed. Our findings suggest that the monoclonal protein produced by MM patients has a very low FcγRIIb binding ability and is incapable of signaling through the inhibitory ITIM pathway. FcγRIIb is expressed on plasma cells. Cross linking of FcγRIIb to induce apoptosis of plasma cells may be a novel therapeutic approach for the treatment of MM patients.

P-039

SERIAL SAMPLE ANALYSIS OF 15 MULTIPLE MYELOMA PATIENTS USING HEAVY/LIGHT CHAIN SPECIFIC IMMUNOGLOBULIN RATIOS (HEVYLITE™). CONTRIBUTION TO THE EVALUATION OF RESPONSE TO TREATMENT

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In multiple myeloma, concentration of monoclonal immunoglobulin by quantification of protein bands in serum protein electrophoresis (SPE) is considered the best biomarker for monitoring and evaluation of response to treatment. A new nephelometric immunoassay (Hevylite™ - Binding Site Group Ltd) allows specific measurement of serum IgGκ, IgGλ, IgAκ and IgAλ concentrations and makes possible calculation of IgGκ/IgGλ and IgAκ/IgAλ ratios (heavy/light chain or HLC ratio). Our aim was to evaluate contribution of Hevylite™ to assess response to treatment. We analysed 114 samples from 15 multiple myeloma patients. In all sera taken at diagnosis, HLC ratio was abnormal. The sum of IgGκ + IgGλ or IgAκ + IgAλ measurements correlated with total IgG or IgA in the 114 samples (R^2 = 0,95). Changes observed in HLC ratios during follow up were correlated with changes in SPE. In 3 patients relapse from partial response was indicated by an increase in HLC ratio 2 months before SPE. No patients achieved complete response and HLC ratio remained abnormal throughout except in 5 patients who achieved very good partial response. Serial samples analysis of these patients showed persistent normalisation of HLC ratio after 6-9 months of treatment, whereas immunofixation was still positive. These preliminary results indicate that it is possible to monitor response to treatment using HLC ratio. However further studies are necessary to define more precisely the interest of this new assays among traditional methods.

P-040

DIFFERENTIAL GENE EXPRESSION PROFILING OF CLONOGENIC MULTIPLE MYELOMA CELLS REVEALS SIGNATURES OF PROLIFERATION AND DIFFERENTIATION

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Cells with greater clonogenic potential and stem cell properties that mediate drug resistance have been identified in the CD138- compartment of multiple myeloma (MM). These CD138- cells can differentiate into CD138+ plasma cells, but the molecular basis of their differences have not been clearly defined. Here we studied the clonogenic properties and expression profile of CD138- cells from MM cell lines RPMI8226 and NCI-H929. CD138- cells contained significant percentage of side population and higher relative levels of ALDH activity, hallmarks of cancer stem cells. ALDH+CD138- cells showed greater clonogenicity than ALDH-CD138+ cells on colony forming assay using MC medium. Upon long term culture ALDH+CD138- cells, but not the ALDH- CD138+ cells produced both CD138+ and CD138- populations, confirming the differentiation potential of CD138- cells to CD138+ cells. Expression profiling revealed that CD138– cells were enriched with genes previously identified in stem cells signatures from other cancers. 125 genes were differentially expressed. Genes involved in cell proliferation (STAG2, RB1CC1 etc), PCRg genes that regulate differentiation (BMI1, SUZ12 etc), signal transducers (RAB18, SHOC2 etc), and regulators of transcription and translation (ZNF146, EIF1AX etc) were differentially expressed. Our data confirm the stem cell properties of CD138- cells and identify genes which are differentially expressed in CD138- cells compared to CD138+ cells in MM. These genes provide insights into the molecular biology of these cells and may provide potential therapeutic avenues.

P-041

INTERLEUKIN-16 IS AN IMPORTANT GROWTH-PROMOTING FACTOR AND A NOVEL DIAGNOSTIC AND THERAPEUTIC TARGET FOR MULTIPLE MYELOMA

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Multiple myeloma is a malignancy characterized by the expansion of a plasma cell clone that localizes to the human bone marrow. Myeloma cells and bone marrow stroma cells both produce soluble factors promoting the survival and progression of multiple myeloma. Interleukin-(IL)-16 is involved in regulating migration and proliferation of normal leukocytes, however, it has been unclear whether IL-16 also plays a role in the pathophysiology of human cancers. We found IL-16 to be strongly overexpressed in the bone marrow of myeloma patients. Myeloma cell lines as well as primary tumor cells from myeloma patients constitutively expressed IL-16 and its receptors CD4 and/or CD9 and spontaneously secreted soluble IL-16. Silencing of IL-16 had an anti-proliferative effect on the tumor cells which could be reversed by the addition of the C-terminal fragment of soluble IL-16. Most importantly, the application of a monoclonal antibody directed against IL-16 had a strong growth-inhibiting influence on myeloma cells. These findings indicate that cytokine IL-16 is an important growth-promoting factor in multiple myeloma and a candidate for novel diagnostic, prognostic and therapeutic applications for this incurable human malignancy.

P-042

SERUM HEAVY/LIGHT CHAIN LEVELS STRONGLY CORRELATE WITH SPE AND DISEASE STATUS IN IGA AND IGG MULTIPLE MYELOMA

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Introduction: Typical features of monoclonal gammopathy(MG) include the presence of monoclonal immunoglobulin(MIG). The latest test which extends the range of MIG examination possibilities is the HevyLiteTM system. *Aim:* The study aimed at comparison of MIG levels obtained by HevyLite™ system and SPE with correlation to disease activity in the group of IgA and IgG-type MG patients. *Methods:* The IgA group consisted of 24 myeloma(MM) patients (18 active, 6 remission) and 7 individuals with MGUS. The IgG group consited of 30 MM patients (27 active, 3 remission) and 1 MGUS individual. The HevyLite™ system (The Binding Site,UK) was used to determine the serum levels of IgAκ, IgAλ, resp. IgGκ and IgGλ. *Results:* The patients in the active disease stage revealed highly pathological levels of the dominant MIG, with suppressed levels of the alternative immunoglobulin, and significant influence on the Igκ/Igλ ratio. When comparing the results of MIG determination by the HevyLite™ and SPE, Spearman's correlation analysis confirmed close correlation of MIG levels regarding IgAκ (r=0.946,p=0.0001), IgAλ (r=0.872,p=0.0001) as well as IgGκ (r=0,970, p=0,00001) and IgGλ (r=0,998,p=0,00001). 8 of 9 patients in remission had normal Igκ/Igλ ratio. *Conclusion:* The HevyLite™ system promisingly complements the set of examinations used routinely by MG monitoring, in particular by confirmation of complete remission of the disease. Supported by LF 2010013, VZ MSMT CR 6198959205.

P-043

HEVYLITE™ IGA ASSAY - A PROMISING TOOL FOR THE DIAGNOSIS AND MONITORING OF MYELOMA PATIENTS

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Hevylite™ IgA (Binding Site) is a new nephelometric assay allowing the IgA kappa (IgAκ) and IgA lambda (IgAλ) measurement. The aim of this study was to determine the performance of Hevylite™ IgA assay, for the diagnosis and follow-up of myeloma patients at different stages. Total IgA, IgAκ, IgAλ concentrations, sPE (serum protein electrophoresis) and sIF (serum immunofixation) were performed at diagno-

sis and during follow-up. At diagnosis, all the myeloma patients (16/16) had an abnormal IgAκ/IgAλ ratio and monoclonal isotype values were often more elevated than the standard IgA level. During follow-up, the patients with positive sPE and sIF had in all cases (7/7) an abnormal IgAκ/ IgAλ ratio. The patients with negative sPE but positive sIF had also an abnormal IgAκ/IgAλ ratio (4/4). Some patients (2/5) with normal sPE and sIF during follow-up had an abnormal IgAκ/IgAλ ratio due to the decreased non monoclonal isotype but with normalized monoclonal isotype. This could indicate a residual disease. In conclusion, Hevylite™ IgA assay appears to be an interesting test, especially when the monoclonal immunoglobulin comigrates with other normal proteins, making impossible a reliable estimation by sPE. In our study, Hevylite™ IgA assay appears as sensitive as sIF. Our results suggest that for high monoclonal IgA concentrations, the values of IgAκ or IgAλ can be used for monitoring. However, at the stage of complete or near complete remission, the IgAκ/IgAλ ratio seems to be more contributive.

P-044

BONE MORPHOGENETIC PROTEINS INDUCE APOPTOSIS BY SMAD-DEPENDENT DOWN-REGULATION OF MYC

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Bone morphogenetic proteins (BMPs) have been shown to induce apoptosis and growth arrest in multiple myeloma. However, the molecular mechanisms behind BMP-induced apoptosis in myeloma cells are unclear. The MYC oncogene is a master regulator of cell growth and protein synthesis and MYC overexpression has been proposed to be associated with the progression of multiple myeloma. We report that down-regulation of MYC and MYC target genes correlated with induction of apoptosis by BMP in primary myeloma cells and cell lines. In contrast, forced expression of MYC abrogated BMP-induced apoptosis, and primary myeloma cells harboring translocations juxtaposing MYC to immunoglobulin enhancers evaded BMP-induced apoptosis. Inhibition of Smad1/5/8 activation using Dorsomorphin abrogated BMP-induced apoptosis. Moreover, we found that BMP-activated Smad1/5/8 bound to the repressive Smad binding element of the MYC promoter, as has previously been shown for TGF-β activated Smad-2 and -3. To transactivate genes, MYC must heterodimerize with its obligate partner MAX. 10058-F4, a small-molecule inhibitor of MYC-MAX heterodimerization, induced apoptosis also in cells resistant to BMP-induced apoptosis. The results suggest that targeting MYC's function as a transcription factor may be an efficient way of inhibiting growth in most multiple myeloma cells.

P-045

TP53 POSITIVELY REGULATES CELL DEATH THROUGH TRAILR2 BUT NEGATIVELY THROUGH TRAILR1 IN MYELOMA CELLS

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Mapatumumab and Lexatumumab are human agonistic antibodies for TRAILR1 and TRAILR2, respectively. Mapatumumab induced cell death more effectively than Lexatumumab in a panel of 30 human myeloma cell lines (HMCLs). Interestingly, sensitivity to Mapatumumab and Lexatumumab was mutually exclusive and related to TP53 status (p=0.006): TP53wt HMCLs (n=9) were sensitive to Lexatumumab (median of death 40%) but resistant to Mapatumumab (median 7%). In contrast, TP53Abn HMCLs (n=21) were resistant to Lexatumumab (median 7%) but sensitive to Mapatumumab (median 44%). TRAILR2 but not TRAILR1 is a p53 target gene. TRAILR2 as well as other p53 target genes, MDM2, CDKN1A, BAX are underexpressed in TP53Abn HMCLs as compared to TP53wt HMCLs (p<0.01). In good agreement, killing by Lexatumumab was correlated to TRAILR2 expression while no correlation was found for TRAILR1 expression and Mapatumumab killing. To activate p53 pathway, we used Nutlin3a, Rita or Melphalan. As expected, TP53wt but not TP53Abn HMCLs were killed by Nutlin3a. In TP53wt HMCLs only, Nutlin3a increased TRAILR2 (n=3, 4-fold increase) and Lexatumumab killing (n=3, 4-fold increase) but did not modify TRAILR1 expression or Mapatumumab killing. Reciprocally, silencing p53 in the TP53wt NCI-H929 decreased Nutlin3a killing, TRAILR2 expression and Lexatumumab killing. Melphalan, but not Rita, gave similar results to Nutlin3a. Thus TRAIL-R pathways are differentially regulated by p53 and TRAILR1 pathway seems very attractive for TP53Abn myeloma cells.

P-046

MYELOMA SELF-RENEWAL IS MAINLY MEDIATED BY IGF1 AND ONLY SUPPORTED BY CD138+ CELLS

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In this study, we explored the mechanisms of myeloma self-renewal of myeloma cell lines and primary myeloma cells in a FCS-free human collagen assay. Seven out of 27 CD138+ cell lines were able to self-renew without growth factors. Self-renewal was mediated by IGF1/IGF1R and/or C-KIT/KITL that both activated ERK1/2 and AKT pathways. Although most if not all cell lines express both IGF1 and IGF1R, only few were able to provide spontaneous colonies suggesting that this loop was not sufficient. Indeed, by micro-array we identified that JAG2 expression correlated to self-renewal (p=0.0002). We further showed that silencing JAG2 impaired both self-renewal and growth in SCID mice. To address the existence of autocrine loops in primary cells, we used microarray data from Arkansas University. Myeloma cells express IGF1R and/or C-KIT (expression is mostly mutually exclusive) at diagnosis. Primary cells express IGF1 but not KITL, suggesting the existence of an in vivo IGF1 loop. On the other hand, we identified JAG2+ cells among CD138+ cells. Clonogenic assays for primary cells were performed in the presence of IL6, IGF1 or KILTL. We separated samples from two patients with leukemic phase into pure CD138+ and CD138- fractions. In both cases, only the CD138+ fraction provided colonies that could be serially replated up to cell line establishment for one case. These data show IGF1 or KITL are clonogenic factors for myeloma cells and argue against a CD138- origin of clonogenic cells.

P-047

RESUMPTION OF THE DRUG-SENSITIVITY IN MYELOMA CELLS THROUGH INACTIVATION OF ABC TRANSPORTERS BY GLYCOLYSIS INHIBITION

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ABC transporters are ATP-dependent efflux pumps for a variety of chemotherapeutic agents to cause drug resistance. ATP production in cancer cells is largely dependent on glycolysis (the Warburg effect). In the present study we explored the effects of inhibition for glycolysis on ABC transporter activity and the susceptibility to chemotherapeutic agents in drug-resistant hematopoietic malignant cells including MM cells. Inhibition of glycolysis by 3-bromopyruvate (3BrPA) mostly suppressed ATP production in MM cells. Of note, 3BrPA preferentially induced cell death in MM cells but not in normal hematopoietic cells in bone marrow samples from patients with MM, suggesting targeting MM cells by glycolysis inhibition. RPMI8226 and KG-1 cells constitutively over-expressed breast cancer resistance protein (ABCG2) and P-glycoprotein (ABCB1), respectively. After passive incorporation of autofluorescence-emitting daunorubicin (DNR), intracellular DNR reten-

tion was analyzed by flow cytometry. Both cells pumped out most of DNR within 2 hours, while DNR was retained in the cells in the presence of 3BrPA. 3BrPA enhanced cytotoxic effects of doxorubicin (Dox) and melphalan on RPMI8226 cells. Furthermore, cytotoxic activity of Dox was restored in combination with 3BrPA against a highly drug-resistant cell fraction isolated from RPMI8226 cells observed as a side population upon Hoechst 33342 staining. These results collectively suggest that glycolysis inhibition is able to target MM cells to deplete ATP, which may inactivate ABC transporters to overcome chemo-resistance in MM cells.

P-048

SENSITIZATION OF MYELOMA CELLS TO A TRAIL-MEDIATED IMMUNOTHERAPY WITH BORTEZOMIB

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Although TNF-related apoptosis-inducing ligand (TRAIL) binds to death receptor (DR) 4 and DR5 to induce tumor-specific apoptosis, TRAIL receptor expression and its downstream apoptotic signaling are down-regulated to cause TRAIL resistance in a variety of malignant tumors. Therefore, sensitization of such malignant cells to TRAIL become a critical issue in TRAIL-mediated immunotherapy. In the present study, we explored the effects of bortezomib (Bor) on TRAIL receptor editing and its downstream signaling in myeloma (MM) cells. Most of MM cells expressed only marginally DR5. Bor markedly up-regulated the surface levels of DR5 along with its mRNA expression in MM cells tested but not in normal peripheral mononuclear cells. Bone marrow stromal cells appeared to reduce surface levels of TRAIL receptors on MM cells. However, Bor was able to up-regulate the DR5 editing even in the presence of bone marrow stromal cells, and enhanced the cytotoxic effects of an anti-DR5 agonistic antibody and recombinant TRAIL on MM cells. Interestingly, Bor decreased the levels of c-FLIP, an inhibitor of DISC, along with activation of caspase-8, suggesting potentiation of DR-mediated extrinsic apoptotic pathway. Furthermore, Bor induced phosphorylation of eIF2alpha, a negative regulator of gene translation and protein synthesis, along with subsequent disappearance of anti-apoptotic proteins including Mcl-1. These results collectively suggest that bortezomib treatment may be able to sensitize MM cells to a TRAIL-mediated immunotherapy.

P-049

A UNIQUE NEW HUMANIZED MOUSE MODEL FOR MULTIPLE MYELOMA (MM): OPPORTUNITIES FOR STUDYING MM IN ITS NATURAL ENVIRONMENT AND PRECLINICAL TESTING

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Here we report the development of a unique model to study the pathobiology of multiple myeloma (MM) by implementing a technology for creating a natural human bone environment in the RAG2-/-γc-/- mouse. Human bone marrow (BM)-derived mesenchymal stromal cells were seeded on BCP-particles and implanted subcutaneously in mice. Within 6 weeks this leads to the formation of ossicles that contain substantial amounts of human bone, with the open spaces filled with mouse hematopoietic cells and blood vessels, creating an environment that strongly resembles human BM. A striking finding was that this humanized environment in the mouse acts as a 3-D natural "niche" for primary MM (pMM) cells. Intrascaffold injection of pMM cells resulted in engraftment and outgrowth of tumor cells in close contact with the human bone layer in the ossicles. In addition, intracardial injection revealed that these pMM cells were also capable of homing to the implanted artificial BM-niches, while no tumor cells were detected in the mouse BM. The outgrowth of pMM in this model is accompanied by an increase in osteoclast numbers on the bone surface, indicating the presence of bone resorption, one of the most important clinical sequelae of MM. Interestingly, by gene-marking pMM cells with luciferase we were able to follow myeloma outgrowth in time, and visualize the effect of treatment. Hence, this novel humanized mouse model provides the first opportunity to investigate primary MM plasma cells in a natural environment, which may lead to better insights in the pathogenesis of this disease.

P-050

IDENTIFICATION OF POTENTIAL PREDICTIVE GENE LISTS FOR MELPHALAN RESISTANCE BY DRUG SCREEN OF B-CELL CANCER CELL LINES

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In multiple myeloma a range of new drugs have been introduced and now challenge conventional therapy including high dose melphalan. Consequently, the generation of a predictive gene list for response to melphalan may have a clinical impact. The hypothesis is that melphalan screens of B-cell cancer cell lines combined with gene expression data may provide predictive value in a clinical setting. Microarray based global gene expressions were generated in 18 B-cell cancer cell lines prior to a melphalan 50% growth inhibition (GI50) screen. Linear discriminant analysis and sparse partial least squares were used to build predictive gene lists of the GI50 values based on the cell line panel. The predictive value of the resistance indices were retrospectively validated in a publicly available clinical data set generated by the University of Arkansas for Medical Sciences. The indices were able to predict a significantly higher risk of relapse and death with an increasing resistance index in the clinical data set. The most sensitive and resistant cell lines, MOLP-2 and RPMI-8226 LR5 respectively, turned out to have high leverage, which indicates that their differentially expressed genes may carry important predictive value. The present study supports that a melphalan resistance index based on a B-cell panel of cancer cell lines is able to predict clinical outcome. The clinical impact of the gene list needs to be functionally and prospectively validated and correlated to known biomarkers in independent data sets to gain insight into the underlying biology of melphalan resistance.

P-051

LOSS OF THE TUMOR SUPPRESSOR CYLD CAUSES ENHANCED NF-KAPPAB AND WNT/B-CATENIN SIGNALING IN MULTIPLE MYELOMA

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In multiple myeloma, the Wnt/b-catenin pathway and NF-kappaB pathway are frequently aberrantly activated, leading to increased tumor proliferation, survival and dissemination. The deubiquitinating enzyme CYLD was originally identified as a tumor suppressor that is mutated in familial cylindromatosis. CYLD is a key negative regulator of NF-kappaB signaling which acts by deubiquitinating tumor necrosis factor (TNF) receptor-associated factor (TRAF2), TRAF6, and NEMO (NF-kappaB essential modulator, also known as IkappaB kinase gamma). It was recently demonstrated that CYLD acts also as a negative regulator of Wnt/β-catenin signaling through a mechanism in which hyperubiquitination of polymerized Dvl drives enhanced Wnt responses. Interestingly, in MM, deletion and missense mutations of CYLD have been reported. Here, we show that CYLD expression is frequently lost in MM tumors and it is strongly correlated with a proliferative gene-expression profile. Functional assays with inducible knockdown of CYLD in MM cells revealed that CYLD silencing increases autocrine and Wnt3a stim-

ulated Wnt signaling and dramatically enhances the NF-kappaB responsiveness of MM cells. Consequently, an increase in survival and proliferation of malignant plasma cells with CYLD knockdown was observed. These findings identify loss of CYLD expression as a potential cause of aberrant Wnt and NF-kappaB pathway activation in MM, enhancing proliferation, survival and dissemination of malignant cells.

P-052

MULTIPLEXED PHOSPHOPROTEIN CELL SIGNALING ANALYSIS PREDICTS PATIENT-SPECIFIC THERAPEUTIC RESPONSE AND/OR OFF TARGET EFFECTS

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Interaction of Multiple Myeloma (MM) cells with bone marrow microenvironment cells has a pathogenetic role in the disease. A great need exists to understand the differential effect of treatment on myeloma as well as non-myeloma cells. We designed an ex vivo study to rapidly screen treatment combinations to predict treatment efficacy. Fresh bone marrow aspirates were subdivided and treated ex vivo with a panel of molecular targeted inhibitors, including combinations thereof, that target a wide range of cellular pathways (autophagy, proteosome, angiogenesis, protein degradation, proliferation/survival, insulin response, and translation). Up to 48 different treatment conditions can be studied from 5mL aspirate. After overnight incubation the samples were placed in a preservative that suppresses fluctuations in kinase pathway proteins. CD138+ plasma cells were separated from CD138- cells via immunomagnetic sorting. Reverse phase protein microarrays were used to quantify 60 cell signaling proteins in both cell populations. Sorafenib, a molecular targeted tyrosine kinase inhibitor which blocks the MAPK pathway, induced a compensatory up-regulation of ERK T202/Y204 in all patient samples except one, who was in remission after treatment. Lenalidomide induced caspase 8 activation and reduction of IL-10 in plasma cell samples. The combination lenalidomide+dexamethasone was as able as bortezomib+dexamethasone to induce p53 phosphorylation in Serine 15 in relapsed patient samples treated with lenalidomide in first line.

P-053

SEROTONIN DYSREGULATION IS INVOLVED IN ABERRANT CROSS TALK OF MULTIPLE MYELOMA MICROENVIRONMENT

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The biologic mechanisms involved in the pathogenesis of multiple myeloma (MM)-induced osteolytic bone disease and the complex network of plasma cells and microenvironment are poorly understood. Circulating monoamine serotonin [5-hydroxytryptamine (5- HT)] is principally stored in platelet-dense granules. Brainstem-derived serotonin positively regulates bone mass following binding to 5-HT2C receptors on ventromedial hypothalamic neurons. This is opposed by platelet-derived serotonin that induces bone lysis and osteoclast activation. In the present work we show that increased circulating-serotonin levels may alter the bone marrow microenvironment to promote active MM. We found an imbalance in the compartmentalization of serotonin associated with presence of MM bone disease, with higher levels of serotonin in platelets for the MM patients compared to the MGUS & healthy controls and concomitant reduction in serum serotonin levels.Bone marrow core biopsies exhibited significant elevation of cellular Serotonin, RANK, MMP-11, TNF?, TNF-R1, and Ezrin Tyr353 in MM patients with active bone disease compared to patients without bone disease. The cellular concentration in CD138+ plasma cells showed a positive trend toward correlation with IL-10, IL-8, IL-6, MMP-6, MMP-9, IL-11, DKK1 and TNFalpha. These data suggest that in the MM bone marrow microenvironment Serotonin may modulate cellular signaling either directly through specific serotonin receptors and transporters or by modulating release of chemokines and cytokines.

P-054

EVALUATION OF ACTIVIN A AND INFLAMMATORY MONOCYTES IN MONOCLONAL GAMMOPATHIES

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Activin-A secretion is potently up-regulated in monocytes by cognate interaction with activated T cells in the bone marrow milieu and plays a functional role in the suppression of inflammation. Inflammatory CD14+/CD16+ monocytes have a wide range of chemokine pathways for recruitment into tumor microenvironment, in which they differentiate into macrophages or alternatively in dendritic cells. We detected Activin A in sera of MM/MGUS/healthy subjects by ELISA and in the same cohort we evaluated the absolute count of monocytes and all subset of inflammatory monocytes, identified by flow cytometry as CD14+CD16+. We observed a significant increase of circulating Activin A in sera of patients affected of MM compared to MGUS/smouldering MM and healthy subjects ($p<0.0001$). Among MM patients, Activin A was significantly increased in presence of osteolytic lesions ($p=0.014$, unpaired t- test). Even though absolute count of monocytes was similar between healthy subjects vs MGUS vs MM, inflammatory monocytes in MM were higher than in other subjects, so suggesting that chronic inflammation is activated in differential manner in gammopathies, characterised by different kind of soluble mediators.Taken together, our findings suggest a role for Activin A in driving the evolution of MGUS in MM through mechanisms immunosuppressive, and contributing to sustain osteolysis. Activin A could be used as surrogate of macrophages activity in bone marrow.

P-055

IMMATURE MYELOID SUPPRESSOR CELLS SUBPOPULATIONS IN MONOCLONAL GAMMOPATHIES

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The immune function in Multiple Myeloma (MM) is impaired consequently to an immunologically hostile microenvironment and cellular defects, differently from MGUS. Myeloid-derived suppressor cells (MDSC) are a heterogeneous mixture of myeloid cells in different maturation stages that accumulate in the secondary lymphoid organs defined in mice as GR1+CD11b+ cells capable of suppressing antigen-specific or nonspecific T cell activation responsible of progression of solid tumours. We identified in flow cytometry an immature subset of Lin-, CD34+, CD33+, CD14-, CD11b+MDSC (imMDSC) with differential behaviour in MM/MGUS. imMDSC were higher in MM compared to MGUS ($p=0,0001$), but no difference was appreciable between MGUS and healthy controls (HC). The mature fraction CD33+, CD14-, CD11b+ MDSC (N-MDSC) was reduced in MM compared to HC, positively correlated with CD4+CD25+FoxP3+ Treg cells, concomitantly reduced in MM. imMDSC levels in MM were related to clinical stage and disease activity. Indeed, patients with complete remission in follow up (>9 months) had similar levels of MDSC compared to MGUS/HC. Taken together, our findings suggest a role for immunological impairment in driving the evolution of MGUS in MM through immunosup-

pressive mechanisms. MGUS does not exhibit an immunological impairment as evident as for MM, suggesting that immune system is able to control the expansion of neoplastic cells.

P-056

BASES OF CANCER SENSITIVITY TO PROTEASOME INHIBITORS: COMPARATIVE PROTEOMICS OF MULTIPLE MYELOMA LINES

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Multiple Myeloma (MM) is the paradigmatic cancer responsive to proteasome inhibitors (PI), but the bases of PI sensitivity are poorly understood. We proposed proteasome capacity and degradative load as critical determinants of PI sensitivity, with highly PI-vulnerable MM cells expressing low immuno-proteasome levels and accumulating poly-ubiquitinated proteins at the expense of free ubiquitin, providing a unique framework to study the bases of PI sensitivity (Blood 2009;113:3040). Here, we deployed a Mass Spectrometric SILAC approach to compare the proteome of PI-sensitive MM.1S cells and resistant U266 cells. The entire proteome of U266 and MM.1S cells was labelled with Heavy (H, LYS8-ARG10) and Light (L, LYS0-ARG0) media (>6 cell cycles). The H/L peak ratio provided a relative quantification in the two cell lines of ~2,000 proteins identified in two biological replicates, with high accuracy and reproducibility. PI sensitivity correlated with increased expression of proteins involved in protein folding and secretion, oxidative stress and deubiquitination. Results were validated by immunoblotting, and differences confirmed in additional MM lines with similar PI sensitivity, strengthening their significance. Moreover, proteasome subunits, including inducible ?-peptidases (LMP2, LMP7, MECL-1), were similarly expressed, despite different proteasome capacity, suggesting reduced proteasome assembly as a mechanism for PI sensitivity. In conclusion, comparative proteomics of MM lines reveals valuable correlates of proteasome stress, of potential prognostic and therapeutic use.

P-057

THE RECONSTRUCTION OF TRANSCRIPTIONAL REGULATORY NETWORKS REVEALS CRITICAL GENES WHICH HAVE IMPLICATIONS FOR CLINICAL OUTCOME OF MULTIPLE MYELOMA

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The bioinformatics analysis of microarray data has improved our understanding of biological complexity of multiple myeloma (MM) but, when applied in attempt to predict clinical outcome, has led to controversial results, with the identification of heterogeneous molecular signatures. Herein, we have reconstructed transcriptional regulatory networks using ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks) and microarray data of 1883 MM patients from seven publicly available datasets. Critical analysis of network components allowed the identification of genes and interactions conserved between datasets and crucial for transcriptional networks, revealing that i) CCND1 and CCND2 were the most critical genes; ii) CCND2, AIF1 and BLNK had the largest number of connections shared among the datasets; iii) robust gene signatures with prognostic power were derived from the most critical transcripts and from their shared primary neighbors. Specifically, a "critical-gene" model, comprising FAM53B, KIF21B, WHSC1 and TMPO, and a "neighbor-gene" model, comprising BLNK and its shared neighbors CSGALNACT1 and SLC7A7, predicted survival in all datasets with follow-up information. The reconstruction of gene regulatory networks in a large panel of MM tumors defined robust and reproducible signatures with prognostic importance, and may lead to identify novel molecular mechanisms central to MM biology.

P-058

PHENOTYPIC AND FUNCTIONAL STUDIES OF SIDE POPULATION CELLS IN MM

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In multiple myeloma (MM) patients who achieve complete clinical remission, their eventual relapse is considered to be due to small populations of persistent tumor cells which are not targeted by established anti-MM strategies. We attempted to identify and characterize a small distinct sub-population with "stem-like" features known as side population (SP) in MM. We observed heterogeneity in the proportion of SP fraction in the panel of MM cell lines and primary MM tumor cells. Importantly, MM SP fraction showed strong clonogenic potential, and increased tumor initiation capacity in vivo. We confirmed that SP fraction contains MM clonotypic cells, however with heterogeneous expression of CD138. We observed that SP fraction express significantly higher levels of ABCG2 transcripts compared to non-SP cells, and further corroborated this finding with functional assays. SP cells had a higher proliferation index compared to non-SP cells, besides coculture of MM cells with bone marrow stromal cells led to increased percentage, viability, as well as proliferation potential of SP cells. We next evaluated whether novel anti-MM therapeutic strategies, such as IMiDs, HDAC inhibitors or compounds implicated in developmental pathways of hematopoietic stem cell regulation, can also effectively target MM SP sub-population. Our studies therefore provide insight towards characterizing of clonogenic and tumorigenic SP cells, providing the framework for new therapeutic strategies targeting subpopulations of MM cells including presumptive stem cells.

P-059

CCL27 ENHANCES MYELOMA GROWTH, ADHESION AND MIGRATION AND MODULATES IMMUNE CELL FUNCTIONS

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The interaction of myeloma cells with the bone marrow microenvironment has a crucial role in the pathogenesis of this disease. Chemokines have currently been shown to be major players in shaping the tumor microenvironment. We found that CCL27, a chemokine which has been correlated with skin disease so far, was produced by several myeloma cell lines. Immuno-histochemical analysis of patient bone marrow confirmed the expression of CCL27 in this niche. Functional assays showed that CCL27 enhances tumor cell proliferation if combined with myeloma growth factor IL-6. Myeloma adhesion to stroma cells as well as migration over bone marrow endothelial cells was enhanced by CCL27 pointing to a role in tumor survival and spread. We further investigated the impact of CCL27 on immune cells. Dendritic cells which were differentiated and matured in the presence of CCL27 (DC-CCL27) exhibited a reduced capacity to activate T cells. Moreover, chemotactic response of dendritic cells and T-cells to other chemokines was altered by the addition of CCL27 which might contribute to altered immune cell distribution and function in multiple myeloma. Finally, in coculture experiments with myeloma cell lines, DC-CCL27 induced enhanced growth of the malignant plasma cells. In summary, we found a novel myeloma-derived chemokine, CCL27, which exhibited autocrine and paracrine potential to interfere with growth, activation and migration of myeloma cells and cells of the tumor microenvironment.

P-060

SYSTEMIC INFUSION OF MESENCHYMAL STEM CELLS HAS A POTENTIAL RISK FOR MULTIPLE MYELOMA DISEASE DEVELOPMENT

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Mesenchymal stem cells (MSCs) give rise to most bone marrow stromal cells that interact with MM cells. However, the direct involvement of MSCs in MM pathophysiology has not been addressed. In the present study, in vitro and in vivo migration assays reveal that MSCs can migrate towards MM sites, and CCL25 is identified as a major MM cell-produced chemoattractant for MSCs. By in vitro co-culture experiments, we found that MSCs favor the proliferation of stroma-dependent MM cells by secreting soluble factors and cell-cell contact. This growth promoting effect was also demonstrated by intrafemoral co-engraftment experiments in the in vivo mouse myeloma model 5T33MM. We also demonstrated that MSCs protect MM cells in vitro against spontaneous and Bortezomib-induced apoptosis. The tumor-promoting effects of MSCs correlates with their capacity to activate MM cells AKT and ERK activities, accompanied with increased expression of CyclinD2, CDK4 and Bcl-XL, and decreased cleaved caspase-3 and PARP expression. In turn, MM cells upregulate IL-6, IL-10, IGF-1, VEGF and DKK1 expression in MSCs. Finally, systemic infusion of in vitro expanded murine MSCs in 5T33MM mice results in a significantly shorter survival as compared to the control group being injected with MM cells without murine MSCs. MSC infusion is a promising way to support hematopoiesis and control of GVHD for patients after allogeneic hematopoietic stem cell transplantation. However, our data suggest that MSC-based cytotherapy should be considered with caution in MM patients.

P-061

THE LOAD/CAPACITY HYPOTHESIS DOES NOT EXPLAIN THE SENSITIVITY OF MYELOMA (MM) CELLS TO THE PROTEASOME INHIBITOR (PSI) BORTEZOMIB (BZB): SILENCING PSMB5 BUT NOT IGGλ OR PSMB8 SIGNIFICANTLY ALTERS SENSITIVITY TO BZB IN VITRO

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M-protein load may sensitize MM cells to Bzb but models thus far have been subclones of a human MM cell line differing 5X with respect to Ig M-protein production (Cancer Res 67:1783) and 4 MM cell lines differing in proteasome (PS) capacity (Blood 113:3040). We used ALMC1 MM cells that produce IgGλ (Blood 112:1931) and decreased M-protein load by knocking down Ig heavy and light chain genes (IgGλ) and decreased PS capacity by knocking down subunits β5c (PSMB5) or β5i (PSMB8). We achieved gene silencing causing no apoptosis and no loss of viability or proliferation in scrambled (scr) or si[Target] cells compared to wildtype (wt) (streptolysin-O, J Immun Meth 333:147). We tested the impact of knockdowns on apoptosis and sensitivity to Bzb. Twenty-four hours after knockdown, M-protein was decreased by one log (flow) and PSMB5 or PSMB8 by > 90% (qRT-PCR). There was no difference in AnnV/PI staining among all knockdowns compared to wt and scr. After 24hr culture with Bzb, there were no differences in sensitivity by MTT assay between wt or scr cells. Si[IgGλ] cells were no less sensitive than scr except at 25nM Bzb (Fig 1). Si[PSMB5] cells were significantly more sensitive than scr or si[PSMB8] cells at all concentrations of Bzb (Fig 2). In ALMC1 cells PS capacity is more important than M-protein load in determining sensitivity to Bzb. We continue these experiments with measurements of functional PS capacity and a focus on whether and how knockdown of Bzb's target PSMB5 or other subunits alters sensitivity to PSIs in ALMC1 and other MM cell lines.

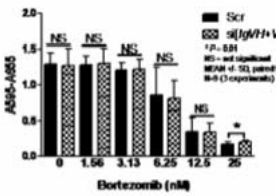

Figure 1

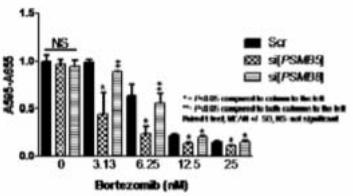

Figure 2

P-062

EPIGENETIC ALTERATIONS AND ABNORMALITIES IN DNA DAMAGE RESPONSE PATHWAYS IN THE PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) OF PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS), SMOLDERING MYELOMA (SMM) AND SYMPTOMATIC MULTIPLE MYELOMA (MM)

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MM is consistently preceded by the precursor states of MGUS and SMM. The aim of this study was to compare epigenetic and DNA damage response pathways in the PBMCs of patients with MGUS, SMM and symptomatic MM. PBMCs were isolated from 12 healthy volunteers (7M/5F; median age: 41 years), 10 patients with MGUS (5M/5F; 68.5 years), 10 with SMM (4M/5F; 64 years), and 32 patients with symptomatic MM (14M/18F; 59 years) who underwent high-dose melphalan and ASCT; of those, 23 achieved a further reduction of paraprotein after ASCT (defined as responders) and 9 did not (defined as non responders). In all subjects, beta-actin, p53 and N-ras genes were transcriptionally active. Importantly, delta-globin gene was silent in all healthy volunteers and MGUS patients, while an induction of the transcription activity of this gene was found in all SMM patients and in 29/32 (90%) of MM patients. Chromatin condensation, gene-specific DNA damage repair efficiency, accumulation of p53 protein, recovery of RNA synthesis as well as induction of apoptosis after ex vivo exposure of PBMCs to melphalan followed the same order: healthy volunteers < MGUS < SMM < symptomatic MM; responders < non-responders to melphalan therapy. We conclude that epigenetic alterations and abnormalities in the DNA damage response pathways can be detected in PBMCs taken from MGUS, SMM and MM patients and may identify possible links with the molecular mechanisms that drive malignant evolution in MM and that characterize the cellular chemosensitivity and subsequent better response to chemotherapy in patients with MM.

P-063

NOTCH PATHWAY DYSREGULATION IS INVOLVED IN IMPAIRED OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS DERIVED FROM MULTIPLE MYELOMA PATIENTS

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It is believed that bone marrow-derived mesenchymal stem cells in MM patients (MM-MSCs) harbor genetic and functional abnormalities. However, regarding the osteogenic differentiation ability of MM-MSCs, conflicting observations were reported. We observed that MM-MSCs, especially those from patients with bone lesions, exhibited significantly decreased alkaline phosphatase (ALP) activity, reduced expression of specific osteogenic markers (OPN, BMP2, OTX and BSP) and impaired matrix mineralization, compared to MSCs from normal donors (ND-MSCs). However, MGUS-MSCs did not show a significantly impaired osteogenesis ability. Previous reports suggested that NOTCH pathway can maintain bone marrow mesenchymal progenitors in a more undif-

ferentiated state by suppressing osteoblast differentiation. Similarly, we found that NOTCH signaling, including NOTCH1, Jagged-1 and downstream genes hes1, hes5, hey1, hey2, heyL, was remarkably suppressed during ND-MSC osteogenesis. However, it was observed that the expression of these NOTCH signaling genes in MM-MSCs did not decrease to the level of ND-MSC (with statistical significance), implicating that the NOTCH pathway remains over-activated in MM-MSCs. Finally, we demonstrated that the NOTCH pathway inhibitor DAPT could significantly enhance the impaired osteogenic differentiation ability of MM-MSCs. In conclusion our data indicate that MM-MSCs exhibit lower osteogenic differentiation ability, and that this impairment is associated with an inappropriate NOTCH pathway deactivation during the osteogenic process.

P-064

THE ANTI-MYELOMA DRUG VORINOSTAT PROMOTES OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS (MSCS)

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We have recently shown that bone marrow-derived MSCs from MM patients exhibited much lower osteogenic differentiation ability compared to normal MSCs. Recent studies indicated that novel anti-MM agents not only target the MM cell directly but also affect the BM microenvironment. Vorinostat is the only HDAC inhibitor drug currently used in clinical phase I/II trials for MM patients. In the present study, we found that Vorinostat increased in bone marrow MSCs from both MM patients and normal donors, in a concentration dependent manner (0-1μM), the activity of alkaline phosphatase (ALP), which is an early marker of osteoblast differentiation. This osteogenesis-promoting effect was also confirmed by PCR analysis for osteogenic markers (OPN, ALP, BSP, BMP2 and OTX) and matrix mineralization. Importantly, we found that Vorinostat upregulates Runx2 expression of MSCs which is a key transcription factor for osteoblastogenesis. Vorinostat increased, even without exogeneous osteogenic stimuli, ac-H3 and p21 expression of MSCs, but suppressed HDAC1 and HDAC4 activity, mimicking natural epigenetic alteration during MSCs osteogenic differentation. We observed that Vorinostat affected in vitro MSCs viability with an IC50 of 15.57μM, while the observed IC50 for myeloma cells RPMI8226, Karpas and U266 was 0.71μM, 0.24μM and 1.29?M, respectively, indicating that MSCs are much more resistant to the action of this drug. The effect of vorinostat on MSCs osteogenesis in vivo is currently under investigation.

P-065

ANALYTICAL EVALUATION OF HEVYLITE IGG, IGA AND IGM KAPPA/LAMBDA ASSAYS USING SPAPLUS INSTRUMENT

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Polyclonal immunoglobulins and co-migration with other serum proteins can make identification of monoclonal proteins difficult by protein electrophoresis. Immunoassays have been developed which can quantify serum Ig'κ/Ig'λ concentrations. Here we report on the analytical performance of HLC assays on the SPAPLUS instrument. Assays were run in accordance with manufacturer's instruction. Intra assay precision was assessed using quality control (QC) and patient samples (n=10). Inter assay precision was assessed using QC samples in triplicate on 10 separate batches. Linearity was assessed at standard dilutions using triplicate measurements of clinical samples.

HLC results for all classes were reproducible with good linearity (r^2 =1) and suggest its suitability for clinical application in identification of paraproteins.

Precision CV % (mg/L)		IgGκ	IgGλ	IgAχ	IgAλ	IgMκ	IgMλ
Intra assay	QC 1	2.9 % (4.56)	2.8 % (3.43)	0.8% (1.72)	1.4% (1.53)	1.72% (0.78)	0.5% (1.1)
	QC 2	0.7% (17.27)	1.0 % (10.36)	1.7% (6.09)	2.3% (5.15)	1.1 % (3.31)	1.1 % (1.96)
	Patient	2.0 % (4.47)	2.0% (7.19)	2.6% (0.58)	2.4% (0.51)	1.5% (0.87)	1.6% (0.38)
Inter assay	QC 1	2.4% (4.66)	1.8% (3.43)	1.5% (1.75)	1.9% (1.55)	2.9% (0.79)	5.7% (0.51)
	QC 2	3.0% (17.99)	8.3% (12.20)	2.1% (6.19)	3.0% (5.30)	3.6 % (3.23)	5.0% (2.00)

	Linearity	Range (mg/L)
IgGκ	0.99x-0.04	1.2-19.0
IgGλ	0.99x-0.02	0.6-10.2
IgAκ	1.00x-0.03	0.5-7.5
IgAλ	1.02x-0.04	0.4-6.1
IgMκ	1.00x-0.02	0.1–2.3
IgMλ	1.01x-0.00	0.1–1.4

P-066

CHARACTERIZATION OF CLONAL CD19+/CD34+ PROGENITOR CELLS IN MULTIPLE MYELOMA

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Bone marrow (BM) CD138-/CD19+ with identical free light chain as plasma cells (PC) have been considered putative Multiple Myeloma (MM) stem cells. In our studies, CD138-/CD19+ population (n=30) represents 2.4 % (0.5% to 3.9%) of whole bone marrow (WBM) and the clonotypic CD138- compartment is 0.7% (0.1% to 2%). The kappa/lambda ratio mean is 1.46 (range 00.1 to 4.56) by flow (n=33). CD138-/CD19+ cells co-expresses CD34 in 10-30% (Pro-B cells) being majority CD34- (Immature B cells). Pro-B cells express CD 20 (30± 2%), CD 27 (80±5), CD45 (99±1), C-kit (25±3), Notch (91±3) and ALDH enzymatic activity (7±2) lacking CD56 expression. Immature B-cells express CD20 (80± 3%), CD27 (13±3), CD45 (99±1), C-kit (3±0.5), Notch (95±2) and ALDH enzymatic activity (7±3) lacking CD56 expression. Flow sorted CD138+ cells did not form colonies, whereas CD138-/CD19+ regardless of CD34 expression grew lympho-plasmacytoid colonies with a low efficiency of 1 in 25,000 in methylcellulose supplemented with 5% PHA-LCM at 2 weeks. Colony efficiency was optimized using conditioned medium from stroma. CD138-/CD19+ differentiated into CD138+ expressing PC with high efficiency (80±5%) compared to HSC (10±5%). CD138-/CD19+ cells were bortezomib and melphalan resistant. We hypothesize that CD138-/CD19+/CD34+ cells contains earlier BM progenitor B cells that differentiate into the malignant PC. Surrogate assays for stem cell activity and xenotransplant models should determine cancer stem cell activity of MM Pro-B cells. Research studies of CD138- cells will allow studies of a potential MRD MM reservoir.

P-067

METABOLIC CHARACTERIZATION OF HUMAN MULTIPLE MYELOMA (MM) CELLS WITH HIGH-RESOLUTION MAGIC ANGLE SPINNING MAGNETIC RESONANCE SPECTROSCOPY (HRMAS-MRS)

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Aims: Whereas knowledge of MM genomics and proteomics have made significant progress, approaches to the MM metabolome are still emerging. We attempted to define the main characteristics of MM metabolite profiles measured in intact MM cells and define metabolomic differences among MM subtypes. *Methods:* MM samples (n = 40) obtained from bone marrow aspirates were analyzed with HRMAS-

MRS. Metabolite profiles were correlated with clinical, genetic and phenotypic characteristics of the cells. *Results:* MM cells are lipid-rich cells in contrast with other high-grade or low-grade lymphoproliferative disorders. This lipidic content (LC) includes saturated and unsaturated fatty-acids with a mean methyl to methylene ratio (M/MR) of 2.8 (SD 0.75). The overall LC was significantly reduced (<0.5 of mean arbitrary units) in patients with aggressive disease (p=0.026), high LDH levels (p=0.011) and IgH translocations (p=0.005). LC did not vary in patients studied at diagnosis and with advanced disease but was diminished in n=5 samples obtained from peripheral blood or effusions (p<0.001). The M/MR correlated with CD56 expression (r=0.047, p=0.011). M/MR<3 was associated with IgH translocations (p=0.031) and aggressive disease (p=0.025). *Conclusions:* MRS metabolite profiles of plasma cells display a greater LC with respect to other lymphoproliferative disorders. In patients with aggressive disease such LC is significantly diminished. Additionally patients with clinically aggressive disease, IgH translocations and high CD56 expression, are characterized by a lower M/MR.

P-068

MULTIPLE MYELOMA SURVIVAL AND CROSSTALK BETWEEN NOTCH1-JAGGED2 AND CD28-CD80/86 PATHWAYS IN DENDRITIC CELLS

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Multiple myeloma is a neoplasm of bone marrow resident plasma cells. Survival of myeloma cells is characterized by their critical interaction with bone marrow stromal cells, which produce IL-6. However, the molecular and cellular components involved in myeloma induced IL-6 production remain largely uncharacterized. At the cellular level, dendritic cells (DC) in the bone marrow microenvironment and at the molecular level the CD28-CD80/86 and Notch1-Jagged2 pathways were separately implicated by us in myeloma induced IL-6 production. While Notch signaling leading to IL-6 production in DC is well understood, the mechanism of "backsignaling" via CD80/86, a ligand with a short cytoplasmic tail, is largely uncharacterized. Inhibiting Notch signaling using pharmacological inhibitors and blocking antibodies leads to a significant decrease in CD28 mediated IL-6 production by DC suggesting crosstalk between the Notch1-Jagged2 and CD28-CD80/86 pathways. We have also found that CD28 mediated crosslinking of CD80/86 leads to activation of P-Akt(Thr 308). Blocking PI3K activity and PKC activation leads to significant downregulation of IL-6 production by DC thus implicating a PI3K-PKC axis downstream of CD80/86. We are examining the effect of Notch pathway on the PI3K-Akt pathway in myeloma induced IL-6 production involving crosstalk. Since IL-6 neutralizing antibodies fail to suppress myeloma proliferation and survival in long term, blocking IL-6 production by targeting crosstalk between these two pathways presents a novel approach in multiple myeloma treatment.

P-069

EVALUATION OF A NEW CONCEPT FOR THE DIAGNOSIS OF IGA MONOCLONAL GAMMOPATHIES : IGA HEVYLITE

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Aim: To evaluate a new assay based upon the ratio IgA kappa / IgA lambda (IgA HLC) to detect monoclonal IgAs and compare it to capillary zone electrophoresis (CZE) and immunofixation-electrophoresis (IFE) *Material and Methods:* 157 sera issued from 122 patients known for having a monoclonal IgA (55 multiple myeloma, 58 Monoclonal gammopathies of undetermined significance, 5 light chain amyloidosis, 4 miscellaneous) were run on Capillarys II™ (SEBIA) and typed IgA kappa or lambda using either automated typing with Capillarys, or IFE performed on SAS III™ (HELENA). IgA Kappa and IgA Lambda were quantified with HevyLite™ immunassay on the Spa PlusTM analyser (The Binding Site). According to the ratio IgA HLC patients were classified IgA Kappa when the ratio was over 1.94, IgA Lambda when under 0.78, and considered as having no M-IgA when it fell within the reference range. *Results:* Of 113 samples with an M-IgA spike, IgA HLC ratio correctly identified 101 samples resulting in a sensitivity of 90 % versus SPE (100%). Of 44 sera with no M-IgA spike and positive IFE only 16 had an abnormal IgA-HLC ratio. 63 % of the samples would have been misdiagnosed with the quantitative assay. *Conclusion:* For samples with no M-spike, IFE remains a more sensitive technique to detect small M-IgAs. Even if CZE appears to be a more sensitive technique, IgA HevyLiteTM provides a sensitive method for identifying M-IgAs when there is an M-spike on electrophoresis.

P-070

DECREASE OF MYELOID AND LYMPHOID DENDRITIC CELLS IN PERIPHERAL BLOOD AND BONE MARROW OF PATIENTS WITH MULTIPLE MYELOMA

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Dendritic cells (DC) are one of the key cell populations in the immune system. In the present study we evaluated DC populations in patients with multiple myeloma (MM). The study group consisted of 35 previously untreated MM patients and control group of 14 healthy donors. Using flow cytometry, we evaluated myeloid and lymphoid DC, as well as the relationships between DC and sub-populations of T lymphocytes, NK cells and NKT cells. As compared to the control group, MM patients had a lower percentage of myeloid DC in peripheral blood (p=0.000001) and bone marrow (p=0.000009) as well as lymphoid DC in peripheral blood (p=0.00005) and bone marrow (p= 0.00005). In patients with advanced MM, the percentage of myeloid and lymphoid DC in bone marrow was lower as compared to the earlier stages (p=0.02, p=0.01, res.). There was a significant inverse correlation between the percentage of myeloid DC (p = 0.0005, R =-0.56) and lymphoid DC (p = 0.003, R =-0.49) in peripheral blood and serum ⨍2-microglobulin, as well as the percentage of plasma cells in bone marrow and the percentage of myeloid DC in bone marrow (p=0.006, R=-0.55). The percentage of lymphoid DC in peripheral blood correlated with the number of CD8+ T cells (p=0.04, R=0.33) and NKT cells (p=0.04, R=0.34). These observations suggest an important role of both myeloid and lymphoid DC in pathogenesis of MM. The progressive reduction of the number of DC might be also responsible for increased susceptibility for infections in MM patients.

P-071

LONGITUDINAL ASSESSMENT OF HIGH RISK T(4;14) MULTIPLE MYELOMA USING NEXT GENERATION WHOLE GENOME SEQUENCING

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To understand genetic events associated with disease progression and development of drug resistance in multiple myeloma (MM), we studied 4 longitudinally collected samples from a single, high risk, t(4;14) MM patient. Samples included diagnosis, first progression on lenalidomide, and second progression on bortezomib. DNA was extracted from blood and CD138+ purified bone marrow tumor cells; whole genome sequencing (WGS) was conducted on the Life Technologies SOLiD™ platform. WGS of 50bp fragment libraries provided >100x109 bases/sample and ~30X aligned coverage. Data aligned to the reference genome (NCBI36/hg18) with BFAST and post-alignment removal of duplicates and recalibration performed with Picard and Genome Analysis Toolkit respectively. SolSNP and Paired Mutation Walker identified 215 novel single nucleotide variants (SNV) in 207 genes and Polymorphism Phenotyping v2 predicted 125 potentially damaging and 7 introducing nonsense mutation SNVs. Only 17 novel SNVs are common to all tumor time points. Diagnostic and second progression shared 7 SNVs while first progression and second progression shared only 4 SNVs. This suggests the presence of multiple clones at diagnosis, which may emerge or regress under treatment selection pressure. WGS has revealed evidence of somatic mutations indicative of the presence of multiple clones

at diagnosis and clonal evolution over time. This supports the use of drug therapies targeting multiple clones and challenges assumptions regarding refractory disease. Evaluation in a larger population is needed to confirm these findings.

P-072

EXTRACELLULAR MATRIX REMODELING AND STROMAL CELL-DERIVED TUMOR PROMOTION IN THE BONE MARROW REFLECT THE PROGRESSION OF MGUS TO MULTIPLE MYELOMA

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MGUS precedes virtually all cases of MM indicating a multistep pathogenesis. Molecular events leading to transition from MGUS to MM are still poorly defined. We hypothesized that the bone marrow microenvironment is critically involved in the pathogenesis of monoclonal gammopathies. We performed a comparative proteome profiling study and investigated the contribution of bone marrow fibroblast precursor cells to disease progression in MM. Primary bone marrow fibroblasts from patients with MGUS and MM were compared to control fibroblasts obtained from hip replacement surgery. Primary cells were cultured for 3 to 5 passages, characterized by FACS analysis, fractionated into cytoplasmic, nucleic and secreted protein fractions and then analyzed using shotgun proteomics. Confirmatory experiments were performed using Western blotting. Strikingly, a group of extracellular matrix (ECM) proteins, ECM receptors and ECM-modulating enzymes was found to be progressively up-regulated from controls to MGUS and to MM. These proteins include laminin 8, lysyl hydroxylase 2, nidogen-2, integrin alpha-5, macrophage mannose receptor 2, PAI-1 and MMP-2. Additionally, the growth factors periostin and stem cell growth factor as well as PDGF-receptor beta showed a similar progression-related pattern. Our results indicate that ECM remodeling and stromal cell-derived tumor promotion in the bone marrow takes place already at the level of MGUS and becomes even more pronounced in MM. Thus, for the first time, marker proteins could be identified indicating a step-wise progression from MGUS to MM.

P-073

PATTERNS OF ALTERED CYTOKINE PROFILES IN MULTIPLE MYELOMA AND MGUS: BIOLOGICAL VARIATION BETWEEN COMPARTIMENTS, ACROSS ISOTYPES, AND DISEASE STATES

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Background: The bone marrow (BM) microenvironment plays a critical role in multiple myeloma (MM), supporting cell survival, expansion and migration. Limited data are available in MGUS. Aim of this study was to characterize cytokine profiles in BM supernatant and peripheral blood (PB) from MGUS and MM pts. *Methods:* Using ultra-sensitive TH1/TH2 multiplex ELISA and Western blot techniques, we determine protein concentrations of the following cytokines: IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, IL-13, IFN-gamma, and TNF-alpha, in the BM supernatant and PB of MM pts, MGUS pts, and controls. *Results:* Compared to controls, we found altered BM supernatant concentrations of TNF-alpha (10.650 vs. 4.980 pg/ml, p=0.016), IL-1beta (0.038 vs. 2.352 pg/ml, p=0.047), IL-8 (11.520 vs. 43.590 pg/ml, p=0.016); in PB we found IL-2, IL-5, IL-6, IL-8, IL-10, and TNF-alpha to be elevated (p<0.05). In MM pts, hierarchical clustering analysis suggested two distinct cytokine profiles; TNF-alpha was increased in both BM and PB, while IL-6 and IL-8 patterns were different in the two compartments. IL-6 was elevated in PB from both MGUS and MM pts; levels were higher in IgA (vs. IgG) (p=0.002). *Conclusions:* Our results provide novel insight regarding altered cytokine profiles in myelomagenesis. Although we found patterns of biological variation between compartments (BM supernatant vs. PB), across isotypes (IgG vs. IgA), and disease states (MGUS vs. MM), hierarchical clustering analysis defined two distinct cytokine profiles.

P-074

MYELOMA-DERIVED MICROPARTICLES CONTRIBUTE TO THROMBOGENICITY AND ANGIOGENESIS EXISTING IN MYELOMA

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Microparticles (MPs) (0.1-1 micron membrane vesicles), shed from cell surfaces upon activation or apoptosis, carry cellular components of their originating cells. Tissue factor (TF), the coagulation initiating factor, and angiogenic factors, including vascular endothelial growth factor receptor (VEGFR) 1-Flt1 and 2-KDR, are major players in the pro-thrombotic pro-angiogenic environment existing in multiple myeloma (MM). Study aims included: isolation and characterization of MM derived MPs and evaluation of their thrombogenic and angiogenic effects on endothelial cells (EC). MPs were isolated from RPMI 8226 MM cell line following the exposure to starvation or IL6(10 or 25 ng/ml) and TNF-α (10ng/ml). MPs were characterized and compared to their parent cells and their thrombogenic and angiogenic impact on EC was evaluated. RPMI 8226 cells expressed high levels of CD38 (95%) and low levels of VEGFR-1 (~5%), irrespective of the stimulant used. However, cell stimulation with TNF-α resulted in increased levels of both TF and flt1 compared to those measured in IL-6 -stimulated cells. Interestingly, MM-MPs expressed lower levels of CD38 (~55%) and higher levels of VEGF2 (~20%) compared to their parent cells. MM-MPs obtained following stimulation with TNF-α, demonstrated the highest level of TF. Remarkably, MM-MPs elicited significant mitigation of human umbilical vein EC (8300-9040 μm^2), which was much higher than that observed without MPs (335 μm^2). In summary, the data obtained suggest the importance of MM MPs in myeloma-related thrombogenicity and angiogenesis.

P-075

COMPARISON OF SERUM FREE LIGHT CHAIN RATIOS WITH STANDARD URINE ANALYSIS IN DIAGNOSIS AND MONITORING OF MULTIPLE MYELOMA

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Background: With the exception of oligosecretory and light chain multiple myeloma (LCMM), serum free light chain (sFLC) measurement is not recommended for monitoring multiple myeloma (MM). Here we present sequential sample data comparing the utility of urine electrophoresis (UPE), urine immunofixation (UFE) and sFLC measurements in MM monitoring. *Methods:* sFLC κ/λ ratios (FLCr) were measured retrospectively in serial serum samples obtained from 182 MM patients enrolled onto the IFM 2007-01 trial. Results for involved FLC (iFLC) and FLCr were compared to historic UPE and UFE data at 4 time points; presentation, post-cycle 2, post-cycle 4 and post autologous stem cell transplant (ASCT). *Results:* At presentation 99% of patients had an abnormal FLCr, with 68% of patients having measurable disease by iFLC (>100mg/L). In contrast, 51% of patients were positive by UFE, with only 45% having measurable disease by UPE (>200mg/L). In 50% of UFE positive intact immunoglobulin MM (IIMM) patients, UFE became negative after 2 cycles of therapy compared to 5% of patients achieving complete response (CR). Similarly in LCMM after 2 cycles of therapy UFE indicated CR in 50% of patients compared to just 10% by FLCr. 8/421 (1.9%) IgG samples were scored with a positive UFE but normal FLCr, 7/8 were positive for intact immunoglobulin only. *Conclusion:* Compared to IFE and FLC, the use of UFE can lead to an over estimation of treatment responses in both IIMM and LCMM. The presence of intact immunoglobulins in urine can make UFE interpretation difficult in a small number of cases.

P-076

NEW INSIGHTS INTO THE ROLE OF ANGIOPOIETINS IN MULTIPLE MYELOMA ASSOCIATED ANGIOGENESIS

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Bone Marrow (BM) angiogenesis correlates with progression and poor prognosis in Multiple Myeloma (MM). BM associated Endothelial Cells (MMEC) are exaggeratedly activated, and the resulting neo-formed vessels are more numerous and aberrant. Angiopoietins (Angs) represent important regulators of angiogenesis and vascular stability via the competitive binding to the common tyrosine kinase receptor Tie2, which is expressed in EC. Ang-1 ensures vascular stability, while Ang-2 destabilizes neo-forming vessels. The balance between Ang-1 and Ang-2 is critical in developing angiogenesis in solid tumors. More recently, high levels of circulating Ang-2 were found to be prognostic in haematological malignancies, including MM. In the present study, we investigated the expression and pro-angiogenic functions of Angs in BM sera from MM patients. Preliminary results indicate that BM levels of Ang-1 and Ang-2, and particularly the Ang-1/Ang-2 ratio, discriminate between patients with MM and MGUS, while VEGF, SDF-1, IL-8 and MCP-1, all involved in angiogenesis, were not significantly different between the two groups. MM plasma cell, in addition to BM macrophages and MMEC from patients with MM, may be sources of Ang-2. Finally, differential contents of Angs in the BM sera of MM patients determined their pro-angiogenic potential, as assessed by specific in vitro assays. Altogether, our results indicate that Angiopoietins could be exploited as molecular targets and biomarkers of ongoing angiogenesis in MM.

P-077

DENDRITIC CELLS LOADED WITH PRETREATING MYELOMA CELLS WITH COMBINATION OF JSI-124 AND BORTEZOMIB CAN GENERATE POTENT MYELOMA-SPECIFIC CYTOTOXIC T LYMPHOCYTES THROUGH RECOVERING DYSFUNCTION OF DENDRITIC CELLS IN MYELOMA PATIENTS

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Background: Signal transducers and activators of transcription 3 (STAT3) is highly activated in multiple myeloma and activated STAT3 is known to promote proliferation of cancer, to suppress Th1 immune responses, and to induce dysfunction of immune cells, including dendritic cells (DCs). We investigated whether pretreating myeloma cells with p-STAT3 inhibitor (JSI-124), bortezomib, or both before loading onto DCs can affect DC function. *Methods:* U266 myeloma cells were pretreated with bortezomib, JSI-124, both or γ-irradiation. Monocyte-derived DCs were loaded with dying myeloma cells 2 h after addition of LPS. The DCs loaded with dying myeloma cells were harvested on day 8. *Results:* Treatment of combination JSI-124 and bortezomib showed the highest expression of Hsp90 and the lowest expression of p-STAT3 on dying myeloma cells. DCs loaded with dying myeloma cells treated by JSI-124 +bortezomib showed the lowest expression of p-STAT3 compared to other treatments. Those DCs were recovered from abnormal cytokine secretions of IL-10, IL-6 and IL-23 without any effect on the production of IL-12p70. DCs loaded with dying myeloma cells treated by JSI-124 + bortezomib could generate the most potent myeloma-specific CTLs compared to other treatments. *Conclusion:* Our data suggested that pretreatment of myeloma cells with combination JSI-124 and bortezomib can recover DC dysfunction from loading the dying myeloma cells through the up-regulation of Hsp90 and the down-regulation of p-STAT3 and inhibitory cytokines, and these DCscan generate to potent myeloma-specific CTLs.

P-078

COMPARISON OF IMMUNONEPHELOMETRY TO CAPILLARY ZONE ELECTROPHORESIS FOR QUANTITATING SERUM ALBUMIN IN PATIENTS WITH OR WITHOUT AN ELECTROPHORETIC M-SPIKE

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Aim: To analyse differences in albumin estimation in patients with or without an electrophoretic M-spike using either immunological (IQ) or electrophoretic quantitation (EQ). *Material and Methods:* Albumin results obtained from the same serum were retrieved retrospectively on 1061 consecutive patient sera analysed between June 1, 2010 and February 1, 2011. Electrophoretic quantitation of both M-spike and albumin was performed on CapillarysIITM (SEBIA). Nephelometric measurement of albumin was performed using BNProspec™ (SIEMENS). For each sample the following ratio was calculated: electrophoretic albumin/ nephelometric albumin. For each M-spike the mobility was assessed according to its location on the electrophoretogram. Each spike was then classified in group1 (beta), group2 (fast gamma), group3 (middle gamma) and group4 (slow gamma). *Results:* Of the 1061 sera, 709 issued from patients without an M-spike and the ratio was 1.011±0.048. Of 252 with M-spike 218 had a spike above 15 g/L and the ratio was1.144±0.166. There was a highly significant difference between them: p< 10-10. Within the population with an M-spike over 15 g/L the ratio was 1.035±0.075 (group1), 1.094±0.109 (group2), 1.184±0.212 (group3) and 1.157±0.137 (group4). Each group differed significantly from the reference population: the slowest mobilities of the M-spikes were associated with the highest overestimations of albumin using electrophoretic quantitation. *Conclusion:* The electrophoretic mobility of an M-spike plays a crucial role in the difference obtained between IQ and EQ of albumin.

P-079

HEAVY/LIGHT CHAIN ASSAY, POTENTIAL NEW TOOL IN MINIMAL RESIDUAL DISEASE ASSESSMENT. A BIOLOGICAL STUDY FROM IFM 2008 TRIAL

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Flow Cytometry (FC), Immunofixation (IF) and Freelight Chain (FLC) assay are currently used for evaluating treatment response in Multiple Myeloma (MM). The aim of the study was analysed sensitivity of the Heavy/light chain ratio (HLC) in Minimal Residual Disease (MRD) assessment, in comparison with IF, serum FLC κ/λ ratio and the FC from bone marrow (BM) aspirations. Both serum and BM samples (1-3 per patient) from 27 patients enrolled in the IFM 2008 trial (15 IgG, 8 IgA and 4 Light Chain MM (LCMM)), were analysed at 3 times: respectively, pre- Stem Cell Transplantation (n=11, MRD1 stage), post-SCT (n=25, MRD2 stages and post-consolidation (n=23, MRD3). HLC and FLC assays (Hevylite and Freelite®, BindingSite) were measured by nephelometry. Six-color FC was performed on FACScantoII (BectonDickinson). IF were performed on Sebia® system. (Fig.1) For 50 intact Ig MM samples, FC and IF showed almost the same sensitivity: 60 and 58% respectively. HLC and FLC were abnormal in 46% and 30% respectively. In details FC MRD1 analysis showed better sensitivity than IF and HLC: 100% vs 80% and 70% respectively. We have not observed significative different sensitivities at MRD2 and MRD3 points between FC, IF and HLC. FLC data showed the same low sensitivity at all points (30%). The HLC ratios were normal in 9 LCMM samples while FC remained positive in 2. We show global aggrement between the different MRD assessments. HLC ratio normalisation seems to prognosticate the patients outcome as well as FC and IF. Further studies must be conducted to evaluate hevylite accuracy in MRD.

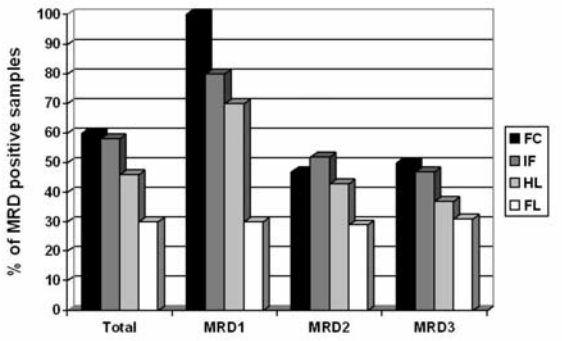

Figure 1 - % Comparison of positive MRD samples of intact Ig Multiple Myeloma patients in Flow cytometer (FC), Serum immunofixation (IF), Hevylite® (HL) and Freelite® (FL). Total (50 samples for IF, HL and FL; 47 samples for FC) MRD1 (10 samples for all techniques) MRD2 (2 samples for IF,HL and FL, 19 for FC) MRD3 (19 samples for IF, HL and FL, 18 for FC)

Flow Cytometry (FC), Immunofixation (IF) and Freelight Chain (FLC) assay are currently used for evaluating treatment response in Multiple Myeloma (MM). The aim of the study was analysed sensitivity of the Heavy/light chain ratio (HLC) in Minimal Residual Disease (MRD) assessment, in comparison with IF, serum FLC / ratio and the FC from bone marrow (BM) aspirations. Both serum and BM samples (1-3 per patient) from 27 patients enrolled in the IFM 2008 trial (15 IgG, 8 IgA and 4 Light Chain MM (LCMM)), were analysed at 3 times: respectively, pre- Stem Cell Transplantation (n=11, MRD1 stage), post-SCT (n=25, MRD2 stages and post-consolidation (n=23, MRD3). HLC and FLC assays (Hevylite and Freelite®, BindingSite) were measured by nephelometry. Six-color FC was performed on FACScantoII (BectonDickinson). IF were performed on Sebia® system. (Fig.1) For 50 intact Ig MM samples, FC and IF showed almost the same sensitivity: 60 and 58% respectively. HLC and FLC were abnormal in 46% and 30% respectively. In details FC MRD1 analysis showed better sensitivity than IF and HLC: 100% vs 80% and 70% respectively. We have not observed significative different sensitivities at MRD2 and MRD3 points between FC, IF and HLC. FLC data showed the same low sensitivity at all points (30%). The HLC ratios were normal in 9 LCMM samples while FC remained positive in 2. We show global aggrement between the different MRD assessments. HLC ratio normalisation seems to prognosticate the patients outcome as well as FC and IF. Further studies must be conducted to evaluate hevylite accuracy in MRD.

P-080

HEAVY/LIGHT CHAIN RATIOS PROVIDE A RAPID AND SENSITIVE ALTERNATIVE TO IFE IN IDENTIFYING HAEMATOLOGICAL MALIGNANCIES

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Background: Novel assays have been produced that make it possible to quantify the Ig'κ and Ig'λ (Heavy/light chain; HLC) concentrations in serum. Here we report on a comparison of the assays with immunofixation (IFE). *Methods:* 1495 patient sera were screened using serum protein electrophoresis (SPE) and free light chain (FLC). Abnormal sera were tested retrospectively by IgG, IgA and IgM HLC assays. *Results:* 198/1495 patients had an abnormal SPE or FLC result, of which 106/198 patients were positive by IFE and 81/198 had an abnormal HLC ratio (HLCr). 24/106 IFE positive patients had symptomatic haematological malignancies (14 Multiple Myeloma [MM], 1 Plasmacytoma, 2 Waldenstrom's Macroglobulinemia [WM] and 7 non-Hodgkins lymphoma [NHL]). Abnormal HLCr were identified in 24/24 patients. Moreover, abnormal HLCr identified 7 additional patients that, at the time of testing, were negative by IFE but who subsequently were diagnosed with a haematological malignancy (1 AL Amyloid, 1 IgAλ MM and 1 IgMκ WM and 4 NHL). 82/106 IFE positive patients had monoclonal gammopathy of undetermined significance (MGUS), 50/82 had an abnormal HLCr. 32 patients had 'normal' HLCr, but were all in the low-low/intermediate MGUS risk category. *Conclusion:* HLC ratios provide a rapid and sensitive alternative to IFE. 7 patients with haematological malignancies were identified by abnormal HLCr when IFE was negative. Patients with low risk MGUS may have normal HLCr and further work is required to look at the utility in MGUS identification and risk stratification.

P-081

THE HEAT SHOCK TRANSCRIPTION FACTOR 1 AS POTENTIAL THERAPEUTIC TARGET IN MULTIPLE MYELOMA

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Experimental evidence suggests that stress response mechanisms are aberrantly activated during malignant transformation. Accordingly, it has been shown that heat shock proteins (HSPs) like HSP90 and HSP70 are frequently overexpressed in multiple myeloma (MM) and protect MM cells from apoptosis. Furthermore, upregulation of several HSPs was reported after pharmacological inhibition of proteasome or HSP90 function indicating a potential role for the development of drug resistance. However, knowledge about regulation of HSPs in MM is still limited. We therefore investigated the role of HSF1 - a key regulator of HSPs. Analyses of biopsies revealed overexpression of HSF1 in primary MM but not in MGUS or in normal plasma cells. HSF1 expression was positively correlated with overexpression of HSP70 and HSP90. Both siRNA-mediated knockdown and pharmacological inhibition of HSF1 led to induction of apoptosis in MM cell lines as well as in primary MM cells. Upon HSF1 inhibition downregulation of several HSPs, like HSP90, HSP72, HSP27 and HSP40 was observed. Furthermore, drug-induced upregulation of HSPs after pharmacological inhibition of HSP90 with NVP-AUY922 or proteasome inhibition with bortezomib was prevented by HSF1 inhibition. In addition, the apoptotic effect of pharmacological HSF1 inhibition was significantly enhanced by the concomitant treatment with NVP-AUY922 or with bortezomib. Taken together, targeting HSF1 may therefore represent an attractive potential therapeutic strategy in MM, in particular in combination with HSP90 and proteasome inhibitors.

P-082

ALTERED GENE EXPRESSION IN MULTIPLE MYELOMA PATIENTS WITH GAIN OF 1Q21 LOCUS

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Chromosome 1 abnormalities namely gain of 1q21 locus is one of the few cytogenetic factors with unfavourable prognostic impact in patients with MM. We analysed gene expression in patients with/without 1q21 gain. The 1q21 gain status was evaluated in 34 patients by FISH and confirmed by arrayCGH (Agilent Human Genome CGH Microarray, 4x44k) when DNA was available. CD138+ cells were separated by MACS. Total RNA was transcribed into cDNA (Ambion WT Sense Target assay), labeled and hybridized to the Affymetrix GeneChip Human Gene ST 1.0 array. Acquisition of Affymetrix array images, RMA normalization algorithm, t-test with Benjamini-Hochberg FDR were performed using appropriate software. The 1q21 gain was detected in 50% (17/34) cases. When comparing expression of patients with/without 1q21 gain, total of 63 transcripts showed altered expression. We found 27 differentialy expressed transcripts with FC<1.5 (22 up, and 5 down), 17 of over-expressed transcripts were mapped exactly to chromosome 1. The most altered expression (FC>2.0, p<0.05) showed increase of UCHL1 (ubiquitin thiolesterase), GPR63 (G-protein receptor), TUBB4 (tubulin), KIF21B (kinesin) and decrease of STAP1, MAML2, FAM13A and PDE4B (phosphodiesterase), respectively. Based on ontology of revealed genes with altered expression, we anticipate that patients with 1q21 gain might have increased microtubules activity and/or dysregulation of G-protein associated signal transduction. This may reflect the pathogenesis of multiple myeloma.

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P-083

GENE AND MIRNA EXPRESSION PROFILES IN PLASMA CELL LEUKEMIAS

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Plasma cell leukemia (PCL) is an aggressive malignancy that can be primary (pPCL) or secondary (sPCL) due to progressive prior multiple myeloma (MM). Genome-wide studies in PCL are still limited. Gene expression profiles (GEP) of purified PCs from 54 MM, 16 pPCL (multicenter GIMEMA clinical trial) patients at diagnosis and 6 sPCLs were generated on Gene 1.0 ST array (Affymetrix). Global miRNA expression (35 MM and 13 pPCL) was generated by means of the miRNA Microarray V2 (Agilent). Unsupervised gene and miRNA expression analyses grouped most of PCLs and MMs in two distinct branches and partly according to the major IgH chromosomal translocations. GEP supervised analysis evidenced 237 differentially expressed genes in PCLs versus MMs: several positively modulated genes in PCLs were involved in cytoskeleton organization, cell adhesion, migration and associated to invasion and metastasis processes in diverse tumors. The comparison of PCL forms revealed in sPCLs the overexpression of transcripts mainly concerned in mitosis, spindle organization and chromosome segregation. Thirty upregulated and 21 downregulated miRNAs were identified in pPCLs vs MMs. Some of the overexpressed miRNAs in PCL samples may have a particular importance in the context of B cell dyscrasias as demonstrated by their involvement in B cell development, lymphoproliferative disorders or in sustaining growth of MM cell lines. In conclusion, transcriptomic analyses of PCL patients may identify molecular alterations characterizing this aggressive PC dyscrasia.

P-084

CIRCULATING MICRORNAS IN MULTIPLE MYELOMA

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Because of the significance of miRNAs in tumorigenesis, circulating miRNAs in blood may be unique biomarkers for minimally invasive diagnosis and for monitoring of cancers. This study was aimed at detecting a distinctive signature of circulating miRNAs for Multiple Myeloma (MM) monitoring. MicroRNAs were isolated from peripheral blood plasma and CD138+ plasma cells of MM patients and healthy donors and analysed by low density array technology (Applied Biosystems). We have characterized a specific circulating miRNA signature that differentiates MM patients from healthy subjects. The altered MM-related miRNA signature in this subset of patients is characterized by increased expression of miR-124,-19b,-135b,-380 and decreased expression miR-187. Targets of these miRNAs include tumor suppressor genes as well as negative regulators of cell proliferation, survival and osteogenesis. To further investigate the clinical relevance of circulating miRNAs in MM in terms of prognosis, we correlated miRNA expression levels with prognostic factors according to ISS and cytogenetic. There was a linear trend that characterizes the patients: decreasing levels of miR-196b and miR-487b correlated with worse prognosis as predicted by ISS I/II/III ($p=0.07$, $p=0.08$). Of note, increased expression of miR-196b significantly contributes to leukemia development. In addition, miR-187 was found less abundant in patients displaying the 17p deletion when compared to other cytogenetic risk group and donors. These results indicate that specific plasma miRNAs can be used for disease monitoring in MM patients.

P-085

INTEGRATIVE GENOMIC ANALYSIS OF PRIMARY PLASMA CELL LEUKEMIA REVEALS STRONG GENE AND MICRORNA DOSAGE EFFECT

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Primary plasma-cell leukemia (pPCL) is a rare variant of plasma cell (PC) dyscrasia characterized by high genomic instability and very poor prognosis. Highly purified PCs from 17 untreated patients (GIMEMA myeloma network) were investigated. By FISH analysis, 13q and 17p deletions were present in 12 and 8 cases; t(11;14), t(4;14) and MAF translocations were found in 4, 1 and 8 patients, respectively. A subset of 13 patients were further investigated for copy number alterations by Mapping 250K NspI Array (Affymetrix). Genome-wide profiling data were concordant with FISH results for the detection of 13q (10 pts) and 17p (7 pts) deletions and identified losses involving chromosomes 1p (5 pts), 8p (4 pts), 14q (5 pts), 16q (5 pts); gains at 1q (8 pts), 7q (4 pts) and 19p (4 pts); and the amplification of 17q21 (6 pts). A near tetraploid karyotype and a hyperdiploid pattern were found in one case, respectively. Mapping information was integrated with the gene (Gene 1.0 ST, Affymetrix) and miRNA (Microarray v2, Agilent) expression profiles of the tumor samples. A non-parametric analysis (Kendall's tau correlation at a $p<0.005$) identified 199 probes whose expression levels strongly correlated with the occurrence of allelic imbalances, mostly (91%) in the previously described altered regions. Furthermore, 23 miRNAs, mainly (69.5%) mapping to 1p (21.7%), 13q (26.1%) and 19 (21.7%), were found as positively correlated at a $p<0.05$. These results highlight a wide gene-dosage effect suggesting that genomic structural abnormalities in pPCL closely reflect in expression imbalances.

P-086

ELEVATED LEVELS OF CYCLIC AMP INDUCE APOPTOSIS IN MULTIPLE MYELOMA CELLS AND INHIBITS TUMOR DEVELOPMENT IN A MOUSE MYELOMA MODEL

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Multiple myeloma is an incurable disease with a strong need for development of new therapeutic strategies. In the present paper we demonstrate that elevation of intracellular cAMP levels kills multiple myeloma cells in vitro and inhibits development of multiple myeloma tumors in vivo. As a model system we primarily used the murine multiple myeloma cells MOPC315, and we show that 50 μM of the adenylyl cyclase activator forskolin more than tripled the percentage of dead cells after 24 hours in vitro. A similar extent of cell death was also induced by forskolin in the human myeloma cell lines U266 and INA-6. The cAMP-mediated cell death presented the typical hallmarks of apoptosis, such as changes in the mitochondrial membrane potential and cleavage of caspase 3, caspase 9 and PARP, but without inducing the levels of p53. By using molecular imaging and a mouse multiple myeloma model of DsRed-labelled MOPC315 cells grown subcutaneously in Balb/c nude mice, we showed that elevation of cAMP by forskolin inhibited the tumor development in vivo. Taken together, our data strongly suggest that compounds activating the cAMP signaling pathway may be therapeutically useful for the treatment of multiple myeloma.

P-087

PROGNOSTIC ROLE OF MIRNOME PROFILING IN MULTIPLE MYELOMA

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MicroRNAs are an abundant class of small non-protein-coding RNAs that function as negative gene regulators in diverse biological processes including cancer. We determined expression of 559 miRNAs by miChip (Exiqon LNA Array probes V9.2) in CD138-purified myeloma cells from previously untreated patients (MMCs, n=67), normal bone marrow plasma cells of healthy donors (BMPCs, n=3 pooled), and human myeloma cell lines (HMCLs, n=10). Gene expression profiling was performed using Affymetrix U133 2.0 DNA-microarrays. We found 22 miRNAs to be significantly up- and 51 down-regulated in MMCs vs. BMPCs, respectively. Expression of 15 miRNAs was associated with event-free survival (EFS), five with overall survival (OS), four of them overlapping. Of these, two miRNAs (miR-659 located at 22q12.1 and miR-590 located at 7q11.23) allowed the delineation of prognostic groups. miR-659 was significantly lower expressed in MMCs compared to BMPCs. Low expression delineates a group with inferior EFS (median 19.7 months vs. n.r., P<.003) and OS (60.3% vs. 55.5% at 60 months, P=.02). This group shows a significantly higher gene expression based proliferation index. High miR-590 expression delineates a group with inferior EFS (median 12.4 vs. 35.5 months, P=.008) and OS (63.5% vs. 25.0% at 60 months, P=.004). Although miR-590 showed no differential expression between BMPCs and MMCs, the 12 MMC samples of the "bad risk"-group showed a spiked expression. In conclusion, we demonstrate the prognostic significance of miRNome profiling in multiple myeloma.

P-088

PLASMA CELL PHENOTYPE CORRELATION WITH CYTOGENETIC AND MORPHOLOGICAL FINDINGS IN MULTIPLE MYELOMA

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Plasma cells (PCs) phenotype could correlate with presence of genetic abnormalities and/or morphological subtypes thus could be related to patient's prognosis as well. The aim of work was to find relation between antigenic profile, cytogenetic aberrations and morphology in MM patients. Analyses were done in 134 newly diagnosed MM patients. Bone marrow PCs were analysed for expression of CD19, CD20, CD27, CD28, CD56 and CD117 by immunophenotyping. FISH on separated PCs was used for analysis of del(13)(q14), del(17)(p13), IGH disruption, t(4;14)(p16.3;q32), 1q21 gain and hyperdiploidy. PC subtypes evaluation was based on the nucleus/cytoplasm (N/C) ratio. Clonal CD19+ PCs were found in 1 patient with del(13)(q14). CD56+ PCs were found in 79.1% (106/134) and correlated with lower expression of CD20 and CD28. CD56+ PCs were more immature than CD56- PCs. CD20+ PCs were found in 8.2% (11/134) and correlated with higher expression of CD28, CD117 and lower expression of CD56. CD27 was less expressed in group with higher number of CD56+ PCs and CD27+ PCs were more mature. CD28 was expressed in 24.6% (33/134). CD28- PCs were more immature than CD28+ PCs. CD117+ PCs was found in 31.3% (42/134) and significantly correlated with hyperdiploidy, negativity for CD117 was associated with del(13)(q14). Result showed CD117 as the only marker corresponding to chromosomal abnormalities. According to morphology assessment majority of PCs was more mature type. Supported by GACR 301/09/P457, MSMT LC06027, MSM0021622434, IGA 10408-3, IGA 10406-3, IGA 10207-3 and GACR P304/10/1395 grants.

P-089

OVEREXPRESSION OF FOXP3 IN BONE MARROW (BM) ASPIRATES HIGHLIGHTS THE IMPORTANCE OF REGULATORY T-CELL SUBPOPULATION AS POSSIBLE THERAPEUTIC TARGET IN MULTIPLE MYELOMA

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Introduction: T regulatory (Treg) cells play important role in the maintenance of self-tolerance and modulation of overall immune response against infections and tumor cells. Th17 cells have a critical function in clearing extracellular pathogens and tumor cells. The balance between Treg and Th17 cells may be essential for maintaining immune homeostasis against tumor cells. The aim of this study was to characterize Treg and Th17 related genes expression in MM. *Material and Methods:* Expression of Foxp3 and ROR-γt, respectively associated to Treg and Th17 subpopulations, was determined by RQ-PCR in BM aspirates of 37 newly diagnosed MM patients and 5 healthy controls. Genes were considered overexpressed when tumor expression level was at least 2 times higher than normal samples. *Results:* Foxp3 was overexpressed in 72% of MM cases. A 5.88-fold increase in Foxp3 expression was observed in MM patients compared to controls (p=0.0476, Mann-Whitney test). There was no difference between ROR-γt expression in MM cases and controls. *Conclusions:* Foxp3 overexpression in more than 70% of MM cases suggest that this gene can be involved in MM pathogenesis and suggest that therapeutic approaches that specifically target Treg cells may provide more focused treatment strategies for the management of MM.

P-090

GENOMIC SCREENING FOR GENES SILENCED BY DNA METHYLATION TO EXPLORE EPIGENETIC BIOMARKERS FOR PREDICTION OF SENSITIVITY TO DEXAMETHASONE IN MULTIPLE MYELOMA

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Multiple myeloma still remains lethal malignancy in spite of development of treatments such as novel drugs including thalidomide, bortezomib, and lenalidomide as well as high-dose chemotherapy combined with stem cell transplantation. The main difficulties in multiple myeloma treatments are drug-resistance. Epigenetic changes such as DNA methylation of 5' CpG islands of the genes play a role in development and progression of various cancers including multiple myeloma. In this study, to screen genes involved in tumorigenesis of multiple myeloma, which are silenced by DNA methylation, we performed cDNA microarray analysis using multiple myeloma cell lines treated with demethylating agent 5-aza-2'-deoxycytidine, and identified RASD1, a dexamethasone inducible gene, as one of targets of epigenetic changes. RASD1 locates in chromosome 17p11.2, in which frequent loss of heterozygosity is detected in various human tumors, and suppresses cell growth. Inactivation of RASD1 was correlated with resistance to dexamethasone, and treatment of multiple myeloma cells with 5-aza-2'-deoxycytidine restored sensitivity to dexamethasone. Methylation of RASD1 was detected in a subset of primary multiple myeloma and methylation levels were increased in after repeated treatment. These findings suggest the involvement of epigenetic gene silencing in multiple myeloma progression and drug-resistance, and the usefulness of demethylation therapy for multiple myeloma treatment. Furthermore, DNA methylation can be an epigenetic biomarker for multiple myeloma.

P-091

LENALIDOMIDE TREATMENT POST AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA DECREASES TERMINALLY DIFFERENCIATED CD4 AND CD8 T CELLS BUT INCREASES NUMBER OF TREG

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Treatment with Lenalidomide (revlimid®), a structural analogue of Thalidomide, plus dexamethasone (Dex) increases time to progression in relapse or refractory multiple myeloma. However, due to its pleiotropic effect, it's not known if its efficacy is due only to a direct tumor toxicity or benefit also of its immunomodulatory effects. Thirty-four myeloma patients were treated with the induction combination bortezomib plus dex, followed by high dose melphalan (140-200 mg/m2) and an autologous transplantation with peripheral blood stem cells. Between 3 to 6 months post autograft, patients were randomized in 2 groups: 12 received 25 mg/day of Lenalidomide for 2 months, 3 weeks per month plus 40 mg of Dex, once a week, then 10 mg/day of Lenalidomide only until relapse. 22, a placebo only. T lymphocyte subpopulation percentage and absolute counts were assessed by multicolor flow cytometry from diagnosis until 18 month after autograft. After Lenalidomide plus Dex treatment, we observed a significant decrease in percentage and absolute counts of CD4+ or CD8+, CD45RA+CCR7- effector T cell subpopulations. Surprisingly, CD4+CD25high or CD4+CD25+CD127-/low Treg increased significantly more in treated patients. No correlation was found with documented infections, relapse or survival. These data suggest that, in vivo, in Human, Lenalidomide plus Dex efficacy on Myeloma tumor is not T cell mediated but this treatment could have a negative impact on T cell immunosurveillance. Additional studies are required to better assess the respective effects of Lenalidomide and Dex on immune function.

P-092

THE ACTIN CYTOSKELETON L-PLASTIN IS INVOLVED IN THE ACQUISITION OF MOLP8 MULTIPLE MYELOMA CELLS RESISTANCE TO DOXORUBICIN.

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The major problem to cure multiple myeloma (MM) is the development of resistance to therapy. We investigate the molecular mechanism underlying the acquisition of MOLP8 cells resistance to doxorubicin (DOX). Doxorubicin resistant MOLP8 cells (MOLP8/DOX) were generated after continuous exposure of cells to increasing concentrations of DOX. These cells display a DOX resistance index of 500 and show a cross resistance to others drugs such as Bortezomib. Gene expression profiling experiments and proteomic approaches yielded 5466 genes and 70 proteins, respectively, differentially regulated in MOLP8/DOX compared to MOLP8 cells. While several genes are known to be involved in MM resistance, others have so far no established function in MM resistance prompting us to investigate their role in this process. The actin cytoskeleton protein L-plastin was found upregulated in MOLP8/DOX cells. It has been demonstrated that the activity of L-plastin depends on its phosphorylation by the protein kinase A and C (PKA and PKC). The overexpression of L-plastin and its phosphorylation level were validated by western blot. To assess the functional involvement of L-plastin in the acquisition of MM resistance to doxorubicin we inhibit the activity of PKA and PKC. Our results provide clear evidence that inhibition of phospho L-plastin restores the sensitivity of MOLP8/DOX cells. Our study identified a novel unexpected cytoskeleton protein involved in the acquisition of MM cells resistance to doxorubicin.

P-093

B-CELL SUBPOPULATIONS FROM NORMAL HUMAN SECONDARY LYMPHOID TISSUES WITH SPECIFIC GENE EXPRESSION PROFILES AND PHENOTYPES: IMPACT ON MYELOMA

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In order to improve insights into the B-cell biology and thereby myelomagenesis we have established a MSCNET standard for multiparametric flow cytometry (MFC) and cell sorting (FACS) for subsequent genetic analysis. The material analysed was fresh tonsils, blood and bone marrow. The method included homogenization, isolation of mononuclear cells, MFC and FACS sorting using a multicolour fluorescence single tube panel of antibodies surface molecules as CD10/20/27/38/45, supplemented with tissue related antibodies. Isolated B-cell subpopulations were evaluated by morphological inspection and single gene expression analysis (qRT-PCR) for transcription factors as well as global gene expression profiling (GEP; GeneChip Human Exon 1.0 ST Array). For example for tonsils, based on the immunophenotypic presentation (including CD3/44/CXCR4 in the panel), B-cell subsets were identified and sorted, naïve, centroblast, centrocyte, memory, and plasmablasts. The identity of the tonsillar subpopulations was verified using qRT-PCR and exon microarray GEP based on the used discriminative phenotypic markers as well as transcriptions factors BACH2, BCL6, PAX5, IRF4, P27, PRDM1 and XBP1. Globally, the B-cell subpopulations identified have distinct gene expression profiles reflecting their functions but also revealing genes with subpopulation-specific exon splicing. In conclusion a combination of surface markers expressed antigens and gene expression analysis of B cell subsets confirm a strong methodology to be used in myelomagenesis and evaluated for prognostic information.

P-094

DISRUPTION OF HEPARAN SULFATE CHAINS PERTURBS B CELL MATURATION AND SURVIVAL OF NORMAL AND MULTIPLE MYELOMA PLASMA CELLS

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The development and antigen-dependent differentiation of B lymphocytes is orchestrated by an array of growth factors, cytokines, and chemokines that require tight spatio-temporal regulation. Heparan sulfate proteoglycans (HSPGs) specifically bind and regulate the bio-availability of soluble protein ligands, but their role in the immune system and its neoplasms has remained largely unexplored. Heparan sulfate modifying enzymes (HSMEs) control HS-chain synthesis and conformation and thereby affect ligand binding. Here we show that deficiency of glucuronyl C5-epimerase (Glce), which controls HS-chain flexibility, impairs B cell maturation and results in decreased plasma cell numbers and immunoglobulin levels. We demonstrate that C5-epimerase modification of HS is critical for binding of a proliferation inducing ligand (APRIL), and that Glce-deficient plasma cells fail to respond to APRIL-mediated survival signals. Furthermore, by employing a novel myeloma xenotransplant model in Rag-2-/-gc-/- mice, we demonstrate that knockdown of the HS copolymerase EXT1 dramatically suppresses the growth of bone marrow localized myeloma in vivo. Our results identify HSPGs as novel players in B cell maturation and and plasma cell survival. Moreover, they show that effective HS-chain synthesis is crucial for the growth and survival of MM cells within the bone marrow environment, thus indicating the HS biosynthesis machinery as a potential treatment target in MM.

P-095

MIR-34A EXPRESSION SENSITIZES MULTIPLE MYELOMA (MM) CELLS TO BORTEZOMIB

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MicroRNA signatures associated with the malignant transformation of plasma cells and with MM molecular subgroups were recently reported. We have investigated the role miRNAs play in MM cells sensitivity to proteasome inhibitors. Microarray profiling, followed by RT-PCR confirmation, of miRNA signatures of MM cell lines with differential sensitivity to Bortezomib (3 fold change in IC50) identified 24 differentially expressed miRNAs and clustered MM cells into 2 distinct groups. In particular, mir-34a had high expression in sensitive cells(S: MM1S, RPMI8226, NCI-H929 and U266 with an IC50 < 1.25nM), in comparison to relatively resistant cells (R: KMS11, INA6 and OPM2 with an IC50 ~ 5nM). Epigenetic silencing of miR-34a through its promoter methylation was identified with bisulfite conversion based PCR in all cell lines with low miR-34a expression. Conversely, and since p53 is reported to activate miR-34a expression, we have correlated miR-34 expression levels with the TP53 mutational status and del(17p.13) (by FISH) in these cells. Mutations in exons 5 and 8 were identified in all cell lines with low miR-34a. In particular del(17p.13) was present in KMS11 cells consistent with their very low or undetectable miR-34a. We last sought to determine if restoration on mir-34a levels would increase sensitivity to Bortezomib. Lentivirus-mediated expression of miR-34a in KMS11 cells, compared to mock control, resulted in a dramatic increase in their sensitivity to Bortezomib. These results support a potential therapeutic role for miRNA manipulation, in particular miR-34a in MM.

P-096

MYELOMA MUTATION DISCOVERY BY EXOME CAPTURE AND NEXT GENERATION SEQUENCING USING ONLY 50 NG OF DNA

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Global analysis of mutations in myeloma samples is possible through targeted capture of exon sequences, but currently requires in excess of 3 μg of DNA. This amount severely limits the number of myeloma and related samples which can be studied. In order to analyse sufficient numbers of samples for recurring mutations a method requiring less DNA is needed. We have carried out a pilot study to define the best method and smallest amount of DNA which can be used and applied to a series of myeloma samples.

We compared SureSelect exome capture (Agilent) using 3 μg of DNA or 60 ng of whole genome amplified (WGA) DNA (Illustra by GE Healthcare) and a variation of the SureSelect exome capture protocol optimised for 50 ng of starting material (low starting amount). The selected DNA was sequenced using 76 bp paired-end reads on a GAIIx sequencer (Illumina) and aligned to the human genome.

Analysis revealed that the low starting amount method, suited to the analysis of 50 ng of starting material, had parity to the 3 μg method and was superior to the WGA method in respect to percentage alignment to the reference genome, as well as error rate and variant discovery, and was applicable to the analysis of clinical material.

Amounts of DNA as low as 50 ng are not a barrier to entry for the use of next generation sequencing technologies and can provide equivalent results to experiments using much larger amounts of DNA. This will allow the analysis of almost all myeloma samples by targeted sequencing and may be used to significantly improve cancer treatment and personalised cancer therapy.

P-097

MULTIPLE TYROSINE KINASE PATHWAYS MODULATE MYELOMA (MM) CELL SUSCEPTIBILITY TO HUMAN NATURAL KILLER (NK) CELLS

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NK cells are primary effectors of the innate immune responses directed against tumor cells. However, transformed cells have also developed mechanisms to evade immune surveillance and the molecular basis for target resistance to immune-mediated lysis is not well understood. RNA interference has been proven to be a powerful tool to perform loss-of function genetic analysis in mammalian cells. To identify new pathways involved in MM resistance to immunologic rejection, we developed an high-throughput cell-cell interaction screen using the TRC1 lentiviral shRNA library. The library targeted 476 kinases, 180 phosphatases and 372 other genes. Each gene was targeted by 5 or more independent shRNAs tested individually using robotic manipulations. IM9 MM cells were transduced with a total of 6,144 shRNAs in more than 30,000 wells and co-incubated with NK cells to identify genes involved in MM resistance to NK activity. We found that 83 genes, belonging to intracellular and cell surface pathways, induced increased MM cell susceptibility to NK cells. In particular, we found that stable suppression of Jak1 and Jak2 in IM9, KM12BM, INA6 and RPMI MM cells induced a significant increase of INF-♀ secretion and lysis from NKL, NK92 or primary NK cells. Importantly, incubation of MM cells with 2 different Jak inhibitors also induced an increased susceptibility to NK lysis. These finding may have important clinical implications, and suggest that small kinase inhibitors, being developed as direct therapeutic anti-tumor agents, may also have important immunologic effects in vivo.

P-098

ROLE OF TORC1 AND TORC2 IN MULTIPLE MYELOMA

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Mammalian target of rapamycin (mTOR) is a downstream serine/threonine kinase of the PI3K/Akt pathway that integrates signals from the tumor microenvironment. Mechanistically, mTOR operates in two distinct multi-protein complexes, TORC1 (Raptor) and TORC2 (Rictor). TORC1 leads to the phosphorylation of p70S6 kinase and 4E-BP1, while TORC2 regulates phosphorylation of Akt and other kinases. In multiple myeloma (MM), PI3K/Akt plays an essential role enhancing cell growth and survival and is activated by the loss of the tumor suppressor gene PTEN and by the bone marrow microenvironment. In this study we have evaluated the role of TORC1 and TORC2 in MM. *Results:* We used INK128, a novel and selective TORC1/2 kinase inhibitor as well as knockdowns of both proteins Raptor and Rictor. We examined the protein expression levels of both mTOR complex and their downstream effectors in MM plasma cells from patients and cell lines. mTOR, Akt, pS6R and 4E-BP1 are constitutively activated in all samples. INK128 induced cell cycle arrest and apoptosis in cell lines and primary plasma cells even in the presence of bone marrow stromal cells (BMSCs). INK128 also showed a significant effect inhibiting cell adhesion in our in vivo homing model. Oral daily treatment with INK128 highly decreased the percentage of CD138+ tumor plasma cells in mice implanted with MM cells and reduced the levels of p-Akt and p-4EBP. These results suggest mTOR1 and mTOR2 are potential therapeutic targets to induce cell cycle arrest, apoptosis and for the disruption of MM cells interaction with the BM microenvironment.

Purpose. Genetic aberrations detected in multiple myeloma (MM) have also been reported in the premalignant conditions, monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM). Our aim was to investigate in depth the level of clonal heterogeneity of recurrent genetic abnormalities in these conditions. *Experimental Design.* Immunoglobulin heavy chain (IGH) translocations, 13q14 and 17p13 deletions, and 1q21 gains using fluorescence *in situ* hybridization (FISH) were evaluated in 90 MGUS, 102 high-risk SMM and 373 MM. To this end, we not only purified plasma cells (PC) for the FISH analysis (purity >90%), but subsequently we examined the correlation between the proportion of PC with cytogenetic changes and the number of clonal PC present in the same sample, as measured by multiparametric flow cytometry. *Results.* We observed a significant difference between the proportion of clonal PC with specific genetic abnormalities in MGUS compared with SMM, and in SMM compared with MM. Thus, the median proportion of PC with IGH translocations globally considered, t(11;14) and 13q deletions was significantly lower in MGUS than in SMM, and in SMM than in MM (IGH translocations: 34% vs. 57% vs. 76%; t(11;14): 38% vs. 61% vs. 81% and 13q deletion: 37% vs. 61% vs. 74% in MGUS, SMM and MM, respectively). For t(4;14) the difference was significant in the comparison between MGUS/SMM and MM and for 1q between MGUS and SMM/MM. *Conclusions.* This study demonstrates that the progression from MGUS to SMM, and eventually to MM, involves a clonal expansion of genetically abnormal PC.

P-099

NEURAL STEM CELL MARKER NESTIN AS A SPECIFIC MARKER FOR PLASMA CELLS OF MULTIPLE MYELOMA PATIENTS

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Introduction and Aims: Nestin, a class VI intermediate filament protein, is considered to be a characteristic marker of multipotent proliferative precursors found in some embryonic and fetal tissues. Unexpectedly, our previous results confirmed nestin levels in mature CD138+ plasma cells (PC) of multiple myeloma (MM) by flowcytometry; significant differences were found between nestin levels in MM and individuals without any hematological malignancy. The purpose of this study was to analyze nestin protein in CD138+PC and CD138-bone marrow mononuclear cells (BM MNC) of MM patients and myeloma cell lines. *Methods:* Nestin protein was detected in CD138+PC (purity >90%) and CD138-BMMNC of 7 MM patients (3M/4F; median age 61 years) and myeloma cell lines (U266, RPMI8226, OPM-2, MOLP-8) by western blots. As a positive control, T98G glioblastoma cell line was used. *Results:* Western blot analyses qualitatively confirmed nestin levels in CD138+PC of MM patients and myeloma cell lines U266 and MOLP-8. Nestin was not found in CD138-BMMNC and myeloma cell line OPM-2 and RPMI8223. *Conclusion:* Our analysis clearly confirmed presence of nestin protein in CD138+PC but excluded nestin in CD138-BMMNC of MM patients. These results indicate that nestin may be a tumor specific marker for MM. Nestin-positive myeloma cell lines (U266, MOLP-8) are suggested as a model for study of nestin functions in PC of MM patients. *Supported with research program MSM of Czech republic Nr. MSM 0021622434 and P304/10/1395.*

P-099 bis

THE PROGRESSION FROM MGUS TO SMOLDERING MYELOMA AND EVENTUALLY TO MULTIPLE MYELOMA INVOLVES A CLONAL EXPANSION OF GENETICALLY ABNORMAL PLASMA CELLS

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P-100

CONTRIBUTION OF WHOLE-BODY MAGNETIC RESONANCE IN THE DIAGNOSTICS OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA

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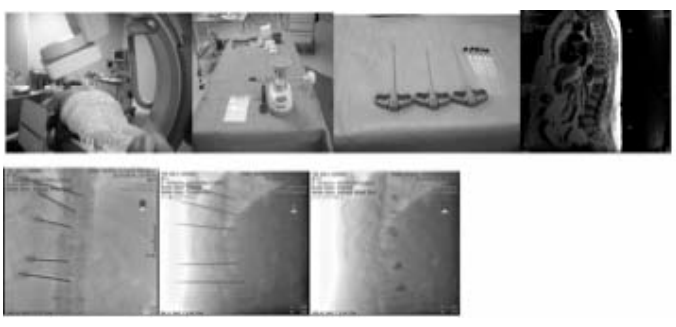
Objective: The aim of the study was to assess the significance of whole-body magnetic resonance (WB-MR) in monoclonal gammopathy of undetermined significance (MGUS) and initial form of multiple myeloma (MM). *Material and Methods:* The analyzed cohort consisted of 28 MGUS individuals, 54 MM patients and 5 patients with solitary plasmocytoma (SP). Whole body coil with sequential acquisition – T2 STIR and T1 was used. *Results:* In the group of 28 MGUS individuals, there were 17 (61%) patients fulfilling the IMWG and/or WB-MR criteria of transformation into MM. In 4/17 (23%) patients, we found more advanced stage when comparing the D-S Plus to D-S stratification system. Nine out of 14 (64%) individuals with transforming MGUS with negative radiological assessment had positive WB-MR findings. The character of WB-MR findings led in 9/17 (53%) patients with MM to the initiation of treatment. The D-S Plus stratification divided the 54 newly diagnosed patients with MM into stages 1-3 (17%, 33% and 50%). In 22% there was a shift into a higher stage using D-S Plus in comparison with D-S, in 9% the shift led to downstaging. In 13% we could trace extramedullary propagation. In 2 of the 5 SP patients we recognized a multifocal form using WB-MR. *Conclusions:* WB-MR is a very contributive imaging method with substantially higher resolution than conventional radiography. It can evaluate the grade and the extent of myeloma bone disease, extramedullary propagation and the solitary form of plasmocytoma, and improves the differentiation of stable MGUS from the malignant transformation into MM.



P-101

PERCUTANEOUS VERTEBROPLASTY IN 35 MULTIPLE MYELOMA PATIENTS: LONG TERM OUTCOME FOLLOW-UP EVALUTATION IN A SINGLE CENTRE

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Purpose: Patients with MM are at high risk for vertebral compression fracture, and use of vertebroplasty (PVT) is expanding in this patient population. Our goal was to evaluate the effectiveness of PVT in patients with symptomatic vertebral fractures that had not responded to conservative treatment. *Methods:* In our centre, 35 patients with 83 multiple myeloma fractures were treated by PVT. Imaging studies, clinical visits and short- and long-term follow-up were assessed by visual analogue scale (VAS) testing of pain. The procedure was performed on fluoroscopy biplanes high resolution guide. The follow-up was conducted with objective review (associate with a Vas reassessment) at 30 days and with MR and CT at 3, 6 and 12 months after procedure. *Results:* Technical success was 94%. The average VAS value pre-PVT was 8.0±2.5, which significantly dropped to 1.5±0.4 by 12 months. There was a low complication rate in our study. During an average follow up to 27.8 months (range: 48-months) vertebral levels previously treated not have shown worsening in morphologically and structurally aspects. *Conclusion:* Percutaneous vertebroplasty is a safe and effective procedure in the treatment of vertebral collapse with disabiliting pain, refractory to medical conservative-therapy, confering lasting pain relief, enhanced mobility, and reduced narcotic use for all of the stages of myeloma associated with painful compression fractures. PVT should be considered the treatment of choice in vertebral fractures with refractory pain.

P-102

ASSOCIATION BETWEEN THE EXPRESSION OF SECRETED FRIZZLED RELATED PROTEIN 3 (SFRP3), DICKKOPF1 (DKK1), AND THE OSTEOLYTIC BONE DISEASE (OBD) IN MULTIPLE MYELOMA (MM)

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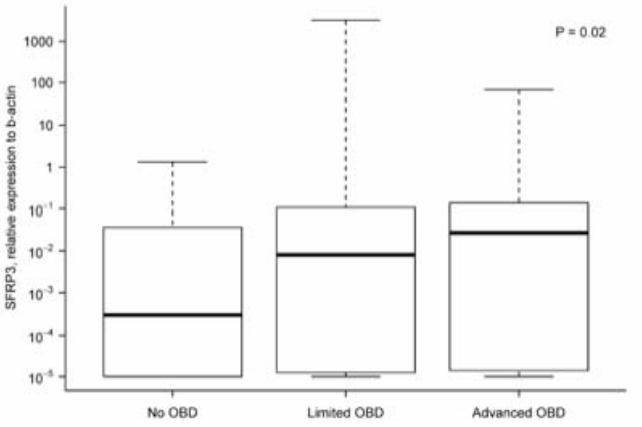
Frizzled related proteins (FRPs) and DKK1 are known soluble inhibitors of the canonical Wnt pathway. SFRP3 is known to be expressed by 60% of primary CD138 MACS isolated MM plasma cells (PC). SFRP2 expression has been found in MM cell lines and primary MM PC, and was found to inhibit mineralized nodule formation and partially alkaline phosphatase activity. DKK1 is known to be produced by MM PC and associates with the presence of osteolytic lesions. *Methods:* Aberrant MM PCs were sorted by fluorescence activated cell sorting. In all cases a PC-purity above 98% was obtained. A cDNA archive was generated by global reverse transcription and amplified as described (Rasmussen et al, BJH 2003). Quantitative PCR was performed using β-actin as internal reference gene. Determination of positivity or negativity was made from a cut-off-value at 10E-05. OBD was evaluated by standard radiographic methods. *Results:* 76% of patients with advanced bone disease (≥2 osteolytic lesions in ≥2 anatomic regions), compared to 76% of patients with limited disease, and 59% of patients with no OBD expressed SFRP3. A significant association with the quantitative expression of SFRP3 was seen with the degree of OBD (Fig. 1, p=0.02). Also an association between DKK1 and OBD was observed as published earlier (p<0.05) (Haaber et al, BJH 2007). A highly significant correlation between the quatitative expression of SFRP3 and DKK1 was observed (p<0.001). Thus, the Wnt-inhibitors SFRP3 and DKK1 are co-expressed in human MM PC and could be some of the multiple factors responsible for OBD in MM.



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INDUCTION OF OSTEOLYTIC BONE LESIONS BY P38 MAPK SIGNALING IN MYELOMA CELLS

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Myeloma cells cause osteolysis, but the underlying mechanism is poorly understood. As p38 mitogen-activated protein kinase (p38) is constitutively activated in myeloma, we hypothesized that p38 activation in myeloma cells might be responsible for myeloma-induced osteolytic bone lesions. Intravenous injection of myeloma ARP-1 and MM.1S cells caused bone lesions in SCID mice. However, once p38 was knocked down by shRNAs in the cells, myeloma was established but failed to cause bone lesions in mice, as measured by radiograph, quantitative u-CT and histological examination. Osteoclast numbers, osteoclast size, cellular nuclear numbers in per osteoclast, and levels of circulating collagen type I and TRAP5b were all reduced in mice injected with myeloma cells with knocked-down p38 as compared with controls. Consistently, we found that tumor p38 had active effects on in vitro osteoclast differentiation and bone resorption, and inhibited osteoblast differentiation and function. By functional studies and protein array analysis, we showed that tumor p38 upregulates MCP-1 and DKK-1 expression and production. MCP-1 enhanced RANK expression on osteoclast precursors and DKK-1 increased RANKL secretion from stromal cells, all of which led to activation of NF-kB and MAPK signaling pathways in osteoclasts. Blocking MCP-1 and DKK-1 by specific antibodies significantly abrogated tumor p38-induced osteoclast activation and bone lesions in vivo. Thus, our results have elucidated a novel mechanism that tumor p38 activity contributes to osteolytic bone lesions in myeloma.

P-104

PERCUTANEOUS VERTEBROPLASTY IN PATIENTS WITH SPINAL MYELOMA LESIONS (SML)

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Background: The therapeutic intervention for SML is based on analgesia, biphosphonates, radiation therapy, percutaneous vertebroplasty (PV) and balloon kyphoplasty. The aim of this study was to evaluate the efficacy and safety of PV in patients with SML. *Patients and Methods:* Patients with SML not responsive to medical treatment were eligible. The presence of fracture or vertebral collapse was evaluated by a rheumatologist and/or radiologist and performed by an interventionist radiologist. In some cases, more than one vertebra was treated in the same procedure. Pain response was evaluated by a qualitative scale at 24 hours, 1 and 6

months after PV. *Results:* Nineteen PV were performed in 15 patients. The most frequent localization of the 38 vertebras treated was L3. The evaluation of pain at 24 hours, 1 and 6 months after PV, showed improvement in 79%, 47% and 37% of cases, respectively. The incidence of cement leakage was 47%. Four out of 15 patients developed severe complications: 1 psoas hematoma, 1 death by respiratory failure of unknown etiology and 2 pulmonary embolism (one patient died because of a cement pulmonary embolism). *Conclusions:* PV is an easy technique for SML not responsive to medical treatment that results in immediate pain relief in 79% of patients. Severe clinical complications secondary to cement leakage can be observed in 4 out of 15 of patients, with some being life-threatening. These results suggest that PV can be useful in acute SML treatment but further studies should be undertaken to confirm the efficacy and the incidence of adverse effects.

P-105

WHOLE-BODY 64-SLICE MULTIDETECTOR COMPUTED TOMOGRAPHY (MDCT) VERSUS CONVENTIONAL RADIOGRAPHY (CR) AND MAGNETIC RESONANCE IMAGING (MRI) IN STAGING OF MULTIPLE MYELOMA (MM)

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Introduction MM is a malignancy characterized by skeletal involvement. CR remains the standard method to detect osteolytic bone lesions, although the false-negative rate (30-70%) is relatively high. The individuation of bone lesions at baseline is mandatory because reflects pivotal prognostic implications. It is universally accepted the superiority of MDCT in comparison with CR for detecting of early osteolysis (< 5 mm), while it is not yet clear whether MDCT is equal to MRI for the assessment of bony metastases. Methods We underwent a MDCT without contrast material and MRI with contrast material 7 patients with MGUS, 11 patients with Symptomatic MM and 25 patients with Asymptomatic MM. CR was the only method employed for radiological screening at diagnosis. Results Patients with MGUS presented completely negative MRI and MDCT. MDCT was positive in all patients with Symptomatic MM, demonstrating in 6/11 patients a more extensive involvement in comparison with MRI; MRI was positive in 8/11 patients with Symptomatic MM. In Asymptomatic MM group, 13 patients with negative CR and 6 patients with negative MRI, showed multiple focal lesions on MDCT; in 7 patients with MDCT and MRI positive, MDCT demonstrated a more extensive involvement in comparison with MRI. Conclusions In our study, in some selected cases, MDCT was superior to MRI. The mayor disadvantage of MDCT is the high doseradiotion delivered to patients. Whole-body low-dose CT techniques are being developed as very attractive and realistic alternative to CR.

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CONSOLIDATION THERAPY WITH BORTEZOMIB, THALIDOMIDE AND DEXAMETHASONE (VTD) REGIMEN AFTER ASCT IN MYELOMA PATIENTS WHO DO NOT RECEIVE BISPHOSPHONATES REDUCES BONE RESORPTION AND IS ASSOCIATED WITH LOW INCIDENCE OF SKELETAL RELATED EVENTS (SRES)

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The effect of VTD consolidation on bone metabolism was evaluated in 42 myeloma patients who underwent ASCT. Patients received 4 VTD cycles (first block, which started on day 100 post-ASCT), were followed without treatment for 100 days and then received another 4 VTD cycles (2nd block). Bisphosphonates were not given during or post-ASCT and throughout the period of VTD. Patients were assessed for SREs, while we also measured serum levels of: osteoclast regulators (sRANKL, OPG), osteoblast inhibitors (Dkk-1, sclerostin), bone resorption (CTX, TRACP-5b) and bone formation markers (bALP and osteocalcin (OC). Before VTD, 16 patients were in CR (9 in sCR), 16 in vgPR and 10 in PR. Despite response, patients had increased Dkk-1, sclerostin, CTX, TRACP-5b and OPG. The first VTD block resulted in a significant reduction of sRANKL/OPG, CTX, TRACP-5b, but also of bALP and OC. This VTD block also improved the status of response in 36% of patients; however alterations of bone markers were irrespective of further response. Before the 2nd VTD block, CTX was further reduced and bALP was increased. The reduction of CTX was continued post-2nd VTD block, but bALP or other markers showed no changes by this VTD block. During the study period, only one patient developed a SRE (i.e. radiation to bone). We conclude that VTD consolidation post-ASCT, without the presence of bisphosphonates, reduces bone resorption and is associated with a very low incidence of SREs. Bortezomib in combination with TD cannot produce a significant bone anabolic effect even in these patients with low myeloma burden.

P-107

HIGH CIRCULATING ACTIVIN-A IN NEWLY DIAGNOSED PATIENTS WITH SYMPTOMATIC MULTIPLE MYELOMA: CORRELATIONS WITH ADVANCED DISEASE FEATURES AND EXTENSIVE BONE INVOLVEMENT AND ALTERATIONS AFTER TREATMENT WITH NOVEL AGENTS

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The circulating levels of activin-A, a member of the TGF-beta superfamily, were evaluated in 85 newly-diagnosed patients with symptomatic multiple myeloma (MM), before and after the administration of novel agent-based therapies. Serum activin-A was measured using ELISA (R&D, MN, USA) along with markers of bone resorption (CTX, TRACP-5b) and formation (bALP, osteocalcin). Circulating activin-A was higher in patients (median: 555 pg/mL, range: 129-2336 pg/mL) than in controls (n=17; 393 pg/mL, 204-899 pg/mL; p<0.001). Activin-A strongly correlated with ISS stage (p-ANOVA=0.002), CTX (r=0.574, p<0.001) and TRACP-5b (r=0.481, p<0.001). Patients with extensive bone disease (>3 osteolyses and/or a fracture) had higher levels of circulating activin-A (618 pg/mL, 211-2043 pg/mL) compared to all others (477 pg/mL, 129-2336 pg/mL; p=0.03). The median survival of MM patients was 63 months. Low levels of activin-A were associated with superior median overall survival: not reached for patients with activin-A of <442 pg/mL (lower quartile, n=24) versus 59 months of all others (p=0.04). Bortezomib-based therapies (n=41) reduced circulating activin-A after 4 cycles of treatment (p<0.01) but no alterations were observed post 4 cycles of IMiDs-based regimens (n=44). We conclude that circulating activin-A is elevated in patients with newly-diagnosed, symptomatic MM and correlates with advanced disease features and high bone resorption. These results reveal activin-A as a possible target for the development of novel anti-myeloma therapies (such as sotatercept, a soluble activin-A receptor).

P-108

SERUM LEVELS OF C-TERMINAL COLLAGEN TELOPEPTIDE TYPE I NOT CORRELATE WITH BONE RESORPTION IN MULTIPLE MYELOMA (MM) PATIENTS

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Introduction: the skeletal involvement is one of the most important clinical characteristics of MM. Collagen type I constitutes 90% of the organic bone matrix and C-terminal telopeptide (CTX-I) together with N-terminal telopeptide are the most abundantly released products from collagen degradation. Recent studies have identified the CTX-I as a specific biological marker of increased bone resorption. We have examined the correlation between CTX-I serum levels and lytic bone lesions recognized on conventional radiography in our MM patients. *Methods:* CTX-I has been investigated in 29 patients with MGUS, in 10 patients with Asymptomatic MM, in 35 patients with untreated Symptomatic MM. The degradation products of CTX-I of type I collagen have been quantified in the serum samples of patients using a commercial immuno-

logic test (ß-CrossLaps/serum Elecsys test). The monoclonal antibodies employed in this test recognize all fragments of type I collagen. *Results:* our results have showed a normal CTX-I serum levels (<0,85 ng/ml) in all study groups. In MGUS group the mean CTX-I serum level was 0,20 ng/ml (range 0,10–0,49), in Asymptomatic MM was 0,26 ng/ml (range 0,15–0,55), in untreated Symptomatic MM was 0,27 ng/ml (range 0,13–0,71). *Conclusions:* our data have demonstrated there was no statistically significant relationship between CTX-I serum levels and skeletal stability. markers of bone turnover have some limitations: genetic and disease variability, diurnal rhythm and many others. This biologic marker must therefore be validated in clinical trials.

P-109

COMPARISON OF IMAGING WITH 18F-FAMT PET/CT AND 18F-FDG PET/CT IN MULTIPLE MYELOMA

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Aims: L-[3-^{18}F]-alpha-methyltyrosine (^{18}F-FAMT) is an amino-acid tracer for positron emission tomography (PET), and uptake has been shown to be related to overexpression of L-type amino acid transporter 1 (LAT1) and proliferative activity in tumor cells. The aim of this study was to assess the diagnostic performance of ^{18}F-FAMT PET/CT compared with 2-[18F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) PET/CT in evaluating patients with multiple myeloma (MM). *Methods:* Twelve patients with MM (3 newly diagnosed, 6 relapsed and 3 remission status after treatment) underwent whole-body ^{18}F-FAMT and ^{18}F-FDG PET/CT within two weeks. The results of these two PET scans were compared using visual analysis and standardized uptake values (SUVs). Magnetic resonance imaging of the spine was also performed to assess bone marrow infiltration patterns. *Results:* In five patients with focal infiltration pattern, ^{18}F-FAMT PET/CT detected 21 lesions and ^{18}F-FDG PET/CT detected 23 lesions. SUVmax for ^{18}F-FAMT and ^{18}F-FDG were 2.49 ±0.96 and 3.37 ±1.01 (mean ±SD, p<0.03), respectively, but no significant correlation was found for the lesion basis. All four patients with diffuse or variegated infiltration patterns showed no increased uptake of ^{18}F-FAMT. Notably, all unspecific ^{18}F-FDG-avid lesions, such as inflammation, were negative on ^{18}F-FAMT. *Conclusion:* Our preliminary data suggest that ^{18}F-FAMT PET/CT provides a useful imaging modality for detecting active myelomatous foci. Further investigations are needed to clarify the cause of the difference between the two PET tracer uptakes.

P-110

SERUM TIMP-1 LEVELS CORRELATE WITH ADVANCED STAGE AND ABNORMAL BONE REMODELING IN MULTIPLE MYELOMA PATIENTS AT FIRST RELAPSE WHO RECEIVE THE COMBINATION OF LENALIDOMIDE AND DEXAMETHASONE (RD) WITH ZOLEDRONIC ACID

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Circulating TIMP-1 levels were evaluated in 36 myeloma patients at first relapse who received RD at the standard dose, according to their renal function. Patients were also given zoledronic acid, 4 mg iv, monthly, both pre- and post-RD. Serum TIMP-1 was determined on day 1/cycle 1 and on day 28/cycle 3 of RD, using ELISA (Oncogene Science, Cambridge, MA, USA) along with serum markers of bone remodeling (CTX, TRACP-5b, bALP and osteocalcin) and osteoblast/osteoclast regulators (Dkk-1, sRANKL and OPG). The mean serum TIMP-1 level of all patients was 251.1 ng/ml (SD 95.4 ng/ml). Only two patients (1M/1F; 5%) had elevated values of TIMP-1 (UNL 459 ng/ml for males and 374 ng/ml for women). Patients had increased levels of Dkk-1, sRANKL, sRANKL/OPG ratio and bone resorption markers (CTX, and TRACP-5b) (p<0.01 compared with 25 healthy controls). Serum TIMP-1 correlated with OPG (r=0.644, p<0.001), creatinine (r=0.572, p<0.001), beta2-microglobulin (r=0.481, p=0.003), TRACP-5b (r=0.449, p=0.006) and Dkk-1 (r=0.444, p=0.007). Patients with ISS-3 disease at diagnosis continued to have higher levels of TIMP-1 at first relapse compared with those with ISS-1 or ISS-2. No significant alterations of TIMP-1 were observed after 3 cycles of RD. TIMP-1 did not predict for survival, both as continuous variable and as dichotomous variable, in this cohort of patients. We conclude that serum TIMP-1 is not elevated in myeloma patients at first relapse although its levels correlate with abnormal bone remodeling and ISS. This may be due to the continuous use of zoledronic acid in our patients.

P-111

THE TYROSINE KINASE INHIBITOR BAFETINIB (INNO-406) INHIBITS OSTEOCLAST FORMATION AND BONE RESORPTION

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Lyn phosphorylation plays an essential role in transmission of inhibitory signals through its phosphorylation of tyrosine residues within the immunoreceptor tyrosine-based inhibitory motifs (ITIM). ITAM–ITIM cross talk is very important for macrophage differentiation and osteoclast formation. To evaluate the effects of bafetinib on osteoclast formation and bone resorption, CD14+ monocytes were treated with 50 ng/ml RANKL and 20 ng/ml MCSF, and bafetinib or zoledronic acid was added into the cells. Bafetinib and zoledronic acid both markedly inhibited osteoclast formation in a concentration-dependent fashion in both monocytes derived from MM patients and normal subjects. Next, we assessed bone resorption using monocytes that were induced with M-CSF and RANKL and cultured on bone slides for 28 days. Bone resorption was determined using toluidine blue staining. Resorption pits were measured and the percentage of the surface area with lacunar resorption by using NIH image-j analysis system. At a concentration as low as 5μM, bafetinib significantly inhibited bone resorption in a concentration dependent fashion (P<0.001). We further examined the effects of bafetinib on NF-κB and JNK signaling pathway. phosphorylation of NF-κB and JNKK was decreased following exposure to bafetinib. Currently, we are evaluating bafetinib on bone resorption in vivo using SCID-hu murine models of MM. These studies suggest that bafetinib may be a new approach to block osteoclast development and reduce bone resorption in cancer patients.

P-112

DOSE-DEPENDENT DUAL EFFECTS OF BORTEZOMIB ON OSTEOBLASTOGENESIS: THE ROLE OF ER STRESS

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Activating transcription factor-4 (ATF-4) is induced by ER stress, and plays a critical role in osteoblast (OB) differentiation as well as cell function and fate including autophagy and apoptosis. In the present study, we explored the roles of ER stress induced by bortezomib (Bor) in OB differentiation with special reference to ATF-4. Bortezomib dose-dependently increased ATF-4 protein levels in bone marrow stromal cells and MC3T3-E1 preosteoblastic cells. Bor at 10 nM or less facilitated OB differentiation by BMP-2 in MC3T3-E1 cells to exhibit mineralized nodule formation. The suppression of ATF-4 expression by siRNA abrogated osteocalcin expression and mineralized nodule formation induced by

Bor, indicating the critical role of ATF-4 in terminal OB differentiation. However, Bor at 20 nM or higher abolished mineralized nodule formation, and dose-dependently induced phosphorylation of eIF2alpha along with reduction at protein levels of beta-catinine and osterix, critical mediators for OB differentiation. These protein reduction may be at least in part due to global suppression of protein translation by the phosphorylation of eIF2alpha. Furthermore, Bor at 50nM induced CHOP, suggesting activation of the ATF4-CHOP pro-apoptotic pathway. These results collectively suggest that excessive ER stress by Bor may hamper OB function and viability despite its cytotoxic activity against MM at higher doses. Therefore, it can be envisaged that optimization of dosing schedules of Bor may make the best use of this important anti-MM agent as a potent inducer of bone formation in MM.

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COMPARISON OF WHOLE BODY DIFFUSION WEIGHTED MAGNETIC RESONANCE IMAGING WITH SKELETAL X-RAY AND MAGNETIC RESONANCE IMAGING OF THE SPINE FOR THE ASSESSMENT OF BONE DISEASE IN MULTIPLE MYELOMA: PRELIMINARY ANALYSIS OF A PROSPECTIVE STUDY

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Bone disease in multiple myeloma (MM) patients is usually assessed by skeletal X-ray (XR) and magnetic resonance imaging (MRI) of the spine (MRIS). Diffusion-weighted MRI (DW-MRI) is an innovative whole-body MRI that detects malignant lesions studying water diffusion in tissues. This prospective study compared DW-MRI with XR and MRIS for the assessment of focal bone lesions in 50 MM patients. Patients performed XR, MRIS and DW-MRI at diagnosis or at relapse, after the treatment and 6 months thereafter (symptomatic MM) or every 6 months for 1 year (asymptomatic MM). MRIS and DW-MRI were done in a single 45-minutes session by a standard 1.5 Tesla MRI scanner. DW-MRI consisted of multiple stacked axial EPI sequences at 4 b-values, evaluated by PET-like MIP and MPR reconstructions at the highest b-value (1000). We report a preliminary analysis of the first exam of the 16 symptomatic MM at diagnosis and the 14 asymptomatic patients enrolled. Median age was 62 years (range, 33-80), 22 patients were ISS stage I and 3 stage III. Median bone marrow infiltration was 40% (range, 10-100%). In symptomatic patients, XR detected a median of 0 (range, 0-14), and MRIS 3 (range, 0-19) focal lesions; DW-MRI detected 22 (range, 0-121) focal lesions, significantly more than XR (p=0.009) and MRIS (p=0.02). Also in asymptomatic patients DW-MRI detected more focal lesions than XR (p=0.009) and MRIS (p=0.01). In conclusion, DW-MRI is superior to XR and MRIS in detecting focal lesions in symptomatic and asymptomatic MM patients. An update of the study will be presented at the meeting.

P-114

PERCUTANEOUS LIMB BONES OSTEOPLASTY , ILEOPLASTY AND SACROPLASTY IN THE TREATMENT OF BONE LYTIC LESIONS IN ADVANCED MULTIPLE MYELOMA

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The aim of this report is to evaluate feasibility, safety and efficacy of limb bones and pelvis osteoplasty under fluoroscopic guidance in patients with mielomatous osteolytic lesions, refractory pain and high risk of pathological fracture. Since November 2004 through February 2011 eighteen consecutive patients with multiple myeloma with osteolysis in the pelvis and limb bones have been treated with percutaneous bone-cement injection with an solution of polymethylmethacrylate into the supracetabular, sacral, femoral, humerus and tibial bone cavity. Before the osteoplasty in all patients was present pain in side of lesions, with oppiods therapy, and immobilization, valued with visual analogue scale score (VAS) system and functional mobility score system. The lesion approach was performed under fluoroscopic guidance using local anaesthesia and conscious sedation in all patients. In four cases, before the PMMA injection, a termoablation with radiofrequency of the tumor was associated. The aim of osteoplasty was analgesic and prevention of the patological fractures. Technical success was achivied in all patients without immediate complicance Complete pain relief occurred whitin 1 month in 15/18 patients and a significant decrease in VAS score occurred in 2/18. During the mean follow-up of months 38 the improvement of the deambulation in as been had in all. Percutaneous osteoplasty of the pelvis and limb bones is feasible and safe in myelomatous patients, is minimally invasive with immediate pain relief, prevention of the pathological fractures and improvement of the ambulation.

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HEPARANASE PROMOTES BONE RESORPTION AND SUPPRESSES BONE FORMATION, RESULTING UNCONTROLLED BONE DESTRUCTION IN MULTIPLE MYELOMA

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90% of patients with myeloma have destructive bone lesions, and its causes remain unclear. In the present study, using SCID-hu and SCID-tibia animal models, we discovered a remarkably enhanced osteolysis in the bones injected with myeloma cells expressing high levels of heparanase (HPSE), as compared to the bones injected with HPSE-low cells. In addition, mice bearing HPSE-high tumors had accelerated osteolysis in distal bones without the presence of metastatic cells. Staining on bone sections revealed a significant increased number of TRAP+ osteoclasts and a decreased number of osteocalcin+ osteoblasts at the primary tumor site and in distal bones of animals bearing HPSE-high tumors. These indicate that heparanase promotes osteoclastogenesis and suppresses osteoblastogenesis within these bones. Furthermore, in vitro studies showed that the expression and secretion of RANKL were significantly enhanced in myeloma cells transfected with human HPSE cDNA or when cells were exposed to recombinant HPSE (rHPSE). RANKL expression was also remarkably elevated when osteoblastic cell lines or human stromal cells were treated with either rHPSE or with medium conditioned by HPSE-high myeloma cells, while the level of OPG was not affected. These findings indicate that 1) HPSE is a major determinant of the osteolytic phenotype in myeloma, 2) the osteolytic action of HPSE is mediated via enhancing the expression and secretion of RANKL by myeloma cells and by promoting the osteoclast-supporting activity of osteoblastic/stromal cells, and 3) HPSE also inhibits bone formation in myeloma.

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BORTEZOMIB STIMULATES OSTEOBLASTIC DIFFERENTIATION VIA INCREASED NUCLEAR VITAMIN D RECEPTOR LEVELS AND ENHANCED VITAMIN D RECEPTOR SIGNALING

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Osteoblast activity is impaired in multiple myeloma, but can be enhanced by proteasome inhibition. The aim of this study was to investigate the effects of bortezomib on primary human mesenchymal stem cells (hMSC) and osteoblasts (hOB) focusing on vitamin D (VD) dependent osteoblastic differentiation, since myeloma cells impaired VD dependent osteoblastic differentiation in our coculture experiments, represented by decreased expression of osteocalcin (OC) and osteopontin (OPN). Bortezomib stimulated osteoblastic differentiation of hMSC and hOB, with increased OC and OPN mRNA expression. Importantly, this effect could be further increased, when VD was added. In hMSC, 5 nM bortezomib led to a 65.9 fold increase in OPN mRNA expression, com-

pared to a 140.6 fold increase by the combination of VD and bortezomib. OC expression was increased 360 fold by bortezomib in the presence of VD, but only 2.77 fold with bortezomib alone. In hOB, similar results were obtained. Bortezomib led to an increase in nuclear VD receptor (VDR) levels in hMSC in western blot analyses. Primary hMSC transfected with a VDR luciferase reporter construct showed a 3.7 fold increase in VDR signalling with BTZ. In summary, our data show that bortezomib stimulates osteoblastic differentiation of hMSCs and hOBs and acts, at least in part, through VDR signalling. This might be of clinical importance given the high prevalence of VD deficiency in myeloma patients. Substitution of vitamin D in multiple myeloma patients treated with bortezomib may be beneficial for bone turnover and needs further clinical evaluation.

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MODULATION OF NON CANONICAL WNT5A/ROR2 SIGNALING PATHWAY IN HUMAN MESENCHYMAL CELLS INCREASES OSTEOGENIC DIFFERENTIATION COUNTERBALANCING THE EFFECT OF MYELOMA CELLS

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Multiple Myeloma (MM) is characterized by the impairment of osteogenic differentiation of mesenchymal cells (MSC). Canonical Wnt signal pathway is critical in the regulation of bone formation even if there is not clear whether MM cells affect Wnt signaling in hMSC. Together to canonical Wnt signaling, a non-canonical Wnt pathway has been identified transduced through FZD receptor and Ror2 co-receptor. Recent evidences suggest that non-canonical Wnt activation by Wnt5a rather than canonical one by Wnt3a, stimulates the osteogenic properties of human MSC through Ror2 activation. The effect of MM cells on non-canonical Wnt signaling and the role of the activation of this pathway on MM-induced osteoblast exhaustion are not known. In this study, first we found that osteogenic differentiation of hMSC significantly increased Ror2 expression and that MM cells inhibit Ror2 and FZD5 expression by hMSC in co-culture. Activation of non-canonical Wnt pathway either by Wnt5a treatment or by both Wnt5a and Ror2 overexpression by lentivirus vectors increased osteogenic differentiation by hMSC. Consistently, Wnt5a and Ror2 overexpression by hMSC blunted the inhibitory effect of MM cells on osteogenic differentiation by hMSC in oc-culture. Finally, these observations were further confirmed showing that Wnt5a or Ror2 silencing in hMSC by siRNA or shRNA trasfection, respectively inhibited the expression of osteogenic markers. Our data indicate that activation of non-canonical Wnt5a/Ror2 pathway in hMSC increases osteogenic differentiation and counterbalances the inhibtory effect of MM cells.

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IMMUNOMODULATORY DRUGS INDUCE DKK-1 EXPRESSION IN HUMAN MESENCHYMAL STROMAL CELLS AND IMPAIR OSTEOGENESIS IN VITRO

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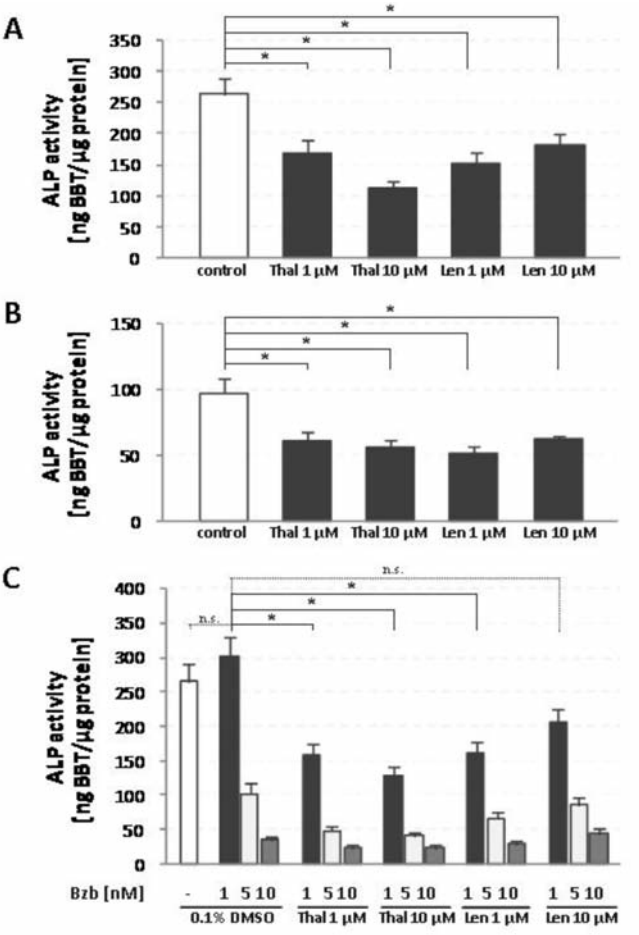
Osteoblastic activity is severely impaired in most MM cases, contributing to the development of myeloma bone disease. Although some drugs reducing osteoclast mediated bone degradation are in clinical use, approaches to specifically augment bone formation are at an early stage of development. Novel anti-MM drugs were not only shown to directly act on MM cells, but to impact the stromal environment as well. Here we investigate the role of IMiDs on bone formation. A model of in vitro osteoblastic differentiation from human BM derived stromal cells (immortalized or primary) was used to study the effect of IMiDs on osteogenesis. Daily treatment with LEN 1 μM resulted in a significant inhibition of osteoblast formation and activation reflected by a decrease of 43% in alkaline phosphatase activity at day 14 (Fig. 1A) and 70.4 % in matrix mineralization at day 21 of osteogenesis. Numbers for THAL 1 μM are 41% and 75.1% respectively. Similar results were achieved when using primary cells (Fig. 1B). Furthermore, both Runx2 and Dlx5 were significantly downregulated by IMiDs. Bortezomib was not able to overcome the inhibitory effects of the IMiDs (Fig. 1C). Treatment with IMiDs resulted in a significant upregulation of the osteoblast inhibitor Dickkopf-1 (DKK-1) in stromal cells, which possibly underlies the inhibitory effects on osteogenesis. In conclusion, IMiDs upregulate DKK-1 expression in mesenchymal stromal cells and inhibit osteogenesis in vitro. Strategies to neutralize DKK-1 may be helpful in overcoming potentially disadvantageous side-effects of therapy with THAL or LEN.



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P-119

A STUDY IN APPLICATION OF DURIE/SALMON PLUS STAGING SYSTEM FOR JAPANESE MYELOMA PATIENTS

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Although Dr. Durie et. al. proposed Durie/Salmon PLUS staging system (D/S PLUS staging system) , not so many studies on usefulness of PET for myeloma so far, comparing with lymphoma. Searching with PubMed, researchers can find one-hundred twenty-three papers on myeloma, but more than ten times of papers in lymphoma. Japan Ministry of Health, Labor and Welfare approved coverage of national medical insurance for using PET/CT for all type of cancers from April, 2010. We studied usefulness of D/S PLUS staging system for Japanese myeloma patients. Forty-one myeloma patients were included in this study, who recieved PET/CT exam from April 2010 to December 2010 in our hospital (MGUS 11, asymptomatic myeloma: 4, symptomatic myeloma: 24, and macroglobulinemia: 2) PET/CT in this study results in accuracy:

79.5%, Sensitivity: 66.7%, Specificity: 100%, PPV: 100%, NPV: 65.2%. Twenty-four cases were detected PET-FL in symptomatic myeloma. Extra-medullary disease was found in four patients by using PET/CT. Distribution of PET-FL number is, 0-4: 15, 5-20: 6, and over 20 lesions : 3 cases, respectively. Four patients in total twenty-four (16.7%) symptomatic myeloma patients were upstaged by D/S PLUS staging system. This is the first report of PET/CT in application of D/S PLUS staging system for Japanese myeloma patients, and displays the usefulness of PET/CT in initial staging of myeloma, especially in distinguishing active/inactive myeloma and identifying EMD. Thus, we are now planning "Japan Myeloma PET Registry (JMPR)" to study for PET/CT usefulness for myeloma.

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CIRCULATING LEVELS OF CATHEPSIN-K IN MULTIPLE MYELOMA PATIENTS

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Cathepsin-K (cat-k) is an osteoclast (OC)-derived cysteine protease, that has been implicated as playing a major role in OC-mediated bone resorption; measurements of its circulating levels may reflect OC activity. Cat-k is a potential target for anti-resorptive treatment in postmenopausal osteoporosis and other skeletal disorders characterized by altered bone remodeling. According to the literature, postmenopausal women with osteoporosis or Paget's disease may show elevated cat-k serum levels, decreasing during biphosphonate treatment. Otherwise, there are no literature data on cat-k circulating levels in multiple myeloma (MM) bone disease, characterized by enhanced OC activity. Aim of this study was to evaluate a possible role of cat-k as biomarker reflecting bone destruction in MM. Therefore, we recruited 96 MM patients (50 M/46 F, median age 72 years): 82 were newly-diagnosed (18 had asymptomatic MM and 64 symptomatic MM), 14 were relapsed/refractory MM. These latter received therapeutic regimens containing bortezomib, and some of them also received lenalidomide combinations and/or monthly biphosphonate. In addition, sera from 30 MGUS patients, and 22 gender- and age-matched healthy controls were tested. Cat-k serum levels were determined by ELISA (Biomedica, Vienna, Austria). No significant differences were detected among our patients, except for those with asymptomatic MM. Indeed, 8/18 patients with asymptomatic MM showed cat-k high levels ($p<0.04$). At present, data on cat-k circulating levels in different populations are however limited and need further investigations.

P-121

NOVEL MOLECULAR IMAGING AS A TOOL TO INITIATE EARLY TREATMENT, MONITOR MINIMAL RESIDUAL DISEASE, AND DETECT EARLY RELAPSE: A PILOT STUDY IN MULTIPLE MYELOMA (MM), SMOLDERING MYELOMA (SMM), AND MGUS

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Background: Despite its poor sensitivity and inability to identify extramedullary disease, skeletal survey remains the gold standard for detection of osteolytic bone disease in MM. Aim of study: to assess novel molecular imaging in MM and its precursors. *Methods:* The study includes 10 MGUS, 10 SMM, and 10 MM pts. All pts are assessed by skeletal survey, fluorine-18 fluorodeoxyglucose and sodium fluoride positron emission tomography/computed tomography (18F-FDG PET/CT, 18F-NaF PET/CT), and dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI). All patients are characterized clinically/molecularly. *Results:* Based on 11 enrolled pts; 3 MGUS and 6 SMM pts were negative by all imaging modalities. In 1 SMM pt,18F-NaF PET had subtle increased uptake in T12 which corresponded to CT, MRI and DCE-MRI abnormalities in this region; 18F-FDG PET and skeletal survey were negative. Bone marrow: 10-20% abnormal plasma cells (aPC) by immunohistochemistry; >99% aPC by flow. Labs: immunoparesis, IgG kappa 1.4 g/dL M-spike, FLC-ratio 12.27. In 1 MM pt 18F-NaF PET and DCE-MRI showed abnormalities not detectable by 18F-FDG PET/CT or skeletal survey. *Conclusions:* Novel molecular imaging techniques detect early bone disease in some high-risk SMM pts; providing new avenues for studies designed to initiate/monitor early treatment. In MM, novel molecular imaging shows abnormalities not detectable by 18F-FDG PET/CT or skeletal survey; suggesting that advanced imaging may be used to monitor minimal residual disease and/or to detect early relapse. Updated results will be presented at the meeting.

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APPEARANCE OF MONOCLONAL PLASMA CELL DISEASES IN WHOLE BODY MRI IN 414 PATIENTS AND CORRELATION WITH DISEASE ACTIVITY

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Focal lesions (FL) detected by MRI have been shown to be of prognostic significance for progression free and overall survival. We retrospectively analyzed results of whole-body MRI (wb-MRI) of 414 unselected and untreated patients with - according to IMWG-criteria - monoclonal gammopathy of undetermined significance (MGUS n=97), smoldering myeloma (sMM n=135), solitary plasmacytoma (sPC n=15) symptomatic multiple myeloma (MM n=156) and AL-Amyloidosis (ALA n=11) who were examined by WB-MRI for routine work-up. WB-MRI was performed on two identical 1.5 Tesla MRI-scanners with body array curls. Assessment of FL was done by two experienced radiologists blinded to the diagnosis of the patients in consensus. We found a median number of 0 (range 0 to 10) FL for MGUS, 1 (range 0 to 23) FL for sPC, 0 (range 0 to >20) for sMM, 2 (range 0 to >20) FL for MM and 0 (range 0 to > 20) FL for ALA, respectively. Correlation of the number of FL with parameters of disease activity revealed a significant correlation with serum M-protein (Spearman's rho=0.17; p=0.009), plasma cell concentration in bone marrow (rho=0.26; p<0.001) and beta2-microglobulin (rho=0.28; p<0.001). The number of focal lesions in WB-MRI increases from MGUS to sMM and MM and correlates with the most important parameters of disease activity. Further investigations and especially comparison with x-ray, computed tomography and Positron Emission Tomography will have to show what kind of changes detected by MRI should lead to the initiation of treatment to prevent further bone destruction and systemic progression of disease.

P-123

INVOLVEMENT OF MOLECULAR FACTORS WITH OSTEOLYTIC BONE LESIONS IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: Osteolytic lesions are an important and debilitating aspect of multiple myeloma (MM). Recently, we have published an MM classification using the gene expression data set obtained from newly diagnosed MM patients included in the HOVON65/GMMG-HD4 trial (Broyl et al., Blood, 116, 2543-2553; 2010). *Aim:* To determine the relation between gene expression in the myeloma cell and occurrence of bone lesions in the HOVON65/GMMG-HD4 trial. *Methods:* The occurrence of bone lesions was determined in the clusters previously defined in the gene expression based MM classification. In addition, gene expression patterns of cases with bone lesions and without bone lesions were compared (GSE19784). *Results:* 64% of cases of this data set presented with 3 or more bone lesions. Clusters with the highest frequency of 3 or more bone lesions were PRL3 cluster (89%), CD-1(82%), PR(75%)

and CTA cluster (74%). Overall, genes associated with presence of 3 or more bone lesions were DKK-1, PTTG1, CDC2, CDCA3, AURKA, BIRC5, MYCBP, RELN, SMYD3 and GINS1. Within the top clusters with bone disease, specific genes were found to be associated with bone lesions. For example, within the PR cluster, AURKA and BIRC5 characterized the cluster and were found to be associated with bone lesions. *Conclusion:* A bone lesion specific gene expression profile was found consisting of known bone modifying genes such as DKK-1 and RELN. In addition, cell cycle and proliferation genes were found to be associated with bone lesions, suggesting a link between myeloma proliferation and bone disease.

P-124

HUMAN MESENCHYMAL STEM CELL-DERIVED PRE-OSTEOBLASTS RESPOND TO TOLL-LIKE RECEPTOR LIGANDS BY UPREGULATING FACTORS PROMOTING OSTEOCLAST ACTIVATION AND BY SECRETING PROINFLAMMATORY CYTOKINES

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Mesenchymal stem cells (MSC) can differentiate into osteoblasts and regulate osteoclasts differentiation by expressing receptor activator of NFkappaB ligand (RANKL) and osteoprotegerin (OPG). Recently, it has been shown that MSC express toll-like receptors (TLRs) and that stimulation of TLRs on MSC induces secretion of cytokines and regulates differentiation. Myeloma patients suffer from frequent infections of both bacterial and viral origin. Thus microbial derived factors can possibly activate TLR-signaling on MSC in these patients. We investigated whether activation of TLR1/2, TLR3 and TLR4 on MSC differentiated in osteogenic direction influenced the expression of RANKL and OPG. We found that MSC treated with the TLR agonists Poly(I:C) (TLR3) and LPS (TLR4) increased the mRNA expression of RANKL in two out of three MSC donors. Poly(I:C) down regulated mRNA OPG expression in the same donors, thereby changing the RANKL/OPG ratio into a more bone destructive state. Moreover, TLR3 activation increased the expression of MIP1alpha, a factor previously shown to activate osteoclasts. Thus TLR signaling in MSC might affect bone homeostasis by promoting osteoclast activation.

P-125

MATRIX METALLOPROTEINSES 13 (MMP13) SECRETED BY MULTIPLE MYELOMA CELLS INDUCES OSTEOCLAST PRECURSOR FUSION BY UPREGULATION OF DC-STAMP RESULTING IN INCREASED BONE RESORPTION

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We explored the role of MMPs in multiple myeloma (MM) and showed that MM cells express MMP13 400-fold higher than other MMPs. Tissue array analysis showed that MMP13 was highly expressed in MM cells but not in normal tissue. ELISA of MM patient sera revealed a strong correlation between MMP13 expression and bone disease. MMP13 was undetectable in healthy donors compared to 87% of MM patients with bone disease. MMP13 enhanced bone resorption in vitro. MMP13 did not increase osteoclast (OCL) numbers but increased OCL size and nuclear number/OCL, suggesting that MMP13 enhances fusion of OCL precursors. To verify this, we tested mononuclear cells (MNC) from mmp-13-/- or wt mice in OCL formation assays. The number of nuclei and average size of OCL decreased significantly ($p<0.0001$) in mmp-13-/- cultures. Addition of MMP13 reversed the fusion defect of mmp-13-/- MNCs. Dendritic cell-specific transmembrane protein (DC-STAMP), essential for cell-cell fusion, was upregulated by exogenous MMP13. Coculture of MM cells with BMSCs significantly ($p<0.001$) increased (up to 14-fold) MMP13 secretion by MM cells. This was blocked by functional inactivation of IL6 with neutralizing antibodies. EMSA showed that IL6-mediated AP-1 activation promoted MMP13 transcription. IL6 increases expression of MMP13 in MM cells that induces DC-STAMP resulting in enhanced fusion of OCL precursors and bone resorption. Our findings are further supported by Stickens et al (2004), who showed, that mmp-13-/- mice exhibit osteopetrosis. MMP13 may represent a novel approach to ameliorate osteolytic lesions in MM.

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THE COMBINATION OF LENALIDOMIDE AND DEXAMETHASONE (RD) REDUCES BONE RESORPTION IN RESPONDING PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (MM) BUT HAS NO EFFECT ON BONE FORMATION: RESULTS OF A RETROSPECTIVE ANALYSIS AND A PROSPECTIVE STUDY ON 205 PATIENTS, ON BEHALF OF THE GREEK MYELOMA STUDY GROUP

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We evaluated retrospectively the effect of RD on bone remodeling of 106 consecutive patients with relapsed/refractory MM. The following bone indices were measured on cycle 1/day 1 and then on day 28/cycle 3 and 6: Dkk-1, sRANKL, OPG, bone resorption markers (CTX, TRACP-5b) and bone formation markers (bALP, osteocalcin (OC). Before RD, patients had increased serum Dkk-1, sRANKL, and bone resorption markers and reduced OC and bALP compared to 44 healthy controls. The objective response was 55%. RD produced a reduction of CTX and sRANKL/OPG only in responders, with no effect on bone formation. To confirm these results, we scheduled a prospective study in which 99 patients received either RD (n=50) or VRD (bortezomib+RD, n=49), based on PN status. VRD reduced Dkk-1 and increased OC after 3 cycles, while it reduced sRANKL/OPG and increased bALP after 6 cycles. These changes were irrespective of treatment response which was similar among treatment arms (63%). In VRD, % Dkk-1 reduction strongly correlated with % increase of bALP. RD reduced CTX only in responders, while it increased Dkk-1; however, responders had a median increase of 9% while non-responders of 91%. No SREs were observed in the VRD arm while two patients treated with RD who had not responded to therapy developed a vertebral pathological fracture. We conclude that RD reduces bone resorption only in responding patients with relapsed/refractory myeloma but has no effect on bone formation. Combination with bortezomib, which enhances bone formation, may be of benefit for the management of bone disease in these patients.

P-127

EVALUATION OF WHOLE-BODY DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING FOR DIAGNOSIS AND MONITORING OF MULTIPLE MYELOMA

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Background: Major recommendations were published for the validation of whole body diffusion-weighted magnetic resonance imaging (DWI) as a biomarker of cancer. Assessments tend to show an interest of DWI in monitoring bone metastases from prostate or lung cancer. The aim of our work is to evaluate DWI as a biomarker for diagnosis and monitoring of multiple myeloma. *Methods:* We performed DWI in 63 patients (10 MGUS, 28 smoldering myeloma and 25 symptomatic multiple myeloma). *Results:* Sensitivity and specificity of DWI for the diagnosis of myeloma by taking into account focal lesions and diffuse infiltration were respectively 90.91% and 97.56%. 25 patients had at least 1 complementary DWI during follow-up. Changes in infiltration and number of focal lesions were correlated with biological response to treatment (weighted kappa = 0.684). 24 patients had a perfusion MRI sequence in

addition to DWI. We observed a correlation between the percentage of enhancement of L1 vertebra in perfusion sequences and the ratio L1 / kidney in diffusion sequences (0,79 - p<0,0001). *Conclusions:* Presence of focal lesions or diffuse infiltration in DWI seems to be a good marker of symptomatic multiple myeloma. Changes in number of focal lesions and diffuse infiltration during treatment could be a useful marker for monitoring response. Further studies are necessary to define more precisely the interest of DWI in multiple myeloma.

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OVER-EXPRESSION OF RANKL IN INVARIANT NKT CELLS IS CHARACTERISTIC OF ACTIVE MYELOMA BUT NOT OF MGUS OR ASYMPTOMATIC MYELOMA (ASM)

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RANKL upregulation in myeloma microenvironment is pivotal for the development of myeloma bone disease. Invariant NKT cells (iNKT) are dysfunctional in MM. Whether they contribute to the RANKL overproduction is not known. Ex-vivo assessment showed that a significantly higher proportion of peripheral blood (PB) iNKT than conventional T-cells from normal donors (ND) (n=51) expressed surface RANKL: median (range): 8.62% (0-57%) vs 0.81% (0.11-14.14); p<0.001. This proportion was 8-fold higher in donors >45yr. Using RQ-PCR, we confirmed that purified iNKT cells (n=7 ND) expressed ex-vivo 2.04-fold (range: 0.5-11.63) more RANKL mRNA than conventional T-cells from the same donors (p=0.028). In patients with active MM (n=41) a higher proportion of PB iNKT expressed surface RANKL than conventional T-cells: 32.04% (3.8-98) vs 2.01% (0.48-10.02) ; p<0.001. Similarly, purified PB iNKT cells expressed 4,38-fold (1.74-7.45) more RANKL mRNA than T-cells from the same patients (n=5; p=0.018;). Compared to age-matched donors (n=33), we found a significantly higher proportion of RANKL expressing PB iNKT cells in MM patients (MM: 32.04% vs age matched ND: 18.75% p=0.013). Furthemore in patients with MGUS or ASM (n=18) the proportion of iNKT cells that expressed RANKL was significantly lower when compared to active MM patients (6.315% vs 32.04 % respectively; p<0.001). RANKL overexpression in iNKT cells in active MM but not in MGUS/ASM, suggest that iNKT cells contribute to osteoclast activation and development of bone disease in MM.

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OSTEONECROSIS OF THE JAW IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH BISPHOSPHONATES

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Introduction: Bone lesions are a major cause of morbidity in multiple myeloma patients. Treatment includes bisphosphonates, which are also associated with avascular osteonecrosis of the jaw (ONJ). Our aim was to evaluate the correlation between bisphosphonates and ONJ. *Patients and Methods:* Of the 39 patients with multiple myeloma treated in our department from June 2004 to December 2009 with i.v. bisphosphonates, two (5 %) developed ONJ. *Results:* The two patients with ONJ had all been treated with pamidronic acid and the diagnosis of ONJ was diagnosed during myeloma treatment. One patient died from sepsis three days after ONJ, the second patient received local invasive treatment with antibiotic, and no progression was noted. All the two patients with O.N.J presented with concomitant risk factors such as paradontopathy and spontaneous avulsion (first patient) or dental extraction (second patient). *Conclusion:* Our results show that ONJ can appear during treatment and that a surgical approach can be used in suitable cases. Closer cooperation is needed among specialists to define guidelines for the prevention of ONJ in patients with myeloma.

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DICKKOPF-1 PROTEIN (DKK1) CONCENTRATION IN SERUM AT DIAGNOSIS IS STRONGLY CORRELATED TO FRACTURES IN PATIENTS WITH MULTIPLE MYELOMA (MM)

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Introduction: DKK-1 protein is an inhibitor of the Wnt signal pathway, crucial to bone metabolism, affecting osteoblast differentiation. MM cell and bone marrow microenvironmental cell interaction is associated with Osteolytic Bone Disease (OBD), characterized by increased bone absorption and inadequate bone formation. *Aim:* To investigate the correlation of DKK-1 serum protein concentration to the extent of OBD in patients with MM . *Patients and Methods:* Sera from 75 MM patients were analyzed at diagnosis.They were 30 females, median age was 66 yrs (42 - 83). Durie-Salmon and International Scoring System stages were evently distributed. OBD was based on X-ray findings and divided into the 4 Durie-Salmon Bone Scale groups. DKK1 was measured by ELISA. Values were compared with disease parameters. *Results:* 11 patients were in BS-0 group with median DKK-1 3383 pg/ml (546-14716), 21 in BS-1 and median DKK-1 1536 pg/ml (751-6966), 21 in BS-2 with median DKK-1 1671 (561-6202) and 22 in BS-3 with median DKK-1 5758 (9-15896). DKK-1 concenrtations were increased in patients with fractures compare to the others (p=0.001) . No other correlations were found. *Conclusions:* DKK-1 increase favours bone fractures in MM

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BORTEZOMIB-CYCLOPHOSPHAMIDE-DEXAMETHASONE FOR RELAPSING MULTIPLE MYELOMA

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Background: In vitro studies have demonstrated synergistic anti-myeloma effects with the combination of bortezomib and alkylating agents. Combinations of bortezomib, cyclophosphamide and dexamethasone (BCD) are rational with the prospect of improved activity and independent toxicity. *Methods:* Between December 2004 and April 2007, we treated 44 patients with relapsing multiple myeloma with the combination of bortezomib 1.3 mg/m2 I.V. on days 1, 4, 8, 11; dexamethasone 20 mg/m2 p.o daily for 4 days beginning on days 1, 9 and 17; and cyclophosphamide 70 mg/m2 p.o. twice daily for 4 days. A second course was given 1 month later. *Results:* Rapid clinical response was observed in 32 patients (73%) including 26 with disease in PR (59%), and 6 with disease in CR (14%). Side effects were uncommon, mild and usually preventable. The median remission time of responding patients was 12 months that contributed, after landmark of 3 months, to a significantly longer median survival for patients with responsive disease (33 months) than for those with unresponsive disease (12 months) (p<0.01). *Conclusion:* Bortezomib-cyclophosphamide-dexamethasone was an effective, well-tolerated combination for treatment of relapsing multiple myeloma.

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CLINICAL AND IMMUNOPHENOTYPIC CHARACTERISTICS OF 63 PLASMA CELL LEUKEMIA (PCL) CASES

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Primary PCL was diagnosed in 37 patients and secondary PCL in 26 patients. Prospective flow cytometric analysis of antigens expression on bone marrow and peripheral blood plasma cells of PCL patients enabled to establish the following immunophenotype of leukemic plasma cell: CD38++, CD138+, CD54+, CD49+, CD29+, CD44+, CD126+, CD19-, CD45-. In one third of patients leukemic plasma cells expressed CD56, CD71, CD117. IgA M-protein was revealed in one case of primary PCL (2.9%) and in 42.4% of secondary PCL. In one case of primary PCL IgE M-protein was detected. Patients were given chemotherapy, in 2 cases combined with autologous stem cell transplantation (ASCT) and in 8 cases with thalidomide, lenalidomide or bortezomib. In 17% of primary PCL patients CR was achieved, in 8% nCR, in 50% PR, in 8% MR and 17% of patients did not respond to treatment. PFS ranged from 2 to 33 months (median 6 months). Median survival of all primary PCL patients was 9 months. Ten patients (27%) survived more than 20 months among them 4 patients treated with bortezomib, 2 ASCT, 1 thalidomide and 1 lenalidomide. In secondary PCL median time to leukemic progression from multiple myeloma was 21 months and median survival was 2.0 months. In 4 cases of multiple myeloma a leukemic transformation occurred following thalidomide therapy. Conclusions. Our findings suggest that combination chemotherapy with bortezomib, doxorubicin and dexamethasone, may be an effective induction treatment for primary PCL, in particular in patients eligible for ASCT, however ASCT produces modest improvement of outcome.

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HIGH RATE OF COMPLETE REMISSION (CR) AND UPGRADED RESPONSE WITH WEEKLY MAINTENANCE BORTEZOMIB POST SINGLE AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANT (PSCT) IN PATIENTS WITH MULTIPLE MYELOMA. RESULTS OF A PHASE II PROSPECTIVE STUDY

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We performed a phase II study investigating the role of weekly bortezomib (bor)/dexamethasone (dex) followed by thalidomide (thal)/dex as maintenance therapy post single autologous PSCT. The objectives were to examine the feasibility, toxicities, CR and PFS. Following autologous PSCT (Mel 200) pts started weekly bor 1.3mg/m2 / dex 40mg x 4 d for 6 mo. followed by thal 50 -200mg /dex 40mgx 4 d for 6 more mo. From 3/2008 to 6/2010, 45 pts were enrolled with med age 54 yrs (29-71), med time from dx 6.1 mo. (3.5 – 145.9), med B2M 1.8 mg/L (1.05 -5.3). ISS stage I/II/III 18/14/8/ 5 N/A, previous treatment: thal based (15), bor (27) and lenalidomide (10), response at PSCT: CR (11), VGPR (11), PR (23). Six pts had ch 13 ∅ and 2 pts t 4;14 (1 with ch 17p del) *Results:* Forty-five pts have been enrolled, 5 pts were unable to start bor due to >= gr II neuro toxicities (4), and low plt (1). Thirty nine pts started bor/dex and 29 pts have completed the planned bor treatment. Twelve pts stopped bor/dex for low WBC (2), PN (4), dx of adrenal cancer (1), MI (1), and relapse (4). With a med F/U of 8.5 mo. (0.2 -24.0) 12 of 41 evaluable pts (29%) achieved CR post PSCT and 17 of 34 evaluable pts (50%) have achieved CR after bor/dex. Eleven of 34 (32%) pts have upgraded their response. Eight pts have relapsed of whom 3 died. Sixteen pts had PN gr I prior to start of bor, 8 pts developed new PN all gr I-II. *Conclusion:* Prolonged weekly bortezomib maintenance therapy is well tolerated and can upgrade response post single autologous PSCT (32%) with no severe peripheral neuropathy.

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EFFICACY OF RETREATMENT WITH IMMUNOMODULATORY (IMID) COMPOUNDS IN PATIENTS RECEIVING INITIAL THERAPY FOR NEWLY DIAGNOSED MULTIPLE MYELOMA

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Objective: To ascertain the degree of response that can be obtained with retreatment with IMiDs in patients (pts) with multiple myeloma (MM). *Pts and Methods:* Among 410 pts with newly diagnosed MM who received either thal-dex or len-dex, 183 pts received IMiDs (thal, len or pom) +/- dex as one of the salvage regimens following relapse, and form the study group. *Results:* The median (range) age at MM diagnosis was 60 (29-80) yrs; 117 (64%) were males. Thal and len were used as initial therapy in 106 (58%) and 77 (42%) pts, respectively. Pts received a median of 2 treatments (range, 1-6) prior to salvage therapy; bortezomib was used as one of the salvage treatment (prior to repeat IMiD) in 41 (22%) pts. The median time to repeat IMiD was 26 mos. SCT was performed in 118 (64%) pts after the initial IMiD therapy. Pts went off first line IMiD due to: SCT (106; 58%), drug toxicity (32; 18%), disease progression (22; 12%), patient's choice (13; 7%) and alternative treatment options (10; 5%). Thal, len or pom were used as salvage therapy in 40 (22%), 129 (70%) and 14 (8%) pts, respectively. The TTP after repeat IMiD is shown in Fig 1. Overall, 67 (37%) pts achieved ≥PR and 73 (40%) pts achieved <PR (stable and progressive disease) to repeat IMiD, and remaining 43 (23%) had non evaluable disease response. *Conclusions:* The lowest and highest response rates were seen with retreatment with thal and pom, respectively (Table 1).

Figure 1: Time to Progression (TTP) after repeat IMiD therapy

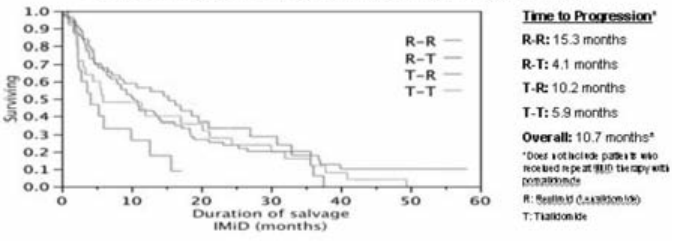

Table 1

	Secondary response to IMiD according to initial IMiD response					
Response to 1st line IMiD therapy	**L-P* N=7**	**L-L* N=55**	**L-T* N=15**	**T-P* N=10**	**T-L* N=71**	**T-T* N=25**
≥VGPR(%)†	4 PR (100)	5≥VGPR (38) 3 PR (23) 3 <PR (23)	1 PR (20) 3 <PR (60)	1≥VGPR(33) 1 PR (33) 1 <PR (33)	1≥VGPR(22) 1 PR (11) 3 <PR (33)	1 PR (25) 3 <PR (75)
PR (%)†	1 PR (33) 1 <PR (33)	4 ≥VGPR(11) 8 PR (23) 13 <PR (37)	1≥VGPR(25) 3 <PR (75)	3≥VGPR(75) 1 PR (25)	4≥VGPR(11) 7 PR (18) 19 <PR (50)	5 PR (38) 6 <PR (46)
< PR (%)†	0	2≥VGPR (29) 4 <PR (57)	1 PR(17) 3 <PR (50)	2 PR (67) 1 <PR (33)	1≥VGPR(4) 9 PR (36) 5 <PR (14)	5 <PR (63)
ORR(>PR)	83%	52%	25%	80%	46%	30%

T: thalidomide; L: lenalidomide; P: pomalidomide; ≥VGPR: very good partial response; PR: partial response; ORR: overall response rate

≥VGPR includes patients with complete response and VGPR

< PR includes patients with stable and progressive disease

*Primary – salvage IMiD combination

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DENIAL OF INTENSIVE THERAPY FOR ELIGIBLE PATIENTS WITH MULTIPLE MYELOMA OLDER THAN 65, SHORTENS SURVIVAL

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Between 2000 and 2010, 73 patients with newly diagnosed multiple myeloma (aged 66-80, median 70) received primary treatment with thalidomide-dexamethasone (TD) (n=36), bortezomib with TD (BTD) (n=20), or with revlimid instead of thalidomide (n=17). Results were compared with 202 contemporary patients (aged 65 or less, median 55) with similar disease who received the same programs. Intensive therapy <1 year (high-dose melphalan supported by ABSC) (HDT) was given to 76% of younger and 40% of older patients (p<.01), with 9 older patients avoiding HDT because of major medical or social factors. Clinical response was defined by standard criteria. Regardless of age, primary response rates were similar, while ultimate response rates were higher with HDT (96%) than without HDT (69%, p<.01), ultimate frequencies of CR being 3X higher after HDT for both age groups. Median survival from initial therapy for all older patients was shorter than for those younger (4.2 vs 5.8 yrs, p=.05), but similar for those who had received HDT (6.3 vs 5.8 yrs, p=.6) or had not received HDT (3.1 vs 4.4 yrs, p=.3). Denial of HDT mainly because of age was observed in 35 older patients for whom median survival after landmark of 1 year was significantly shorter than that of 29 similar patients who received HDT (4.1 vs 6.3 yrs, p=.01). The shorter survival of older patients by approximately 2 years was attributed mainly to the arbitrary denial of HDT for patients who qualified for such treatment by standard criteria.

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IGD MULTIPLE MYELOMA A DESCRIPTIVE REPORT OF 17 CASES:SURVIVAL AND RESPONSE TO THERAPY

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Background: Immunoglobulin D multiple myeloma (MM) is rare and has a poorer prognosis than other MM isotypes. Design and Methods. Seventeen patients (pts) diagnosed from 1993 to 2009 with IgD MM were selected from six institutions of Multiple Myeloma Latium-Region GIMEMA working group. *Results:* Median age was 55 yrs, 14 patients had bone lesions, 4 a serum creatinine > 2 mg/dl, 1 serum calcium > 12 mg/dl, 11 had lambda light chains, 5 stage III of ISS, 4 with chromosomal abnormalities. Six pts received conventional chemotherapy (CT): 5 melphalan + steroids based regimens. Eleven underwent autologous stem cell transplantation (5 single and 6 tandem ASCT): 6 received bortezomib and/or thalidomide as first-line chemotherapy and 5 VAD. Thalidomide maintenance was used in 2 pts: 1 in high dose therapy (HD) and 1 in CT group; bortezomib was used in 1 patient after HD. At a median follow up of 35 (range 19-48) and 24 months (range 7-148) for pts treated with CT and HD, respectively, the overall response rate was 83% and 90%, the median overall survival was 34 months (95% CI 18-50 months) and 57 months (95% CI 5-108 months) and the median Progression Free Survival was 42 (95% CI 28-55) and 56 months (95% CI 4-107). A relapse was observed in 27.3% of pts treated with HD and in 66.7% undergone CT. *Conclusions:* Despite the retrospective analysis and the small number of pts our study showed that the use of HD-therapy seems to improve also the prognosis of IgD MM patients. Treatment options including new drugs, before and after SCT, may further improve the outcomes of these patients.

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IMPROVED PROGRESSION FREE SURVIVAL AND OVERALL SURVIVAL WITH CYTOREDUCTIVE AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) PRIOR TO REDUCED INTENSITY CONDITIONING (RIC) ALLOGRAFT IN A PLANNED TANDEM FASHION (AUTO-ALLO) IN COMPARISON TO UPFRONT EARLY RIC ALLOGRAFT WITHOUT PREVIOUS ASCT (EARLY RIC) IN PATIENTS WITH MULTIPLE MYELOMA, ON BEHALF OF THE MULTIPLE MYELOMA SUBCOMMITTEE OF THE CHRONIC LEUKEMIA WORKING PARTY, EBMT

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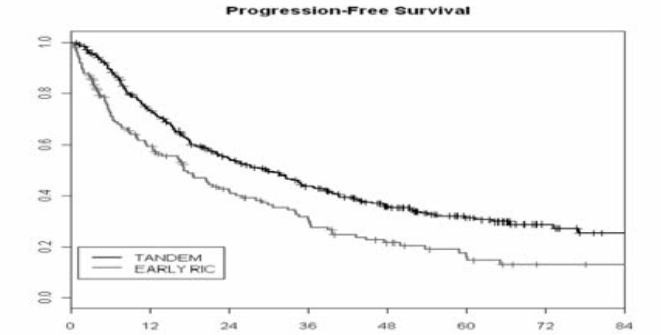
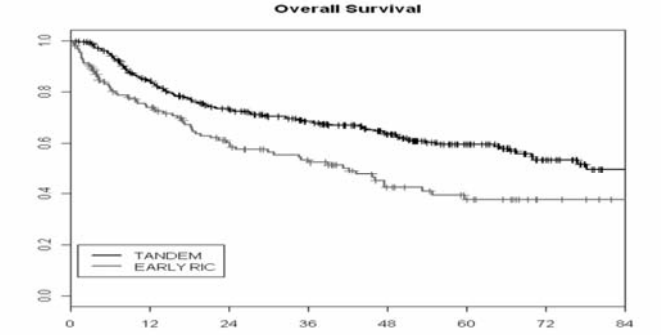
Reduced intensity conditioning (RIC) allografts after ASCT in tandem fashion (auto-allo) have reported long term disease control in multiple myeloma pts. It is not clear if early RIC allograft without preceding ASCT can produce the same outcome. The objective of this study was to compare the results of auto-allo to early RIC allograft to address if ASCT is needed and allograft candidates can undergo RIC allograft directly without ASCT morbidities. Study: We analyzed the EBMT database from 1998 to 2007 and identified 504 pts with 356 pts assigned to auto-allo and 148 pts to early RIC allograft, all within 1 yr. from dx. In the auto-allo group 253 had their planned allograft, 88 had no allograft and 15 had a second ASCT. No pts in RIC group had previous ASCT. There were no significant differences between the 2 groups except age (med 51 RIC vs. 53 auto-allo P 0.03), calendar yr. ('98-'02 51% RIC vs. 33% P <0.001), time from dx (9 mo. RIC vs. 6 mo. auto-allo P 0.0001) and CR (17% RIC vs. 9% auto-allo P 0.008). *Results:* On ITT basis best response occurred in the auto-allo group (62% vs. 47%) with significantly better 5 yr. PFS (31% vs 17% early RIC P<0.001) and 5 yr. overall survival (60% vs 37% early RIC P<0.001). NRM at 1 yr. was lower in the auto-allo group (9% vs. 18% p<0.001). Log rank test showed better outcome in the auto-allo group was independent of the calendar yr. *Conclusion:* This large retrospective study suggests cytoreductive ASCT prior to RIC allograft is associated with better outcome in multiple myeloma patients who are RIC allograft candidates.





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RESULTS OF PRE- AND POST-AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) WITH THREE INDUCTION REGIMENS IN MULTIPLE MYELOMA (MM): SUPERIORITY OF VTD (BORTEZOMIB/THALIDOMIDE/DEXAMETHASONE) OVER TD AND VBMCP/VBAD PLUS BORTEZOMIB (VBMCP/VBAD/V).

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Introduction: A randomized phase III trial comparing TD vs. VTD vs. VBMCP/VBAD/V in patients <66 years with de novo MM followed by ASCT with MEL-200 was performed. Primary end points: response rate after induction and after ASCT. *Patients and Methods:* 386 patients entered the study (VTD: 130, TD: 127, VBMCP/VBAD/V: 129). 21% had high-risk cytogenetics. *Results:* The CR rate was significantly higher with VTD (35%) compared to TD (14%) and VBMCP/VBAD/V (22%) (p=0.0001 and p=0.01, respectively). In patiens with high-risk cytogenetics, the CR rate was significantly greater with VTD when compared with TD (35% vs. 0%, p=0.002) and with VBMCP/VBAD/V (35% vs. 22%, p=0.02). On an intention to treat basis, the post-ASCT CR rate was higher with VTD as compared to TD (46% vs. 24%, p=0.004) and VBMCP/VBAD/V (46% vs. 38%, p=0.1). The estimated overall survival (OS) at 4 years was 76% with no significant differences among the 3 arms. After a median follow-up of 27 months, the progression-free survival (PFS) was not reached with VTD versus 27 and 38 months with TD and VBMCP/VBAD/V, respectively (p=0.006). Patients with high-risk cytogenetics had a significantly shorter OS and PFS with all treatment arms. *Conclusions:* Induction with VTD resulted in a significantly higher CR rate in both the overall series and in patients with high-risk cytogenetics. The post-ASCT CR rate was higher with VTD than with TD and with VBMCP/VBAD/V. In the overall series VTD resulted in a significantly longer PFS. Finally, patients with high-risk cytogenetics had shorter PFS and OS with the three induction arms.

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PAD IS EFFECTIVE IN MYELOMA PATIENTS WITH POOR RESPONSE TO VAD AT DIAGNOSIS

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Introduction: The combination of bortezomib with adriamycin/dexamethasone (PAD) is a highly effective induction agent with response rates of up to 95% (Oakervee, et al, BJH 2005; 129:755-62). We have previously shown the superior efficacy of PAD chemotherapy over VAD or VAD-like regimens (VAMP, C-VAMP, Z-DEX etc.) by comparing response to PAD given following relapse to the response previously obtained with VAD or VAD-like regimen in the same patients. *Materials and Methods:* This was a phase 2 study with 3 cohorts, of 23 patients. Cohort 3 was for patients refractory to VAD and who proceeded directly to PAD chemotherapy. *Results:* 23 patients (6 females, 17 males, median age 62 years) achieved less than PR to a median number of 4 (range 2-6) courses of VAD or VAD-like therapy (C-VAD 2, VAD 15, Z-DEX 6). Following PAD therapy 2 patients achieved CR and a further 2 patients achieved VGPR based on their PAD protein level at the time of starting PAD therapy. A further 12 patients achieved PR, When compared to the responses achieved by VAD therapy, the exact McNemar significance probability was p=0.0005. The fall in median paraprotein levels post-VAD was 23.6% and after PAD was 56.5% (using pre-PAD protein level for comparison) (p=0.0002 using the Wilcoxon signed rank test). *Conclusion:* Using a novel trial design, requiring only small numbers of patients we have shown that PAD is superior to VAD using two independent statistical methods.

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EFFICACY OF BENDAMUSTINE IN RELAPSED/REFRACTORY MYELOMA PATIENTS: RESULTS FROM THE FRENCH COMPASSIONATE USE PROGRAM

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Bendamustine (Ben) is a cytotoxic with structural similarities and no cross-resistance to alkylating agents and antimetabolites. It's active against myeloma cell lines and in both de novo and relapsed/refractory (R/R) patients (pts) with MM. The French compassionate-use program (CUP) started in 2007. To receive Ben, pts with MM had to have R/R after Bortezomib or Lenalidomide therapy.The recommended dosage was 120-150 mg/m^{2}d1-2 in combination with Prednisone every 28d. We here report the results of Ben therapy in 110 pts with R/R MM enrolled in this CUP.Pts were67male and43female, with a median age of 63y(34-83), ISS stage I36%, stageII40%, stageIII24% of the cases. Pts received amedian number of4lines of priortherapy. 60%of them received priorASCT.All pts received priortreatment with alkylating agents, Bor and corticosteroids, while 85% and 52% of them were previously treated with Len and thalidomide, respectively. Themedian number of Ben cycles infused was 3(1–13).The initial dosage varied from 60 to150 mg/m^2,d1-2 every4w, according to physician's choice. A total 419 cycles of Ben were delivered. Hematological toxicities were the most adverse-side effects. The ORR (evaluated according to EBMT criteria was 30% (33 patients, 2 complete and 31 partial responses. 20% of patients experienced stable disease, and 50% of the pts progressed on Ben. With a median FU of 1 y, the median TTP of the entire population was 167d (0-511). In this population of R/R MM patients, both RR and DOR are encouraging. Other series of Ben used in combination with other drugs in less heavily pretreated or denovo MM pts are warranted.

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HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA WITHOUT GROWTH FACTORS: EXPERIENCE FROM A DEVELOPING COUNTRY (ORAN, ALGERIA)

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EHU 1ER NOVEMBRE, ORAN, ALGERIA

The need for growth factors after autologous stem cell transplant (ASCT) has been investigated recently. Data from developing countries are scanty. We analyzed and updated our experience on 28 consecutive patients with multiple myeloma (MM) treated with ASCT without receiving growth factors after transplant.

Among the 28 patients 11 was females and 17 was males. The medi-

an age was 55 years (range, 37–64 years). Before transplant, patients received chemotherapy using VAD (vincristine, adriamycin and dexamethasone, n=10) or bortezomib-dexamethasone (n=17). The median number of CD34+ cells was 3,86x106/kg (range, 1,05 -8,62). High-dose melphalan (200 mg/m^2) was used for conditioning and followed after 24 hours by reinfusion of autologous non-frozen hematopoietic stem cells, which had been stored for 24 hours at 4°C. All patients received prophylactic ciprofloxacin,fluconazole and acyclovir.

Median time to achieve neutrophils ≥ 500/µl was 12 days [range 10-17] and median time to achieve an unsupported platelet count≥20 000/µl was 13 days [range, 11 - 28].

After ASCT , 87% of patients responded. Grade II–III mucositis was the major regimen related toxicity. The median follow-up was 07 months (range,1-20 months). Estimated overall survival and EFS at 20 months were 96.5%±0.05% (s.e.) and 93%±0.05% (s.e.), respectively.

We conclude that it is feasible and reasonable to perform ASCT for MM without administering growth factors and the procedure is easy to perform without requiring costly growth factors.

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BORTEZOMIB PLUS DEXAMETHASONE (VD) FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) PRODUCES MOLECULAR REMISSIONS (MOLR) IN 23 % OF PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM)

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Background: The depth of complete remission (CR) may be critical for the long-term outcome of patients with MM. Thus, MolR is proposed as the new goal of treatment. The aim of this study was to explore the response rates after VD induction followed by ASCT, including minimal residual disease (MRD) assessment in patients with at least near-CR response. *Patients and Methods:* Until Dec 2010, 35 symptomatic MM patients with a median age of 61 years with informed consent have been included. Study protocol consists of induction with four cycles of VD followed by ASCT. MRD was assessed by allele specific quantitative PCR using pretreatment sample with proportion of myeloma cells determined by flow cytometry as the reference. *Results:* After induction 9 patients (26%) were in nCR/CR; 2 of them were PCR negative (sensitivity 0.003%-0.01%) and 4 PCR positive (range 0.002%-0.2%). 16 patients (46%) were in VGPR/PR, and 7 (20%) had less than PR. Three patients are not yet evaluable. Two more patients were PCR-negative (sensitivity <0.001%) after mobilisation. 18 patients have underwent ASCT. Three months after ASCT,12 patients (34%) are in nCR/CR, 4 in VGPR/PR, 10 not yet evaluable, 9 out of study. Of the 12 patients in nCR/CR, 6 are PCR-negative (sensitivity 0.001%-0.007%) and 6 PCR-positive (range 0.003%-0.09%). Two more patients have achieved PCR-negativity later. *Conclusion:* Even with the relatively short median follow-up time PCR-negativity has already been achieved in 23% of the patients. Further studies will be necessary to determine the long-term outcome associated with MolR.

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MAINTENANCE THERAPY OF BORTEZOMIB-DEXA(BZDX) FOR MULTIPLE MYELOMA

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Object: This is a pilot study for the prospective trial of BD-Maintenance therapy for relapsed or refractory MM patients. The purpose is to explore whether or not this maintenance therapy can lead a long-term survival with good QOL. Material and Method: From Sep/2008, 35 MM patients who achieved better than partial response by previous 2 or 3 regimens of chemotherapy or ASCT followed by 1-4 courses BD therapy were enrolled for the BD-maintenance therapy with informed consents. Age 45-86 y.o., IgG-type; 21, IgA-type; 8, BJP-type; 5 and IgD-type; 1.Cytogenetic abnormalities were 11 cases (31.4%) with 7 cases of complex abnormalities. Patients received dexamethazone (20mg/body) daily for two days every two or four weeks with bortezomib, 1.3mg/㎡. TTP was the primary efficacy endpoint. *Results:* IgG-type; 6/21, IgA-type; 4/8, BJP-type; 1/5 and IgD-type; 1/1 were diagnosed as refractory with Thalidomide, second ASCT and CHOP-like regimens. The adverse events of BzDx Maintenance, which are asthenic conditions, peripheral neuropathy, thrombocytopenia, were all Grade-1 and well tolerated. Discussion: A long-term survival with good QOL is the most important goal for the elderly/low genetic risk MM patients. The BD-Maintenance therapy over one year were effective for this group (27/35 cases). However, the other group of patients in rapidly relapsing with complex cytogenetic abnormalities (7/35 cases) may need more intensive combination chemotherapy following several months of the BD-Maintenance therapy.

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T-CELL DEPLETED ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH BUSULFAN, MELPHALAN, AND FLUDARABINE CONDITIONING FOLLOWED BY POST TRANSPLANTATION DONOR LYMPHOCYTE INFUSIONS FOR PATIENTS WITH RELAPSED MULTIPLE MYELOMA AND HIGH-RISK CYTOGENETICS

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We report results of a pilot study of 13 pts, using T-cell depleted allo HSCT (TCD HSCT) from HLA compatible donors for pts with relapsed myeloma within 12 mos following auto HSCT who also had high-risk cytogenetics [t(4;14), t(14;16), del17p by FISH and/or del13q by karyotyping]. Pts underwent TCD HSCT with busulfan (0.8mg/kg x 10 doses), melphalan (70mg/m^2 x 2 days), fludarabine (25mg/m^2 x 5 days) and rabbit ATG (2.5mg/kg x 2 days). T-cell depletion was performed by positive CD34 selection (Isolex) followed by rosetting with sheep erythrocytes, achieving < 10e3CD3+/kg for all grafts. Pts were eligible to receive low doses of donor lymphocyte infusions (DLI) (5x10e5 – 1x10e6 CD3+/kg) no earlier than 5 mos post TCD HSCT. Four pts are in continuous complete remission (CR) at 31, 25, 15 and 13 mos following allo TCD HSCT. Two pts with stable minimal residual disease for 26 and 24 mos before progression were treated with DLI. Three pts with refractory myeloma achieved CR for 12, 11 and 8 mos post allo TCD HSCT, developed progression of disease and received DLI. 12/13 pts were without signs of GvHD, one pt had possible superimposed gut GvHD following fulminant C diff colitis. Four pts died, one at 2 mos with oseltamivir-resistant H1N1 infection; one at 4 mos due to respiratory failure secondary to infection of unknown etiology, one at 5 mos due to status epilepticus, and one at 6 mos due to acute cerebral hemorrhage. A phase II clinical trial with TCD HSCT and DLI for patients with relapsed multiple myeloma and high-risk cytogenetics is now being performed at MSKCC.

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A FIRST-IN-MAN, PHASE I, MULTICENTER, OPEN-LABEL, DOSE ESCALATION STUDY OF KW-2478, A NOVEL HSP90 INHIBITOR, IN PATIENTS WITH B-CELL MALIGNANCIES

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Introduction: KW-2478 is a novel non-ansamycin, non-purine inhibitor of Hsp90. The effects of Hsp90 inhibitors on pathways needed for cell survival make them potential candidates for treatment of B-cell malignancies, eg, multiple myeloma (MM) and B-cell non-Hodgkin's lymphoma (NHL). This study investigated KW-2478 in patients (>18y) with

relapsed/refractory MM or NHL to determine its safety, tolerability, and PK/PD profiles. *Methods:* Patients received escalating doses of IV KW-2478 (14-176 mg/m^2/day), over 60 min on Days 1-5 of a 14-day cycle. Safety, including visual ophthalmological examination and electroretinogram, was assessed. Blood samples were collected for PK and PD (Hsp70 induction) on Days 1 & 5 (cycle 1). *Results:* The study enrolled 27 patients (22 MM, 5 NHL), median age: 63y. No DLTs or retinal toxicities were observed. Mean duration of therapy was 6.3 cycles. Overall, 21 (77.8%) patients experienced KW-2478 related toxicities; 10 (37%) patients experienced ≥grade 3 toxicities. KW-2478 related toxicities included grade 4 thrombocytopenia (1), grade 2 hypertension (1), and 2 episodes of grade 3 QTc interval prolongation (1). Plasma concentrations peaked at the end of the infusion, then decayed in a biphasic manner with a half-life ~6 hours. Cmax and AUC0 ∞ values increased in a dose-dependent manner. Hsp70 induction occurred at doses ≥71 mg/m^2. *Conclusions:* KW-2478 was well tolerated with no DLTs or retinal toxicity at doses up to 176 mg/m^2. A predictable PK profile was demonstrated. No CR or PR was observed but 8 patients had SD for ≥3 months.

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REAL-LIFE USE AND RESPONSE RATE OF BORTEZOMIB IN MULTIPLE MYELOMA: A UK RETROSPECTIVE OBSERVATIONAL STUDY

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In October 2007 NICE, the UK Special Health Authority, recommended bortezomib (BTZ) monotherapy for patients with multiple myeloma at 1st relapse, under the conditions of the Velcade Response Scheme (VRS) where the full cost of BTZ is rebated if patients achieve <PR after 4 cycles. The objectives were to assess BTZ treatment patterns, response rates achieved and the level of implementation of NICE guidance. A retrospective observational chart review was carried out in 9 UK centres, in the last 20 consecutive patients who had the opportunity to complete ≥4 cycles of BTZ. Two thirds of patients were younger than 70 years old. Most patients received BTZ in combination (94%, 134/143) with dexamethasone (Dex) (65%, n=87) or with cyclophosphamide and Dex (28%, n=40). The median number of cycles given was 5 (range 2-10). Response to BTZ was assessed in 155 patients out of 160. Based on serum M-protein, response at cycle 4 was: CR=10%, VGPR=9%, PR=57%, MR=10%, SD=9%, PD=5%. Based on serum free light chain assay, 48% of patients (14/29) reached ≥PR increasing to 97% (28/29) based on clinicians' assessment. The higher PR rate determined by clinicians was primarily explained by other clinical parameters being taken into account such as bone marrow, serum creatinine. A claim was made in most of the cases where a PR had not been achieved. No submitted claim was rejected. In clinical practice the use of BTZ resulted in most patients achieving ≥PR leading to few VRS claims being made.

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KINETICS OF NEUTROPHIL AND PLATELET RECOVERY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION PREDICTS OVERALL SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Autologous stem cell transplantation (ASCT) is the gold standard as first-line treatment in young patients with multiple myeloma (MM). Prognostic factors have been usually related to patient characteristic and disease stage. Few investigations on the role of platelet and neutrophil recovery after ASCT have been addressed. The aim of this study was to investigate the prognostic influence of the kinetics of peripheral blood recovery on progression-free survival (PFS) and overall survival (OS) after ASCT. *Methods:* One hundred and ninety one patients (109M/82F; median age 55 years) underwent melphalan-based ASCT in our institution. The median follow-up after ASCT was 4 years. Peripheral blood recovery was assessed as the day when the neutrophils reached 500 (N500) and 1000x109/L (N1000) and platelet count 20,000 (P20) and 50,000x109/L (P50) after CD34+ infusion. Patients were classified in two groups according their recovery above or below the median. *Results:* N1000 (Fig. 1), P20 and P50 predicted for a longer OS ($p<0.05$). No significant association with the number of infused CD34 cells was observed. PFS was also correlated with N1000 (Fig.2) ($p=0.001$) and there was a trend for N500 ($p=0.053$), with no impact of P20 and P50. A stratification model showed 3 prognostic stages according to the achievement of complete remission and early N1000 after ASCT. *Conclusion:* Early neutrophil recovery was associated with significantly longer PFS and OS, while platelet recovery was only associated with OS.

Figure 1. Overall survival according to median 1,000x10^9/L neutrophils recovery

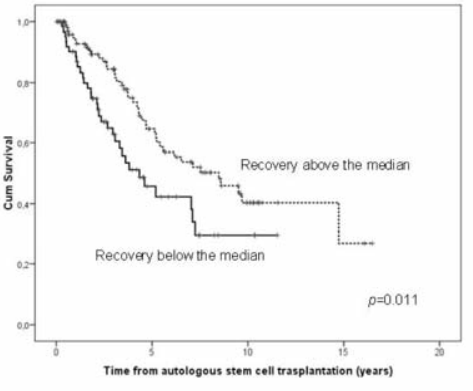
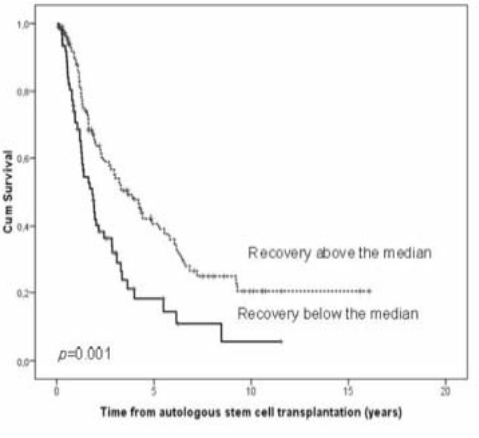


Figure 2. Progression-free survival according to 1,000x10^9/L neutrophils recovery



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PHASE 2 MULTICENTER, RANDOMISED, OPEN LABEL STUDY OF 2 MODALITIES OF POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE (POMD) IN PATIENTS WITH MULTIPLE MYELOMA (MM), REFRACTORY TO BOTH LENALIDOMIDE AND BORTEZOMIB (DOUBLE REFRACTORY). IFM 2009-02

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Background: MM patients double refractory (progression on or within 2 months of end of treatment) or intolerant to bortezomib and lenalidomide have less than 8 month's life expectancy (Kumar. ASCO 2010). Pomalidomide was developed as either 4 mg on 21 days of each 28-days-based regimen (Richardson. ASH 2010) or continuously at 2 mg 28 days of each 28-days, with some increased to 4mg (Lacy. ASH 2010). The addition of dexamethasone improved response rates. IFM2009-02 aimed to determine efficacy and toxicity of 2 modalities of Pomd in double refractory patients. Method: 84 progressive and double refractory MM were included. The primary objective was response rate (≥ PR) to Pomd using IMWG response criteria. The response and FISH cytogenetic analysis were assessed centrally. Pomalidomide was given orally at either 4 mg daily on days 1–21 of each 28-days or continuously on days 1–28 of each 28-days. Weekly dexamethasone was given orally at 40 mg. All patients received prophylaxis against venous thromboembolism. *Results:* There was 43 patients in arm 21/28 and 41 in arm 28/28. The Table summarized the response rates, survival and toxicity profiles. Toxicity was primarily myelosuppression and similar in both arms; no occurrence of neuropathy and no thromboembolic events have occurred.*Conclusion:* Pomalidomide and dexamethasone is active and well tolerated in heavily pre-treated double refractory MM patients.

Arms	21/28	28/28
Prior line of therapy, N (range)	4 (1-8)	4 (1-8)
T(4;14), %	0	7
Del17p, %	12	10
Follow up, months	7	7
N cycle in 2009-02, median	5	5
ORR (PR and better), %	42	39
≥VGPR	7	5
Stable disease, %	20 (46.5)	21 (51)
Overall population		
Time to Progression (TTP), months	7	9.7
TTP in del17p and t(4;14), months	9.7	7
Survival at 6 months, %	88	85
≥ Grade 3 adverse events		
Hematological, %	23.5	26.5
Non hematological, %	12	9

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A NOVEL STATISTICAL METHODOLOGY DEMONSTRATES THAT PAD IS SUPERIOR TO VAD-TYPE CHEMOTHERAPY

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Introduction: Bortezomib with adriamycin and dexamethasone (PAD) is a highly effective induction combination with response rates of up to 95% (Oakervee et al, BJH 2005; 129:755-62). In this study patients who had received VAD or a VAD-like regimen (VAMP, C-VAMP, Z-DEX) acted as their own controls and were given further treatment using the PAD regimen and compared using appropriate statistical methods. *Methods:* This was a Phase 2 study with 3 cohorts of 23 patients. Cohort 1 was patients treated with VAD and auto transplanted, cohort 2 similar patients but not transplanted and cohort 3 was patients refractory to VAD who proceeded directly to PAD without any intervening chemotherapy. The paraprotein level at the start of each type of treatment (VAD or PAD) was used for estimation of response. *Results:* Using EBMT criteria with addition of VGPR, 7 patients in cohort 1 achieved CR after PAD compared to one patient achieving CR after VAD. Using the exact McNemar significant probability comparison of all responses gave p=0.0078 and using the Wilcoxon Signed Rank Test to compare reductions in paraprotein (or Bence-Jones protein) p=0.093 was achieved. A combined analysis of group 1 and 2 together gave similar results. In cohort 3 similar comparisons gave an exact McNemar significance of p=0.0005 and the Wilcoxon Signed Rank test p=0.002 in favour of the PAD therapy. Data on overall survival and toxicities will be presented. *Conclusion:* PAD was demonstrated to be significantly superior to VAD particularly in the refractory group. This type of study is an alternative to large phase 3 studies.

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BORTEZOMIB (VELCADE)-THALIDOMIDE-DEXAMETHASONE (VTD) IS SUPERIOR TO THALIDOMIDE-DEXAMETHASONE (TD) IN PATIENTS WITH MULTIPLE MYELOMA (MM) PROGRESSING OR RELAPSING AFTER AUTOLOGOUS TRANSPLANTATION

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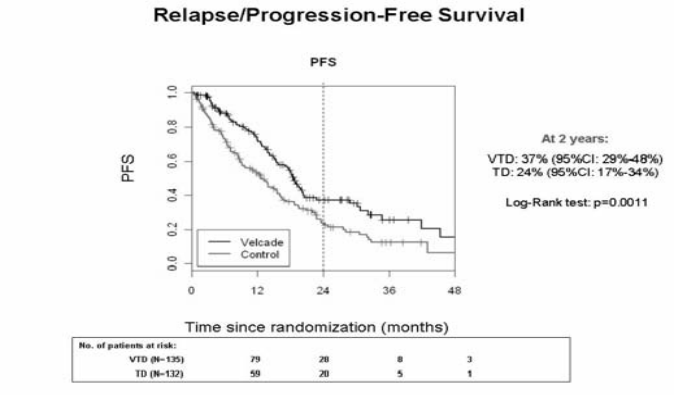
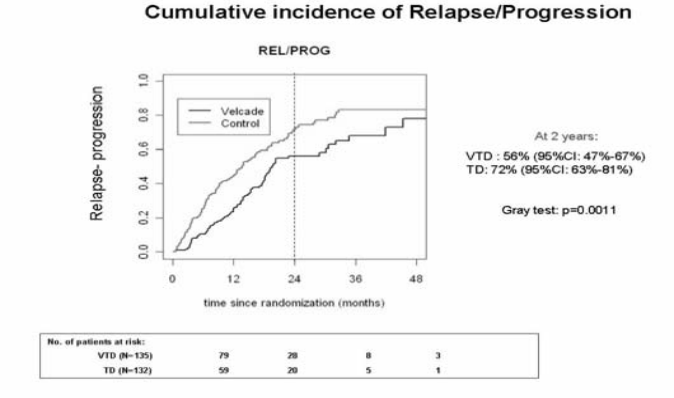
An IFM and EBMT prospective, randomized, open label phase III, multicenter study, comparing VTD (arm A) with TD (arm B) for MM patients in first progression/relapse after at least one autologous transplantation. Primary end point:TTP. Bortezomib 1.3 mg/m2 on days 1, 4, 8 and 11, followed by a 10-day rest period (8 cycles) and then on days 1, 8, 15 and 22, followed by a 20-day rest period (4 cycles). In both arms:thalidomide:200mg/day for 1 year, dexamethasone:40 mg/day for 4 days every 3 weeks for 1 year. 267 patients (135 in arm A, 132 in arm B) and 157 events. Median age:61 years (range 29-76), ISS: I in 56 %, II in 27 %, III in 17 %, previous autologous transplants:one in 71 vs 69 patients and two or more in 64 vs 63 patients(A and B). Median follow-up:27 mo, median TTP:19.5 vs 13.8 mo (A and B), with a CI of relapse/progression at 2 years of 56% vs 71% (p=0.0011). Median PFS:18.6 vs 12.7 mo with a CI at 2 years of 37% vs 23% (A vs B, p=0.0011). First two years OS:72% vs 68% (p=0.18). First year CR and CR+PR:32% vs 12% and 90% vs 69% with VTD and TD (p=0.0001,and p=0.0001). Mean number of treatment cycles for the first 8 cycles:6.25 vs 6.88 and for the 12 cycles, 7.56 vs 9.93 (VTD and TD). Treatment discontinuation due to toxicity: 48 patients (VTD= 36, TD=12). 33 death during treatment period (VTD= 14, TD= 19). Thrombo-embolism; 6.6% vs 5.2%, p=ns while thrombocytopenia:16% vs 7%, p= 0.025 (events >= grade 3,VTD vs TD). VTD resulted in significantly longer TTP and PFS in patients relapsing after ASCT with an acceptable toxicity. Protocol EUDRACT number: 2005-001628-35.





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ISS IS ASSOCIATED WITH RESPONSE ONLY AMONG PATIENTS RECEIVING MELPHALAN-PREDNISOLONE AND WITH SURVIVAL REGARDLESS OF THE TREATMENT TYPE :THE UPDATED RESULTS OF THE TURKISH MYELOMA STUDY 95-001.

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Following the publication of the initial results (Eur J Hemat 2011) the impact of prognostic factors will be presented. Patients: 115 who were randomized to MP(n: 57) or MPT(n:58), received. MP+/- Thalidomide: 100 mg/d continuously for a maximum of 12 months. Statistics: "SPSS 15.0 was used for data analysis: comparisons were assessed by chi-square test and Fisher's exact test for the categorical variables. OS and PFS rates were compared between treatment arms with Kaplan-Maier test. Age, ISS, creatinine, light chain disease, LDH, Hb, albumin, treatment arm and response at month 12 were included in the model for multivariate - Cox regression analysis. *Results:* Response> VGPR was observed more frequently in the MPT arm: response at mo 12 compared to mo 6 in the MPT arm: 39.2 % vs 21.6 % (p= 0.012) vs the MP arm : 25% vs 14.3% (p= 0.125). Addition of Thalidomide increased PFS at 2 yr 49.7 vs 30.5 % (p=0.045) significantly, but not OS (64 vs 58.9 % , p=0.186). Predictors of response at mo 12 were: treatment arm (p=0.08, OR: 2.511),ISS (p=0.013, OR: 0.306) and serum albumin (p=0.018, OR: 1.101)(Table.1). Advanced ISS was associated with lack of CR (12 month) in the MP arm. Addition of Thalidomide improved response (CR/VGPR) moderately among patients with advanced stages(Table.2). The increment in response of ISS-III MPT patients lead to a borderline PFS and OS benefit. *Conclusion:* This study validates the value of ISS as a predictor of survival in both groups. In addition, we were able to demonstrate an association between ISS and response in the MP arm.

Table 1: Results of multivariate analysis (two models)

	P	Hr	95%	
Age > 75	0,033	2,131	1,061	4,280
Creatine > 2 mg/dL	0,004	4,047	1,563	10,478
ISS	0,040	1,952	1,031	3,696
Hb	0,039	0,769	0,600	0,986
Albumin	0,013	0,928	0,875	0,984
Traetment (MPT)	0,072	0,499	0,234	1, 065
Ig light chain	0,090	0,545	0,270	1,098
Age > 75	0,018	2,399	1,165	4,940
Creatine > 2 mg/dL	0,002	4,295	1,687	10,934
ISS	0,001	2,535	1,428	4,501
Ig light chain	0,019	2,427	1,153	5,108
LDH	0,009	1,003	1,001	1,005
Response at mo12 (≥ VGPR)	0,000	0,030	0,006	0,141

Table 2: Impact of ISS on response, progression or death rates in both treatment arms

		ISS I	ISS II	ISS III	p
CR	MP	28,6%	23,5%	0%	0,021
	MPT	30,0%	12,5%	14,3%	N.S.
	p	N.S.	N.S.	N.S.	
> VGPR	MP	42,9%	35,3%	9,5%	0,036
	MPT	30,0%	56,3%	33,3%	N.S.
	p	N.S.	N.S.	N.S.	
> PR	MP	42,9%	64,7%	23,8%	N.S.
	MPT	70,0%	75,0%	42,9%	N.S.
	p	N.S.	N.S.	N.S.	
Progression	MP	40,0%	52,4%	47,6%	N.S.
	MPT	40,0%	26,3%	36,4%	N.S.
	P	N.S.	N.S.	N.S.	
death	MP	20,0%	33,3%	71,4%	0,003
	MPT	20,0%	26,3%	54,5%	0,036
	P	N.S.	N.S.	N.S.	

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OUTCOMES IN PATIENTS WITH MULTIPLE MYELOMA FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION USING INTRAVENOUS BUSULFAN AND MELPHALAN: A MATCHED COMPARISON TO A DOUBLE AUTOLOGOUS TRANSPLANT STRATEGY

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In this case-matched study, we analyze the efficacy of intravenous busulfan (ivBu: 3.2 mg/kg in a single daily dose, days -5 to -3) and melphalan (Mel: 140 mg/m^2, day -2) as conditioning regimen before autologous stem cell transplantation (ASCT) in 51 patients with newly diagnosed MM. Their clinical outcome was compared with that of a control group of 102 pair mates included in the Spanish PETHEMA/GEM2000 study of tandem transplant for patients not achieving at least nearCR (nCR) after the first ASCT (control group (CG)). Patients were transplanted between 2002-2005 and between 2005-2009 in the CG and ivBuMel group, respectively. Controls were matched with respect to sex, age, Durie-Salmon and ISS stage at diagnosis, and disease status at transplant. In CG standard melphalan 200 mg/m^2(Mel200) was the conditioning regimen administered in the 1st ASCT and Mel200 or a combination of cyclophosphamide, carmustine, and etoposide was administered to those patients undergoing a second ASCT. Transplant-related mortality was 4% and 5% in the ivBuMel and CG, respectively. CR/nCR rate was 51 % in both groups. At 5 years, PFS was 42±8% in the ivBuMel and 24±4% in the CG (P = 0.1). For patients achieving CR/nCR after ASCT, the 5-years PFS rate was 49±11% and 28±6% in ivBuMel and CG, respectively (P = 0.3). Results of this case-matched study show that the use of ivBuMel conditioning regimen before ASCT in patients with MM is associated with low transplant-related morbidity and mortality and a high anti myeloma efficacy that compares favorably with a tandem transplant strategy.

P-153

PREVIOUS THALIDOMIDE THERAPY MAY NOT AFFECT LENALIDOMIDE RESPONSE AND OUTCOME IN RELAPSE OR REFRACTORY MULTIPLE MYELOMA PATIENTS.

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Introduction: Lenalidomide is a thalidomide analogue, designed to have improved efficacy and tolerability over the parent drug. The aim of this retrospective analysis is to evaluate the impact of thalidomide therapy on lenalidomide response and outcome in relapse or refractory multiple myeloma patients. *Patients and Methods:* A total of 106 relapsed or refractory multiple myeloma patients received lenalidomide 25 mg plus dexamethasone as salvage therapy; 80 patients progressed on thalidomide treatment (thalidomide-resistant, TR) and 26 patients discontinued thalidomide in at least partial remission (thalidomide-sensitive, TS). Median time from diagnosis to thalidomide treatment was 56 versus 55,5 months in the TR and TS groups, respectively (P = .40) with a median duration of therapy of 12 and 10 months (P= .15). Overall, 58.7% and 65.4% (P = .55) of TR and TS patients underwent autologous bone marrow transplantation and 69.2% and 72.5% (P = .8) received bortezomib-based regimens before lenalidomide therapy. *Results:* Overall response rate was similar among TR and TS patients (56.2% vs 61.5%, P= .63) including at least VGPR rates of 16.2% and 11.5%; similarly, there was no difference in PFS (median 10 vs 12 months, P= .12) and OS (median 17 vs 18.5 months, P= .50) among the TR and TS groups, respectively. *Conclusion:* Lenalidomide may be equally effective in heavily pre-treated multiple myeloma patients who are thalidomide-resistant or -sensitive to a previous therapy. Lenalidomide may overcome thalidomide resistance in previously thalidomide-exposed MM patients.

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EARLY VERSUS LATE AUTOLOGOUS STEM CELL TRANSPLANT IN PATIENTS RECEIVING NOVEL THERAPIES FOR MULTIPLE MYELOMA

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Introduction: Autologous stem cell transplant (ASCT) with high-dose therapy (HDT) is an effective treatment modality for newly diagnosed multiple myeloma (MM). However, with the improved responses seen using the novel agents (thalidomide, bortezomib, or lenalidomide) for initial treatment, the benefit of ASCT in the upfront setting has not been established. *Methods:* We compared the outcomes of 167 MM patients who received initial novel agent and underwent ASCT within 12 months of diagnosis (early gp, N=102) to those who received ASCT at a later date (late gp, N=65). All patients received melphalan preparative regimen. *Results:* Baseline characteristics were not statistically different between the two groups. The overall response rate was similar in both groups (p=.056), but with a statistically significant proportion of patients in the early gp obtaining CR (50% vs. 28%, p=0.0066). For the early vs. late gps respectively, the median PFS was 28 mos vs. 18 mos with 1, 3, and 5 year PFS of 80, 32, and 25% vs. 66, 28, and 23% (p=0.11, Fig.1), while the median OS from time of diagnosis was not reached vs. 75 mos, (p=0.45, Fig. 2) with 1, 3, and 5 year OS of 96, 90, and 63% vs. 100, 82, and 63%. Univariate and multivariate analysis for PFS and OS are listed in Tables 1 and 2. *Conclusions:* There was no difference in PFS or OS between the early and late transplant groups in patients receiving novel agents. Patients going to ASCT with less than VGPR at > 12 months from diagnosis had the worst outcomes. Randomized trials are needed to verify these results.

Fig. 1 PFS

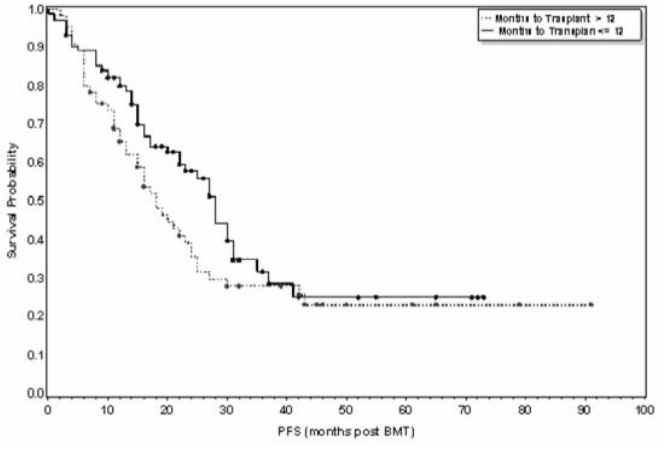

Fig. 2 OS

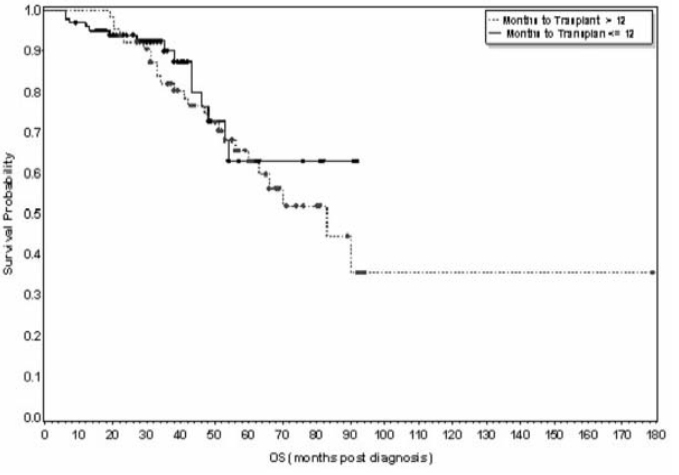

Table 1: Univariate Analysis for Progression Free Survival (PFS) and Overall Survival (OS)

PFS variable	HR	p-value
Number of prev therapy >1 vs 1	1.60	0.02
Pre-transplant response Others vs. CR+VGPR	1.67	0.018
Durie Salmon Stage 3 vs.1 or 2	1.80	0.033
Complex karyotype No vs. yes	0.63	0.042
Del 13 karyotype No. vs. yes	0.56	0.035
Del 13 FISH No vs. yes	0.58	0.0092
High risk FISH/karyotype No vs. yes	0.65	0.041
IPS Stage 1 vs. 2/3	1 vs. 2: 0.89; 1vs. 3: 0.49; 2 vs. 3: 0.56.	0.038
OS Variable		
Del 13 karyotype No vs. yes	0.44	0.045
Del 13 FISH No vs. yes	0.49	0.027
HD No vs. yes	0.36	0.018

Table 2: Multivariate Analysis for PFS

	HR	95% CI		p-value
Number of prev therapy >=2 vs. 1	2.00	1.18	3.40	0.010
Del 13 FISH No vs. Yes	0.57	0.33	0.99	0.047
IPS Stage				
1 vs. 3	0.54	0.27	1.08	0.045
2 vs. 3	0.49	0.27	0.89	
1 vs. 2	1.10	0.55	2.22	

P-155

OUTCOME OF HIGH DOSE MELPHALAN WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANT IN MYELOMA ASSOCIATED WITH AL AMYLOIDOSIS

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Background: The impact of concurrent AL amyloidosis on the prognosis of myeloma is not well known. High-dose melphalan followed by autologous hematopoietic stem cell transplant (auto HCT) has shown significant activity in both MM and AL amyloidosis. *Methods:* We identified 41 patients with concurrent MM and AL amyloidosis. Patient characteristics are summarized in Table 1. *Results:* Median age at auto HSCT was 56 yrs (39-77), median follow up of 58.7 mos from the time of diagnosis. Median time from diagnosis to auto HCT was 8.9 mos (2.7-102.4 mos). Post transplant hematologic responses (HR) were:>CR=10(24%), >VGPR=16(39%), >PR=33(80.5%), >stable disease= 40(97.6%). Of the 15 evaluable patients, organ responses (OR) were: PR=5 (33.3%), ≥SD=7(46.7%). No correlation was seen between OR and HR. The 100-day TRM was 0 and 1-year TRM was 2.4%. Median PFS and OS from diagnosis were 49.8 and 96 mos, respectively. In multivariate analysis, creatinine ≥ 2mg/dl (p=0.043) and hemoglobin <10g/dl (p=0.093) was associated with a shorter PFS. *Conclusion:* In this analysis the outcome of patients with concurrent MM and AL amyloidosis was comparable to patients with MM alone.

Table 1: Disease characteristics.

Variable	N (%)
Involved amyloidosis site	
Bone marrow	4 (9.7%)
Kidney	1 (2.4%)
Subcutaneous tissue	34 (82.9%)
Heart	2 (4.8%)
Gastrointestinal tract	3 (7.3%)
Tongue	1 (2.4%)
Lung	1 (2.4%)
Bone Marrow Plasma cell category	
<30%	13 (31.7%)
>30%	28 (68.3%)
Hemoglobin <10 g/dl	15 (36.6%)
HB ≥10	26 (63.4%)
Serum Creatinine	
Creatinine <2 mg/dl	31 (75.6%)
Cr ≥2 mg/dl	10 (24.4%)
Beta 2 microglobulin	
<3.5 mg/l	23 (56%)
3.5-5.5 mg/l	6 (14.6%)
≥5.5 mg/l	12 (29.3%)

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SURVIVAL IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH CYCLOPHOSPHAMIDE, BORTEZOMIB AND DEXAMETHASONE (CYBORD): FAILURE OF BORTEZOMIB IN HIGH-RISK DISEASE.

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Background: The three drug induction regimen of cyclophosphamide, bortezomib and dexamethasone (CyBorD) produces high response rates in newly diagnosed multiple myeloma (Leukemia 2009; 23:1337-1341; Blood. 2010 Apr 22;115(16):3416-7). The secondary goals of this trial were overall (OS) and progression free (PFS) survival. *Patients and Methods:* 63 untreated symptomatic patients were enrolled on this Phase II trial. Data was frozen as of January 29, 2011. Survival was calculated using the Kaplan Meier method. *Results:* With a median follow-up of 27.4 months, the 2 year PFS is 61% (95%CI: 49-76). Patients were stratified into high-risk groups by mSMART criteria, cytogenetic 13 deletion, del 17, and t(4;14): Response rates (≥ PR) in these high risk groups were similar to the entire group (88%, 89%, 90%, and 83%, respectively). Progression free survival however was shorter in all high risk groups (49% (95%CI: 32-74), 49% (95%CI: 30-79), 36% (95%CI: 15-87), and 0% (95%CI: 0-46), respectively) with the shortest in t(4;14) group. Interestingly, the t(4:14) group had no apparent long term benefit from this bortezomib based induction. The 2 year OS for all patients however is excellent at 86% (95%CI: 77-96). *Conclusions:* CyBorD is one of the most active induction regimens for newly diagnosed patients with multiple myeloma but the addition of a proteasome inhibitor in induction did not improve the poor prognosis in high risk patients. Employing this novel agent in consolidation or maintenance strategies will be necessary to change the course of high risk cytogenetic disease.

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THE IMPACT OF FRONTLINE RISK-ADAPTED STRATEGY ON THE OVERALL SURVIVAL (OS) OF PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM): A POPULATION STUDY IN SINGAPORE (SG)

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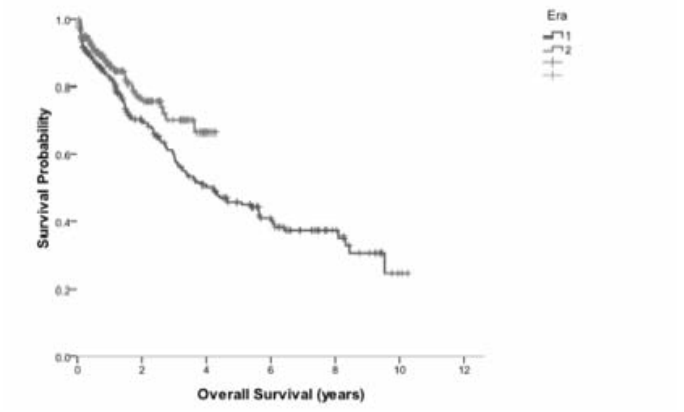
Background: MM is clinically heterogeneous and risk stratification is vital for prognostication and informing treatment. We evaluate the survival data of MM patients (pts) managed in SG, overall, and with respect to treatment eras before (era 1) and after (era 2) the incorporation of bortezomib (BTZ) as frontline therapy for high-risk MM. *Methods:* From

the consolidated database of 3 main tertiary centers treating MM, we study the OS of 483 consecutive and previously untreated MM pts from 2000 to 2009. From 2006, BTZ became incorporated in the frontline for high-risk pts (defined by any adverse factors: ISS stage 3, karyotypic del13 or non-hyperdiploidy, or del17p, t(4;14) or t(14;16) on i-FISH). *Results:* Pts and disease characteristics were comparable between the 2 eras. At a median follow-up of 1.5 years, 0% and 26% of pts had received frontline BTZ, while 24% and 21% received BTZ at relapse in eras 1(n=262) and 2 (n=221) respectively. Overall, ISS, cytogenetics, age, presenting platelet count and eventual response discriminating of the OS (median= 4.7 years) on univariate analyses. The median OS for era 1 and 2 were 4.2 years and NR respectively (p=0.02). On Cox regression multivariate analysis stratified by era, a non-hyperdiploid karyotype was the single most significant adverse prognostic factor in era 1 (p=0.03), while attainment of ≥VGPR superceeded all baseline parameters as the most significant prognosticator in era 2 (p<0.001). *Conclusions:* Our study suggests that BTZ in frontline, rather than sequential use may be better able to overcome adverse risk factors.



P-158

VENOTHROMBOEMBOLISM (VTE) RISK IN ASIAN MULTIPLE MYELOMA (MM) PATIENTS RECEIVING IMMUNOMODULATORY AGENTS (IMIDS) IS LOWER, BUT CORRELATES WITH CUMULATIVE EXPOSURE

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Background: The risk of VTE in Asian MM patients (pts) receiving IMIDs is unknown. We sought to evaluate the incidence and risk factors of VTE in MM pts and explore the role of anti-platelet agents (APA) in prevention of VTE. *Methods:* From a comprehensive MM registry in a tertiary institution, pts with objectively confirmed VTE were identified. Pts characteristics and exposure to iMIDs and APA were compared against other pts in the registry. Cumulative thalidomide (Th) exposure is measured in mg-month (dose x duration). Thromboprophylaxis was not mandated in this pt population. *Results:* 232 of 320 consecutive and previously untreated MM pts were exposed IMIDs from 2000 to 2009. 17 VTE events (7.4%) were reported. The median time to VTE from MM diagnosis was 16 months (range, 2 -134). 12 of 17 pts developed VTE during maintenance or salvage treatment. Among pts exposed to Th(N=230), median cumulative dose was 3250mg-mth (range, 100 - 37,100). VTE risk was 2.7% for cumulative dose < 3250mg-mth, compared with 11.7% for patents receiving >3250 mg-mth (P=0.01). 72 pts (31%) were on APA for other indications while undergoing MM treatment. Interestingly, there was a near significant trend of a higher VTE risk for pts receiving concomitant APA and Th (8/72)(P=0.057). *Conclusions:* VTE risk in Asian MM pts is lower than Western series. Events appear to occur later in disease course and were significantly influenced by the cumulative exposure to iMiDs. Concomitant use of an APA did not preclude the risk of developing VTE. An optimal prophylaxis strategy needs to be defined and validated.

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A PHASE I TRIAL OF ZEVALIN RADIOIMMUNOTHERAPY WITH HIGH-DOSE MELPHALAN (HDM) AND AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) FOR MULTIPLE MYELOMA (MM)

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Although HDM is the standard conditioning for pts with MM undergoing ASCT, it is not sufficient for cure. In an effort to improve upon this standard, we added escalating doses of Y-90 Zevalin (a murine IgG1 kappa monoclonal antibody Ibritumomab conjugated to Y-90) to HDM in a standard 3+3 phase I trial design. Y-90 is a beta-emitting radioisotope with a path length of 5 mm allowing it to target CD20+ cells and bystander cells within 5 mm. Eligibility included being a candidate for ASCT. Consenting pts received: day -22, rituxan 250 mg/m2 with In2B8 for scanning; day -14, rituxan 250 mg/m2 with escalating doses of Y90-Zevalin; day -2 and -1, melphalan 100 mg/m2/day; day 0, >2 x 10(6) CD34/kg stem cells; and GM-CSF until ANC engraftment. The 6 dose levels (DL) were (in Gy): 10; 12; 14; 16; 18 Gy; and 20. DLT was defined as:non-hematologic gr 4 toxicity other than constitutional or GI; sustained pulmonary or liver toxicity; and delayed engraftment. Responses were according to IMWG criteria. 55% of the 27 accrued pts had ASCT as primary therapy. There was 1 DLT at DL 4, including CMV viremia, delayed engraftment, and hepatic failure. Only 3 of 6 pts at DL 6 have completed their DLT observation. Gr 3-5 AEs occured in 100% of pts. Non-heme gr 4 AEs were seen in 22%. Median time to ANC >=500 & plts>=50 was 10.5 and 13 days. Confirmed response rates were: CR, 4; VGPR, 4; continued VGPR, 2, and continued PR, 7. Median PFS for pts proceeding to early & delayed ASCT were 30 (95%CI 10-NR) and 19 mo (95%CI 3.1-22), respectively.

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SINGLE AGENT LENALIDOMIDE OR NEWLY DIAGNOSED MYELOMA WITH ON-DEMAND DEXAMETHASONE: A PHASE 2 TRIAL

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Background: Lenalidomide (Len)- Dexamethasone (Dex) combination is a commonly used initial therapy for multiple myeloma (MM). Len is an immunomodulatory drug and use of Dex may impair this effect. *Patients and Methods:* Patients (Pts) with previously untreated symptomatic MM were treated with Len (25 mg/day) for 3 weeks during a 4 week-cycle. Dex was added at 10 mg weekly if a partial response (PR) was not seen after 3 cycles, with dose increased by 10 mg weekly every cycle until a PR was seen or a maximum of 40 mg weekly was reached. Dex 40 mg weekly was added at any time for disease progression. *Results:* Thirty-eight pts with a median age of 66 (45-78) years, were enrolled. Overall, 26 (68%) patients had Dex added at a median time of 3.3 months. Hematological toxicity was the main adverse event observed, with 45% of patients having a grade 3 or 4 event. Fatigue was the most common non-hematological toxicity. The three-cycle response rate (>PR, without Dex) was 29%. The overall confirmed response (>PR) rate, including those with Dex added per protocol, was 58% (VGPR or better of 21%), and a median time to response of 3.7 months. There have been 11 progressions, 8 while on study. The 1-year overall survival is 91%. *Conclusion:* Len as a single agent with Dex added based on response is a feasible strategy that will allow avoidance of Dex related side effects, especially where coexisting illnesses make steroid side effects difficult to manage.

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IMPACT OF GENE EXPRESSION PROFILING BASED RISK STRATIFICATION IN PATIENTS WITH MYELOMA RECEIVING INITIAL THERAPY WITH LENALIDOMIDE AND DEXAMETHASONE

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Background: Multiple myeloma is a heterogeneous disease in terms of outcome, which is primarily driven by various genetic abnormalities. Detection of specific chromosomal abnormalities by FISH and metaphase cytogenetics allows risk stratification in multiple myeloma (MM); however, gene expression profiling (GEP) based signatures may enable more specific risk categorization. *Patients and Methods:* we examined the utility of GEP-based risk stratification among patients with MM undergoing initial therapy with lenalidomide in the context of a phase 3 trial. GEP was performed on CD138 selected myeloma cells obtained prior to initiating therapy, using Affymetrix U133 Plus high-density oligonucleotide arrays. Risk status according to GEP was determined using the GEP 70 signature as reported by University of Arkansas. *Results:* among 45 patients studied by GEP at baseline, 7 patients (16%) were high-risk using the GEP70 signature. The median overall survival of this group was 19 months while not reached for the remaining 38 patients (hazard ratio = 14.1). In contrast, the OS for patients considered high-risk by FISH was 39 months vs. not reached for rest; HR = 5.8. The C-statistic for the GEP risk stratification was 0.74, compared to 0.70 for FISH classification. Here we demonstrate the prognostic value for GEP risk stratification in a group of patients primarily treated with novel agents.

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ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH MULTIPLE MYELOMA AT RELAPSE : A SINGLE CENTRE EXPERIENCE

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Introduction: We retrospectively reviewed the outcomes of MM patients allografted at our centre between 1988 and 2010. *Methods:* 23 patients (15 males and 7 females) were included. Their median age at transplantation was 48 (range, 24-57) years and the median interval from diagnosis to allo-HSCT was 18 (range, 5-78) months. Sixteen (70%) patients had IgG, 3 (13%) IgD, 1 (4%) IgA and 3 (13%) light chains MM. Almost all patients (91%) were Salmon-Durie stage III and 12 patients (52%) were ISS stage III. Most of the patients (n=18, 80%) were allografted at first relapse (n=11, 48%). Thirteen (56%) patients had received novel drugs and 14 (60%) had undergone an autologous transplantation prior to allo-HSCT. *Results:* Almost all patients (n=20, 87%) were transplanted with an HLA identical sibling donor. Twelve (52%) patients received a myeloablative (MAC) and 11 (48%) a RIC. At time of allo-HSCT, 9 (40%) patients were in CR/VGPR and 13 (60%) in PR/SD (1 missing). After allo-HSCT, 20 (92%) patients reached CR/VGPR. The incidence of acute grade>2 and chronic GvHD were 30% (SE + /- 20%) and 57% (SE +/- 22%), respectively. The incidence of transplant-related mortality (TRM) was 23% (SE +/- 20%). At 5 years, the progression free (PFS) and overall survival (OS) were 34% (SE +/-21%) and 59% (SE +/- 23%), respectively. *Conclusion:* Our series suggest that high-risk patients may substantially benefit from allo-HSCT at relapse. Still, chronic GVHD and transplant related mortaliy remain the major limitations that likely compromise the allo-HSCT risk-benefit balance.

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LENALIDOMIDE, ADRIAMYCIN AND DEXAMETHASONE (RAD) IS SAFE AND EFFECTIVE IN NEWLY DIAGNOSED MYELOMA PRECEDING RISK-ADJUSTED STEM CELL TRANSPLANTATION

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Background: Introduction of the "novel compounds" into up-front treatment of multiple myeloma (MM) resulted in early, deep response. High-dose melphalan (HDM) is a standard in consolidating patients (pts) up to 65 years (y) while allogeneic SCT has yielded conflicting results. We decided to explore RAD (lenalidomide, adriamycin and dexamethasone) in first-line MM after having shown its high efficacy in relapsed/refractory disease. *Patients and Methods:* This phase-II trial was designed to include pts up to 65 y with symptomatic MM. RAD induction consists of four 4-week cycles (len 25 mg d1-21; adriamycin 9 mg/m²*day, d1-4; dex 40 mg d1-4, d17-20) followed by mobilization of peripheral blood stem cells (PBSC). Consolidation utilizes two transplants (T): HDM and auto PBSCT is followed by RIC-allo PBSCT in pts featuring at least one cytogenetic/serologic risk factor (RF). Subjects without any RF do receive a second cycle HDM and all participants 12 months of len maintenance. *Results:* 114 pts (median age 55 [range, 30-66] y) have been enrolled by 15 centers so far. As of 1/2011, 78 severe adverse events (SAEs) have been reported in 49% of pts. 54% of SAEs were treatment-emergent. Most frequent events were infection (n=15); fever (n=13); and venous thrombosis (VTE; n=7). 36 pts are currently evaluable for post-induction response: ORR is 84% with 14% CR/sCR. *Conclusions:* Our preliminary results suggest RAD to be a well tolerated and effective induction protocol. As of yet, no treatment-related deaths were observed and VTE incidence was low.

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A PHASE I TRIAL TO EVALUATE SAFETY OF T CELLS ARMED WITH BISPECIFIC ANTIBODY TARGETING MULTIPLE MYELOMA (MM) CANCER STEM CELLS (CSC) TO BOOST POST AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) ANTI-MYELOMA IMMUNITY

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MM may be derived from MMCSC. CD20 protein on MMCSC provides a target for ex vivo expanded activated T cells (ATC) armed with anti-CD3 x anti-CD20 bispecific antibody (BiAb). The study was done to determine the feasibility, safety, and the development of anti-myeloma immunity before and after ASCT. We targeted CD20 on MMCSC prior to ASCT with CD20Bi armed ATC (aATC) to determine whether anti-MMCSC immunity could be induced by 2 immune therapy (IT) infusions of 1010 aATC 1 week apart and transferred via the stem cell product. Twelve patients (pts) are enrolled after induction therapy. Nine pts have received IT, ASCT, and are evaluable for safety, blood and bone marrow (BM) phenotyping, cytotoxicity, and IFNγ Elispots. Nine pts are alive at a median 159 days after ASCT (115-360). Two pts have relapsed. There were no dose-limiting toxicities associated with aATC infusions. One pt developed a catheter related blood clot. All pts engrafted between days 11 - 23. Colony forming assays and phenotyping of BM and peripheral blood for CD20+/CD138- MMCSC, plasma cells, and B cells did not

change. IFNγ EliSpots directed at Daudi [CD20+ target], K562 [NK target] and RPMI 8226 [a CD20- MM target] were significantly higher after IT in 7 pts compared to before IT ($p < 0.03$). The k/l ratio in CD20+ B cells decreased in 5/6 pts. Immune testing show that aATC infusions induced immune responses in 7/9 pts prior to ASCT and suggest that these immune responses could be transferred in the stem cell product.

Pt #	Age	Sex	M Protein	Durie Salmon-Stage	Disease Status@ ASCT	Stem Cells Collected x 10^6/ Kg	Neutrophil Engraftment (Days)*	Post-ASCT Follow-up (Days)*
1	66	F	IgG	I A	VGPR	6	11	360
2	42	M	IgG	II A	CR	7.6	13	319
3	55	F	IgA	III A	PR	10.3	17	248
4	49	F	IgG	III A	PR	14	23	187
5	59	M	IgA	III A	CR	7.9	17	150
6	41	F	IgA	III A	VGPR	7.2	13	159
7	66	M	IgG	III A	PR	20.9	21	122
8	67	F	IgG	I A	PR	8.2	12	117
9	66	F	IgG	II B	PR	8	18	115

*** post ASCT, CR=Complete Remission, VGPR=Very Good Partial Response, PR=Partial Response**

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BORTEZOMIB-BASED INDUCTION THERAPY OVERCOMES ADVERSE PROGNOSTIC EFFECT OF 1Q21 AMPLIFICATION ON RESPONSE RATES IN NEWLY-DIAGNOSED MYELOMA

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Background: Amplification of chromosome 1q21 genes occurs in 40% patients with myeloma and predicts for lower response to novel agents. We assessed if a PAD combination overcomes adverse effects of amplification on response in newly-diagnosed myeloma. *Methods:* A phase II trial included 107 newly-diagnosed transplant-eligible patients, stratified by 1q21 amplification. All received four 21-day cycles of PAD induction: bortezomib 1.3mg/m2 D 1,4,8,11; doxorubicin 20mg/m2 D1,4; dexamethasone 20mg D1,2,4,5,8,9,11,12. Responders proceeded to high-dose melphalan (200mg/m2) based ASCT. The primary endpoint was Overall Response Rate (ORR) after 4 cycles of PAD induction. *Results:* Amplification of 1q21 occurred in 28% (28/100) of evaluable patients. ORRs are shown in the Table.

Response	After 4-cycles		3-months after ASCT	
1q21	NOT amplified n=61	Amplified n=20	NOT amplified n=56	Amplified n=20
sCR	8.2%	15%	10.7%	25.0%
CR	9.8%	15%	14.3%	15.0%
VGPR	24.6%	15%	33.9%	30.0%
PR	45.9%	55%	33.9%	25.0%
SD	11.5%	-	5.4%	-
Clinical Relapse	-	-	1.8%	-
ORR	88.5%	100%	91.2%	100%

After a median 184 days, 4/107 patients have progressed (1 with amplification), 7 died (1 considered treatment-related), and 11 ceased therapy with Adverse Events (AEs). Grade 3/4 AEs include peripheral neuropathy (8%), constipation (6%), back pain (6%), neutropenia (5%). *Conclusions:* Four cycles of PAD therapy induced similar, high response rates in both 1q21 amplified and non-amplified patients with acceptable toxicity. We are following patients to determine if progression-free survival is shorter with 1q21 amplification.

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EFFICACY OF BORTEZOMIB PLUS DEXAMETHASONE VERSUS BORTEZOMIB MONOTHERAPY IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (MM): INTERIM RESULTS FROM AN INTERNATIONAL ELECTRONIC OBSERVATIONAL STUDY

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Introduction: the international, non-interventional electronic VELCADE® (bortezomib [Vel]) observational study (eVOBS) is ongoing to prospectively assess clinical and health economic outcomes of Vel-based therapies for relapsed/refractory MM in a clinical practice setting. Only pts who received Vel monotherapy or Vel + dexamethasone (Vel–Dex) during the 3-yr study period were included in this analysis. *Methods:* all administered Vel doses and concomitant treatments (except investigational therapies) were permitted. Investigator-defined responses were based on EBMT, SWOG, or M-protein criteria, and outcomes were analyzed using Kaplan–Meier methodology. *Results:* 432 pts received Vel (n=106) or Vel–Dex (n=326). Median follow-up was 8.6/11.5 months for Vel/Vel–Dex, respectively. CR rate was higher, and time to CR shorter for Vel–Dex compared to Vel. ORR and OS did not differ between groups in unadjusted or inverse probability weighting (IPW)-adjusted analyses (Table); however, there were trends for improved PFS and TTP with Vel–Dex. *Conclusions:* addition of Dex to Vel appears to improve CR rate in relapsed/refractory MM pts. At current follow-up, Vel–Dex did not appear to prolong OS compared to Vel; however, it was associated with trends for improved PFS and TTP. These results may require confirmation after longer follow-up and in a larger cohort.

	Response, %		Hazard ratio, Vel vs Vel–Dex	
	Vel	Vel–Dex	Unadjusted	IPW-adjusted
CR	**8.2**	**24.1**	**3.51 p=0.0071**	**2.42 p=0.0272**
ORR	68.4	72.7	1.13 p=0.45	1.25 p=0.1799
PFS	—	—	0.80 p=0.2195	0.73 p=0.0758
TTP	—	—	0.82 p=0.3261	0.82 p=0.3393
OS	—	—	1.15 p=0.5342	0.93 p=0.7127

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EXTENDED RESULTS OF SOUTHWEST ONCOLOGY GROUP PROTOCOL S0232: DURABLE RESPONSES ACHIEVED WITH LENALIDOMIDE (L) PLUS HIGH-DOSE DEXAMETHASONE (D) AS FIRST-LINE THERAPY FOR MULTIPLE MYELOMA

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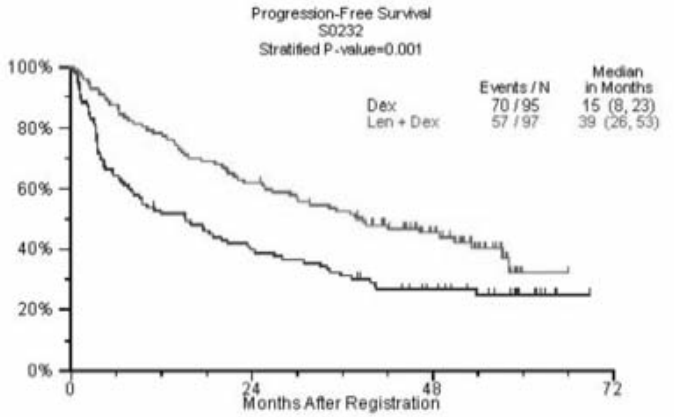
Lenalidomide plus dexamethasone (LD) is approved for previously treated myeloma. Here, we report extended results for protocol S0232

(LD vs D, frontline). Of 192 pts enrolled, 97 were treated with three 35-day induction cycles (L 25 mg/d x 28d and D 40 mg/d on days 1-4, 9-12, 17-20) followed by 28-day maintenance cycles (L 25 mg/d x 21d and D 40 mg/d on days 1-4, 15-18). Ninety-five pts received the same schedule of D plus placebo, with crossover to LD for progression. Of 65 pts on LD with cytogenetics data, 15 had cytogenetic abnormalities (CA). Median (Med) follow-up from initial registration is 45.4 mos. The overall response rate with LD was 78% (63% >VGPR; 13% CR), vs 43% (16% >VGPR) with D (p<0.001). Med progression free survival (PFS) for LD arm was 39 mos, vs. 15 mos for D (p=0.001). Estimated PFS and OS at 48 mos are 36% (LD, 46%; D, 27%) and 64% (LD, 71%; D, 57%), respectively. LD pts without CA had med PFS of 53 mos (48-month estimates: PFS 55%, OS 76%). 40 pts crossed over from D to LD, with 39 mos med post-crossover follow-up; med PFS and OS are 19 and 43 mos. The overall incidence of thrombotic events on LD (initial + crossover) was 25.0% (21% if on aspirin prophylaxis). Nine pts (5 LD, 4 D; p=NS) developed second cancers (8) or MDS (1). In summary, frontline LD induces durable, high quality responses, particularly in pts without baseline CA.



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DEVELOPMENT OF AN EPIDEMIOLOGICAL MODEL FOR ENROLMENT IN A LARGE, INTERNATIONAL, MULTICENTER, OBSERVATIONAL STUDY OF PATIENTS WITH MULTIPLE MYELOMA (MM) TREATED IN ROUTINE CLINICAL PRACTICE

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There is a lack of objective information on everyday clinical practice and outcomes in MM treatment. The prospective, non-interventional MMY4046 study has been designed to provide an accurate picture of routine treatment and resource utilization practices for MM patients during therapy, independent of drugs used, in 24 countries in Europe and Africa. A multi-staged site and patient selection enrolment model was developed to minimize selection bias and maximize the representative nature of the population. Enrolment is stratified by country, region, and practice type. Types of sites treating MM patients within each country and region, and the proportion of patients treated at specific site types, were identified using regional data and market segmentation data. GLOBOCAN 2002, the most recent comprehensive single source of country-specific MM prevalence data worldwide at the time of protocol development, was used to obtain overall population estimates of MM prevalence, and the number of patients to be enrolled in each country and region was determined proportionately. A total of 2987 MM patients will be enrolled at 279 sites, with highest enrolment planned in Germany (546 patients, 41 sites), Italy (500 patients, 57 sites), Russia (224 patients, 27 sites), Ukraine (224 patients, 22 sites), Spain (219 patients, 26 sites), and France (180 patients, 22 sites). All consecutive eligible patients treated at each site will be invited to enrol in MMY4046, up to the site's assigned enrolment target. Full details of this epidemiological enrolment model will be presented and discussed.

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PHASE 1/2 TRIAL OF KW-2478 PLUS BORTEZOMIB IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: RESULTS FROM THE PHASE 1 PORTION

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Introduction: KW-2478 is a novel non-ansamycin, non-purine inhibitor of Hsp90. KW-2478 + bortezomib (BTZ) had synergistic antitumor activity in preclinical studies. This study investigated the combination of KW-2478 + BTZ in a Phase 1/2 trial in patients (≥18 y) with relapsed and/or refractory multiple myeloma (MM). Preliminary results from Phase 1 of the study are reported here. *Methods:* Patients received BTZ (1.0 and 1.3 mg/m^2) combined with KW-2478 (130 and 175mg/m^2) on Day 1, 4, 8 & 11 of a 21 day cycle. Dose escalation proceeded sequentially via 3 cohorts of 3 patients and a 4th cohort of 6 patients to the maximum planned dose of BTZ 1.3 mg/m^2 and KW-2478 175 mg/m^2. Safety assessments included ECGs, chemistry/haematology and slit lamp retinal examination. Blood samples were collected for PK and PD (Hsp70 induction, a marker for Hsp 90 inhibition) on D 1 & 11 (cycle 1). *Results:* The study enrolled 15 patients, median age: 67 y. The most common toxicities observed were diarrhoea, constipation, pain, nausea and fatigue. One DLT (vasovagal reaction) was observed in cohort 4. There have been one CR, one VGPR, and one PR observed to date. Terminal half-lives for KW-2478 and BTZ averaged about 6 h and 13-22 hours, respectively. Hsp70 induction occurred at both doses of KW-2478. *Conclusions:* Preliminary results suggest that the KW-2478 + BTZ combination was well tolerated with Hsp70 induction at all dose combinations. PK profiles for KW-2478 and BTZ in combination were comparable to each agent administered alone. Updated Phase 1 safety and efficacy data will be presented.

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STUDY OF DOSE INTENSITY (DI) OF LENALIDOMIDA AS SALVAGE THERAPY IN PATIENTS WITH RELAPSE/REFRACTORY MULTIPLE MYELOMA

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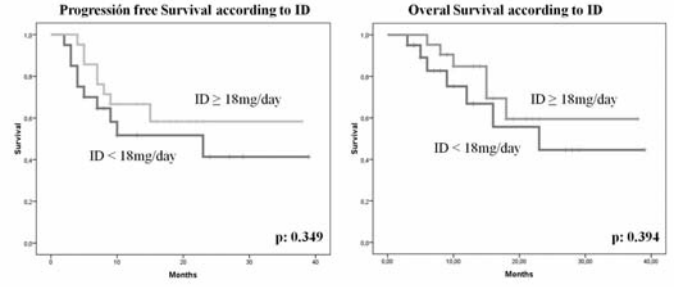
Up to 40% of patients treated with lenalidomide suffer from grade 3-4 hematological toxicity being necessary dose reductions. Until now there are no studies of dose intensity (DI) in response. *Aim:* compare response, PFS and OS according to DI of lenalidomide in patients with

relapse MM. *Results:* DI of lenalidomide was calculated in 41 patients at relapse or refractory MM as mg per month, and in order to better comprehension is expressed as mg/day of 21-day cycle. With a mimimun of 2 cycles, response, PFS and OS has been calculated. Patients with renal failure has been excluded. Median time from diagnosis to treatment was 34 months (3-174). Half of the patients received at least 3 lines of treatments (1-6). Duration of treatment with lenalidomide was 8 months (range: 2-39). Globally, 83% of patients reach ≥PR (27% complete response); 14% remain with stable disease. Median PFS and OS were 9 (2-39) and 13 months (3-39), respectively. Focusing on DI, median dose of lenalidomide was 18 mg/day/cycle (5.25-25). For patients receiving > or ≤ 18 mg/day of lenalidomide, no differences were observed in median time to best response (7.2 vs. 5.5 months; p: 0.320), response (>= PR: 85,7% vs. 85%; p: 0,645) , PFS (25,5 vs 21 month; p: 0,345) and OS (28 vs. 24 months, p: 0,534). Although a trend toward better outcome could be observed for patients receiving higher doses.

We did not observe differences on outcomes for patients receiving higher DI of lenalidomide, although a trend toward longer PFS and OS could be observed for those receiving higher DI.



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PHASE II TRIAL OF SYNCOPATED THALIDOMIDE, LENALIDOMIDE AND WEEKLY DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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We conducted a Phase II trial of syncopated thalidomide (200 mg qhs days 1-7, 15-21) lenalidomide (25 mg q d days 8-14, 21-28 for the 1st cycle and 50 mg on the same schedule for the second and subsequent cycles) with weekly dexamethasone (40 mg) in patients with newly diagnosed multiple myeloma. The patients received either ASA or warfarin for DVT prophylaxis. Twenty-two patients were enrolled with a median age 62 (range 39-80); ISS 1 (n = 9), ISS 2 (n = 8), ISS 3 (n =4), NA (n =1). The median number of cycles administered was 3.5 (range 0.5-7) with the intention that patients would proceed to ASCT following 4 cycles of therapy. Best response < 4 cycles: VGPR (n =1), PR (n = 12), MR/SD (n = 5), PD (n = 2) and TE (n = 2) for an overall response rate > PR 65%. Two patients achieved a VGPR > 4 cycles. Nine patients have completed ASCT, 10 are eligible and 3 were deferred [early death (1), age (2), refused (1)]. Study discontinuation was for PD (n =2), SD (n = 3), and 1 each for early death, transient hypercalcemia and non-compliance. Toxicities G > 3 deemed study related include: neutropenia (n = 2), hyperglycemia (n =2), UTI (n = 2) and 1 each for thrombocytopenia, elevated LFTs, CHF, pneumonia (early death). We conclude that although well-tolerated, syncopated thalidomide and lenalidomide to decrease toxicity associated with the individual agents and to increase myeloma cell targeting resulted in modest activity.

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CHEMOMOBILIZATION WITH INTRAVENOUS PLERIXAFOR

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Phase III trials have demonstrated improvement in stem cell mobilization for multiple myeloma (MM) patients(pts) treated with subcutaneous plerixafor(administered of 0.24mg/kg 9-11 hours pre-apheresis) and G-CSF. The strategy of chemomobilization with plerixafor could potentially further increase stem cell yield. In addition intravenous administration (IV) may result in a quicker rise in CD34+ cell counts thereby allowing same day collection. This phase I trial determined the safety and efficacy of administering IV plerixafor ten days following cyclophosphamide (CY) chemomobilization. Pts received 1.5g/m^2 CY followed by G-CSF (10mcg/kg) 24 hrs later. At day ten post-CY, pts received IV plerixafor (0.16mg/kg and 0.24mg/kg). Plerixafor was given 4-6 hours pre-apheresis, CD34+ counts were obtained pre-plerixafor and two hours post-plerixafor. Successful mobilization was defined as collection of ≥5 x 106 CD34 + cells/kg in two or fewer days. 6 pts have been enrolled, 3 at each dose level. Median age 60.8 (range, 43.4 – 68.9)There were no DLTs at the highest dose level. Grade 3/4 plerixafor related adverse events included anemia, hyperuricemia, hyponatremia. Successful mobilization was observed in 4/6 pts. All pts collected a minimum of 5X10 6 CD34 + cells/kg. Peripheral blood CD34 count increased median 2.5-fold post-plerixafor. A median of 9.8 X 106 (range 5.7-58.8) CD34+ cells/kg were collected. All pts have undergone transplant and engrafted.Chemomobilization and IV plerixafor was well tolerated and mobilized adequate cells for transplant in MM pts.

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PROPHYLACTIC INTRAVENOUS IMMUNOGLOBULIN DURING AUTOLOGOUS HEMOPOIETIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA IS NOT ASSOCIATED WITH REDUCED INFECTIOUS COMPLICATIONS

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Patients with multiple myeloma undergoing autologous hemopoietic stem cell transplantation (ASCT) are at high risk for infectious complications. Peri-transplant intravenous immunoglobulin (IVIG) has been used with the aim of reducing these risks. METHODS. Retrospective review of peri-transplant IVIG use in 266 ASCT for myeloma from 2000 to 2009 at a major metropolitan referral centre for hematological malignancies. Data on infectious complications were identified from case records, pathology and radiology reports. RESULTS. There was no difference between those receiving peri-transplant IVIG (n=130) or not (n=110) with regard to total BSI (35.4% vs 31.8%, p=0.59), pneumonia (17.7% vs 14.5%, p=0.60), urinary tract (1.5% vs 1.8%, p=1.0) or gastrointestinal infections (4.6% vs 10.0%, p=0.13) in the overall cohort. When analyzed according to pre-transplant therapy (conventional chemotherapy vs novel agents) there was no significant difference in infectious complications between those who did or did not receive peri-transplant IVIG. In a subgroup of patients treated earlier in the study period that was enriched for those treated with pre-transplant conventional chemotherapy, there were fewer ASCT complicated by multiple and polymicrobial BSI. *Conclusions:* our study did not show a benefit for the use of peri-transplant IVIG to reduce infectious complications in a large cohort of patients with myeloma undergoing ASCT. In the absence of data supporting efficacy in this context, there appears to be no benefit in the routine use of IVIG for this purpose.

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BORTEZOMIB, MELPHALAN, PREDNISONE AND THALIDOMIDE FOLLOWED BY BORTEZOMIB AND THALIDOMIDE (VMPT-VT) AS INITIAL TREATMENT OF MULTIPLE MYELOMA (MM) PATIENTS OLDER THAN 65 YEARS: UPDATED FOLLOW-UP AND PROGNOSTIC FACTORS.

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Bortezomib-melphalan-prednisone-thalidomide followed by maintenance bortezomib-thalidomide (VMPT-VT) was compared with standard VMP in newly diagnosed elderly myeloma patients (pts). 511 pts

were randomized to receive 9-6-week cycles of VMPT-VT (N= 254; induction: bortezomib 1.3 mg/m^2 days 1,4,8,11,22,25,29,32, cycles 1-4 and days 1,8,22,29 on cycle 5-9; melphalan 9 mg/m2 days 1-4, prednisone 60 mg/m2 days 1-4, thalidomide 50 mg continuously; maintenance: bortezomib 1.3 mg/m^2 every 14 days and thalidomide 50 mg/day) or VMP alone (N=257).In March 2007, protocol was amended with weekly infusion of bortezomib in both arms. Response rates were higher in VMP-VT: 42% of complete remission (CR) vs 24% (p< 0.0001). After a median follow-up of 32 months, the 3-year progression-free survival (PFS) were 51% in VMPT-VT and 32% in VMP (p< 0.0001). The 3-year overall survival were 85% vs 80%, respectively (p=0.35). CR was a strong predictor of longer PFS in both groups (P < 0.0001). VMPT-VT do not add any significant advantage in pts ≥ 75 years and in those with high risk disease (cytogenetic abnormalities and ISS 3). The 1-year landmark analysis of PFS demonstrated that VT reduced the risk of disease progression of 52% (p< 0.0001). This advantage was less evident in pts ≥75 years and those with high-risk disease. VT had favorable safety profile with <5% grade 3-4 toxicities. In conclusion, VMPT-VT prolonged PFS with a 3-year PFS of 51%;higher dose-intensity seemed to be less effective in pts ≥75 years; maintenance therapy with VT further improved PFS with a good safety profile.

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COMPARISON OF BORTEZOMIB (VC) CUMULATIVE DOSE, EFFICACY, AND TOLERABILITY WITH THREE DIFFERENT BORTEZOMIB-MELPHALAN-PREDNISONE (VMP) REGIMENS IN PREVIOUSLY UNTREATED MULTIPLE MYELOMA (MM) PATIENTS (PTS) INELIGIBLE FOR HIGH-DOSE THERAPY (HDT)

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VMP has substantial efficacy in pts with untreated MM ineligible for HDT. Various dosing schedules of Vc 1.3 mg/m^2 have been used in three phase 3 studies of VMP. In VISTA, 340 pts received Vc BIW for 4 6-wk cycles, then QW for 5 6-wk cycles. In GEM05 (>65), 130 pts received Vc BIW for 1 6-wk cycle then Vc QW for 5 5-wk cycles, plus 3-yrs Vc maintenance. In GIMEMA MM-03-05, Vc was given either per VISTA (BIW, 63 pts) or QW for 9 5-wk cycles (190 pts). Rate of pts completing early 'induction' cycles (4 in VISTA/GIMEMA, 6 in GEM05) was similar, at 61.8% in VISTA vs 69.2% in GEM05 and 74.6%/80.5% in GIMEMA BIW/QW; however, 37.4% vs 58.7%/65.3% received Vc for all 9 cycles in VISTA vs GIMEMA BIW/QW. Median cumulative Vc dose delivered (mg/m2) in early 'induction' was similar in VISTA (29.4), GEM05 (32.9), and GIMEMA BIW (29.6), but lower in GIMEMA QW (20.8). Median overall cumulative Vc dose delivered in all pts in cycles 1-9 was similar in VISTA (38.5) and GIMEMA BIW/QW (42.1/40.3); this was due to the lower number of Vc dose modifications required with GIMEMA QW. Response rates were 71%, 80%, and 87%/80% in VISTA, GEM05, and GIMEMA BIW/QW, with 30%, 20%, and 26%/23% CR. After similar median follow-up, no differences were seen in PFS in GEM05 and GIMEMA BIW/QW. Grade 3-4 peripheral neuropathy rate was 13% in VISTA and 14% in GIMEMA BIW, but was reduced to 7% in GEM05 and 2% in GIMEMA QW. Use of optimized Vc dosing schedules in the VMP regimen in GEM05 and GIMEMA QW resulted in high efficacy (comparable with VISTA) with a favorable toxicity profile.

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COMBINED TREATMENT WITH DONOR LYMPHOCYTE INFUSION AND BORTEZOMIB IN RELAPSED MULTIPLE MYELOMA AFTER ALLO-SCT

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To improve the response rate of 30-50% after DLI in relapsed allogeneic transplanted myeloma patients, we investigated the addition of bortezomib in a prospective phase II study. Patients were treated with bortezomib 1.3 mg/m2, standard schedule, without dexamethasone. At day 21 of the second cycle a DLI was given consisting of 1x107 T cells/kg. Two to 4 weeks after DLI the 3rd and 4th cycle were given. Patients in CR stopped further treatment, if response was PR or better bortezomib was given for a maximum of 8 cycles. Patients with < PR and without GvHD received a second DLI consisting of 1x108 T cells/kg followed by 2 cycles. Blood samples were collected for analysis of T-cell subsets. Due to slow accrual only 5 patients were included; 3 men and 2 women, median age 58 years (range 45-68). All patients were treated with 1 DLI, and 2 received 2 DLIs. Total given bortezomib cycles was between 2-8. Maximal response rate on protocol treatment was 80%, including 1 CR. Median PFS was 6.8 months (range 4,3-21,5) and median OS not reached with a median follow up of 2 years. Adverse events for bortezomib were similar as observed in patients without allo-SCT and 1 patient developed limited cGvHD of the skin. In the analyzed blood samples there was a slight decrease of both CD4 and CD8 IFNʒ and CD4 FOXP3 cells during treatment but in other T-cells (CD4 IL17+ and NK) no changes were detected. Bortezomib treatment after allo-SCT is feasible and no enhanced toxicity was observed. Despite high response rates the PFS was only 6.8 months indicating that no durable remissions were induced.

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BORTEZOMIB-BASED THERAPY AS INDUCTION REGIMEN OF AN AUTOGRAFT PROGRAM IN FRONT-LINE TREATMENT OF MULTIPLE MYELOMA (MM) WITH END STAGE RENAL DISEASE (ESRD).

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Renal failure is a common complication of newly diagnosed MM, accounting up to 50% of patients (pts). The proportion of pts requiring dialysis is between 5 and 10%; they have a poor prognosis with decreased response, shorter survival, and early mortality. We evaluated the efficacy and feasibility of Bortezomib-based therapy as induction regimen of an autograft program in 17 pts undergoing dialysis with de novo MM. Patient eligibility criteria included: newly symptomatic MM, end stage renal disease, age <65 years, WHO performance status 0-3, absence of cardiac or hepatic dysfunction. Treatment schedule included 4 Bor-based regimen cycles, stem cells mobilization and autologous stem cell transplantation (ASCT). The IMWG criteria were used for response definition; toxicity was graded according to NCI-CTC criteria. At time of this report, all pts were available for response to Bor-induction (≥PR 94%, ≥VGPR 41.1%) and experienced mild toxicity, 16 collected adequate numbers of stem cells (median of 4.6x106 CD34/kg), 1 failed mobilization and was withdrawn from the autograft program. Twelve pts performed ASCT with a prompt bone marrow recovery and manageable toxicities. After a FUP of 26 ms 8/12 pts are still alive and in remission. Our experience suggests that a Bor-based therapy is well tolerated and represents an effective option as preparatory regimen before ASCT for MM young pts in ESRD. ASCT with low dose of melphalan is an attractive alternative for this subgroup of pts, although further studies are warranted to establish a comprehensive safety and efficacy profile.

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SMOLDERING MULTIPLE MYELOMA (SMM) AT HIGH-RISK OF PROGRESSION TO SYMPTOMATIC DISEASE: A PHASE III, RANDOMIZED, MULTICENTER TRIAL BASED ON LENALIDOMIDE-DEXAMETHASONE (LEN-DEX) AS INDUCTION THERAPY FOLLOWED BY MAINTENANCE THERAPY WITH LEN ALONE VS NO TREATMENT

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In this phase III trial, SMM pts at high-risk of progression were randomized to receive early treatment vs no treatment to evaluate if it prolongs the TTP to active disease. Len-dex arm received an induction with 9 four-weeks cycles of len at 25 mg on d 1-21 plus dex at 20 mg on d 1-4 and 12-15, followed by maintenance until progression with len at 10 mg on d 1-21 every month plus dex in the case of biological progression). 119 out of the 125 recruited pts were evaluables. On an ITT analysis (n=58), the ORR during induction was 81%, including 7% sCR, 7% CR, 10% VGPR and 57% PR. If we select 47 pts who completed the induction, the ORR was 92%, including 9% sCR, 9% CR and 13% VGPR. After a median of 7 cycles of maintenance (1-21), the sCR increased to 13%. After a median f/u of 22 m (4-39), 5 pts progressed to active disease in the Len-dex arm and 10 pts have developped biological progression, but with the addition of dex the disease remain controlled (median of 10,5 m). In the no treatment arm, 28 out of 61 pts progressed to active MM. Median TTP from inclusion was 25 m for the abstention arm vs median not reached in the treatment arm (p=0.0001)(HR: 8; 95%CI=3-21). Toxicity was manageable and one patient developped prostate cancer in Len-dex arm, 16 m after inclusion. In conclusion, this analysis shows that in high-risk SMM pts, delayed treatment resulted in early progression to symptomatic disease, with a significant prolongation of TTP in treatment arm; moreover, biological progressions occurring under maintenance have remained controlled over a prolonged period of time.

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RANDOMIZED PHASE II TRIAL OF A COMBINATION OF BORTEZEMIB WITH HIGH DOSE MELPHALAN, ARSENIC TRIOXIDE AND ASCORBIC ACID

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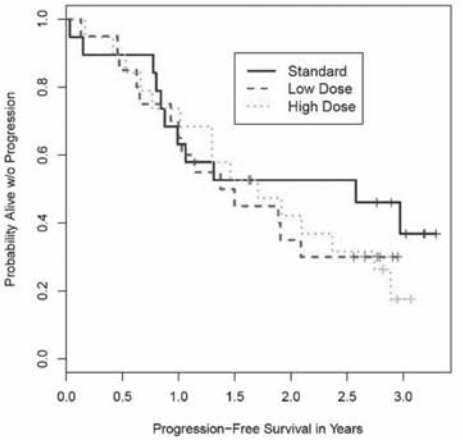
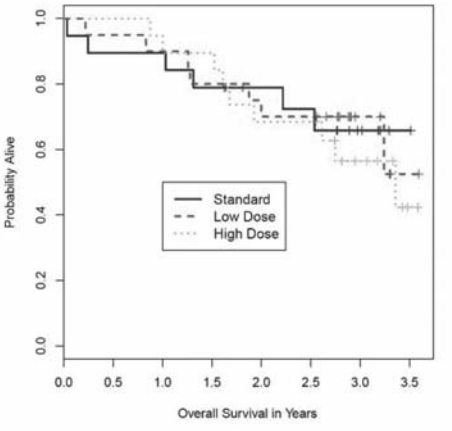
Background: Bortezomib is an active agent in newly diagnosed or relapsed multiple myeloma, and has synergistic activity with melphalan. We conducted a randomized phase II trial to determine the safety and efficacy of adding bortezomib to a preparative regimen of arsenic trioxide (ATO), ascorbic acid (AA) and melphalan. *Methods:* among 60 patients enrolled between October 2006 and September 2007, 58 went on to receive auto HCT, with a preparative regimen of melphalan 200 mg/m^2 IV, AA 1000 mg/day IV x 7 days and ATO 0.25 mg/kg IV x 7 days. Patients were randomized to 3 groups; no bortezomib (group 1), bortezomib 1 mg/m^2 x 3 doses (group 2), and bortezomib 1.5 mg/m^2 x 3 doses (group 3). Primary endpoints were complete response (CR), NCI grade 4 toxicity, and 90-day treatment-related mortality (TRM). Secondary endpoints were PFS and OS.

Figure 1. Progression-free survival by treatment group.



Results: median follow up in all surviving patients was 36 months (range 20 to 43). CR rates in arms 1, 2 and 3 were 20%, 10% and 10%. Grade 3–4 non-hematologic toxicity and TRM was similar in 3 random groups. Median OS has not been reached in any of the 3 arms. Median progression-free survival (PFS) times were 17.8, 17.4 and 20.7 months, respectively. PFS and OS were significantly shorter in patients with high-risk cytogenetics (p=0.0002 and 0.0001) and relapsed disease (0.0001 and 0.0001) regardless of the treatment group. *Conclusions:* Allthough safe, and well tolerated, adding bortezomib to a preparative regimen of ATO, AA and high dose melphalan did not provide a significant improvement in CR rate, PFS or OS in bortezomib groups.

Figure 2. Overall survival treatment group.



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TACKLING EARLY MORBIDITY AND MORTALITY IN MYELOMA: ASSESSING THE BENEFIT OF ANTIBIOTIC PROPHYLAXIS AND ITS EFFECT ON HEALTHCARE ASSOCIATED INFECTIONS

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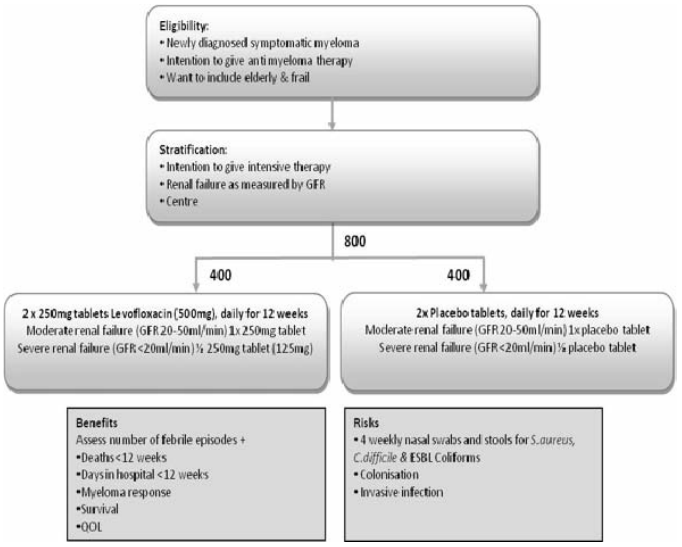
This National Institute of Health Research HTA Programme Study will recruit 800 UK patients starting in Summer 2011. The trial will assess the risks, benefits and cost effectiveness of levofloxacin in newly diagnosed symptomatic myeloma by a prospective, multi-centre, randomized, double-blind, placebo-controlled trial. The trial tests if levofloxacin once daily for 12 weeks 1) reduces the rates of febrile episodes, hospitalisation, pneumococcal bacteremia and death 2) increases response to anti-myeloma therapy 3) improves quality of life and overall survival. Further the trial tests if levofloxacin affects the carriage of and/or infection by three important groups of bacteria; Clostridium difficile, S. aureus (including MRSA) and ESBL coliforms. 1) Is the carriage of these organisms increased in patients receiving levofloxacin compared to those receiving placebo? 2) Is the carriage of these organisms associated with later invasive infections? 3) Does levofloxacin increase the rate of invasive infections by these three groups of organisms?



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WHATEVER HAPPENED TO THE BIRD (CLARITHROMYCIN[BIAXIN®], LENALIDOMIDE [REVLIMID®] AND DEXAMETHASONE)? AN UPDATE AFTER 6 YEARS OF FOLLOW UP WITH FOCUS ON SECOND PRIMARY MALIGNANCIES

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Background: Lenalidomide (Rev) has markedly improved survival in MM. Insight into its long-term effects raised concern for SPM including MDS/AML. BiRd has proved effective in symptomatic, newly diagnosed MM. Of 72 pts, 90.3% had objective response, 38.9% complete response, and 73.6% at least very good partial response. This was compounded by the observation of deepening responses to prolongation of Rev-based therapy beyond induction. All pts were followed. *Methods:* charts were reviewed focusing on overall survival, progression of disease, time to progression, and development of SPM. Pts remained on BiRd until progression of disease, intolerable side effects, or stem cell transplant. *Results:* follow up shows overall survival of 82.2% (95% CI 70.7, 89.5) at 4 years. Eleven pts are on study, 47 received second line therapy. New diagnoses of SPM were seen in 11/68 pts (incidence of 16%) after 31 cycles (mean, range 3-68) of Rev (BiRd). Six were skin cancers (4 BCC, 2 SCC), 2 colon, 1 prostate, 1 pancreas and 1 metastatic melanoma (after 11yr NED). No one developed MDS/AML. Mean time to SPM diagnosis was 35 months (range 5-64). Only 7/11 pts were on active Rev therapy. *Conclusion:* BiRd is highly effective in newly diagnosed MM. In our treatment-naïve pts, no MDS/AML was seen, in contrast to reports of Rev as 3rd or 4th line therapy (Reece, Goswami) or post-transplant maintenance (Attal , McCarthy). Incidence of SPM is similar to 2010 SEER data for non-MM individuals of similar age. As survival in MM improves, so will our understanding of long-term effects of novel agents.

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EFFICACY AND SAFETY OF BORTEZOMIB-MELPHALAN-PREDNISONE AS FIRST-LINE TREATMENT IN PATIENTS WITH MULTIPLE MYELOMA INELIGIBLE FOR TRANSPLANTATION

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Background: Bortezomib plus melphalan and prednisone (VMP) is significantly better than melphalan plus prednisone alone for elderly patients with untreated multiple myeloma (MM).Our study reports the efficacy and safety of (VMP) schedule as first-line treatment in MM patients not eligible for high-dose therapy, in daily clinical practice. *Patients and Methods:* We analyzed 62 patients between July 2007 and November 2010, treated with nine 6-week cycles of VMP: bortezomib (1.3 mg/m2 days 1, 4, 8, 11, 22, 25, 29 and 32, cycles 1-4 and days 1, 8, 22 and 29, cycles 5-9), with melphalan (9mg/m2) and prednisone (60mg/m2) days 1-4, cycles 1-9.Median age was 71 years (59-90) with 25 males (40%). Response was evaluated using the EBMT criteria. Adverse events were graded according to the NCI CTCAE v3.0. *Results:* With a median follow-up of 9.5 months (1-24), response was assessed in 48 patients (77%) after receiving a median of 4.25 cycles (range 1-9). Overall response rate was 83 % (31% CR, 52% PR) with 2% MR, 8% SD and 6% progression. Toxicity was evaluated in all patients. Grade 3-4 adverse events: neutropenia 12%, anemia 7%, thrombocytopenia 7% and 10% gastrointestinal symptoms. Peripheral neuropathy was observed in 45%, 13% grade 3-4. 39% required dose adjustment of bortezomib, 24% of melphalan and 7% of prednisone. 22 patients (36%) discontinued treatment, 14 for toxicity. Overall survival was 87%. *Conclusions:* VMP can be considered a good therapeutic option in patients with newly diagnosed MM not candidates for intensive therapy, given its efficacy and good tolerance.

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BORTEZOMIB PLUS MELPHALAN AND PREDNISONE IN PREVIOUSLY TREATED PATIENTS WITH MULTIPLE MYELOMA

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Background: Bortezomib plus melphalan-prednisone (VMP) is an effective therapy as first-line treatment in patients with multiple myeloma (MM) ineligible for stem cell transplantation. *Aim:* To evaluate the efficacy and safety of VMP regimen for patients with MM previously treated. *Patients and Methods:* We treated 32 patients with relapsed/refractory MM with nine 6-week cycles of VMP: bortezomib (1.3mg/m2 days 1,4,8,11,22, 25, 29, 32, cycles 1-4; and days 1, 8, 22, 29, cycles 5-9) plus melphalan (9mg/m2) and prednisone (60mg/m2) days 1 to 4, cycles 1-9. Median age was 70 years (range 42-83) and included 15 male (47%). Response was assessed using IMWG criteria. Adverse events were graded with NCI-CTC, v3.0. *Results:* After a median follow-up of 6 months (1-35) response rates could be evaluated in 20 patients (63%) with a median of 3.5 cycles (1-9) administered. Median number of prior lines therapies were 2 (1-6).Previous schedules received were: alquilant agents 45%, bortezomib 48%, IMiDs-containing regimens 33% and ASCT 12%.Overall response rate was 70% (20% CR, 5% nCR, 15% VGPR and 30% PR).Toxicity was evaluated in all the patients. Grade 3-4 adverse events were anemia 16%, neutropenia 16%, thrombocytopenia 16% and gastrointestinal symptoms 9%. Peripheral neuropathy was reported in 41%, including 16% grade 3-4. Ten patients (31%) discontinued treatment, 4 because adverse events. OS was 87.5%. *Conclusion:* VMP schedule is safe and effective in previously treated MM patients

and could be considered as alternative for patients with relapsed/refractory MM.

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EXPLORATORY ANALYSIS OF PROGNOSTIC FACTORS FOR TANDEM TRANSPLANTS FOR MULTIPLE MYELOMA (MM) IN BMT CTN0102

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BMT CTN 0102 MM trial compared melphalan 200mg/m2 (MEL200) auto-auto with auto-allo using MEL 200 followed by sibling allogeneic transplant (AlloTx) with 2 Gy TBI, assigned according to donor availability among patients with MM after initial therapy. The primary endpoint was 3-year progression-free survival (PFS) in pts with standard risk (SR) MM (absence of deletion 13q and ♀2 microglobulin <4.0mg/L). 625 patients with SR were enrolled (auto-auto, n=436; auto-allo, n=189). The groups differed by age (median auto-auto 55y vs. auto-allo 53y, p <0.01) and race (more African Americans in auto-auto (18% vs 10%, p=0.03). Three year PFS was 46% auto-auto and 43% auto-allo (p=0.67) and overall survival (OS) was 80% auto-auto and 77% auto-allo (p=0.19). Exploratory multivariate analysis (MVA) comparison between treatment arms revealed that assignment to auto-allo was associated with poorer OS (HR 1.45 p= 0.02) but not PFS (HR 1.14 p=0.26). Among all factors analyzed, disease status, gender, age, CMV, Durie Salmon (DS) stage significantly impact survival outcomes. The effect of treatment was different by DS stage (interaction p<0.01). Among patients with DS I/II, HRs for treatment failure and overall mortality were 1.8 (p<0.01) and 2.9 (p<0.01). Corresponding HRs for DS III were 0.9 (p=0.6) and 1.1 (p=0.5). In conclusion, auto-allo was associated with higher treatment failure and mortality in patients with early DS. Further investigation in AlloTx for MM should focus on patients with high risk of relapse and mortality from disease progression.

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BORTEZOMIB INDUCTION FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN PATIENTS WITH MULTIPLE MYELOMA (MM): ACHIEVING RESPONSE TO BORTEZOMIB INDUCTION AND CR POST-ASCT ARE BOTH IMPORTANT PROGNOSTIC FACTORS.

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In MM, the association between the response to induction before ASCT and long-term outcome is less clear but the situation may change with the introduction of novel agents. We assessed whether the extent of response to bortezomib induction leads to improvement in outcomes. We retrospectively assessed 178 MM patients who received bortezomib-containing induction therapy (BTZIT) followed by ASCT in 24 institutions throughout Korea. Records of these patients were reviewed using the Korean Myeloma Registry database (www.myeloma.co.kr). Forty nine (27.5%) received bortezomib as front-line therapy and 129 (72.5%) as second-line treatment. After BTZIT, the response rates in this selected series of patients were 38.8% CR, 10.7% VGPR, 41.0% PR, 7.3% SD and 2.2% PD; the corresponding post-ASCT rates were 70.6% CR, 10.6.% VGPR, 11.9% PR, 3.8% SD and 3.1% PD. Eighty (44.9%) patients were treated with maintenance therapy. At a median follow-up of 29.3 months, the 2-year OS and EFS rates were 77.9% and 71.0%, respectively. Multivariate analysis showed that factors independently predictive of OS and EFS included achievement of BTZIT response ≥PR (P=.0001 and P=.019, respectively) and the treatment with maintenance therapy (P=.008 and P=.0001, respectively). Post-ASCT CR vs. ≤VGPR was also an independent prognostic factor for OS (P=.007). At least PR to BTZIT and CR after ASCT were predictive of survival. These findings suggest that patients who responded to BTZIT may benefit from ASCT due to an enhanced quality of response. Maintenance therapy can also affect patient outcomes.

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THE DOSE OF INFUSED NKT CELLS IN THE AUTOGRAFT DIRECTLY CORRELATES WITH LYMPHOCYTE RECOVERY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN MULTIPLE MYELOMA (MM)

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Early lymphocyte recovery after ASCT in MM is an important predictor of survival. The dose of infused lymphocytes in the autograft directly correlates with early lymphocyte recovery (Porrata LF et al. Leukemia 2004;18:1085, Hiwase DK et al. Biol Blood Marrow Transplant 2008;14:116). However, limitations of previous studies are the lack of lymphocyte subset analyses. Thirty-two patients with MM who underwent ASCT were examined to investigate the correlation between infused cell populations and lymphocyte subsets at engraftment. The cell populations of infused autograft and lymphocyte subsets of peripheral blood at engraftment were examined by flow cytometry. Immunophenotyping was performed for the T cell panel (CD3/CD4/CD8), B cell (CD19), and natural killer (NK) cells (CD56/16). By Spearman correlation coefficients, we identified a correlation between absolute lymphocyte count (ALC) at engraftment and each lymphocyte subset at engraftment. The cell dose of infused NKT cells ($CD3^+CD56^+CD16^+$) was significantly associated with $CD3^+$ (rs = 0.435, P = 0.013), $CD4^+$ (rs = 0.455, P = 0.009), $CD8^+$ (rs = 0.399, P = 0.024), $CD19^+$ (rs = 0.392, P = 0.027), and ALC (rs = 0.395, P = 0.025) at engraftment. On the contrary, the dose of infused $CD34^+$ cells was not associated with changes of any lymphocyte subsets. In addition, the recovery of $CD4^+$ cells was significantly associated with the dose of infused $CD3^+$ and $CD8^+$ but not CD4+ cells. Our data suggest that a certain number of NKT cells as well as the number of $CD34^+$ cells should be aimed for successful ASCT in MM.

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LENALIDOMIDE AS POST TRANSPLANT CONSOLIDATION-MAINTENANCE THERAPY IN ELDERLY MULTIPLE MYELOMA PATIENTS: UPDATED RESULTS OF A PHASE II STUDY.

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Bortezomib-dexamethasone-doxorubicin (PAD) induction, followed by reduce-intensity autologous transplantation proved to be safe and effective in elderly newly diagnosed multiple myeloma (MM) patients (pts). Lenalidomide is less neurotoxic than thalidomide and represents an optimal agent to include in maintenance strategies. In this analysis, efficacy and safety end-points of post transplant lenalidomide consolidation and maintenance were updated. 102 newly diagnosed pts received PAD induction followed by tandem melphalan 100 mg/m2 and stem-cell support and consolidation with four 28-day cycles of lenalidomide-prednisone (LP), followed by lenalidomide maintenance (L) until relapse. LP-L therapy improved post transplant responses: VGPR rate raises from 82% to 92%, CR rate from 38% to 71%: 1 patient improved from SD to PR, 1 from SD to VGPR, 1 from PR to VGPR, 2 from PR to CR, 16 from VGPR to CR. After a median follow-up of 3 years, the 3-year progression-free survival (PFS) was 66%, the 3-year time-to-progression was 73% and the 3-year overall survival (OS) was 85%. For pts who achieved CR 3-year PFS and OS were 81% and 100%, respectively, patients with low risk cytogenetic abnormalities and who achieved CR, have 3-year PFS of 100%. LP-L treatment was well tolerated, grade 3–4 adverse events included neutropenia (31%), thrombocytopenia (15%), pneumonia (8%), cutaneous rash (7%). This sequential approach improved depth and rate of response, was well tolerated, and is a valid treatment strategy for elderly MM pts eligible for reduced-intensity ASCT.

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BENDAMUSTINE-BORTEZOMIB-DEXAMETHASONE FOR RELAPSED/REFRACTORY MYELOMA

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Bendamustine - an alkylating substance with purine analogue like activities - exerts high activity when combined with bortezomib in myeloma. Here we evaluated the efficacy of the triple combination in patients with relapsed/refractory myeloma. 20 patients have been enrolled so far, data documentation is available for 15 patients. Median age 62 yrs (range 46-86), male/female: 6/9, ISS stage I: 2, stage II: 5, stage III: 8, Previous treatment lines 1-2: 10, 3-4: 4 or >4: 1. 10 of 15 patients had previously been exposed to bortezomib. *Treatment regimen:* Bendamustine 70 mg/m2 day 1+4, Bortezomib 1.3 mg/m2 days 1, 4, 8 and 11, Dexamethasone 20 mg on days 1, 4, 8 and 11, repeated every 4 weeks. Responses are presently evaluable in 10 patients (≥ 2 cycles completed and documented). ORR was 60% with 2 patients (20%) achieving nCR, 1 (10%) PR, and 3 (30%) MR, respectively. Median time to response was 87 days. Responses were observed in 5/10 patients previously exposed to bortezomib therapy. Grade 3/4 toxicities were observed in 14 documented patients as follows: Anemia 3 (21%), thrombopenia 6 (43%), leukopenia 1 (7%), acute kidney injury 2 (14%), diarrhea 1 (7%), and sepsis 1 (7%). Interestingly, no grade 3/4 polyneuropathy had been observed. *Conclusion:* The BBD combination exerts significant activity (ORR: 60%) in heavily pretreated relapsed/refractory myeloma with a favorable toxicity profile. Updated results will be presented at the meeting.

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BORTEZOMIB AND STEM CELL TRANSPLANTATION (SCT) IN MYELOMA PATIENTS WITH DIALYSIS-DEPENDENT RENAL FAILURE

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Patients with multiple myeloma (MM) and dialysis-dependent renal failure have particular poor outcomes. Bortezomib has been reported to be safe and efficacious in patients with renal impairment. In this retrospective case analysis, we evaluated 24 consecutive patients requiring hemodialysis at the time of first-line induction therapy followed by stem cell mobilization, high-dose therapy and autologous SCT treated at our center between 2002 and 2010. With respect to MM subtypes, 10 patients had Bence-Jones, 10 IgG, 2 IgA, 1 IgD, and 1 hyposecretory MM. For induction therapy, 10 patients received PAD (bortezomib, doxorubicin, dexamethason) and were compared to 14 patients who received VAD or a VAD-like induction regimen. Patient characteristics were comparable between the two groups. No significant differences were seen in the duration of dialysis-dependency, recovery of renal function, or ß2-microglobuline. However, the overall response rate (PR or better) was significantly better after PAD induction prior to SCT (90% vs. 36%, p=0,018), as well as at day +100 post SCT (100% vs. 58%, p=0,04). The estimated median event-free and overall survival has not yet been reached in the PAD group compared to 28 months and 35 months, respectively, without bortezomib. In conclusion, these data confirm the promising efficacy of bortezomib in first-line induction therapy followed by high-dose therapy and autologous SCT for patients with end-stage renal failure.

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A SALVAGE TREATMENT CONTAINING NOVEL AGENTS CONSOLIDATED BY ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED-INTENSITY CONDITIONING IMPROVES OUTCOME OF MULTIPLE MYELOMA PATIENTS FAILING AUTOLOGOUS TRANSPLANTATION

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The use of allogeneic stem cell transplantation (allo-SCT) in multiple myeloma (MM) is controversial due to mortality and morbidity related to procedure and availability of novel antimyeloma drugs. We investigated the role of reduced-intensity-conditioning (RIC) allo-SCT in MM patients who relapsed after autologous SCT, were treated with a salvage therapy based on novel agents and underwent a search for a donor. Our study was structured similarly to an intention to treat analysis and included only those patients undergoing a HLA-typing immediately after the failure of auto-SCT. Seventy-five of 169 patients (44%) found a donor (donor group) and were compared with 96 patients (56%) not having a suitable donor (no-donor group). Sixty-eight underwent RIC allo-SCT (24 siblings, 44 unrelated). Two-year cumulative incidence of non-relapse mortality (NRM) was 22% in the donor group and 1% in the no-donor group ($p<0.0001$). Two-year PFS was 42% in the donor-group and 18% in the no-donor group ($p<0.0001$). Two-year OS was 54% in the donor-group and 53% in the no-donor group ($p=0.329$). In multivariate analysis, lack of a donor was a significant unfavorable factor for PFS, but not for OS. Lack of chemosensitivity after salvage treatment and high-risk karyotype at diagnosis significantly shortened OS. In patients who underwent allo-SCT, the development of chronic GVHD had a significant protective effect on OS. This study provides evidence for a significant PFS benefit of salvage treatment with novel drugs followed by RIC allo-SCT in relapsed MM patients having a donor.

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PHASE I-II STUDY OF MELPHALAN, THALIDOMIDE AND PREDNISONE (MPT) COMBINED WITH ORAL PANOBINOSTAT IN PATIENTS WITH RELAPSED/REFRACTORY MM

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In MM Panobinostat (Pan) showed additive/synergistic activity with steroids and/or immunomodulatory drugs. This multicenter, phase I-II study explored the combination MPT (Melphalan 0.18 mg/kg for 4 days, Prednisone 1.5 mg/kg 4 days, Thalidomide 50 mg/day) with Pan 15 mg p.o. thrice weekly for 3 weeks in a 28-day cycle to assess safety profile and activity in patients with relapsed/refractory MM. Study was designed according to the Briant and Day method. The protocol was amended after 13 patients were enrolled due to an excessive and persistent grade 3-4 hematologic toxicity; therefore, Pan was reduced to 10 mg. Median age of 24 patients enrolled was 71.5 years and 21% had received 2 or more prior lines of therapy. Using Pan 15 mg, grade 3-4 thrombocytopenia, neutropenia and non-hematological adverse events occurred in 46%, 69% and 31% of patients, respectively. In the 11 patients receiving Panobinostat 10 mg, grade 3-4 thrombocytopenia decreased to 18% but rate of neutropenia was longer high (72.5%). Three patients (27%) had grade 3-4 non-hematological toxicity. Dose adjustment was necessary in 9 patients, while 6 patients interrupted the protocol because of side effects. One patient died on study due to sepsis. At least PR was observed in 12 patients (50%) including 4 VGPR. Ten patients had SD and 2 progressed. Notably, response was obtained also in 2/7 patients who had progressed during bortezomib or IMIDs. In conclusion, MPT-Pan exerts an encouraging anti-myeloma activity but different schedules of administration should be explored to reduce haematological toxicity.

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FREE LIGHT CHAIN RATIO AS A MARKER OF COMPLETE REMISSION

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Introduction: Stringent complete remission (sCR) in multiple myeloma (MM) has been defined as complete remission (CR) with normal free light chain ratio (FLCr). However only a few studies have so far incorporated FLCr as the marker of the outcome in MM patients. *Aim:* Goal was to evaluate CR patients and compare the relapse rate in the group with normal FLCr and abnormal FLCr. *Patients and Methods:* From 2008 we incorporated FLCr as a marker of CR in our MM patients. We included patients who remained in CR from previous time and evaluated for sCR. Patients from two institututions were included (Brno and Hradec Kralove). Free light chains were evaluated using FreeLite (Binding Site) and also ELISA method (BioVendor). *Results:* 63 MM patients in CR were included. When FreeLite was used, 31 patinets fulfilled criteria for sCR and 32 did not. When ELISA was used, 49 patients met criteria for sCR and 14 did not. During the 2-year follow-up, only 4 relapses occured. 3 patients from sCR group relapsed versus one patient from non-sCR group. Many discordant results exist when both methods are compared - 27 samples showed different ratios (normal or abnormal). *Conclusion:* So far only a few relapses occured, however it seems that in both groups the relapse rate seems to be the same regardless of FLCr status. Further longer follow-up is needed to confirm the significance of FLCr. Standardisation of methods used for FLCr evaluation is needed if we are to incorporate FLCr as a standard of CR evaluation. Supported by IGA MZ CR NS/10387-3 and NS/10406-3 and by research project MZO00179906.

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BORTEZOMIB RETREATMENT IN PATIENTS (PTS) WITH RELAPSED MULTIPLE MYELOMA (MM) IN SWITZERLAND

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Background: Several studies have shown that bortezomib (Vc) retreatment in pts with relapsed/refractory MM is effective, even in heavily pretreated pts. *Methods:* Retrospective survey conducted at 26 Swiss centers. Adults with relapsed MM who had responded to prior Vc treatment (≥PR) were eligible to receive Vc retreatment, monotherapy or in combination. From 2005–2009, data from pt forms and clinical records were collected to evaluate the efficacy and safety of Vc retreatment in pts with MM. Pts were heavily pretreated and received a mean of 3 prior therapies (range 1–11) before initial Vc treatment. *Results:* 42 pts were response-evaluable: mean age at retreatment was 66 yrs. 86% of pts were treated with Vc monotherapy and 14% received combination therapy for retreatment. Pts received a mean of 4 cycles of Vc retreatment. One-third of pts achieved CR/nCR to initial Vc therapy. At retreatment, ORR was 64% (95% CI 48, 78); 3 pts had repeat CR. Median duration of second response in 35 evaluable pts was 12.6 months. Following Vc retreatment, median TTP was 10.5 months and median TFI was 5.7 months. Median OS after Vc retreatment was 1.7 yrs. 19 AEs were reported; the majority were mild-to-moderate. Most common AEs: nervous system (12%), heme-lymphatic (7%), and general (7%) disorders. *Conclusions:* This retrospective survey in the Swiss clinical setting demon-

strates that Vc retreatment is effective and well tolerated in pts with relapsed MM who have responded to prior Vc therapy. This survey suggests that initial Vc treatment does not appear to cause Vc resistance.

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A PHASE I TRIAL OF IPH-2101, A NOVEL ANTI-INHIBITORY KIR ANTIBODY, IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) cells upregulate MHC class I as ligands to inhibitory killer immunoglobulin-like receptors (KIR) on natural killer (NK) cells. IPH-2101, an anti-KIR antibody, enhances NK cell function against cancer by disrupting inhibitory KIR/ligand relationship. *Methods:* A dose-escalation study was conducted in previously treated patients (pts) with MM in 7 cohorts (0.0003 to 3mg/kg IV q28-d for up to 4 cycles) in a 3+3 design (with a 7-pt extension at 3mg/kg in pts with one prior therapy). Pharmacokinetic (PK), pharmacodynamic (PD), safety and biologic data were collected. *Results:* 32 pts (med age = 61, prior therapies = 1-7) were treated. Full KIR saturation over dosing interval was achieved without DLT. The median number of IPH-2101 doses received was 2, 31% of pts received all 4 doses. 12% of AEs were reported (mild or moderate) "possibly" or "probably" related to IPH-2101. 1 SAE occurred (acute renal failure). PK and PD data closely approximated preclinical models, IPH-2101 had no effect on NK cell maturation but enhanced ex vivo NK cell cytotoxicity against MM. 12 pts had stable disease. *Conclusions:* IPH-2101 is safe and tolerable with doses that achieve full KIR saturation over the dosing interval. Cmax and KIR occupancy correlate, and IPH-2101 enhances NK cell cytotoxicity against MM. 38% of pts achieved a best response of stable disease. Phase II studies in maintenance after autologous SCT and in smoldering MM as well as a phase I/II trial of IPH-2101 and lenalidomide are underway. Final results will be presented.

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THE THERAPEUTIC EFFECT OF LENALIDOMIDE IS ENHANCED AFTER ALLOGENEIC TRANSPLANTATION

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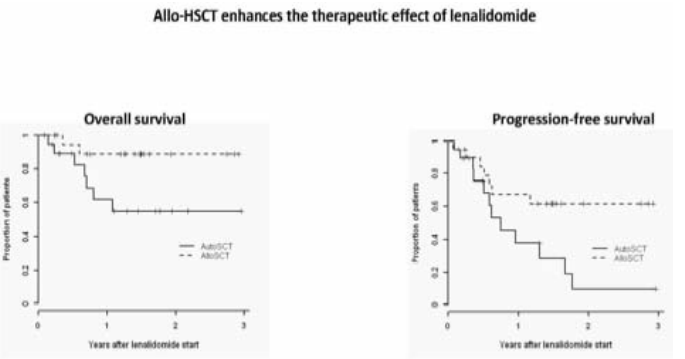
Immunomodulatory properties of Lenalidomide (Len) may enhance the graft-versus-myeloma (GVM) effect after allogeneic hematopoietic stem cell transplantation (Allo-HSCT). To verify this hypothesis, we are conducting a case-matched analysis of Len after autologous stem cell transplantation (Auto-HSCT) or Allo-HSCT. We present the data from 20 patients in each group. Baseline characteristics between Auto and Allo patients were similar, including number and type of previous treatments. Eighteen of 20 Allo patients received Allo-HSCT after the first line. Sixteen (80%) Auto and 19 (95%) Allo patients received bortezomib in previous lines; 14 (70%) Auto and 12 (60%) Allo patients were previously treated with thalidomide. Len was always combined with dexamethasone. Best responses for Auto and Allo patients were as follows: 2 vs. 2 CR, 4 vs. 7 VGPR, 3 vs. 4 PR, 4 vs. 3 SD, 7 vs. 4 PD. Time to the best response was 2.5 months (range 1-6) for Auto, and 3.5 months (range 1-19) for Allo patients. One year and 2 year progression-free survival were 37% and 9% for Auto patients, and 67% and 61% for Allo patients (p=0.03), respectively. Two years overall survival was 55% for Auto and 89% for Allo patients (p=0.03) (Figure). Similar results were observed regardless of previous thalidomide treatment. No unexpected toxicities were reported. Two (10%) patients had a worsening of a pre-existent extensive chronic GVHD. In conclusion, the preliminary data of our study support the hypothesis that Len is synergistic with the GVM effect, still retaining a favourable toxicity profile.



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A PHASE 3, RANDOMIZED, INTERNATIONAL STUDY (MMY-3021) COMPARING SUBCUTANEOUS (SC) AND INTRAVENOUS (IV) ADMINISTRATION OF BORTEZOMIB IN PATIENTS (PTS) WITH RELAPSED MULTIPLE MYELOMA (MM)

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Bortezomib (VELCADE®, Vc) is administered as a 3- to 5-sec bolus IV injection. SC administration may be a good alternative for pts with poor venous access. This multicenter study compared SC and IV Vc in relapsed (1-3 prior lines of therapy) MM pts. 222 pts were randomized 2:1 to SC (reconstituted to a final concentration of 2.5 mg/mL in normal saline) or IV (1 mg/mL) Vc 1.3 mg/m^2 on days 1, 4, 8, 11 (21-day cycles); after 4 cycles; if <CR (without progression), oral Dex 20 mg could be added (day of and after Vc). Response was assessed by EBMT criteria plus nCR/VGPR. A 32-pt substudy assessed PK/PD. Efficacy was comparable with SC and IV Vc (Table). Vc exposure (AUC) was equivalent and proteasome inhibition (AUE) comparable, while Cmax was lower and Tmax longer with SC vs IV Vc. There were fewer grade ≥3 AEs (57% vs 70%), including peripheral neuropathy (PN) events (6% vs 16%; p=0.03) with SC vs IV Vc; 31% vs 43% of pts had Vc dose reductions and 22% vs 27% discontinuations due to AEs. SC injection site reactions were reported as AEs in 6% of pts. In conclusion, SC and IV Vc showed similar efficacy and equivalent systemic exposure in pts with relapsed MM. SC Vc appeared to have an improved safety profile, with a significant reduction in PN compared with IV Vc.

	IV, n=73	SC, n=145
ORR (CR+PR) after 4 cycles, %	**42**	**42**
CR/nCR, %	14	12
≥VGPR,%	16	17
ORR after 8 cycles (±Dex), %	**52**	**52**
Median TTR (responding pts), mo	**1.4**	**1.4**
Median DOR, mo	**8.7**	**9.7**
	n=74	**n=148**
1-year OS, %	**76.7**	**72.6**
Median TTP, mo	**9.4**	**10.4**
	n=14	**n=17**
Mean AUC_{last}, ng.h/mL	151	155
Mean C_{max}, ng/mL	223	20.4
Median T_{max}, hr	0.03	0.50
Mean AUE_{72}, %.hr	1383	1714

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CLINICAL PHARMACOKINETICS (PK)/PHARMACODYNAMICS (PD) OF INTRAVENOUS (IV) AND ORAL (PO) MLN9708, AN INVESTIGATIONAL PROTEASOME INHIBITOR, IN FOUR PHASE 1 MONOTHERAPY STUDIES

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Introduction: Investigational drug MLN9708 is an orally bioavailable, potent, reversible, and specific inhibitor of the 20S proteasome in phase 1 trials of hematologic malignancies and solid tumors. We report preliminary PK/PD data for IV and PO dosing on a twice-weekly (Days 1, 4, 8, 11; 21-day cycle) and weekly (Days 1, 8, 15; 28-day cycle) schedule. MLN9708 completely hydrolyzes to pharmacologically active MLN2238 in aqueous solution. *Methods:* Preliminary PK/PD data collected after the first and last doses of MLN9708 in Cycle 1 from two IV (n=34) and two PO studies (n=14) were analyzed using noncompartmental methods (WinNonlin software v5.2). PK samples were analyzed using LC/MS/MS with a LLOQ of 0.5 ng/mL; PD samples were analyzed for 20S proteasome chymotryptic-like activity using a fluorometric-based assay. *Results:* With both IV dosing schedules, MLN2238 showed multi-exponential plasma disposition and similar PK, but less accumulation with weekly dosing (2-fold on day 15 vs. 3-fold on day 11). Following PO dosing, MLN2238 was rapidly absorbed (T_{max} ~1 hr) with t1/2 (similar to IV) of 5–7 days. At the 1.76 mg/m^2 MTD for the IV twice-weekly schedule, E_{max} was ~60% at 0.08 hr. PD effect was immediate and dose dependent, as indicated by no hysteresis in individual PK/PD plots at all doses. For PO dosing, E_{max} appeared to be ~60–72% at the 2.23 mg/m^2 dose (time to E_{max}: 0.5–2 hr). *Conclusions:* Dose-dependent increases in drug exposure and 20S proteasome inhibition were observed with both IV and PO dosing, supporting continued clinical evaluation of MLN9708.

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A COMPARISON OF IMMUNOFIXATION, SERUM FREE LIGHT CHAIN AND IMMUNOPHENOTYPING FOR RESPONSE EVALUATION AND PROGNOSTICATION IN MULTIPLE MYELOMA

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Purpose: Investigate the impact of immunophenotypic response (IR) versus complete response (CR) and CR plus normal serum free light chain (sFLC) ratio ("stringent CR") in elderly multiple myeloma (MM) pts treated with novel agents. *Methods:* From a total of 260 elderly newly diagnosed MM pts included in the GEM05>65y trial, 102 pts achieving at least a PR with ≥70% reduction in M-protein after 6 induction cycles were simultaneously analyzed by immunofixation (IFx), sFLC and multiparameter flow cytometry (MFC). *Results:* 43% of patients achieved CR, 30% "stringent CR" and 30% IR. Patients in "stringent CR" showed no significant survival advantage compared with those in CR, while pts in IR showed significantly increased PFS and TTP than those in "stringent CR" or CR; this was confirmed by multivariate analysis (HR=4.1; P=.01 for PFS). Discrepancies between techniques were relatively common; notably, in all 7 pts achieving IR but remaining IFx positive the M-component disappeared in follow-up analysis. In contrast, MFC positive pts who were IFx negative (n=20) showed a tendency towards early reappearance of the M-protein (median: 3 months). Similarly, in 5 of 11 "stringent CR" but MFC positive pts, symptomatic disease progression was recorded at a median of 13 months after induction. *Conclusions:* Achieving an IR translates into superior PFS and TTP compared with conventional CR or "stringent CR". These techniques provide complementary information and thus, an effort should be made to refine response criteria in MM.

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THE PROGNOSTIC IMPACT OF SFLCR-ISS COMBINATION IS CONCERVED IN MM PATIENTS TREATED WITH NEW AGENTS BUT NOT IN THOSE THAT UNDERWENT ASCT

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Introduction: Improved power of prognostic models combining sFLCR and ISS has been shown. It is however unknown whether such models remain prognostic in patients receiving new agents or who underwent autologous bone marrow transplantation (ABMT). *Aims:* To evaluate the prognostic impact of sFLCR-ISS prognostic model in a multicenter study including 350 MM patients at presentation. *Patients and Methods:* 77% of patients were symptomatic and required treatment, 21% underwent ASCT, 69% received novel agents. sFLCR values and ISS were significant for survival in multivariate analysis. A prognostic model combining ISS and sFLCR was generated and separated 3 patients' groups. The low-risk had "low" sFLCR (below or equal to median) and ISS stage 1, the high-risk "high" sFLCR and ISS stage 3 and the intermediate risk group had any other combination. Survival significantly differed among groups (p<0.00001). The model improved sFLCR and ISS prognostication; it kept its prognostic power in patients that received new agents (p<0.001) but failed in those that underwent ASCT. *Conclusions:* The combination of sFLCR with the ISS improves ISS prognostication and can be easily used in everyday practice for risk-stratification of patients not eligible for ASCT. This finding is of important value given that, MM is mainly a disease of the elderly. For patients eligible for ASCT, that remains possibly the most potent modality, other prognostic approaches are needed.

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LENALIDOMIDE (REVLIMID), BORTEZOMIB (VELCADE) AND DEXAMETHASONE (RVD) FOR HEAVILY PRETREATED RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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The combination of revlimid (rev), velcade (vel), and dexamethasone (dex) (RVD) has shown excellent efficacy in relapsed/refractory (rel/ref) multiple myeloma (MM) patients (pts). The aim of our study is to assess the efficacy and toxicity profile when len is combined with vel and dex for rel/ref MM. Methods We retrospectively reviewed the records of all pts with rel/ref MM treated with RVD at our center between 03/09-01/11. Twenty-one pts received at least 1 full cycle of RVD as described

by Anderson K, et al. Primary endpoints were response rate, time to progression and toxicity. Responses were assessed according to modified EBMT criteria. Results Median age was 57 years and 61% were female. In many instances, pts previously treated with len had len added to btz + dex at progression (n=6), or pts previously treated with btz had btz added to len + dex, at progression (n=5). After a median of 4.6 cycles (1-14), VGPR was seen in 4.8%, PR in 33% and SD in 14% (ORR of 52.4%). Disease progression was seen in 16 pts at a median of 3.9 months (1-13.6 mon). Six pts experienced grade 3/4 adverse events, including anemia, neutropenia, muscle weakness, and pneumonia. No patient experienced worsening of peripheral neuropathy. Conclusions The ORR for our patient population was 52.4% with no patients achieving CR and the median TTP was also short at 3.9 months. Although RVD has been shown to be effective, our data suggests that responses/duration to RVD are affected by very advanced disease stage at relapse and the extent of prior treatment with novel agents.

Table 1. Clinical characteristics of 21 patients with relapsed/refractory multiple myeloma treated with RVD.

Clinical Characteristics	Median	Range	%
Age (yrs)	57	37-71	
Hemoglobin (g/L)	111.3	71-155	
Creatinine (mmol/L)	99.76	36-383	
Beta2-microglobulin (mmol/L)	227.4	119-1440	
Lactate dehydrogenase (U/L)	169	89-255	
Males	4 (2.7)	1 (0.7)	38.1
Females			61.9
IgG			52.4
IgA			23.8
IgM			4.8
Light chain			19
Kappa (mg/L)	583	5.3-3460	
Lambda (mg/L)	576	5.1-5300	
Kappa			57.1
Lambda			42.9
BPMC*	48%	6-95%	
M-spike serum (g/L)	28	0-77	
M-spike urine (g/d)	0.62	0-7.9	
Prior therapies	3	1-6	
ASCT			90.5
Thalidomide			61.9
Lenalidomide			85.7
Bortezomib			76.2

*Bone marrow plasma cells.

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15 YEARS OF SINGLE CENTER EXPERIENCE WITH STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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Introduction: Autologous stem cell transplantation (ASCT) became standard of care for patients with multiple myeloma (MM) under the age of 65 years. We routinely perform ASCT for newly diagnosed MM since 1996 in our department. *Patients and Methods:* We retrospectively analyzed all 285 transplants in 185 patients done for MM from January1996 till December 2010. 270 transplants were autologous and 15 were allogenic. Median age of patients was 57 years (27-75). We analyzed overall survival (OS) and progression-free survival (PFS) regarding conditioning, stage, complete or very good partial remission (CR, VGPR) achievement, renal impairment, single vs. double transplant. *Results:* Estimated 10-years survival of the whole set of patients is 39% (median survival 55 months). Patients with renal impairment show same OS as those without (p=0.22). We observed better outcome in terms of overall survival in patients treated with new drugs (p=0.001). Reaching CR or VGPR was surprisingly not translated into better OS (p=0.30) and EFS (p=0.10). Also stage of the disease and whether single or double transplant was used did not make any significant difference in the outcome. *Conclusion:* Stem cell transplantation greatly improved outcome of patients with MM. Poor outcome of allogenic transplantation in our group of patients is related to high transplant related mortality (20% vs. 0%) and unexpected high relapse rate. There is an obvious trend towards better survival, when new drugs are incorporated at any time in the course of the disease as an addition to ASCT.

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VERY GOOD PARTIAL RESPONSE AND COMPLETE RESPONSE PREDICT SUPERIOR OVERALL SURVIVAL AND PROGRESSION FREE SURVIVAL AFTER SINGLE AUTOLOGOUS STEM CELL TRANSPLANT IN PATIENTS WITH MULTIPLE MYELOMA

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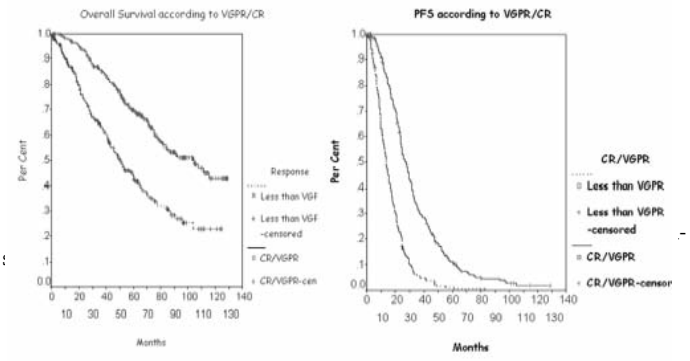
In multiple myeloma (MM), the impact of complete response (CR) and very good partial response (VGPR) achievement has been shown mostly after introduction of high dose therapy (HDT) supported by autologous stem cell transplant (ASCT). Recently, the IFM group reported the impact of achievement of CR and VGPR in double ASCT. The purpose of this study is to confirm the prognostic value of CR/VGPR in a large group of patients treated with single ASCT. Methods All consecutive patients who underwent single ASCT at our Institution between 01/00-12/06 were evaluated. Response to therapy was assessed according to the IMWC including VGPR. Progression Free Survival (PFS) and Overall Survival (OS) were measured from transplant date to the date of death or last follow-up. OS and DFS were analyzed using the Kaplan-Meier Method. The Cox proportional hazard model was used to assess CR and VGPR. All p-values were 2-sided and statistically significant if <0.05. Results Six hundred and twelve patients were identified for the study; their median age was 56 years (30-73) and 40% were female. All received induction therapy before ASCT. The median OS and PFS were significantly better for patients who achieved CR/VGPR, 103 months versus 50.6 months, and 27.3 months vs 13.5 months respectively. Multivariate analysis shows CR/VGPR as an independent prognostic factor for OS and PFS (Fig 1 and 2). In conclusion, VGPR/CR remains a simple and



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PERIPHERAL BLOOD STEM CELL COLLECTION IN PATIENTS UNDERGOING INDUCTION THERAPY WITH LENALIDOMIDE BASED REGIMENS: FAILURE RATES AND SALVAGE APPROACHES

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Objective: To describe the failure rates and salvage approaches in peripheral blood stem cell collection in patients (pts) with Multiple Myeloma undergoing induction therapy with lenalidomide. Patients: 224 pts seen between July 2004 and December 2009 receiving initial therapy with lenalidomide were studied. *Results:* 245 collection attempts were made from among 224 pts. 21 (9.8%) pts attempted to remobilize after failing to reach the goal with the first attempt. For the initial collection attempt, the median duration of lenalidomide therapy prior to collection was 4 months (range; 1, 26) and the mobilization strategies were GCSF in 151 (67%), Cyclophosphamide + GCSF in 29 (13%), and GCSF + AMD in 44 (20%) patients. Overall 15 pts (7%) failed to reach the peripheral CD34 cell counts required to initiate apheresis, and among those starting apheresis 6 pts failed to collect at least 2 million CD34 cells/kg; a cumulative failure rate of 9%. 21 pts reattempted stem cell mobilization; GCSF, Cyclophosphamide + GCSF, GCSF + GM-CSF, GCSF + AMD in 5, 8, 3, and 4 pts respectively. All pts collected at least 2 million CD34 cells/kg and 14 pts (70%) collected more than 4 million

CD34 cells/kg. The median CD34 cells collected with the second attempt was 5.4 million/kg (range; 2, 19.5) bringing the median total collection to 9.6 million/kg (2.6-19.6). *Conclusion:* While the failure rate of stem cell collection in pts receiving initial therapy with lenalidomide is 10%, majority of them can be salvaged with a second collection to proceed to a stem cell transplant if so desired.

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SECOND AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) AS SALVAGE THERAPY FOR MULTIPLE MYELOMA: IMPACT ON PROGRESSION FREE AND OVERALL SURVIVAL

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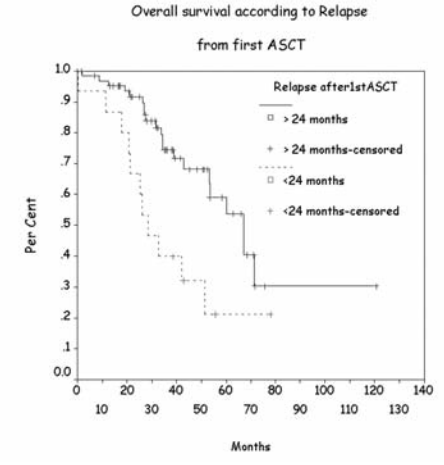
The role of a second ASCT as salvage therapy outside the setting of tandem ASCT is unclear, particularly with the availability of novel agents to treat relapse MM. Methods We retrospectively reviewed all MM patients who received a 2nd ASCT as salvage therapy at our center between 03/92 –12/09. Results Eighty-one patients received a 2nd ASCT for relapsed MM. The median time to relapse after first transplant was 39 months. All patients received re-induction therapy before second ASCT. Conditioning for second ASCT consisted of MEL+TBI +/- Etoposide in 2, BU/Cy in 1 and MEL in the rest. Overall response after 2nd ASCT was seen in 78 (97%), including: CR in 6 (8)%, VGPR 32 (40%) and partial response were seen in 40, (50%). The median time to relapse post-2nd ASCT was 19 months (3.7-79). Median progression-free survival (PFS) based on the time to myeloma relapse after first ASCT was 9.83 months (relapse ≤ 24 months), 16.70 months (relapse >24 but <36) and 21.17 months (relapse >36 months) (p=0.08). Median overall survival (OS) was 28.47 months (relapse ≤ 24 months), and 71.3 months (relapse of >24 months) (p=0.006). (Fig 1) Conclusions Second ASCT is feasible and safe salvage therapy with early deaths of less than 2% in our group. The greatest benefit was observed in patients whose time to progression was >24 months after the first ASCT, with subsequent remission lasting over 1 year, and a significant improvement in OS. A second ASCT should be considered in patients whose disease relapses > 24 months after first ASCT.



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EFFECTS OF CYTOGENETICS ON RESPONSES AND SURVIVAL IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (R/R MM) TREATED WITH SINGLE-AGENT CARFILZOMIB (CFZ)

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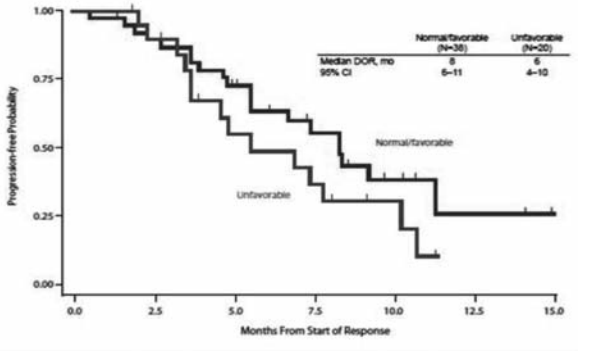
Introduction: This was a retrospective analysis of effects of abnormal cytogenetics (poor prognostic factors) in a phase 2b study (PX-171-003-A1) of single-agent carfilzomib (CFZ; a highly selective proteasome inhibitor) in pts with R/R MM. *Methods:* Pts with R/R MM received CFZ 20 mg/m^2 IV on days 1, 2, 8, 9, 15, and 16 in a 28-day cycle for cycle 1 then 27 mg/m^2 for each cycle for ≤12 cycles. 229/266 enrolled pts (86%) had metaphase cytogenetics for hypodiploidy or chromosome 13 deletions (200/266 pts; 75%) and/or fluorescence in situ hybridization (FISH) analyses (205/266 pts; 77%) for del 17p13, t(4:14), or t(14;16) chromosomal abnormalities. Overall response rate (ORR; partial response or better) and clinical benefit response (CBR; ORR + minimal response) were assessed. *Results:* Pts had a median of 5 prior regimens; 99.6% had prior bortezomib. 71/229 pts (31%) had ≥1 cytogenetic abnormality. For the 71 pts with an abnormality, ORR was 28% vs 24% in the 158 pts with no abnormality. CBR was 32% vs 37%, respectively. 47/71 (66%) pts had abnormalities detected via metaphase cytogenetics, 44 (62%) by FISH, and 20 (28%) by both methods. Median duration of response (DOR) for ORR pts with ≥1 abnormality was 6 mo (95% CI 4-10) vs 8 mo (95% CI 6-11) in pts with no abnormality. *Conclusions:* CFZ activity in these heavily pretreated pts with R/R MM was durable and comparable in the absence or presence of a cytogenetic abnormality, consistent with data from another phase 2 study of CFZ (PX-171-004).

Figure 1. Kaplan-Meier analysis of duration of response by cytogenetic status



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A PHASE I/II STUDY OF ORAL MELPHALAN (MEL) COMBINED WITH PANOBINOSTAT (PAN) FOR PATIENTS WITH RELAPSED OR REFRACTORY (R/R) MULTIPLE MYELOMA (MM)

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Background: Our previous preclinical studies have shown that PAN, a potent histone deacetylase inhibitor, markedly enhances the anti-myeloma effects of melphalan in vitro and in vivo. *Methods:* Thus, we initiated a Phase I/II, open-label, dose-escalation study to treat R/R MM patients using oral PAN every Monday, Wednesday and Friday in combination with oral MEL (0.05 mg/kg) on days 1-5 of a 28 day cycle. After amendments to dose & schedule because of toxicity, PAN 15 mg is now being administered with oral MEL 0.05 mg/kg on days 1, 3 & 5 of a 28-day cycle in the current cohort. *Results:* To date, 25 (of 40 planned) patients have been enrolled with a median of 4 (4-17) prior regimens. Sixteen were previously treated with MEL. To date, 4 patients (16%) have shown

objective responses to this combination with 2 complete (8%) & 2 partial (8%) responses. An additional 11 (44%) patients have had stable disease while 10 (40%) have progressed while on study. Fourteen patients experienced grade 3 or 4 adverse events, including: reversible neutropenia (n=7), reversible thrombocytopenia (n=9), reversible worsening anemia (n=1), and one case each of a forearm rash, fatigue/weakness, hypokalemia, and hyponatremia. *Conclusions:* The combination of PAN & low-dose oral MEL has shown encouraging responses in heavily pretreated patients with relapsed/refractory MM. An expanded Phase II part of the trial will be conducted using this oral combination treatment once the MTD has been determined from the current Phase I portion of the trial.

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PHASE (PH) 1B EVALUATION OF THE SAFETY AND EFFICACY OF A 30-MINUTE IV INFUSION OF CARFILZOMIB (CFZ) IN PATIENTS (PTS) WITH RELAPSED AND/OR REFRACTORY (R/R) MULTIPLE MYELOMA (MM)

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Introduction: CFZ is a novel, highly selective, epoxyketone proteasome inhibitor. In MM pts, single-agent CFZ shows significant activity at up to 27 mg/m^2 given over 2–10 min. In rats, 48 mg/m^2 was the LD50 as an IV bolus, but nonlethal with minimal toxicity as a 30-min infusion. *Methods:* We conducted a dose escalating trial of CFZ as a 30-min IV infusion to pts with R/R MM. CFZ is given on days (D) 1, 2, 8, 9, 15, 16 of a 28-day cycle (C). C1 D1–2 doses are 20 mg/m2, with subsequent escalation to 36, 45, 56, or 70 mg/m^2. Dexamethasone (4 mg for <45 mg/m^2, 8 mg for >45 mg/m^2) is given prior to infusion. Responses are determined by IMWG Criteria. PK/PDn were performed on C1D1 and C2D1. *Results:* 20 pts have been enrolled (4 at 36 mg/m^2; 3 at 45 mg/m^2; 11 at 56 mg/m^2; 2 at 70 mg/m^2). Median number of prior regimens: 4 (1-9). Median duration of treatment: 4 C (range 1–13+). DLTs were seen in both pts at 70 mg/m^2. 11 pts enrolled at 56 mg/m^2, with 1 DLT (Gr3 hypoxia/fever). Responses for 17 evaluable pts are shown below. 3 pts at 20/56 mg/m^2 were not evaluable for efficacy (pts were withdrawn due to: DLT after 3 doses; Gr4 NTP after 2 doses; and Gr4 TCP after 3 doses). Cmax (30-min vs 10-min) is ~4-fold lower. Proteasome inhibition: >90% at >36 mg/m^2. Increased immunoproteasome inhibition was seen comparing 56 and 20 mg/m2. Common toxicity: nausea, fatigue, chills, pyrexia, and vomiting. *Conclusions:* CFZ as 30-min IV infusion is highly active and well tolerated at 20/56 mg/m^2 (recommended dose for R/R MM via 30-min infusion). Enrollment continues at 20/56 mg/m^2.

Best response	20/36 mg/m^2 (n=4)	20/45 mg/m^2 (n=3)	20/56 mg/m^2 (n=8)	20/70 mg/m^2 (n=2)
VGPR	1		3	
PR	1	1	5	1*
MR				1*
SD	2	2		
*Re-treated at lower doses				

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PROGNOSIS OF MULTIPLE MYELOMA (MM) PATIENTS AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN THE LAST DECADE. COMPARISON OF TWO COHORTS WITH DIFFERENT INDUCTION TREATMENT APPROACHES

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Background: The use of bortezomib (btz) or IMID-based induction regimens has improved pre-SCT remission rates in MM. However, in randomized trials, overall survival (OS) has not improved substantially. *Patients and Methods:* Data of two cohorts of pts with a newly diagnosed MM, treated in a single centre and submitted to ASCT were collected. Cohort 1 (C1: 1999-2005) received a dex, alkylators and anthracyclin-based induction before ASCT and btz and/or IMIDs at relapse. Cohort 2 (C2: 2005-2009) received IMIDs and/or btz upfront before ASCT. Post-ASCT CR rates, time to progression (TTP), event-free survival (EFS), time to next treatment (TNT) and OS were compared. *Results:* Out of 141 potential ASCT candidates diagnosed during both periods, 88 received an ASCT after induction (N=49 in C1 and N=39 in C2). Both cohorts were comparable. Median time from diagnosis to ASCT was 34 weeks (range 14-80 weeks) without significant differences between cohorts. Post-SCT CR rates were 35% for C1 and 61% for C2 (p=0.025). During the first year post-ASCT 6 patients died due to toxicity or infection (12%) and 3 relapsed (6%) among 49 pts at risk in C1. In C2, 1 patient died due to progression (2%) among 39 pts at risk. After a median follow-up of 6 yr for C1 and 3 yr for C2, differences in outcome are shown in the table. *Conclusion:* In our experience, patients with MM who received dex, alkalating agents and anthracyclins as first line induction presented an increased risk of death during the first year post-ASCT and inferior survival compared with those receiving bortezomib or thalidomide.

	Cohort 1		Cohort 2		
	Median (years)	95% CI	Median (years)	95% CI	p-value
TTP	2	1.5-2.7	3.4	1.8-5	0.243
EFS	1.8	1.1-2.4	3.4	1.8-5.1	0.049
TNT	1.9	1.3-2.4	3.6	2.3-4.9	0.034
OS	4.1	1.1-7	NA*	NA*	0.02
			*projected post-ASCT for C2 at 4 years was 83% (95%CI 68-98%).		

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PHASE (PH) 1B/2 DOSE-RANGING STUDY OF CARFILZOMIB (CFZ) IN COMBINATION WITH LENALIDOMIDE (LEN) AND DEXAMETHASONE (LODEX) IN RELAPSED-REFRACTORY MULTIPLE MYELOMA (R/R MM)

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Introduction: CFZ, a specific, irreversible epoxyketone proteasome inhibitor, shows durable single-agent activity and favorable side effect profile in patients (pts) with R/R MM. This ph 1b/2 study evaluated the maximum tolerated dose (MTD) of CFZ in combination with LEN and loDex (CRd) in pts with relapsed or refractory MM. An expanded cohort received CRd at highest dose. *Methods:* Escalating doses of IV CFZ (Days [D] 1, 2, 8, 9, 15, 16), LEN (po D1–21), and loDex (D1, 8, 15, 22) were administered in 4 wk cycles (Table 1) to adult pts with relapsed or refractory MM (1–3 prior regimens). Adverse events (AEs) were assessed by NCI-CTC (v3.0), responses by IMWG Uniform Response Criteria. *Results:* MTD was not reached. 81 pts (50 at the highest dose) were response-evaluable. Initial responses generally occurred within the first 2 cycles and improved with continuing therapy. Responses were observed at all dose levels and included 9 nCR/CR (11%), 19 VGPR (23%), 25 PR (31%), and 6 MR (7%). Median DOR has not been reached (>14 mo). Most AEs were reversible and manageable. Most common AEs ≥ Grade 3 were primarily hematologic (Table 2). 24 pts discontinued treatment due to AEs. *Conclusions:* The combination of CFZ (≤27 mg/m2), full dose LEN, and loDex was well-tolerated in MM pts with 1–3 prior regimens, including BTZ or immunomodulators. At highest doses tested, ORR was 78% and

prolonged administration led to no new or overlapping toxicities. ASPIRE, an ongoing ph 3 open-label, international, multicenter trial, is comparing CRd to Rd in pts with relapsed MM.

Table 1. Carfilzomib/Lenalidomide/Dexamethasone dosing and Reposness by Cohort.

Cohort	Doses			Best response, n				
	CFZ mg/m2	LEN mg	Dex mg	nCR/CR	VGPR	PR	MR	SD
1 (n=6)	15	10	40	-	2	1	-	2
2 (n=6)	15	15	40	-	1	-	2	2
3 (n=8)	15	20	40	1	1	2	1	1
4 (n=6)	20	20	40	1	2	1	1	-
5 (n=6)	20	25	40	-	2	2	1	-
6+Expansion (n=52)	27*	25	40	9	11	19	1	4

*CFZ 20 mg/m2 IV on C1D1, 27 mg/m2 thereafter.

Table 2. Incidence of adverse events of grade 3 or higher*

Adverse Event	≥ grade 3, ≥5% n(%)
Neutropenia	39 (46)
Thrombocytopenia	19 (23)
Anemia	14 (17)
Hyperglycemia	10 (12)
Hypophosphalemia	10 (12)
Lymphopenia	9 (11)
Hyponatremia	7 (8)
Fatigue	6 (7)
Leukopenia	5 (6)

*Regardless of relationship to study drug; date cutoff 11 Oct 2010

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CARFILZOMIB (CFZ) IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (R/R MM): SUMMARY OF SAFETY AND EFFICACY DATA UPON LONG-TERM TREATMENT

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Introduction: CFZ is a novel, selective proteasome inhibitor without cumulative toxicity (including neurotoxicity) in 6-9 month toxicology studies. We report on the updated clinical experience with long-term treatment (>12 cycles) with CFZ in MM. *Methods:* Pts treated in phase 1 or 2 MM trials were eligible to enroll in an extension study after 12 cycles. Pts initially receive CFZ on Days 1, 2, 8, 9, 15, and 16 every 28-days; dosing frequency could be reduced to alternate weeks and dose increased to a maximum of 56 mg/m^2. Time of administration: 10min or 30min depending on dose. Pts continued treatment until PD, unacceptable toxicity, or withdrawal of consent. *Results:* As of 31 Jan 2011, 78 MM pts had enrolled. Rollover was highest in 2 phase 2 trials: 29/266 (11%) in 003-A1 (R/R MM pts) and 38/129 (29%) in 004 (bortezomib-naïve pts, 1-3 prior regimens). 61 (78%) MM pts remain on study at a median dose of 27 mg/m^2 (range: 15–56). Median total duration of treatment (original+extension): 18 mo (29 pts: original schedule; 21 pts: intermittent schedule). Longest total duration: >30 mo. Off-study: 13 pts (PD); 1 pt (investigator discretion); 1 pt withdrew consent; no withdrawals due to toxicity. 2 pts had dose reduction due to toxicity; 15 pts had the CFZ dose increased; 2 added len; 4 added 40 mg/wk dex. Clinically significant cumulative toxicities were not observed. *Conclusions:* CFZ can be safely administered to MM pts for extended therapy using the original or intermittent schedule. Maintenance CFZ sustains disease control and provides excellent long-term tolerability.

Baseline parameters

Median time since initial diagnosis	6 years
Unfavorable cytogenetics/FUSH	16%
Median # orior regimens	3 (range: 1-12)
Prior bortezomib	60%

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CARFILZOMIB (CFZ) PRODUCES A HIGH SINGLE-AGENT RESPONSE RATE WITH MINIMAL NEUROPATHY EVEN IN RELAPSED MULTIPLE MYELOMA (MM) PATIENTS (PTS) WITH HIGH-RISK DISEASE

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Introduction: CFZ, a selective epoxyketone proteasome inhibitor, produces potent, sustained inhibition and lacks many bortezomib (BTZ)-associated off-target activities. Durable single-agent activity has been seen in relapsed/refractory MM pts, including those with advanced disease or significant comorbidities. PX-171-004 is an ongoing phase 2 study of CFZ in pts with relapsed or refractory MM after 1–3 prior regimens. This is an update on BTZ-naïve pts on study, including those with poor prognosis at baseline. *Methods:* Pts in 2 sequential cohorts received IV CFZ at 20 mg/m^2 on Days 1, 2, 8, 9, 15, 16 of every 28-day cycle (C); pts in Cohort 1 remained at 20 mg/m2; pts in Cohort 2 escalated to 27 mg/m^2 for C2–12. Primary endpoint was overall response rate per IMWG criteria. Subgroup analyses were performed by baseline criteria including ECOG performance status, ISS disease stage, cytogenetics, and refractory status. *Results:* Data are available for 123 BTZ-naïve pts. Responses according to dose and baseline measurements are detailed below. The most common treatment-emergent AEs (all cause) were fatigue (60%), nausea (45%), and anemia (40%). There were no discontinuations for PN. 49 pts completed 12C of therapy. 27 pts con-

tinued on extended treatment protocol PX-171-010; no cumulative toxicities have been noted. *Conclusions:* CFZ achieves high response rates in BTZ-naïve pts with relapsed MM across treatment groups. CFZ continues to demonstrate long-term tolerability with minimal PN, even in pts with comorbid conditions who may benefit from a steroid-sparing regimen.

Table 1.

Dosing group	N	CR n (%)	VGPR n (%)	PR n (%)	MR n (%)	SD n (%)	ORR n (%)
20 mg/m2	59	2 (3)	8 (14)	15 (25)	10 (17)	13 (22)	25 (42)
20-27 mg/m2	64	1 (2)	17 (27)	16 (25)	6 (9)	12 (19)	34 (53)
Overall	**123**	**3 (2)**	**25 (20)**	**31 (25)**	**16 (13)**	**25 (25)**	**59 (48)**

Table 2.

Baseline measurement	N (%)	ORR n (%)
ECOG PS		
0	51 (41)	27 (53)
1-2	72 (59)	32 (44)
Cytogenetics or FISH		
Normal/favorable	88	44 (50)
Unfavorable	16	6 (38)
Refractory status		
No	80	44 (55)
Yes	43	15 (35)
ISS stage		
I, II or unknown	92	44 (48)
III	19	8 (42)

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CONDITIONING FOR AUTOLOGOUS STEM CELL TRANSPLANTATION BY COMBINING BORTEZOMIB AND DEXAMETHASONE WITH HIGH-DOSE MELPHALAN (BD-HDM) IS FEASIBLE IN YOUNG JAPANESE MULTIPLE MYELOMA PATIENTS

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Multiple myeloma is still an incurable disease. However, autologous stem cell transplantation is recommended for younger patients with newly diagnosed multiple myeloma because a high complete response (CR) rate is achieved. Patients with CR after autologous stem cell transplantation are assumed to gain improvement of overall survival and progression-free survival. Bortezomib has shown a synergistic effect with melphalan and no prolonged hematologic toxicity. Recently, it was reported that the CR rate was improved after autologous stem cell transplantation by combining bortezomib with melphalan for the conditioning regimen. In our phase 2 study, 10 patients were enrolled from February to November 2010. They received bortezomib (1 mg/m^2 x 4), dexamethasone (16 mg x 4), and melphalan (200 mg/m^2) as their conditioning regimen (BD-HDM). No toxic deaths occurred. Neutrophils (ANC>0.5x 10^9/L) and platelets (>20 x 10^9/L without transfusion) recovered after a median of 5 days (range: 4-6 days) and 7 days (range: 4-8 days), respectively. No patient showed exacerbation of peripheral neuropathy. Three patients achieved CR and three obtained VGPR. These results suggest that BD-HDM is a safe and promising conditioning regimen for young Japanese multiple myeloma patients. We conclude that a prospective multicenter trial of BD-HDM combined with suitable induction therapy and consolidation therapy should be performed in the future.

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PRELIMINARY RESULTS OF A PHASE I TRIAL OF INTRAVENOUS MV-NIS FOR RELAPSED, REFRACTORY MULTIPLE MYELOMA (MM)

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MV-NIS is an oncolytic measles virus expressing the human sodium-iodide symporter (hNIS) whose activity can be monitored by noninvasive imaging of radioiodine. The Phase 1 trial escalates with 3-pts/dose level. Cohort 1 includes viral titers: 106, 107 108 and 109 TCID50 of MV-NIS. Cohort 2 pts start treatment with CTX 10 mg/kg 2 days prior to MV-NIS at MTD/100. Correlates include measurement of immunity, CD46 expression, & MV infectivity. PK & biodistribution are tested by Q-RT-PCR of MV-N in blood, urine, & gargle samples and by serial nuclear imaging. Cohort I is completed with 12 pts receiving 1 dose of MV-NIS at 106, 107 108 or 109 TCID50 of MV-NIS. No DLT has been observed, and 4 pts have been treated on Cohort 2. The gr 3-4 AEs at least possibly related to Rx were: lymphopenia (n=1), neutropenia (n=2) anemia (n=1), and thrombocytopenia (n=1). Gr 1-2 AEs included neutropenia (n=5), thrombocytopenia (n=3), nausea (n=2). Other gr 1-2 AEs include 1 of each: infusion related rigors, anemia, vomiting, diarrhea, prolonged aPTT, fever, and rash. There have been no MM responses, but 123I scans have been positive in 3 pts demonstrating proof of principle of NIS. Pts' MM cells expressed higher numbers of surface CD46 molecules and were preferentially infected by MV compared to BM stromal cells ex vivo. All pts had low baseline humoral immunity against MV but developed anti-IgG MV titers by 6 wks. MV-N has been amplified from gargle specimens, blood and urine. The virus is capable of replicating before being cleared by the immune system. Accrual continues.

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A PHASE II STUDY OF PEGYLATED LIPOSOMAL DOXORUBICIN (PLD), BORTEZOMIB, DEXAMETHASONE AND LENALIDOMIDE (DVD-R) FOR PATIENTS WITH RELAPSED/REFRACTORY (R/R) MULTIPLE MYELOMA (MM)

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Background: Our previous studies have shown that modifying the dose & schedule of PLD in combination with low-dose bortezomib (BORT) and dexamethasone (DEX) maintained its efficacy and improved its tolerability in MM. Lenalidomide (LEN) in combination with BORT and DEX shows high response rates but is not well tolerated. *Methods:* A single-arm multi center phase II study for R/R MM patients to evaluate the combination of intravenous 40 mg DEX, 1.0 mg/m^2 BORT, 4.0 mg/m2 PLD on days 1, 4, 8, & 11, & 10 mg LEN daily on days 1-14 of each 28-day cycle. Patients were treated to maximum response plus 2 additional cycles or to a maximum of 8 cycles of therapy without disease progression. *Results:* Thirty (of 40 planned) patients have been enrolled to date with efficacy data evaluable on 27 patients. Patients were heavily pretreated with a median of 3 (1-17) prior regimens. Nineteen patients (70%) have shown objective responses to the DVD-R regimen: 5 complete response (19%), 4 very good partial responses (15%), 4 partial responses (15%), & 6 minimal responses (22%). An additional 4 patients showed stable disease and 2 progressed on study. Fifteen patients experienced grade 3 or 4 adverse events as follows: 3 reversible neutropenia, 4 pneumonia, 6 reversible anemia, 3 thrombocytopenia and only one patient with grade 4 reversible thrombocytopenia. Eight (27%) have developed treatment-emergent peripheral neuropathy with no cases of stomatitis or hand-foot syndrome. *Conclusions:* DVD-R regimen is a well tolerated regimen with high response rates for pretreated patients with (R/R) MM.

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VORINOSTAT TREATMENT OVERCOMES LENALIDOMIDE-DEXAMETHASONE RESISTANCE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Phase I data for the lenalidomide (R), dexamethasone (D), and vorinostat (Z) combination have been encouraging. However, clinical experience with RDZ in RD-refractory patients is limited. We pres-

ent a single institution experience in consecutive patients with RD-refractory multiple myeloma (MM) who received RDZ. *Methods:* This is a retrospective chart review of patients receiving oral Z 300-400 mg once daily (d 1–7 and 15–21), R 10–25 mg (d 1–21), and D 20–40 mg weekly (d 1, 8, 15, 22) in a 28-day cycle. Overall response rate, duration of response (DOR), and the safety and tolerability of RDZ was assessed. *Results:* All patients (N=25) were relapsed/refractory to RD, and 23 patients had prior SCT. The median number of prior regimens was 4 (range, 2–11). The ORR was 28%, including 6 PR and 1 ≥VG PR; 5 MR and 8 SD. The clinical benefit rate (ORR+MR+SD) was 80%, and median DOR of 3 months (0 – 30) and median OS was 9 months (2 – 33). In patients with high-risk disease, the ORR was 11%. Most commonly reported toxicities were diarrhea (48%), fatigue (28%), nausea/vomiting (24%), weakness (12%), and constipation (12%). Grade 3/4 neutropenia (44%) and thrombocytopenia (32%) were manageable. *Conclusions:* The convenient oral RDZ regimen is well tolerated in patients with RD-refractory MM. These results demonstrate the ability of Z to overcome resistance to RD.

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VORINOSTAT OVERCOMES RESISTANCE IN PATIENTS WITH MULTIPLE MYELOMA REFRACTORY TO BORTEZOMIB, LENALIDOMIDE AND DEXAMETHASONE

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Background: Several large multinational phase II and III trials combining the first-in-class oral histone deacetylase inhibitor vorinostat (Z) with the proteasome inhibitor bortezomib (V) are ongoing. We present experience in patients with multiple myeloma (MM) refractory to V, lenalidomide (R), and dexamethasone (D; VRD) who received Z in addition to VRD. *Methods:* A retrospective chart review of patients who received oral Z 300 or 400 mg once daily (d 1–7 and 15–21), R 10–25 mg (d 1–21), D 20–40 mg once weekly (d 1, 8, 15, 22), and intravenous V 1.3 mg/m2 (d 1, 4, 8, 11) in a 28-day cycle. Overall response rate (ORR; ≥partial response [PR]), duration of response (DOR), and safety and tolerability of VRDZ were assessed. *Results:* All patients (N=9) were refractory to VRD. The median number of prior regimens was 5 (range, 2–10), including 7 patients with prior SCT. The ORR was 44%, which included 2 patients with PR and 2 with VGPR or better; 4 patients had minimal response (MR). The clinical benefit rate (ORR+MR+SD) was 89%. Median DOR was 3 months (0 - 5) and median overall survival was 4 months (3 - 21). Commonly reported toxicities (all grades) were fatigue (56%), constipation (56%), weakness (44%), diarrhea (22%), and nausea/vomiting (11%). Grade 3/4 toxicities included neutropenia (66%) and thrombocytopenia (89%). No patients discontinued therapy due to toxicity. *Conclusions:* These results suggest that the addition of Z to the VRD regimen may overcome resistance in patients with refractory MM.

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MAINTENANCE THERAPY IN MYELOMA PATIENTS: THE ROLE OF THALIDOMIDE

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Thalidomide has established role in the induction treatment of multiple myeloma. The aim of study was to analyse results of thalidomide as maintenance. The study included 130 de novo patients: 1) 50pts eligible for ASCT (29 male/21 female, mean age 53yrs); 2) 80 elderly pts (45 male/35 female, mean age 68yrs); with common distribution according to the myeloma type, clinical stage and ISS score. First group was treated with VAD (35/50pts) and CTD (15/50pts), followed by HD-Melphalan and ASCT. Second group was treated with MP (30/80pts), MPT (35/80pts), and Thal-Dex (15/80pts). The maintenance was alfa-Interferon (aIFN) after VAD (10/35pts) and MP (30/30pts); Thalidomide after VAD/CTD (35/50pts) and MPT/Thal-Dex (50/80pts) with median duration of 16m. Thalidomide maintenance after CTD significantly improved 3-yrs probability of relapse-free survival (VAD+HDT+aIFN: 25% vs. VAD+HDT+Thal: 37% vs. CTD+HDT+Thal: 51%, P<0,0025) and overall survival (VAD+HDT+aIFN: 70% vs. VAD+HDT+Thal: 77% vs. CTD+HDT+Thal: 85%, P<0,0039); as well as the progression-free survival (MP+aIFN: PFS 25% vs. MPT+Thal: PFS 54% vs. Thal-Dex +Thal: PFS 50%, P<0,003) and overall survival (MP+aIFN: 29% vs. MPT+Thal: 48% vs. Thal-Dex +Thal: 51%, P<0,0025) after MPT/Thal-Dex. Predominant adverse event in a both group of pts was peripheral neuropathy recorded in 58/85pts (68%) with grade 3-4 toxicity in 10/72pts, and thrombosis recorded in 4/50pts. *Conclusion:* Thalidomide as maintenance therapy significantly improves treatment results in myeloma patients with limitations due to toxic effects.

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EARLY RESPONSE TO BOTEZOMIB COMBINED CHEMOTHERAPY CAN BE HELPFUL FOR PREDICTION OF PROGRESSION FREE SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA WHO WERE INELIGIBLE FOR STEM CELL TRANSPLANTATION

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Background: Response to treatment has been associated with improved survival in multiple myeloma (MM). The use of novel agents changed achievement of CR rates compared with conventional chemotherapy. The purpose of our study is to show influence of early response after treated with bortezomib combined chemotherapy to survival in patients with newly diagnosed MM who are ineligible for stem cell transplantation. *Methods:* We assessed response at least forth cycles before next chemotherapy by international myeloma working group response criteria. We divided into good response group (A group) which were included showing more than very good partial response (VGPR) and poor response group (B group) which were partial response (PR) or less than PR. Endpoints were comparision of progression free survival (PFS) and overall survival (OS) between A and B groups. *Results:* We retrospectively analyzed 119 patients registered data for our study from the Kore-

an Multiple Myeloma Working Party (KMMWP) performed a nationwide registration of MM patients. 14 patients were in sCR or CR (11.8%), 45 were in VGPR (37.8%), 34 were in PR (28.6%), 14 were in SD (11.8), and 12 were in PD (10.1%). 3 years PFS of A group and B group were 53.0% and 19.8%, respectively (p-value < 0.001). 3 years OS of A and B group were 64.8% and 53.7%, respectively (p-value 0.106). *Conclusion:* Early response at least forth cycles before next chemotherapy might be helpful for prediction of PFS in patients who were ineligible stem cell transplantation.

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DURABLE RESPONSES AFTER DONOR LYMPHOCYTE INFUSION IN PATIENTS WITH RESIDUAL MULTIPLE MYELOMA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Objective: The graft-versus-Myeloma(GVM) effect mediated by donor lymphocyte infusions (DLI) is well described in multiple myeloma (MM). We evaluated the role of DLI in patients (pts) with MM, who had residual or recurrent disease after allogeneic hematopoietic stem cell transplantation (allo HCT). *Methods:* We identified 23 pts with MM, who received DLI collected from their original donors at UT-MD Anderson Cancer Center between 7/1996 -6/2008. Eight pts had residual disease (RD) and 15 ps had recurrent or progressive disease (PD) after allo HCT. *Results:* Nineteen pts (82%) received DLI from matched related donors. Median age at DLI was 50 yrs. A total of 36 DLI doses were administered (70% received only one DLI). Median interval between allo HCT and the first DLI was 8.2 months. The median DLI dose was 33x 10^6 $CD3^+$ T cells (range 5 - 148 x 10^6). Median follow up in surviving pts was 24 months. Pts who had DLI for PD were less likely to get CR or VGPR (7% vs. 50% for RD; p=0.03). Compared to RD, PD pts had shorter PFS (5.2 vs. 11.9 months) and shorter OS (7.6 vs. 28.3 months; p=0.03). Acute graft-versus-host disease (GVHD) was seen in 6 (26%) pts, with median time to onset at 30 days from DLI. None of the pts developed chronic GVHD. All pts who had either CR or VGPR survived more than 2 yrs. *Conclusions:* This is the largest single institution study to evaluate the role of DLI for MM. DLI was safe and effective. DLI for RD had a significantly better response and OS than for PD. The role of DLI needs to be further explored in prospective clinical trials.

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PHASE II TRIAL OF BORTEZOMIB-BASED THERAPY WITHOUT ASCT IN NEWLY DIAGNOSED PATIENTS WITH T(4;14) MULTIPLE MYELOMA

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The PFS of newly-diagnosed MM pts with t(4;14) is 8-9 months and median overall survival (OS) 18 mos following a single ASCT without novel agents. We designed a non-transplant bortezomib-based phase II protocol for these pts with DBD induction x 4 (Doxil® + bortezomib + dexamethasone), CyBor-P post-induction x 8 cycles (weekly oral cyclophosphamide 300 mg/m^2 + bortezomib 1.5 mg/m^2 days 1,8,15 + prednisone 100 mg q 2 days q28 days), and maintenance dexamethasone 40 mg/wk. Between 02/08-01/11, 330 pts were screened in 8 Canadian centers; 36(11%) were t(4;14)-positive by FISH but 5 were asymptomatic, 1 refused and 6 were ineligible; 24 have been entered onto study. Median age was 59 yrs (45-69), serum β2-microglobulin 302 nmol/L (43-1695) and albumin 36 g/L (28-48). Using modified Uniform criteria, best response in 20 evaluable pts is: sCR in 6 (30%), CR in 4 (20%), VGPR in 6 (30%), PR in 2 (10%) and stable disease in 2 (10%). Median F/U is 14 mos (1-29); 5 have progressed at median of 3 mos on study. Four pts have died (progression in 3 and complex medical problems/consent withdrawal in 1 in VGPR). SAEs were reported in 4 pts and 6 developed grade 2 peripheral neuropathy Actuarial 1-yr PFS is 68% (95%CI 47-97%) and OS 80% (95%CI 63-100%). We conclude: 1) the incidence of t(4;14) by FISH in newly-diagnosed MM pts is 11%; 2) 14% of these are asymptomatic; 3) this bortezomib-based regimen is well-tolerated; 4) the overall response rate (sCR + CR + VGPR + PR) is 90%; 5) the 1-year PFS and OS with this approach compare favorably with ASCT, although longer F/U is required.

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PATTERNS OF PROGRESSION IN MULTIPLE MYELOMA (MM) AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)

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Background: Most multiple myeloma(MM)s relapse after autologous stem cell transplantation(ASCT), and timing of M-protein appearance and clinical relapse is various. We attempted to describe clinical features according to speed of progression in MM after ASCT. *Methods:* 122 MM patients received single or tandem ASCT as a part of initial treatment between 1998 and 2009 at Samsung Medical Center. Among 107 patients achieved ≥VGPR, 70 patients with progressive disease(PD) after ASCT were included, and were divided into two groups by the interval 6 months from PD to clinical relapse. *Results:* The median OS and PFS of 107 patients was 72.6 and 26.6months. The interval from PD to clinical relapse was <6 months in 55 patients(median 0.2months) and ≥6months in 14 patients (median 13.4months). OS and PFS from ASCT were worse in rapidly progressive group than in slower progressive group(median OS 52.9 vs. not reached, p=0.006; median PFS 16.7 vs. 34.0months,p=0.018), however DFS was not different in two groups. OS from the 1st salvage treatment was worse in rapidly progressive group(median OS 24.9 vs. 44.3months, p=0.028). The proportion of patients treated with novel agent before ASCT was higher in rapidly progressive group(29.1% vs. 0%, p=0.029). Other characteristics at diagnosis including stage, anemia, azotemia, hypercalcemia, and bone lesion and response before and after ASCT were not associated with the speed of progression. *Conclusions:* The speed of progression is heterogenous in MM patients. Rapidly progressive disease was associated with earlier progression after ASCT and worse OS.

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THE REGIMEN OF LENALIDOMIDE (LEN), THALIDOMIDE (THAL), AND DEXAMETHASONE (DEX) IS ACTIVE AGAINST RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (RRMM) AND OVERCOMES LENALIDOMIDE RESISTANCE

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Background: Thal may help overcome mechanisms of resistance to len, providing the rationale for a phase I/II study. *Methods:* Patients (pts) with RRMM with ≥1 line of therapy were enrolled in 3 cohorts (C), C1: Len 15 mg/Thal 100 mg/Dex, C2: 25 mg/100 mg/Dex, and C3: 25 mg/200/Dex. Dex was dosed in pulse fashion at 40 mg for cycles 1-2, and then weekly in cycles 3+. *Results:* 36 patients were enrolled to date with a median of 3 lines of therapy (range: 1-10). There were no dose limiting toxicities (DLT) in C1, 1/6 DLT in C2 and 2/8 DLT in C3. The DLT in C2 was due to steroid toxicity. DLT in C3 included one patient with grade (G)3 rash due to thal and asymptomatic G2 atrial fibrillation (AF), and a second pt with G3 hypertensive crisis and volume overload due to dex. No G3/4 peripheral neuropathy has been observed. Among 27 patients evaluable for efficacy, the overall response rate (ORR; ≥partial response (PR)) was 78%, 6 pts with at least near-complete remission (nCR), 4 very good partial remission (VGPR), 11 PR, and 2 minor responders (MR). Among the 13 evaluable patients with Len refractory disease

the ORR was 62%, with 1 MR, 6 PR, 1 VGPR, and 1 nCR. *Conclusions:* The regimen of len/thal/dex is tolerable with dosing of len at 25 mg on days 1-21 of every 28-day cycle, thal at 100 mg daily, and pulse dose dex. An ORR of 78% has been described to date, including robust and durable responses in patients with prior len resistance. These findings support further exploration of this regimen in the phase II and III settings, and possibly its use in a newly diagnosed population.

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CLINICAL EFFICACY OF A BORTEZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE COMPARED WITH BORTEZOMIB, CYCLOPHOSPHAMIDE, THALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Backgrounds: Toxicities of salvage regimen due to combination bortezomib and thalidomide interrupt the consecutive treatments. Therefore, we compared the clinical responses and toxicities between bortezomib, cyclophosphamide and dexamethasone (Vel-CD) combination and Vel-CTD in patients with relapsed or refractory MM. *Methods:* Seventy-six patients received at least 2 cycles of treatment with Vel-CTD (bortezomib 1.3 mg/m2 i.v. on D1, 4, 8 and 11; cyclophosphamide 150 mg/m2 orally on D1-4; thalidomide 50 mg/day orally every day; and dexamethasone 20 mg/m2 i.v. on D1, 4, 8 and 11) and 50 patients with Vel-CD, which is the same regimen except thalidomide. *Results:* Eighteen (41%) and 16 (21%) of patients had undergone a previous autologous stem cell transplantation in Vel-CD and Vel-CTD, respectively (p=0.04). The overall best response of Vel-CD group and Vel-CTD group were 86% and 89.5% (p>0.05). There was no difference in time to progression (p=0.27) and overall survival (p=0.93). In non hematologic toxicities profiles, Vel-CTD group (13%) showed the higher proportion of grade 3 or more sensory neuropathy compared with Vel-CD group (2%) (p=0.03). The rates of treatment discontinuation due to adverse events in Vel-CD and Vel-CTD were 6% and 11%, respectively (p=0.38), and dose adjustment in Vel-CD and Vel-CTD were 34% and 50%, respectively (p=0.08). *Conclusion:* Vel-CD combination therapy in patients with relapsed or refractory MM is an effective and more tolerable salvage regimen compared with Vel-CTD in the aspect of comparable response rate, less toxicities.

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MOLECULAR REMISSION AFTER BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE (VTD) COMPARED WITH THALIDOMIDE-DEXAMETHASONE (TD) AS CONSOLIDATION THERAPY FOLLOWING DOUBLE AUTOLOGOUS TRANSPLANTATION (ASCT) FOR MULTIPLE MYELOMA (MM): RESULTS OF A QUALITATIVE AND QUANTITATIVE ANALYSIS

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The high rate of complete response (CR) effected by novel agents as induction and consolidation therapy in MM has renewed interest in the evaluation of minimal residual disease (MRD) after these combined treatment strategies. We present the results of a molecular sub-study to the phase 3 GIMEMA trial of VTD vs. TD incorporated into ASCT for newly diagnosed MM. By study design, patients (pts) randomized to VTD or TD as induction therapy received two 35-day cycles of consolidation with VTD or TD starting 3 months after ASCT(s). Aim of the study was to compare the activity of VTD consolidation with that of TD by qualitative and quantitative PCR. At this time, 67 pts who were in confirmed CR or near CR before the start of consolidation were included in the study and were analyzed for MRD detection. Qualitative PCR at day 0 showed that MRD before the start of VTD consolidation was undetectable in 13 out of 35 pts (39%); the corresponding value before the start of TD consolidation was 31%. Analysis at day +70 revealed an upgrade in PCR-negativity from 31% to 48% with TD and from 39% to 64% with VTD (p=0.008, McNemar's test). Real-time quantitative PCR analysis confirmed a median 1 log reduction in tumor burden with TD vs. a median 5 log reduction with VTD at day +70 (p=0.03, Wilcoxon test). In conclusion, consolidation therapy with VTD significantly increased the rate of molecular remissions and significantly reduced the burden of residual myeloma cells persisting after ASCT. Supported by: Progetto di Ricerca Finalizzata Orientata (to M.C), BolognAIL, Fondazione Carisbo.

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LENALIDOMIDE-DEXAMETHASONE (LD) FOR REVERSAL OF ACUTE LIGHT CHAIN-INDUCED RENAL FAILURE (ARF) IN MULTIPLE MYELOMA (MM). RESULTS FROM A PHASE II STUDY

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This phase 2 trial evaluates the efficacy of Lenalidomide (L)–Dexamethasone (D) in restoring renal function and tumor control in light chain-induced acute renal failure (LC-ARF). 18 pts with LC-ARF as formerly defined (JCO 2010) have been enrolled so far (no data as yet in 2). Baseline data from 16 pts: Median age 68 yrs (range: 47-87), ISS stage III: 16. 15 (94%) presented with de novo MM and 1 (6%) with PD; median GFR 18 ml/min (range 6.1-27.6 ml/min). L was given from d 1-21 with dose adaptation according to GFR. D 40mg was administered on d 1-4, 9-12, 17-20 during cycle 1; thereafter 1x/week. Cycles were repeated q 4 weeks. 14 pts have completed ≥2 cycles and 12 are evaluable for tumor response as yet. 7 pts achieved nCR (58.3%), 1 (8.3%) PR and 4 (33.3%) MR; CR-MR (100%). Median time to response was 133 days. Grade 3&4 leucopenia, thrombopenia, and anemia were seen in 6%, 6% and 25%, respectively. Other common grade 3&4 toxicities were infection (19%), cardiac dysfunction (25%), pulmonary embolism (6%) and weakness/fatigue (6%). 2 pts died due to infection (12%). Median GFR increased to 26.8 ml/min (range 11.3-74 ml/min). In 7 pts with nCR median GFR improved from 9.4 (6.1-27.6 ml/min) to 30.6 (11.3-74 ml/min). In pts with PR/MR median GFR increased to 24.6 (16.8-40.7 ml/min). Renal response was categorized as formerly defined (JCO 2010). 2 pts achieved CRrenal and 8 PR/MRrenal, respectively, yielding an ORRrenal in 10 (71%) of 14 pts. 3 of 6 dialysis dependent pts became dialysis independent. *Conclusion:* LD resulted in ≥PR in 67%, ≥MR 100% and ORRrenal in 71%.

Stage of renal failure at baseline (GFR)	Number of pts	Best renal response (number of pts)			
		CR^{renal}	PR^{renal}	MR^{renal}	No response
Stage III 30-59ml/min	0	0	0	0	0
Stage IV 15-29ml/min	7	2	0	2	3
Stage V <15ml/min	7	0	3	3	1

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BENDAMUSTINE IN COMBINATION WITH THALIDOMIDE AND DEXAMETHASONE IS AN EFFECTIVE SALVAGE REGIMEN FOR ADVANCED STAGE MYELOMA

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Multiple myeloma (MM) patients respond well to treatment but subsequently relapse, eventually exhausting all options. Bendamustine is a novel alkylating agent with a benzimidazole ring, giving it a unique mechanism of action. Experience of its use in combination with other drugs is limited. Thus, bendamustine was given to a small group of patients as part of a compassionate use programme. 23 relapsed/refractory patients (median age 65y) were treated with a combination of bendamustine, thal and dex (BTD). Patients received 60 mg/m^2 bendamustine on d1, 8 (and 15), 50-200 mg thal daily and dex on d1, 2, 8, 9, 15, 16, 21, 22 of each 28-day cycle. The patient group was heavily pre-treated (median lines of previous therapy=5). All had received previous thal and the majority had received bortezomib (n=21), lenalidomide (n=20) and high dose therapy (n=19). 9 patients had baseline grade 3/4 pancytopenia. The median number of cycles administered was 3 with a median cumulative bendamustine dose of 390 mg/m^2. WHO grade 3/4 haematological toxicities occurred in a further 11 patients and grade 3/4 infection occurred in 6 patients, consistent with alkylating agent therapy. The majority of non-haematological toxicity was related to dex, thal, or to progressive MM. Clinical benefit was seen in 61% of patients [CR n=1, PR n=5, MR n=4, SD n=4] with a median time to best response of 3 months. Responders had a median OS of 15m, compared to 4m for non-responders. We conclude that BTD is a useful salvage regimen for extensively pre-treated patients with prior exposure to bortezomib and lenalidomide.

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SECOND PRIMARY MALIGNANCIES (SPM) IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH HIGH-DOSE MELPHALAN AND AUTOLOGOUS TRANSPLANTATION AND MAINTENANCE TREATMENT: LONG TERM FOLLOW- UP OF 3 SUCCESSIVE HOVON AND GMMG RANDOMIZED TRIALS

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High-dose Melphalan (HDM) followed by autologous stem cell transplantation (ASCT) has significantly improved progression-free survival (PFS) and overall survival (OS) in transplant eligible patients with multiple myeloma (MM). Post transplant consolidation and maintenance treatment may further increase PFS and occasionally OS. Randomized trials have investigated several drugs for maintenance treatment including Interferon alpha-2a, Thalidomide, and more recently Lenalidomide and Bortezomib. In 3 prospective trials (IFM 99-02, UK MMIX and HOVON-50) maintenance with Thalidomide was associated with increased PFS but not OS. Similarly, maintenance with Lenalidomide (IFM 2005-02) did improve PFS but as yet not OS. Recent reports on an increased incidence of second primary malignancies (SPM) with Lenalidomide maintenance in the post-transplant setting raise questions on the therapeutic benefit of maintenance treatment and ask for a balanced evaluation of survival benefit against the risk of SPM. We have investigated the incidence of SPC in 3 consecutive randomized HOVON (HOVON-65 trial in cooperation withGMMG) trials for newly diagnosed patients treated with HDM and ASCT followed by different maintenance strategies. In the HOVON-24 trial (1995-2000), 441 eligible patients were included, of whom 303 were randomized after induction with VAD between single (Intermediate dose Melphalan, 2 x 70 mg/m2 without ASCT) or double (IDM + Cy/TBI with ASCT) intensive therapy followed by Interferon-α-2a (IFN-α-2a) as maintenance until relapse in both arms. (Segeren et al., Blood 2003;101:2144-2151). In the HOVON-50 trial (2001-2005), 540 eligible patients were randomized between induction therapy with Vincristin, Adriamycin, Dexamethasone (VAD) (arm A) or Thalidomide-AD, TAD (arm B), followed by HDM/ASCT. Maintenance with either IFN-α-2a (arm A) or thalidomide (arm B) was given until progression/relapse (Lokhorst et al., Blood 2010;115:1113-1120). In the HOVON-65/GMMG-HD4 study 827 eligible patients were randomized between VAD (arm A) or Bortezomib-AD (PAD) (arm B) induction followed by single or double HDM and maintenance with Thalidomide (arm A) or Bortezomib (arm B) for 2 years (Sonneveld et al., Blood 2010, ASH abstract #40). In the HOVON-24 trial Cy/TBI + ASCT was not associated with a higher incidence rate (IR) of SPM (0.6 vs 0.4 SPM per 100 patient years, Table 1). The type of SPM was myelodysplasia (MDS, n=3), lymphoma (NHL, n=2) and solid tumors (n=2). The time to SPM was not statistically significant between both treatment arms, hazard ratio (HR) = 1.33, 95% confidence interval (CI) = 0.30-5.96. The first event occurred after 14 months. In the HOVON-24 trial 138 patients were not randomized either due to proceding towards allogeneic transplantation or due to premature discontinuation from the study. Three cases of Non Hodgkin Lymphoma were observed in patients who received subsequent allo-SCT, which were all EBV-related lymphomas. The types of SPC in the HOVON-50 and HOVON-65/GMMG-HD4 trials are also specified in Table 1. In the HOVON-65/GMMG-HD4 trial two malignancies of the skin were reported (1 melanoma in arm A and 1 basal cell carcinoma in arm B). In addition, adenocarcinoma of the prostate, colon and ovarium, non-small cell lungcancer and bladder carcinoma in the Thalidomide arm, and adenocarcinoma of the colon and mamma and a non-seminoma testis in the Bortezomib arm occurred. The time to SPC in HOVON-50 was not different between 2 treatment arms with the first event at 5 days and an increase in incidence until 96 months in both arms. Similarly, no differences of time to SPC was observed between treatment arms in HOVON- 65/GMMG-HD4 , with the first event at 3 months and an increase in incidence until 47 months of FU. In conclusion, there is no difference in the incidence of SPM between the two treatment arms of 3 successive HOVON and GMMG randomized trials, investigating either the role of myeloablative transplantation or different maintenance treatments. The lowest incidence of SPM sofar was observed in the Bortezomib treated HOVON-65/GMMG-HD4 patients (0.3 per 100 patient years). However, longer FU is needed to exclude the development of late SPM in bortezomib treated patients.

Table 1. Incidence Rates and type of SPM.

Study	Arm A	Arm B
HOVON-24	3/148*	4/155*
MDS	1	2
Solid tumor, except skin	1	1
B-cell malignancy	1	1
Time to SPM (mo), median (range)	72 (48-75)	45 (14-88)
HR (95% CI)	1	1.33 (0.30-5.96)
IR (number SPM/100 pts. Years)	3/7.34 = 0.4	4/7.27 = 0.6
HOVON-50	22/270**	19/270**
MDS/AML	1	4
Solid skin tumor	8	4
Solid tumor, except skin	12	10
B-cell malignancy	1	1
Time to SPM (mo), median (range)	44 (0-96)	52 (3-97)
HR (95% CI)	1	0.80 (0.43-1.48)
IR (number SPM/100 pts. Years)	22/11.93 = 1.8	19/12.26 = 1.5
HOVON-65/GMMG-HD4	11/4143***	4/413***
MDS/AML	3	0
Solid skin tumor	1	1
Solid tumor, except skin	6	2
B-cell malignancy	1	1
Time to SPM (mo), median (range)	30 (3-47)	26 (11-29)
HR (95% CI)	1	0.34 (0.11-1.08)
IR (number SPM/100 pts. Years)	11/11.83 = 0.9	4/12.39 = 0.3

*A intermediate dose melphalan, IDM, followed by IFN-α-2a maintenance; *B IDM + Myeloablative cyclophoshamide/TBI + ASCT followed by IFN-α-2a maintenance; **A HDM followed by IFN-α-2a maintenance; **B HDM followed by thalidomide maintenance; ***A HDM followed by thalidomide maintenance; ***B HDM followed by bortezomib maintenance.
HR indicates hazard ratio; CI, confidence interval; and IR, incidence rate.

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LENALIDOMIDE AND PREDNISONE (RP) FOLLOWED BY LENALIDOMIDE, MELPHALAN AND PREDNISONE (MPR) IN NEWLY DIAGNOSED ELDERLY MULTIPLE MYELOMA PATIENTS: A MULTICENTER, OPEN LABEL STUDY

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Melphalan-Prednisone-Lenalidomide (MPR) showed promising results in newly diagnosed multiple myeloma (MM). This 2-stage phase II study evaluates safety and efficacy of Lenalidomide-Prednisone (RP) induction, followed by MPR consolidation in unfit elderly pts with newly diagnosed symptomatic MM. Pts with low blood count, abnormal performance status, hepatic, renal, cardiac or pulmonary functions were included. Pts received 4 RP courses (Lenalidomide 25mg/day for 21 days, plus Prednisone 50mg 3 times/wk continuously) followed by 6 MPR cycles (Melphalan 2mg and Prednisone 50mg 3 times/wk continuously, plus Lenalidomide 10-15mg/day for 21 days every 4 wks) and RP maintenance (Lenalidomide 10mg/day for 21 days and Prednisone 25mg for 3 times/ wk). MP was combined with Lenalidomide 15mg (12 pts) or 10mg (12 pts); 22 additional pts received 10mg. A total of 46 pts (median age 75 yrs) were enrolled and 36 pts were evaluable after a median of 7 cycles and a median follow-up of 8.5 months. During RP, the most frequent grade 3-4 hematological adverse events (AEs) were neutropenia (19%), anemia (11%), thrombocytopenia (6%). During MPR, AEs were neutropenia (42%), and thrombocytopenia (4%). Non-hematologic toxicities (cutaneous rash and infections) were more frequent during RP compared to MPR. After RP, at least partial response (PR) rate was 67%, at least very good partial response (VGPR) was 17%. After 2 MPR, PR rate increased to 72%, including 22% of ≥VGPR.Conclusions.RP induction followed by MPR consolidation showed a good safety profile with reduced toxicity in unfit elderly MM.

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EFFICACY AND SAFETY OF THREE BORTEZOMIB-BASED INDUCTION REGIMENS FOLLOWED BY WEEKLY BORTEZOMIB MAINTENANCE THERAPY IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS (PTS) INELIGIBLE FOR TRANSPLANT: RESULTS OF THE PHASE 3B UPFRONT STUDY

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Introduction: The multicenter, phase 3b UPFRONT study compares the efficacy and safety of 3 bortezomib (Vc)-based induction regimens, VcD (Vc-dexamethasone), VcTD (Vc-thalidomide-dexamethasone) and VcMP (Vc-melphalan-prednisone), followed by weekly Vc maintenance in newly diagnosed MM pts ineligible for HDT-SCT. *Methods:* Pts were randomized (1:1:1) to receive 8 induction cycles followed by 5 maintenance cycles. Efficacy and safety were assessed after 100 pts in each arm had the opportunity to complete 13 cycles. Responses were based on IMWG criteria. *Results:* All three induction (I) regimens were active (Table). Vc maintenance (M) was associated with modest increases in ≥VGPR rates in all 3 arms, with little additional toxicity. Grade ≥3 AEs were highest for VcTD (86% vs 74% VcD and 80% VcMP). Post-induction, grade ≥3 PN rates were 15% VcD, 26% VcTD, and 20% VcMP; during maintenance, grade ≥3 PN rates were low (5%, 6%, and 2%, respectively). Median follow-up was 13.4 months. PFS appeared similar between treatment arms (p=0.51) in the ITT population (n=502). Vc maintenance is tolerable and effective in sustaining responses, with a trend to improving response depth, in newly diagnosed transplant-ineligible MM pts. Pts continue to be followed for progression/survival.

	VcD (n=99)		VcTD (n=93)		VcMP (n=99)	
	I	I+M	I	I+M	I	I+M
Median cycles received, n (range)	9 (1–13)		6 (1–13)		7 (1–13)	
Mean Vc doses received/planned, %	76	73	63	77	69	85
Pts receiving Vc maintenance, %	56		33		43	
ORR, %	68	71	78	79	71	73
CR+nCR	24	31	36	38	31	34
≥VGPR	36	39	44	47	40	44
PR	32	32	34	32	31	29
Median PFS, months	13.8		18.4		17.3	

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PEGYLATED INTERPHERON ALPHA-2B (PEGINTRON®) IN A WEEKLY DOSE AS MAINTENANCE TREATMENT AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA (MM)

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Introduction: The favourable results observed with lenalidomide as maintenance treatment post-autologous PBSC transplant in MM have reopen the debate of this approach. We present interim results of a study with Pegintron as maintenance treatment after APBSCT. *Patients and Methods:* Between 05/2003 y 03/2007, 34 MM patients, 57 (44-65), were included. Pegintron®, (Schering-Plough/MSD) was administered sc in PR or CR after APBSCT. Initial dose was 15 µg/w x 2, escalated to 25 µg/w and to 35 µg/w.Maintenance was continued during at least 5 years or until toxicity, relapse or progressión. *Results:* Interval to treatment was 4 m (1-12). Median dose of Pegintron was 15 mcg/w. 4 patients have finished the 5 years of maintenance with sustained CR and 5 keep on therapy. 25 patients (73%) have suspended, 20 (58%) for progresión or relapsed, 3 (8%) for toxicity and 2 (5 %) for personal decision. Median EFS post- APBCT was 38 m (10-58) and median survival was 52 m (20-81). One case of grade > 3 SAE (subclavia VTP) was observed. Mild bone pain, "flu-lyke syndrome" and mild thrombopenia and neutropenia, were the most common adverse effect reversible when adjusting dose. *Conclusions:* Maintenance with Pegintron could be efficacious after APBSCT in MM. Toxicity was acceptable and no relevant negative effect was showed on graft. This formualtion could be an alternative to standard interpheron. Longer follow-up and more experience are needed along with the evaluation of the potential synergy in this setting with immunomodulatory drugs as lenalidomide and other new drugs.

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VMPT (BORTEZOMIB, MELPHALAN, PREDNISONE AND THALIDOMIDE) FOLLOWED BY MAINTENANCE WITH BORTEZOMIB-THALIDOMIDE (VT) IS ACTIVE AND WELL TOLERATED IN NEWLY DIAGNOSED PATIENTS WITH MULTIPLE MYELOMA (MM) WITH MODERATE RENAL IMPAIRMENT (RI) INELIGIBLE FOR AUTOLOGOUS STEM CELL TRANSPLANTATION: RESULTS FROM A COHORT ANALYSIS OF A PHASE III RANDOMIZED CONTROLLED TRIAL

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Purpose: We assessed efficacy, safety and reversal of RI in previously untreated MM by VMPT-VT vs VMP. *Patients & Methods:* Pts received nine 6-wk cycles of VMPT or VMP, followed by maintenance with VT until progression. After 139 pts, both VMPT-VT and VMP induction schedules were emended from twice to once/wk. Pts with creatinine level >25 mg/L were excluded. *Results:* In the VMPT-VT/VMP arms, 6%/7.9%, 24.1%/24.9% and 69.8%/67.2% pts had GFR<30, 31-50, and >50 mL/min, respectively. ORRs were higher and PFS longer with VMPT vs VMP across all renal cohorts, but in pts with severe RI no difference in OS was observed. Within the VMPT-VT and VMP arms, ORR and CR rates as well as PFS were similar across renal cohorts. Only pts in the VMPT arm with severe RI showed reduced OS. RI was reversed in 16/62 (25.8%) pts receiving VMPT-VT vs 31/77 (40.3%) receiving VMP. By logistic multivariate analysis, male sex (p=0.022) and less severe RI (p=0.003) significantly predicted RI recovery, while therapy had no influence. Within the VMP arm only pts achieving renal response showed longer OS. In both arms, pts with GFR<50 mL/min reporting grade 3/4 hematologic or AEs were statistically higher than pts with GFR>50 mL/min. A significant higher rate (p=0.042) of treatment interruption was observed in pts with GFR<50 mL/min treated with VMPT-VT compared to similar pts treated with VPM. *Conclusion:* VMPT-VT was superior to VMP for MM cases with moderate RI, however VMPT-VT showed no improvement in pts with severe RI. Moreover, the VMPT-VT schedule had no advantage in terms of RI reversal over VMP.

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MINIMAL RESIDUAL DISEASE (MRD) ASSESSMENT BY MULTIPARAMETER FLOW CYTOMETRY (FCM) IN A MULTICENTER RANDOMIZED PHASE 2 STUDY IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM): FEASIBILITY AND CORRELATION WITH RESPONSE AND SURVIVAL OUTCOMES

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Routine use of multidrug combinations incorporating novel agents has led to deep responses in patients with MM, rarely seen with traditional therapies. This has highlighted the need for more sensitive measures of disease burden, and, with previous studies correlating improved outcomes with MRD negative status, has led to increasing use of FCM- or PCR-based MRD assessment. In the context of the multicenter phase 2 EVOLUTION trial evaluating combinations of bortezomib, lenalidomide, cyclophosphamide and dexamethasone in newly diagnosed MM, we performed FCM-based assessment of MRD at the time of suspected complete response (CR). Bone marrow (BM) aspirate was placed in a fixative (Cytochek; for maintaining surface expression of antigens) and sent to a single institution for analysis. Samples were analyzed within 48 hrs using a panel of antibodies against CD138, CD38, CD45, CD19, CD56, kappa and lambda on a FACS CantoTM flow cytometer. An algorithmic gating strategy was used; if no clonal plasma cells (PC) were detected at thresholds of <100 events or <0.01% of total analyzed events, the sample was considered MRD negative. Among the 158 pts treated, 97% had a baseline FCM sample analyzed. In the expansion cohorts, 35 pts achieved CR, of whom 28 (80%) had successful FCM analysis of bone marrow at time of CR. FCM allowed a more sensitive disease assessment, with 15 (54%) patients classified as CR by conventional methods being classified as MRD positive. An assessment of progression-free survival and overall survival of these patient groups will be presented.

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IMMUNE FUNCTION IN MULTIPLE MYELOMA - PLENTY LEFT TO WORK WITH

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Multiple myeloma (MM) is thought to profoundly impair humoral and cellular immunity (CI) but there has been no detailed evaluation of the relative impact of MM and therapy. We analysed CI on day (d) 1 cycle (c) 1 and d1c4 in two clinical trials; LITVAC (LV) n=19 untreated MM patients (pts) received low dose lenalidomide (LDL) 15mg d1-21 and dexamethasone (dex) 20mg weekly; REVLITE (RL) n=25 relapsed MM pts received LDL and dex 20mg D1-4, 8-11 and 14-17. Lymphocyte (Ly) subsets were enumerated by flow cytometry and proliferative capacity (PC) was determined by CFSE dilution in a mixed ly reaction (MLR). NK function was assessed by cytotoxicity against K562 cells. All data were compared to age-matched controls (AC). At study entry LV and RL pts had reduced mean CD3 ($p<0.05$, 0.01), CD4 ($p<0.01$) and Treg ($P<0.01$) compared to AC. CD4 lys were lower in RL vs LV pts ($P<0.01$). On d1c4 of RL there was a rise in Tregs ($P<0.01$). B cells were reduced on d1c1+4 in RL and d1c4 in LV pts ($P<0.01$). The PC of LV and RL CD3, 4 and 8 lys on d1c1+4 was similar to AC. Three LV and 3 RL pts had pronounced spontaneous proliferation on d1c1 that normalised by d1c4. NK function was reduced on d1c1 LV vs RL and AC ($P<0.05$). By d1c4 NK function in LV pts had improved but was reduced in RL pts vs AC ($p<0.05$). CI in MM pts at diagnosis and relapse is relatively intact, with specific reductions in CD4, 19 and Treg. The immune defect in MM is largely due to reduced CD4 T cell numbers directly related to active disease and exposure to high dose dex. MM immunotherapies should focus on CD4 T cell augmentation.

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HIGH RATE OF DURABLE RESPONSES IN A PHASE I/II TRIAL OF THE HISTONE DEACETYLASE INHIBITOR ROMIDEPSIN COMBINED WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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We present results of a phase I/II study assessing the tolerability and clinical efficacy of a histone deacetylase inhibitor, romidepsin (romi), proteasome inhibitor, bortezomib (bz), and dexamethasone (dex) in the treatment of Multiple Myeloma in subjects who had previously received at least one prior therapy. We employed a novel accelerated dose escalation schedule initially enrolling one patient per dose level. On first instance of dose limiting toxicity or second instance of moderate toxicity, the study reverted to a standard 3 + 3 design. A total of 25 patients were enrolled. Median lines of prior therapy was 2 (1-3). ISS at enrolment was 1 (52%) 2 (28%) and 3 (20%). The maximum tolerated dose defined as bz 1.3mg/m^2 (D1, 4, 8 and 11) dex 20mg (day of and day after each dose of bz) and romi 10mg/m2 (day 1, 8 and 15) on a 28 day cycle. As expected, thrombocytopenia (64%) was the most common grade ≥ 3 haematological toxicity with predictable recovery prior to the start of the next cycle. Peripheral neuropathy, mainly sensory, occurred in 76% (n = 19) with 20% (n = 5) experiencing > grade 3 bz-related neuropathy that did not improve with dose interruption. Maintenance romi 10mg/m2 (D1 and 8 of a 28 day cycle) was feasible, with 12 patients receiving a median of 7.5 (range: 1-29) cycles. An overall M-protein response of minimal response (MR) or better was seen in 18/25 patients (72%); 2

(8%) CR (bone marrow confirmed), 13 (52%) PR and three MR with a median time to progression of 7.2 (range: 5.5-19.6) months. This promising regimen warrants further evaluation.

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FINAL RESULTS FROM THE MULTI-CENTER, RANDOMIZED, PHASE 2 EVOLUTION STUDY OF COMBINATIONS OF BORTEZOMIB (V), DEXAMETHASONE (D), CYCLOPHOSPHAMIDE (C), AND LENALIDOMIDE (R) IN PREVIOUSLY UNTREATED MULTIPLE MYELOMA (MM)

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Combinations of V and D with either C or R have shown significant activity in phase 2 studies. We designed this randomized phase 2 trial to concurrently evaluate these regimens (VDC, VRD) along with a combination of all four drugs (VDCR) in untreated MM. Patients were randomized to 1 of 4 treatment groups receiving up to eight 21-d cycles of induction with VDCR (V 1.3 mg/m^2 d 1, 4, 8, 11; D 40 mg d 1, 8, 15; R 15 mg d 1–14; C 500 mg/m2 d 1, 8), VDR (VD as in VDCR, R 25 mg d1–14), VDC (VDC as in VDCR), or VDC-mod (as in VDC plus a d 15 dose of C); followed by V 1.3 mg/m2 (d 1, 8, 15, 22) for four 42-d maintenance cycles in all arms. Best response was PR or better for 88, 85, 75 and 100% including a CR rate of 24, 24, 22 and 47%, respectively, for the VDCR, VDR, VDC and VDC-mod arms. Hematological toxicities, peripheral neuropathy, fatigue and GI disturbance were the most common AEs, with a 17, 19, 12 and 6% AE-related discontinuation rate, respectively. With a median follow up of 15–22 months for the 4 groups, the 1-year overall survival was 92% for VDCR and 100% for the others. Discontinuation rates and time to discontinuation were similar between the different arms, with 19–30% of patients completing maintenance. In conclusion, all regimens were highly active and generally well tolerated. VDC-mod was particularly effective although the numbers enrolled in this arm were small (n=17). Addition of C to VDR added toxicity with no substantial improvement in response. Further study of VDC-mod and VDR is warranted.

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LENALIDOMIDE, BORTEZOMIB, AND DEXAMETHASONE (RVD) IN COMBINATION WITH VORINOSTAT AS FRONT-LINE THERAPY FOR PATIENTS WITH MULTIPLE MYELOMA (MM): INITIAL RESULTS OF A PHASE 1 STUDY

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The combination of lenalidomide (R), bortezomib (V), and dexamethasone (D) (RVD) in newly diagnosed MM patients is well tolerated and associated with a high overall response rate. Preclinical studies have demonstrated that vorinostat (Vor), an HDAC inhibitor, is synergistic with bortezomib, IMiD® compounds and dexamethasone. The aim of this study is to determine the tolerability and efficacy of the combination of RVD with vor, provided by Merck and Co. Inc, in newly diagnosed patients with MM. Patients received the standard RVD regimen with vor in 4 dose levels. 17 pts (median age 55, 71% men, 47% ISS Stage II/III) have been enrolled to date with 4 pts in dose levels 1 and 2, 6 pts in dose level 3, and 3 in dose level 4. One DLT (syncope) occurred in dose level 1, and 1 DLT occurred in dose level 2 (asymptomatic grade 3 elevation of ALT). No DLTs occurred in dose level 3. All 3 patients in dose level 4 had DLTs (thrombocytopenia, syncope and dyspnea/sudden death). Hence, the MTD was dose level 3. Peripheral neuropathy occurred in 8 patients (3 grade 2 and 1 grade 3). No episodes of study related grade 3 fatigue, nausea, or vomiting, have occurred. Sixteen patients are evaluable for response. All have responded to study therapy with 7 nCR/CR (41%), 4 VGPR (24%) and 5 PR 29%) within four cycles. The combination of RVD with vor up to 300mg has been generally well tolerated to date. Vor of 400mg in combination with full dose RVD is not tolerated. Efficacy is promising with 65% of patients achieving at least a VGPR within four cycles. Accrual to dose level 3 is ongoing.

Dose Level	Assigned Therapy
Level 1	25 mg lenalidomide daily on days 1-14 followed by 7-day rest every 21 days 1.3 mg/m^2 bortezomib daily on days 1, 4, 8 and 11 20 mg dexamethasone daily on Days 1, 2, 4, 5, 8, 9, 11, 12 100mg vorinostat daily days 1-14 followed by 7 day rest every 21 days
Level 2	25 mg lenalidomide daily on days 1-14 followed by 7-day rest every 21 days 1.3 mg/m^2 bortezomib daily on days 1, 4, 8 and 11 20 mg dexamethasone daily on Days 1, 2, 4, 5, 8, 9, 11, 12 and 200mg vorinostat daily days 1-14 followed by 7 day rest every 21 days
Level 3	25 mg lenalidomide daily on days 1-14 followed by 7-day rest every 21 days 1.3 mg/m^2 bortezomib daily on days 1, 4, 8 and 11 20 mg dexamethasone daily on Days 1, 2, 4, 5, 8, 9, 11, 12 and 300mg vorinostat daily days 1-14 followed by 7 day rest every 21 days
Level 4	25 mg lenalidomide daily on days 1-14 followed by 7-day rest every 21 days 1.3 mg/m^2 bortezomib daily on days 1, 4, 8 and 11 20 mg dexamethasone daily on Days 1, 2, 4, 5, 8, 9, 11, 12 and 400mg vorinostat daily days 1-14 followed by 7 day rest every 21 days

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BORTEZOMIB AND DEXAMETHASONE FROM CYCLE 1 AS TREATMENT AND MAINTENANCE FOR MULTIPLE MYELOMA RELAPSE (THE BOMER TRIAL) SIGNIFICANTLY IMPROVES RESPONSE AND TIME TO PROGRESSION: A MATCHED ANALYSIS OF BOMER VS APEX

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No large prospective trial of bortezomib (Btz)/dexamethasone (Dex) from cycle (C) 1 in relapsed (r) multiple myeloma (MM) has been published, despite common use and data showing that adding Dex to Btz in patients (pts) with SD or PD improves overall response (OR). While Btz maintenance (mnt) improves time to progression (TTP) in untreated pts, there is limited data in rMM. 20 sites enrolled 100 rMM pts (Karnofsky≥60, platelets≥50÷109/L and eGFR≥20mL/min) requiring 2nd line therapy (or 3rd line if prior thalidomide (Thal) and/or Dex monotherapy) in a Phase 2 study of IV Btz (1.3mg/m2; Day (D)1,4,8+11) plus PO Dex (20mg; D1,2,4,5,8,9,11+12) for stable disease received Btz (1.3mg/m2;D1) and Dex (20mg;D1,2) q14d until progression. 35/100 pts completed at least 1 C of mnt. A prospectively defined matched analysis of the 1o endpoint OR and 2o endpoints (Complete Response (CR) and TTP) with the Btz-only arm of APEX was performed. The algorithm matched 90 BoMeR and APEX pts for prior (P) Dex, P Thal, time since last therapy, P transplant, and # of P regimens. Toxicity during induction was similar to APEX (Harrison, ASCO 2010). OR by EBMT criteria (CR, PR, MR) was 62% BoMeR vs 42% APEX (odds ratio (OdR) 0.44 (0.244-0.806)), >PR 56% BoMeR vs 36% APEX (OdR 0.44 (0.242-0.804)), with >50% (100%) reduction in M-protein in 76% (30%) of BoMeR vs 51% (18%) APEX pts (n=67). The median TTP was signifi-

cantly longer in BoMeR (300 days) vs. APEX (154 days) (p<0.001). The BoMeR protocol significantly improved OR, M-protein reduction and TTP for rMM pts vs APEX.

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SEQUENTIAL HIGH-DOSE DEXAMETHASONE AND RESPONSE ADOPTED PAD (BORTEZOMIB, ADRIAMYCIN, DEXAMETHASONE) OR VAD (VINCRISTINE, ADRIAMYCIN, DEXAMETHASONE) INDUCTION CHEMOTHERAPY FOLLOWED BY HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR NEWLY DIAGNOSED MULTIPLE MYELOMA; OPEN-LABELLED, MULTICENTER PHASE 2 STUDY (KMM-94 STUDY)-INTERIM ANALYSIS

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Many different types of induction treatment regimens still have been used. We evaluate the efficacy and safety of the short course of high dose dexamethasone (HD dexa) and the response adopted 4th PAD or 2nd VAD induction chemotherapy. Newly diagnosed MM patients from 22 institutions received 2nd cycles of HD dexa. The patients with less than PR to HD dexa received 4th cycles of PAD chemotherapy. The primary endpoint was CR + near CR rate after ASCT. Among 83 patents enrolled from Nov 2009, 19 patients (23%) have been dropped out. This trial will be continued until total 210 patients will be enrolled. The trial is registered on NCI website, number NCT01255514. 83 patients (42 male, 41 female) were enrolled (median age; 56). 26 patients were D-S stage II and 51 were stage III. 15 patients had ISS stage I, 41 had stage II and 27 had stage III. 24 patients had abnormal cytogenetics. There were 32% del13, 7% del17, 18% t(4;14), 13% t(14;16) and 27% t(11;14) in FISH analysis. Among the 64 evaluable patients, CR + PR rate was 44% (28/64) after 2nd HD dexa. 28 patients (44%) received subsequent VAD chemotherapy and 33 patients (52%) received PAD chemotherapy. Among the 42 patients finished VAD or PAD, CR + PR rate was 81% (34/42). 29 patients finished ASCT until now. 11% (7/64) treatment related deaths were observed. The cause of death was disease progression (n=3) and infections (n=4). The short course of HD dexa and the response adopted PAD or VAD induction chemotherapy followed by ASCT showed acceptable response and toxicities. We will report update results of this trial.

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PHASE IB DOSE-ESCALATION STUDY OF ORAL PANOBINOSTAT AND IV BORTEZOMIB IN PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS

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Panobinostat (PAN) is an oral pan-deacetylase inhibitor with synergistic anti-myeloma activity in combination with the proteasome inhibitor bortezomib (BTZ). This phase Ib study of relapsed or relapsed and refractory multiple myeloma (MM) has completed enrollment (N=62). In the dose escalation phase, 47 patients (pts) received PAN thrice weekly every week (wk) with BTZ (1.0 or 1.3 mg/m2 IV Days 1, 4, 8, 11) and optional dexamethasone (DEX) in cycle (C) 2 onwards every 3 wks. The MTD was 20 mg PAN+1.3 mg/m2 BTZ. In the dose expansion phase, 15 pts received the MTD of PAN+BTZ, with a modified PAN schedule (2 wks out of 3) for upfront management of main adverse events (AEs), and DEX starting at C2. Best responses in the escalation phase (median of 2 [1-10] prior therapies) including 15 BTZ-refractory pts are presented (Table 1). Response (≥MR) was observed in 36/47 (76%) of pts and 10/15 (66%) of BTZ-refractory pts. In 6 pts ongoing on PAN alone (BTZ stopped after C8), median therapy duration is 436 days (324-786). Common grade 3/4 AEs included: thrombocytopenia (n=39), neutropenia (n=28), asthenia (n=12), anemia (n=10), with no treatment-related mortality. The dose expansion phase opened in Aug 2010 and the new regimen is expected to improve tolerability and therapy duration. Updated data will be presented and are relevant to the ongoing phase 2 and 3 PANORAMA trials with same dose and schedule. The combination of PAN and BTZ has a predictable and manageable safety profile with promising activity, as reflected by ≥PR of 64%, which was durable and included BTZ-refractory MM.

Table 1: Response in dose escalation phase (n = 47)

Cohort	1	2	3	4	5	6
Panobinostat dose	10 mg	20 mg	20 mg	30 mg	25 mg	20 mg
Bortezomib dose	1.0 mg/m²	1.0 mg/m²	1.3 mg/m²	1.3 mg/m²	1.3 mg/m²	1.3 mg/m²
# of patients: Total (BTZ- refractory)	7 (4)	7 (5)	8 (2)	7 (0)	9 (2)	9 (2)
CR		1 (0)	2 (0)	1 (0)		2 (0)
VGPR	1 (0)		1 (0)		2 (0)	1 (0)
PR		3 (3)	2 (1)	4 (0)	6 (2)	4 (0)
MR	1 (1)	1 (1)	2 (1)	1 (0)		1 (1)
SD*	3 (1)	1 (1)	1 (0)	1 (0)		1 (1)
PD	1 (1)					
NE	1 (1)	1 (0)			1 (0)	

Data cut-off as of 14-Jan-2011

*SD and MR patients are listed separately.

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ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN FIRST LINE MULTIPLE MYELOMA: DOES IT STILL EXIST? RESULTS FROM A MULTICENTRE STUDY

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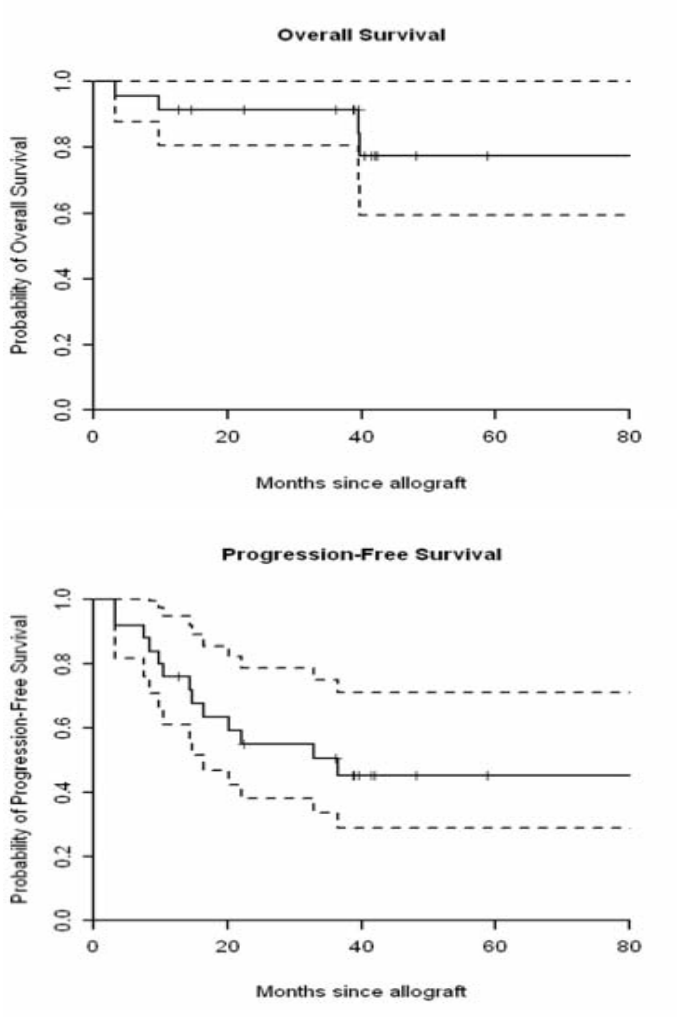
This study concerned MM patients (pts) prospectively allocated to receive a tandem auto-allo-HSCT for being of bad prognosis. They

received RIC followed by allo-HSCT after achieving at least a PR to auto-HSCT. RIC regimen in majority combined Fluda. 30mg/m²/d (d-5→d-1), Busilvex IV 3.2mg/kg/d (d-4, d-3) and ATG 2.5mg/kg/d (d-2, d-1). This analysis included 25 pts, 18 males and 7 females, median age 51 years [28-67], there were 15 IgG, 6 IgA and 4 light chains MM, 14 pts had del13, 7 had del17 and 17 had high level of beta2µ; 7 pts had the 3 factors combined and 6 pts had 2 factors combined . For induction therapy, 16 pts received VAD and 9 patients received Vel.D. Pts received auto-HSCT after a median time of 5.5 months [3.6-15.3] from diagnosis. All pts were in PR after auto-HSCT and before allo-HSCT. Allografts came from 16 identical siblings, 8 matched (10/10) and 1 mismatched (9/10) unrelated donors. At Day 90, 10 pts were in CR, 15<CR among them 9 received Velcade, 6 received other treatments including DLI. There were 8 acute GVHD (7 grade II & 1 grade III) and 11 chronic GVHD (3 lim. and 8 ext.). At the last follow-up, 10 pts were in durable CR1 post allo-HSCT. After a median follow-up of 40 months [3-125], the median OS was not reached with a 6 years probability of 70% [53-95], the median PFS was 36 months [16-125], with a 6 years probability of 45% [29-71], TRM was 8% at 2 years and reached 12% at 4 years. According to these very promising results, we should reconsider the allo-HSCT as a 1st line treatment for MM especially for pts with poor prognostic factors.



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LINES OF THERAPY VS NUMBER OF DRUGS AS PREDICTORS OF PROGRESSION FREE SURVIVAL (PFS) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Previously, Stadtmauer et al (EJH 2009) demonstrated that lenalidomide plus dexamethasone (Len+Dex) significantly prolonged PFS and overall survival when used at first relapse vs use as later line of therapy. This analysis evaluates the impact of the number of lines of therapy (NoL) vs the number of drugs (NoD) on PFS. *Methods:* Data presented is based on pooled data from 2 phase 3 registration trials, MM009/010. Median follow-up was 48 months in surviving patients. Cox regression analyses were used to evaluate the contribution of NoL and NoD on PFS in univariate and multivariate models. The definition of lines of therapy was previously described by Stadtmauer et al. *Results:* 353 pts received Len+Dex. Univariate analysis showed both variables were predictors of PFS; however, only NoD was statistically significant (p=0.02). Neither the average NoD per year since diagnosis nor the average NoL per year since diagnosis were significant predictors of PFS. In a multivariate analysis, NoD was highly significant as a predictor for PFS (p<0.001). Important covariates (p<0.05) factored in the multivariate analysis included number of prior stem cell transplants, albumin, percent cellularity, ISS, and time since diagnosis (p<0.06). Similar results were observed for the placebo+Dex arm. *Conclusion:* These data suggest that the number of prior drugs received is a stronger predictor of PFS than lines of therapy regardless of when these drugs were administered. Len+Dex shows the greatest benefit when used early in the disease course and when fewer drugs are previously administe

Variable	Hazard ratio (95% CI) p-value		
	Univariate	Average per year since diagnosis	Multivariate
NoL	1.13 (0.99, 1.29) p=0.07	1.07 (0.84, 1.38) p=0.58	–
NoD	1.18 (1.03, 1.35) p=0.02	1.10 (0.88), 1.38) p=0.42	1.35 (1.15, 1.59) p<0.001

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PANORAMA 2: A PHASE II STUDY OF ORAL PANOBINOSTAT IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED AND BORTEZOMIB-REFRACTORY MULTIPLE MYELOMA

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Introduction: Current bortezomib (BTZ)-based therapies do not provide benefit for many patients (pts) with multiple myeloma (MM) and novel combinations are urgently needed. Panobinostat (PAN) is an oral pan-deacetylase inhibitor (pan-DACi) with synergistic anti-myeloma activity in combination with BTZ via dual inhibition of the aggresome and proteasome pathways. PANORAMA2, a multicenter US-based, single-arm phase II trial was initiated to evaluate the efficacy of PAN + BTZ + dexamethasone (DEX) in pts with relapsed and BTZ-refractory MM to assess if PAN can sensitize pts to BTZ-based therapy. *Methods:* Adult pts with relapsed and BTZ- refractory MM are eligible. Treatment is comprised of two phases. Phase 1 consists of 8 three-week cycles of PAN (20 mg D1, 3, 5, 8, 10, 12, i.e. thrice weekly (TIW), 2 wks on 1 wk off) + BTZ (IV 1.3 mg/m2 D1, 4, 8, 11) + DEX (20 mg on day of and day after each BTZ dose). Pts demonstrating clinical benefit can proceed to treatment phase 2 which consists of 4 six-week cycles of PAN (20 mg TIW, 2 wks on 1 wk off, and repeat) + BTZ (IV 1.3 mg/m2 D1, 8, 22, 29) + DEX (20 mg on day of and day after each BTZ dose). A Simon two-stage design will be used to test for the primary endpoint of ORR (≥ PR). At least 4 responses are required in Stage 1 (N=24) to proceed. *Results:* As of Jan 24, 2011 enrollment to the first stage has completed (N=24). Response assessment is ongoing.

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LONG TERM OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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We reviewed the outcome of allogeneic stem cell transplantation (SCT) performed for advanced stage multiple myeloma (MM) from 1988 to 2010 at our institution. Forty-one patients (24 males, 17 females), median aged 51y (range 36-67) were transplanted using either bone marrow (n=16) or peripheral stem cells (n=25), from a familial (n=36) or an alternative (n=5) matched donor, after multiple induction therapies (median 2, range 0-5), including autologous SCT (n=15), followed by either a myeloablative (n=10) or a reduced intensity conditioning (RIC)(n=26). At day 100, 42% were in complete response (CR). Grade 2-4 acute graft-versus-host disease (GvHD) occurred in 17 (42%), and extensive chronic GvHD in 14 of them (34%). Three out of 13 showed at least a partial response to DLI. The median follow-up of the surviving patients was 6.8y. The 10y overall (OS) and progression-free survival (PFS) probabilities rates were 35+/-10% (SE) and 11+/-9% (SE) respectively. Cumulative incidence of progression/relapse and treatment-related mortality were 89% (SE 9%) and 25% (SE 7%). There was no statistically significant different outcome between RIC and myeloablative conditioning. Chronic GvHD and the achievement of CR after SCT were not significantly associated with better OS nor PFS. Our observation suggests that allogeneic SCT, even performed in heavily pretreated advanced stage MM, may result in long-term survival and prolonged response in a minority of patients.

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LONG-TERM PROGRESSION-FREE (PFS) AND OVERALL SURVIVAL (OS) WITH TANDEM AUTOLOGOUS TRANSPLANT (TASCT) AFTER HIGH-DOSE INDUCTION WITH MELPHALAN (MEL) AND BUSULFAN/CYCLOPHOSPHAMIDE (BU/CY), OR A NOVEL REGIMEN OF MEL AND TOTAL MARROW IRRADIATION (TMI), FOLLOWED BY MAINTENANCE WITH INTERFERON A-2 (IF) AND/OR THALIDOMIDE (THAL).

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Higher complete (CR) and very good partial response (VGPR) rates predict for improved PFS and OS. We report on 144 pts treated between 5/94 and 3/10, who were enrolled on sequential TASCT trials: Mel 150 mg/m2 [cycle 1 (C1)], and oral Bu 16 mg/kg and Cy120 mg/kg (cycle 2[C2]; 46 pts) Mel followed by iv Bu (12.8 mg/kg and Cy120 mg/kg (68 pts), and recently, Mel 200 mg/ m2 (C1) followed TMI (C2) of 1.2 Gy - 1.8Gy: 30 pts). Maintenance consisted of IF, IF and Thal, or Thal. Pts with responsive or stable MM, ≤ 66 yrs old, with ≤ 40% marrow involvement, with a creatinine clearance of ³ 70 cc/min were enrolled. The median age was 53 yrs (range, 29-66); 102 (71%) of pts were diagnosed with stage III MM. The median time from diagnosis to TASCT was 8 mos (range, 2-73); 91% of pts received both C-s at a median of 2.5 mos (range, 1.0-5.0). CR and VGPR rates were 4 and 13% prior to TASCT, and 47% and 15% after TASCT and prior to maintenance. Treatment-related mortality [TRM] was 7% in pts treated with oral Bu/Cy, 1.2% in pts treated with iv Bu/Cy, and 0 in pts treated with Mel and TMI. Three-yr PFS is 38% ((95%CI, 29-46%) for all pts treated with Bu/Cy and 34 % ((95%CI, 17-52%) after Mel/TMI, and OS is 72% ((95%CI, 63-79%) and 79% (95%CI, 56-91%) respectively; 7-yr PFS and OS for all pts treated with Bu/Cy is 16% ((95%CI, 10-24%) and 44% (95%CI, 35-53%). At 3-yrs, PFS and OS with Mel/TMI TASCT followed by IMid maintenance are comparable to Mel/Bu/Cy TASCT, and the novel regimen - pending further evaluation- may also provide an alternative to tandem Mel -based TASCT.

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PHASE 1 STUDY OF ELOTUZUMAB PLUS LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Elotuzumab, a humanized monoclonal IgG1 antibody targeting human CS1, is highly and uniformly expressed on multiple myeloma (MM) cells with limited expression in normal tissues. In preclinical studies, lenalidomide enhanced the activity of elotuzumab. This phase 1 study evaluated the maximum tolerated dose (MTD), safety, and efficacy of elotuzumab in combination with lenalidomide and dexamethasone in patients with relapsed/refractory MM. In 3 escalating dose cohorts, patients were treated with 5, 10, and 20 mg/kg elotuzumab IV days 1, 8, 15, and 22 q 28 days for 2 cycles and days 1 and 15 of subsequent cycles; lenalidomide 25 mg PO days 1–21; and dexamethasone 40 mg PO weekly. After the first 5 patients received up to 6 cycles of therapy, the protocol was amended to allow treatment until disease progression. During the dose escalation phase, MTD was not reached. For 28 treated patients the most common grade 3/4 adverse events (AEs) were neutropenia (36%) and thrombocytopenia (21%). Infusion reactions appeared to be the primary elotuzumab-related AEs; 2 patients experienced 3 serious infusion-related reactions (1 patient with a grade 4 hypersensitivity reaction and 1 with two grade 3 stridor events) during the first treatment cycle. A PR or better was achieved by 82% of patients, including 95% of lenalidomide-naïve patients. The median time to progression has not been reached for the 20 mg/kg cohort at a median of 12.7 months follow-up. Updated data will be presented at the meeting. A phase 2 study of this combination is ongoing.

Best Confirmed Response (IMWG) After ≥2 Cycles of Therapy

Parameter	All	Lenalidomide-Naïve	Prior Thalidomide	Refractory to Most Recent MM Therapy
Patients, n*	28	22	16	12
ORR (≥ PR), n (%)	23 (82)	21 (95)	15 (94)	10 (83)
ORR 95% CI, n (%)	63–94	77–100	70–100	52–98
CR, n (%)	1 (4)	1 (5)	0	0
VGPR, n (%)	10 (36)	9 (41)	7 (44)	5 (42)
PR, n (%)	12 (43)	11 (50)	8 (50)	5 (42)
SD, n (%)	3 (11)	1 (5)	1 (6)	2 (17)
PD, n (%)	2 (7)	0	0	0

*Twenty-nine patients were enrolled; 1 patient discontinued prior to receiving any treatment.

CI, confidence interval; CR, complete response; IMWG, International Myeloma Working Group; MM, multiple myeloma; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease; VGPR, very good partial response.

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CLINICAL EXPERIENCE WITH LENALIDOMIDE AND DEXAMETHASONE (LEN + DEX) FOR THE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (MM): SAFETY AND EFFICACY DATA FROM THE TURKISH COMPASSIONATE USE PROGRAM (CUP)

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Background: Len + Dex is generally well tolerated and improves survival in patients (pts) with relapsed/refractory MM. Prior to its Turkish approval in March 2010, this compassionate use program provided Len to previously treated MM pts and obtained additional safety data. *Methods:* From Apr'09-Oct'10, 45pts with ≥ 1 prior MM therapy were given Len 25mg/d (D1-21) plus Dex 40mg/d (D1-4, 9-12, and 17-20 for cycles 1-4; D1-4 thereafter) until progression or unacceptable toxicity. Lower Dex doses were used to improve tolerability at investigator discretion. Serum leukocyte, neutrophil, platelet, and Hgb levels were assessed at baseline and prior to subsequent cycles. *Results:* 42pts were evaluable, median follow-up was 4 months. Median age was 60. 67% had ISS stage II/III and 40% had serum β2-M levels ≥ 3.5mg/dL. Pts received a median of 3 prior therapies, including bortezomib (78%) and thalidomide (49%). 45% experienced G3/4 adverse events (AEs), including neutropenia (16%), thrombocytopenia (13%), and anemia (7%). Only 4% had febrile neutropenia of any grade. No peripheral neuropathy was reported. 30% received platelet/red blood cell transfusions and 25% used growth factors. 80.6% used aspirin or LMWH for prophylaxis. 20 of 29 (69%) evaluable pts have already achieved ≥ PR in a median of 12 weeks. *Conclusions:* Preliminary data were consistent with previous reports. G3/4 neutropenia were low, possibly due to adjusted Dex doses and/or growth factor use. Len is an established treatment for relapsed/refractory multiple myeloma.

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PHASE 1 DOSE-ESCALATION STUDY OF INVESTIGATIONAL AGENT MLN9708, AN ORAL PROTEASOME INHIBITOR, IN PATIENTS (PTS) WITH RELAPSED OR REFRACTORY (REL/REF) MULTIPLE MYELOMA (MM)

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Introduction: MLN9708 is an investigational, orally bioavailable, potent, reversible, and specific 20S proteasome inhibitor. MLN9708 is active in solid tumor and hematologic xenograft models. IV and oral formulations are in phase 1 trials. This study is assessing the safety and MTD of oral MLN9708 in pts with rel/ref MM. *Methods:* Adults with rel/ref MM after ≥2 prior therapies, which must have included bortezomib, thalidomide/lenalidomide, and corticosteroids, received MLN9708 on Days 1, 4, 8, 11 every 21 days. Starting dose was 0.24 mg/m^2, escalated using a 3+3 scheme based on DLTs in cycle 1. Toxicity was graded by NCI-CTCAE v3. Response was assessed by modified EBMT criteria. *Results:* To date, 26 pts were enrolled: 3 each at 0.24, 0.48, 0.8, 1.2, and 1.68, 7 at 2.0, and 4 at 2.23 mg/m^2. Pts received a median of 4 cycles (1–12+); 9 are ongoing. DLTs of Gr 3 rash and Gr 4 thrombocytopenia occurred at 2.23 mg/m^2; MTD was established as 2.0 mg/m^2. Common AEs (≥30%) included fatigue, diarrhea, and nausea. Twelve pts had Gr 3/4 AEs; thrombocytopenia (n=6), neutropenia, and non-cardiac chest pain (n=2 each) were seen in >1 pt. Five pts had Gr 1 paraesthesia/neuropathy and one had a Gr 2 event (unrelated). Six pts had dose reductions due to AEs with no discontinuations. SAEs were seen in 9 pts with one on-study death (unrelated). One pt at 1.2 mg/m^2 had a PR and 16 had SD; response evaluation is ongoing. *Conclusions:* These data suggest oral MLN9708 is generally well tolerated and has early signs of activity. Enrollment at MTD continues to further characterize efficacy.

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AUTOLOGOUS TRANSPLANTATION IN RELAPSED MYELOMA

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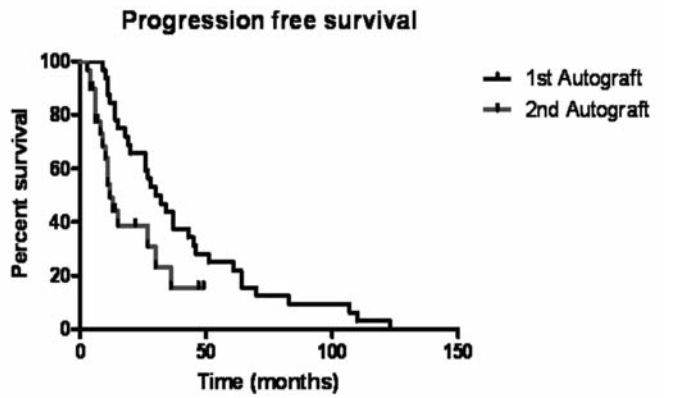
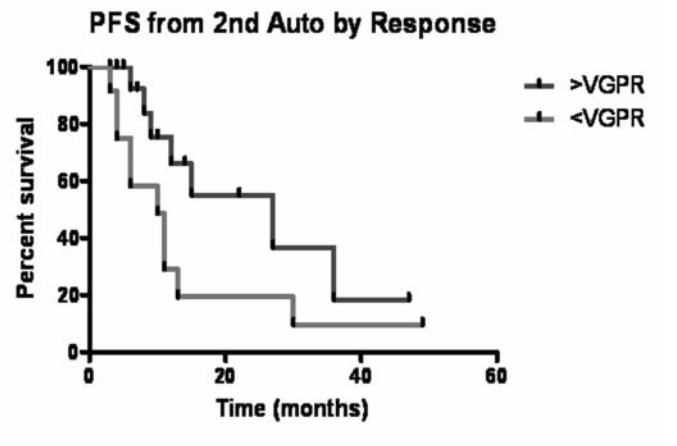
Introduction: Induction chemotherapy followed by autologous transplantation (ASCT) is the gold standard therapy for medically fit myeloma patients. However, all patients will eventually relapse and the optimal treatment at relapse remains to be determined. In particular use of a second ASCT following salvage chemotherapy is contentious in the era of novel therapies. We report a retrospective analysis of second ASCT for relapsed myeloma. *Results:* 33 patients underwent a second ASCT at relapse following salvage chemotherapy (bortezomib based 15/33, lenalidomide based 5/33, thalidomide based 7/33, other 6/33) at a median of 45 months (range 15-126) from 1st ASCT. Median time to neutrophil engraftment was similar but platelet engraftment was significantly longer in the 2nd ASCT group (19.5 v 15 days, p=0.016). No difference was observed in post ASCT overall response rate (>VGPR: 64% v 60%). Progression free survival was significantly longer following 1st ASCT (31 v 12 months, p=0.0175). PFS >18 months following 1st ASCT did not predict for better PFS post 2nd ASCT (15 v 10 months, p=ns) but VGPR response or better following 1st ASCT was significantly associated with gaining VGPR or better after 2nd ASCT (p<0.0001). Patients with VGPR or better following 2nd ASCT had a trend toward better PFS (27 v 10 months, p=0.06). 1/33 patients died within 100 days following 2nd ASCT. *Conclusions:* Salvage including a 2nd ASCT is a safe and effective treatment approach, particularly for patients responding well to 1st ASCT. PFS compares favourably to a non-transplant-based salvage approach.





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EXPRESSION OF MYELOID CELL LEUKEMIA-1 (MCL1) DEMONSTRATED BY USING IMMUNOHISTOCHEMISTRY ON THE BONE MARROW PLASMA CELLS FROM 78 PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA ASSOCIATED WITH LESS RESPONSE RATES AND SHORTER DURATION OF RESPONSE TO VELCADE/DEXAMETHASONE SALVAGE TREATMENT

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Expression of myeloid cell leukemia-1 (MCL1) has been demonstrated to confer resistance of myeloma cells (MCs) to bortezomib (Velcade®) in vitro. However, its clinical role is still unclear. We therefore examined the expression of MCL1 on MCs by immunohistochemistry (IHC) staining on bone marrow (BM) sections from 78 relapsed and/or refractory myeloma patients before commencement of Velcade+Dexamethasone (VD) as the salvage treatment. The positive MCLl staining on IHC was determined by three reviewers. The MCs of 46 patients (59%) were stained positive for MCL1 (Group P), which of the remaining 32 patients (41%) were negative (Group N). Comparing to Group N, the patients in Group P had less overall response rates (ORR), terms of minimal response or better (59% vs. 49%, respectively; p=0.468), and much shorter duration of response (median, 23m vs. 8.5m, respectively; p=0.012). From the preliminary results, we suggest that expression of MCL1 in MCs associated with less response to VD salvage treatment and significant shorter duration of response than the patients whose MCs were stained negative for MCL1. More patients enrolled are required to validate the observation.

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A COMPARISON OF THE EFFICACY AND SAFETY OF LOW-DOSE LENALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE (RD) WITH FULL-DOSE LENALIDOMIDE + DEXAMETHASONE (RD) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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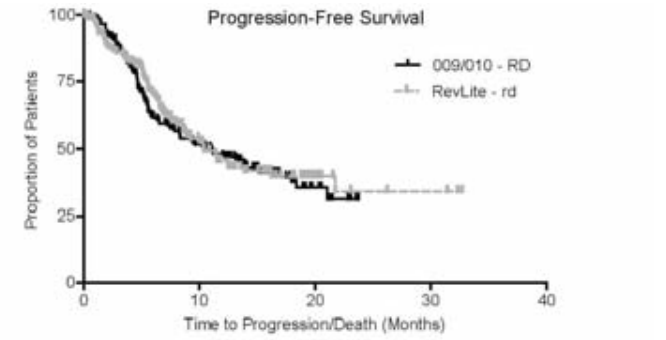
Background: MM009/010 established RD as a standard of care for RRMM. The phase 2 RevLite study evaluated efficacy and safety of a lower dose of both drugs (rd) in RRMM pts at high risk for myelosuppression, including the elderly. We compared efficacy of rd to a subset of MM009/010 RD pts. *Methods:* 150 pts received 28-day cycles (C) of rd: 15mg lenalidomide, D1-21 (C1-4; C5+ if ≥stable disease [SD]) and 20mg dexamethasone (D1-4, 9-12, 17-20 [C1-4] and D1-4 [C5+] if ≥ SD) until progression. MM009/010 treatment has been described. 255 MM009/010 RD pts with ≥1 of the following RevLite eligibility criteria were included in the analysis: age 60+, creatinine clearance (CrCL) ≤60 mL/min, platelets ≤75x10^9/L). *Results:* Baseline characteristics were similar except for prior thalidomide (rd 65% vs RD 36%). With a median follow-up of 9.2 (rd) and 14.8 (RD) months (mos), PFS (rd 10 vs RD 11 mos; P=0.64) and TTP (rd 12.5 vs RD 13 mos, P=0.36) were similar. OS was not significantly different for rd vs RD (25 mos vs not reached, P=NS). Overall response rates were similar (rd 67% vs RD 60%). In pts with CrCL ≤60 mL/min, PFS (8.5 vs 7.4 mos), TTP (10.3 vs 9.5 mos) and OS were similar (15.8 mos vs not reached) for rd vs RD. Pts receiving rd had lower incidence of grade 3/4 AEs vs RD, including neutropenia (19% vs 38%), thrombocytopenia (4.7% vs 13.7%) and thrombotic events (2% vs 13%). *Conclusion:* These data suggest that the low-dose rd regimen is an option in elderly pts with RRMM or pts at high risk of myelosuppression, achieving similar efficacy with less toxicity vs RD.



Study (Regimen)	N	Events (%)	Censored (%)	Median Months (95% CI)
009/010 (RD)	255	117 (46)	138 (54)	11.01 (7.88-15.11)
RevLite (rd)	150	69 (46)	81 (54)	10.38 (8.54-16.23)

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LENALIDOMIDE (R), BORTEZOMIB (V), AND DEXAMETHASONE (D) IN PATIENTS (PTS) WITH RELAPSED (REL) AND RELAPSED/REFRACTORY (REL/REF) MULTIPLE MYELOMA (MM): EFFICACY AND SAFETY DATA AFTER 3 YEARS OF FOLLOW UP IN A MULTICENTER PHASE II TRIAL

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RVD was evaluated in a phase II trial in pts with rel and rel/ref MM; here we report data after a median follow up of 2.9 yrs. Eligible pts had 1-3 prior therapies, age ≥18 yrs, KPS ≥60%. Pts received up to eight 21-d cycles of R 15 mg/d (d1-14), V 1.0 mg/m^2 (d1,4,8,11), D 40/20 mg/d (cycles 1-4), 20/10 mg/d (cycles 5-8; d1,2,4,5,8,9,11,12). Responding pts could receive maintenance RVD. 64 pts were treated; median age was 65 yrs, 66% were male, 42% had rel/ref MM, 44% ECOG PS 0, and 59% had DSS III MM at diagnosis. Median no. of prior therapies was 2. As of Jan 2011, pts received a median of 11 cycles (range 1-60); 66% of pts received ≥8 cycles. Median treatment duration was 7.9 mos (range 0.4-45.5); 18 pts were on study for >1 yr and 3 remain on study at 45-59 cycles. 48 (75%, 90% CI 65, 84) pts were alive without PD at ≥6 mos. 41 (64%) pts had ≥PR and 51 (80%) ≥MR; 16 (25%) pts had CR/nCR. 56 pts have PD, of whom 33 have died; 2 pts died without PD. Median PFS is 9.5 mos (95% CI 7.3, 11.7), with respective 12- and 24-mos PFS of 36% and 16%. Median OS is 29 mos (95% CI 26, 35) with 12- and 24-mos OS of 88% and 66%. Treatment-related AEs included sensory PNY (64%), fatigue (50%), neutropenia (42%), and diarrhea (41%); G3/4 AEs (≥10%) were neutropenia (30%), thrombocytopenia (22%), and lymphopenia (11%). No G3/4 sensory PNY was seen and only 1 pt had DVT. RVD combination therapy was effective and well tolerated in pts with rel and rel/ref MM, with 29 (45%) pts still alive after a median follow up of nearly 3 yrs.

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DOSE-ESCALATION PHASE 1 TRIAL OF ELOTUZUMAB GIVEN WITH FIXED-DOSE BORTEZOMIB IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Elotuzumab, a humanized monoclonal antibody targeting human CS1, is highly and uniformly expressed on multiple myeloma (MM) cells with limited expression in normal tissues. In preclinical studies, bortezomib enhanced the activity of elotuzumab. This phase 1 study evaluated the maximum tolerated dose (MTD), safety, and efficacy of elotuzumab with bortezomib in patients with relapsed/refractory MM. Patients received 4 escalating doses of elotuzumab (2.5, 5, 10, and 20 mg/kg IV, days 1 and 11) with bortezomib (1.3 mg/m^2 IV, days 1, 4, 8, 11) q 21 days. After cycle 2 or 3, patients with disease progression were given dexamethasone 20 mg PO (days 1, 2, 4, 5, 8, 9, 11, 12). Patients with ≥ stable disease after 4 cycles continued treatment until disease progression or unexpected toxicity. No dose-limiting toxicities were observed in the dose-escalation phase and an MTD was not reached. Among 28 patients enrolled and treated, the most common grade 3/4 adverse events (AEs) were lymphopenia (25%), fatigue (14%), neutropenia, thrombocytopenia, hyperglycemia, and peripheral neuropathy (11% each). Most elotuzumab-related AEs appeared to be infusion reactions; 1 patient experienced a grade 3 hypersensitivity reaction. Among 27 evaluable patients, 48% had ≥ partial response and 63% ≥ minimal response. Two of 4 bortezomib-refractory patients had a partial response. Median time to progression was 9.46 months for all patients and 5.78 months for patients with prior bortezomib. Two patients remain on study. These results support further study of this combination.

Best Response (EBMT) After ≥2 Cycles of Therapy in Evaluable Patients*

Parameter	All	Prior Bortezomib	Refractory to Prior Bortezomib	Refractory to Last Line of Therapy
Patients, n	27	11	4	12
Objective response rate (≥ PR), n (%)	13 (48)	5 (45)	2 (50)	5 (42)
Clinical response rate (≥ MR), n (%)	17 (63)	5 (45)	2 (50)	7 (58)
CR, n (%)	2 (7)	0	0	1 (8)
PR, n (%)	11 (41)	5 (45)	2 (50)	4 (33)
MR, n (%)	4 (15)	0	0	2 (17)
SD, n (%)	8 (30)	5 (45)	1 (25)	4 (33)
PD, n (%)	2 (7)	1 (9)	1 (25)	1 (8)

*Patients who completed at least 2 cycles of therapy or progressed earlier.
CR, complete response; EBMT, the European Group for Blood and Marrow Transplantation; MR, minimal response; PD, progressive disease; PR, partial response; SD, stable disease.

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EFFICACY OF LENALIDOMIDE THERAPY IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: INFLUENCE OF NUMBER AND TIMING OF PRIOR ASCT(S)

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Our myeloma program has assumed it is preferable to maximize the use of 1 or 2 ASCT(s) early in the course of multiple myeloma (MM) therapy, and reserve lenalidomide (len) for subsequent relapse, but this approach has never been formally evaluated. We retrospectively examined 129 patients treated with len-based regimens for relapsed MM between 2003 - 2008 to determine if their prior exposure to ASCT had an impact on their response, PFS or overall survival (OS) after len. The len-treated patients were subdivided into 4 groups: 1) pts with no prior ASCT; 2) pts with a single prior ASCT; 3) pts with a second salvage ASCT; 4) pts with planned tandem ASCT. Pts in group 1 were older, had higher beta2M levels, and had less often received prior bortezomib. The median F/U was 7 yrs from diagnosis (3.2–15.7) and 2.7 yrs (0.2–6.9) from start of len. Response (CR+VGPR+PR) rates to len among the 4 groups did not differ: 52%, 61%, 68%, 63% for groups 1, 2, 3 and 4 respectively. Duration on len was significantly longer for the tandem ASCT group. Median PFS (mos) was 6.4, 7, 9.6 and NYR (p=0.02), while the median OS (mos) was 10, 14, 29 and NYR (p=0.005) after len for groups 1,2,3 and 4, respectively. We conclude: 1) the deferral of len until after 2 ASCTs – with the second for salvage or part of a tandem approach – is not detrimental in terms of the anti-myeloma efficacy of this important MM drug; 2) a strategy of tandem ASCT followed by len for relapse yielded the best overall results. The contribution of pt selection bias on these results is being assessed.

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CRD COMBINATION TREATMENT WITH CARFILZOMIB, LENALIDOMIDE (REVLIMID®), AND LOW DOSE DEXAMETHASONE IS HIGHLY ACTIVE IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM): PHASE I/II MMRC STUDY

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Introduction: Combination of Carfilzomib (Cfz), Lenalidomide (Len), and Dexamethasone (Dex) shows promising activity and is well tolerated in relapsed/refractory MM, including limited peripheral neuropathy (PN), which provided rationale for this study in new MM. *Methods:* Three dose levels were evaluated in Phase (Ph) I using TITE-CRM algorithm. Cfz was the only escalating agent at 20, 27 and 36 mg/m2 on days 1,2,8,9,15,16, with Len 25 mg days 1-21 and Dex 40/20 mg weekly (cycles 1-4/5-8) in 28-day cycles. All patients (pts) receive 8 initial cycles followed by CRd maintenance. ASCT candidates collect stem cells after 4 cycles then resume treatment. Responses are assessed by IMWG criteria with addition of nCR. *Results:* The study enrolled planned 35 pts in Ph I, 4 at level 1, 14 at level 2 and 19 at level 3. MTD was not reached and maximum-planned dose level 3 was selected for treatment of additional 18 pts in Ph II. Toxicities were mostly mild, most common anemia (55%), thrombocytopenia (51%) and hyperglycemia (58%). PN was limited to G1/2 (10%), even after prolonged treatment. G3/4 AEs included thrombocytopenia (10%), neutropenia (7%), DVT/PE (7%) and dyspnea (7%). After a median of 6 cycles (range 1-13), best response rates are: ≥PR 96%, ≥VGPR 70%, CR/nCR 55%. Responses are rapid and improving with ≥nCR/VGPR/PR in 24/40/96%, 36/59/100% and 67/82/100% after 2, 4 and 8 cycles, respectively. No pts progressed and all are alive. *Conclusions:* CRd is highly active and well tolerated. Response and safety data from the combined Ph I/II study will be presented at the meeting.

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PHASE 2 STUDY OF ELOTUZUMAB IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA

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Elotuzumab is a humanized monoclonal IgG1 antibody targeting human CS1, a cell surface glycoprotein. CS1 is highly and uniformly expressed on multiple myeloma (MM) cells, with limited expression on natural killer (NK) cells and CD8+ T-cells. A phase 1 study of elotuzumab, lenalidomide, and dexamethasone demonstrated an 82% response rate and did not identify a maximum tolerated dose in patients with advanced MM. In this ongoing multicenter phase 2 study, patients with 1–3 prior therapies were randomized 1:1 to elotuzumab 10 or 20 mg/kg IV (days 1, 8, 15, and 22 q 28 days in first 2 cycles; days 1 and 15 of subsequent cycles) with lenalidomide 25 mg PO (days 1 to 21) and dexamethasone 40 mg PO weekly. Treatment continued until disease progression or unacceptable toxicity. Patients received methylprednisolone, diphenhydramine or equivalent, ranitidine or equivalent, and acetaminophen prior to each elotuzumab infusion. The primary objective was to evaluate efficacy. Sixty-three patients were randomized and have been treated; 81% achieved at least a partial response and 37% at least a very good partial response (Table). Results were similar despite prior thalidomide, prior bortezomib, ≥2 prior therapies, and β2 microglobulin ≥3.5 mg/L. The most common grade 3/4 adverse events were neutropenia (14%), lymphopenia (14%), and thrombocytopenia (13%). One patient had a grade 3 elotuzumab infusion-related reaction (rash). Further clinical study of this combination using 10 mg/kg elotuzumab is warranted. Updated data will be presented during the meeting.

Best Confirmed Response (IMWG Criteria)

	Elotuzumab		
Parameter	10 mg/kg	20 mg/kg	Total
Patients, n	31	32	63
ORR (≥ PR), n (%)	28 (90)	23 (72)	51 (81)
sCR/CR, n (%)	3 (10)	2 (6)	5 (8)
VGPR, n (%)	10 (32)	8 (25)	18 (29)
PR, n (%)	15 (48)	13 (41)	28 (44)
SD, n (%)	3 (10)	7 (22)	10 (16)
PD, n (%)	0	0	0
Not evaluable, n (%)	0	2 (6)	2 (3)

CR, complete response; IMWG, International Myeloma Working Group; ORR, objective response rate; PD, progressive disease; PR, partial response; sCR, stringent complete response; SD, stable disease; VGPR, very good partial response.

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GENETIC VARIATION IN THE P2X7 RECEPTOR AND THE ASSOCIATION TO RISK OF DISEASE, EFFECT OF HIGH-DOSE TREATMENT AND BONE DISEASE IN MULTIPLE MYELOMA

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The P2X7 purinergic receptor is an ATP-gated cation channel. The receptor may be involved in immunogenic cell death caused by chemotherapy. Furthermore, the receptor is involved in the osteoclast activity and survival. In a retrospective population-based study of 348 myeloma patients undergoing Auto-SCT between 1994 and 2004, we tested the functional important P2RX7 gene polymorphisms 1068G>A, 1096C>G, 1405A>G, 151 +1g>t, 1513A>C, 1729T>A, 253T>C, 474G>A, 489C>T and 946G>A for association with risk of disease, effect of Auto-SCT and extent of bone disease and vertebral fractures. No association was found to risk of disease when compared to the Danish Osteoporosis Prevention Study cohort. With adjustment for prognostic factor such as ♀2microblobulin, creatinine and stage, no association was found to time-to-treatment failure and overall survival for any of the SNPs, the 5 most common haplotypes and risk stratification according to gain- or loss of function of the receptor. In a small population, homozygous variant carriers of the gain-of-function allele 1405A>G, who did not carry any loss-off-function allele, were at increased risk of vertebral fractures (p=0.01). No convincing association with polymorphism in P2RX7 gene and risk of disease and effect of high-dose treatment was found. An association with vertebral fractures was found for homozygous variant carriers of the gain-of-function allele 1405A>G, who did not carry any loss-off-function allele and suggests further studies on the importance of the P2X7 receptor activation in myeloma bone disease.

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THE USE OF NOVEL AGENTS IN PATIENTS WITH MULTIPLE MYELOMA INITIALLY TREATED WITH ALLOGENEIC STEM CELL TRANSPLANTATION CORRELATES WITH A SIGNIFICANT PROLONGATION OF BOTH OVERALL AND POST RELAPSE SURVIVAL

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Background: The use of allogeneic stem cell transplant (alloHSCT) in multiple myeloma (MM) was initially pursued as potentially curative therapy but has since fallen out of favor. Ongoing care of these patients, especially after relapse, remains a challenge. Here we present our experience of novel agent (NA) use in patients with MM initially treated with alloHSCT. Patients: 108 patients underwent an allografting procedure for MM at our center between 1989 and 2009. The 58 patients who have relapsed made up our primary cohort for analysis. 36 patients received NAs and only 4 received them prior to transplant. Endpoints examined were post relapse survival after the first relapse from the initial SCT procedure (PRS), overall survival (OS) and progression free survival (PFS) from the time of the initial transplant. *Results:* The median OS of the entire cohort was 71.7 months (m). The effect of NA exposure was examined in the cohort of relapsed patients. In this population the median OS, PRS and PFS was 62.8m, 31.5m and 18.8m respectively. Exposure to NAs correlated with improvements in PRS (42.3m vs 10.5m, p = 0.003, figure 1a) and OS (92.1m vs 22.8m, p = 0.025, figure 1b). No significant difference was noted in PFS (21.9m vs 12.1m, p = 0.86). *Conclusions:* Ongoing management of relapsed patients with multiple myeloma in the post allo-SCT setting remains a significant challenge. This retrospective study demonstrates that the use of NAs is both safe and effective in this patient population. NA use correlates with improved OS likely driven by the positive impact on PRS.

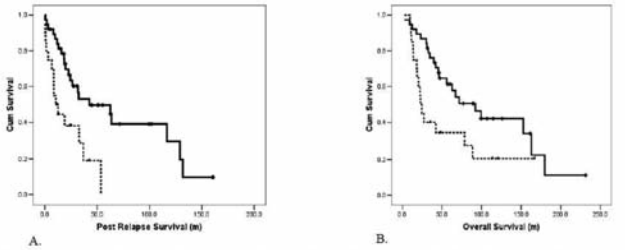

Figure 1) Kaplan-Meier curve of PRS (A) and OS (B) for patients who received an allo-SCT based on NA exposure. (▬) NA and (▪▪▪) no NA.

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MAINTENANCE THERAPY WITH LENALIDOMIDE IN PATIENTS WITH MULTIPLE MYELOMA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION; A DOSE - FINDING - STUDY

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Allogeneic stem cell transplantation (ASCT) is a curative option for multiple myeloma (MM) patients. ASCT with dose-reduced intensity conditioning is associated with higher relapse and progression rate compared with transplantation after a myeloablative conditioning regime. There is a need to continue post-transplant strategies. Lenalidomide is an effective drug in treatment of MM-patients. Aim of our study is to examine: is a maintenance therapy with Lenalidomide possible and the tolerable dosage to find. A total of 24 patients with MM after ASCT were investigated to Lenalidomide maintenance until now. Treatment starts between 100 and 180 days after ASCT. 3 patients started with 5mg/d for four cycles. In this group DLT not appear. The next higher dose level was 10 mg/d Lenalidomide for a total of 6 patients. DLT were observed in 3 patients, which were caused by an acute pancreatitis in one case, elevated liver enzymes CTC grade III, allegeable to liver GvHD in one case and renal insufficiency in one patient. Because three patients in 10 mg cohorte developed DLT, the maximum tolerate dose has been declared as 5 mg. 15 additional patients filled this dose level (5 mg/d). In this cohorte 6 DLT was observed: 3 with GvHD, 1 with elevated liver enzymes and 1 with thrombopenia Grade IV. Our *Conclusion:* 5 mg Lenalidomide as a single agent is the maximum tolerate dose if used early after ASCT (day 100 180) for MM patients. Lenalidomide has immune modulatory properties that might increase the Graft versus myeloma effect and the risk of GvHD.

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IMPACT OF CYTOGENETICS ON THE OUTCOMES IN MULTIPLE MYELOMA PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Purpose: High-risk (HR) cytogenetics (CG) confer poor prognosis in multiple myeloma (MM) patients. We evaluated the impact of HR CG abnormalities on the outcome of patients receiving allo HCT for MM. *Patients and Methods:* 149 patients received allo HCT for MM between 1985 and 2010 at our institution. Pretransplant CG studies were available on 112 of these patients. HR CG features, defined as the presence one or more of the following; CG: hypodiploidy, monosomy of chromosome 13, or deletion 13q; FISH: deletion of p53 (locus 17p13) or IgH translocations, t(4;14), or t(14;16) were identified in 36 patients (Table). The outcomes of these HR patients were compared to the patients without HR CG (SR, standard risk). *Results:* In HR CG group, 26 (79%) had del 13 and 17 (47%) had del 17p, either alone or in combination with other abnormalities. 5 (14%) patients had a hypodiploid clone as the sole HR feature. 100-day TRM was 27% and 5% in HR and SR groups, respectively (p=0.013). Overall response rate in HR group was 67% (CR=6%). 2-yr PFS and OS were 12% and 20% and 32% and 52% in HR and SR groups, respectively (p=0.0005 and 0.0004) (Fig). Nine patients were alive at last follow up in HR group with 5 in remission. The causes of death were recurrent disease (N=15), second malignancy (N=1), acute or chronic GVHD (N=5), infection (N=5), and unknown (N=1). *Conclusion:* HR CG abnormalities are associated with high TRM and poor survival in MM patients undergoing allo HCT. However, approximately 15% of patients in this high-risk cohort were alive and in remission nearly 2 years after allo HCT.

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AN OPEN-LABEL, PHASE 1/2 TRIAL OF BENDAMUSTINE PLUS BORTEZOMIB IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (MM)

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This phase 1/2 study assessed the efficacy and safety of bendamustine plus bortezomib in relapsed/refractory MM. Pts ≥18 yrs with relapsed/refractory MM received bendamustine IV at 50, 70, or 90 mg/m^2 (days 1, 4) plus bortezomib 1.0 mg/m^2 (days 1, 4, 8, 11) for up to eight 28-day cycles. Dose-limiting toxicity (DLT) was assessed after cycle 1. Maximum tolerated dose (MTD) was determined by a 3+3 approach, with MTD cohort expansion in phase 2. Endpoints were objective response rate (ORR), duration of response (DOR), time to progression (TTP), and safety. Pts (n=38; median age, 67; median prior therapies, 3.5) received a median of 3 treatment cycles (range 1-9). Study treatment is ongoing in 14 pts (median cycles 4 [range 1-7]). No DLT was observed; thus, bendamustine 90 mg/m^2 plus bortezomib 1.0 mg/m^2 was studied in phase 2. Grade 3/4 adverse events in ≥10% of pts were neutropenia (34%), thrombocytopenia (21%), and anemia (11%). Grade 3/4 infection occurred in 3 pts (8%); grade 3 renal failure in 2 pts (5%). No grade 3/4 peripheral neuropathy (PN) was observed; grade 1/2 PN was reported in 10 pts (26%; 8/10 had baseline PN). In 36 evaluable pts, ORR was 47%, including 1 very good partial response, 6 partial responses, and 10 minimal responses; ORR was 52% in pts treated at 90 mg/m^2 (n=27). In pts with prior bortezomib (n=27) or prior alkylators (n=26), ORR was 37% and 40%. Overall, clinical benefit rate (response+stable disease) was 94%. Median DOR and TTP have not been reached. Bendamustine plus bortezomib was well tolerated and effective in heavily pretreated MM pts.

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PHASE I TRIAL OF PLERIXAFOR AND BORTEZOMIB AS A CHEMOSENSITIZATION STRATEGY IN RELAPSED OR RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Introduction: Plerixafor (Mozobil®), a potent CXCR4 inhibitor, is approved in combination with G-CSF to mobilize hematopoietic stem cells for autologous transplantation. Another area of investigation consists of exploring whether disruption of the CXCR4 pathway could potentiate the effect of chemotherapy. *Methods:* Eligibility criteria included patients with relapsed or relapsed/refractory MM with any prior lines of therapy including bortezomib. Patients received plerixafor at the recommended dose sc on days 1, 2, 3, 6, 10, and 13 of every cycle. *Results:* Nineteen patients have been treated to date, three in each cohort. The median age is 60, the median lines of prior therapy is 2. All but three patients out 19 have received prior bortezomib. The median number of cycles on therapy was 3 (1-9). To date, there have been no dose-limiting toxicities. Grade 3 possibly related toxicities include lymphopenia (30%), hypophosphatemia (15%), anemia (8%), and hyponatremia (8%). Eighteen patients are evaluable for response, including 1 (6%) complete remission (CR) and 2 (11%) minimal response (MR), with an overall response rate including MR of 3 (17%) in this relapsed/refractory population. We also examined the number of plasma cells, CD34+ HSCs, and other accessory bone marrow cells. Analysis of these samples indicates de-adhesion of plasma cells. *Conclusions:* The combina-

tion of plerixafor and bortezomib is very well tolerated and encouraging responses in this relapsed/refractory population. This study was supported by R01CA133799-01, and by Genzyme.

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MOLECULAR REMISSION AFTER BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE (VTD) AS CONSOLIDATION THERAPY FOLLOWING DOUBLE AUTOLOGOUS TRANSPLANTATION (ASCT) FOR T(4;14)(P16;Q32) POSITIVE MULTIPLE MYELOMA (MM) PATIENTS: RESULTS OF AN INTEGRATE GENOMIC AND EXPRESSION ANALYSIS

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Translocation t(4;14) is associated with a poor clinical outcome in MM patients treated either with conventional or high-dose chemotherapy. Recently, it has been reported that novel agents may overcome the poor prognosis related to this cytogenetic abnormality. We present the results of an integrate genomic and expression analysis performed on 41 patients harboring t(4;14) and enrolled in the VTD arm of the phase 3 GIMEMA trial of VTD vs. TD incorporated into ASCT for newly diagnosed MM. Aim of the study was to investigate the prognostic heterogeneity of t(4;14) positive, VTD-treated patients. On an intention-to-treat basis, the rate of responder (CR+nearCR, R) to VTD induction therapy was 41%. By merging the list of genes differentially expressed in t(4;14) positive patients who either responded or failed to respond to VTD (as detected by GEP), and the list of genes included into regions with copy number alterations (as detected by aCGH), we obtained a list of 5541 probesets with a p value <0.05. Genes located on chr. 1, 19, 3, 9 and 7 resulted most frequently differentially expressed in R vs. non-R patients, as a consequence of chromosome amplification; on the contrary genes located on chr. 14, 13 and 22, resulted most frequently differentially expressed in R vs. non-R patients, as a consequence of chromosome deletions. The Wnt signaling pathway resulted the most significantly differentially affected pathway in t(4;14) positive responder vs. non responder VTD-treated patients. Supported by: Progetto di Ricerca Finalizzata Orientata (to M.C), BolognAIL, Fondazione Carisbo.

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D(T)PACE AS SALVAGE THERAPY FOR AGGRESSIVE OR REFRACTORY MULTIPLE MYELOMA (MM)

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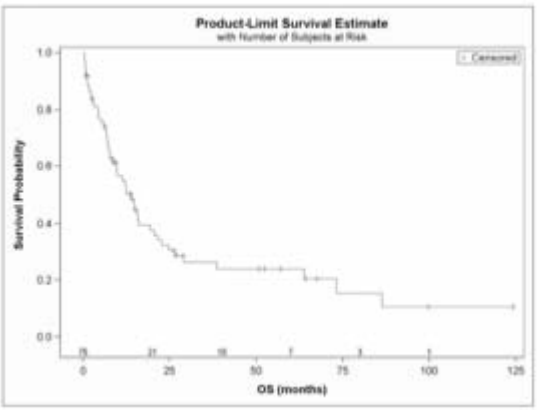
Background: Dexamethasone ± thalidomide with infusion of cisplatin, doxorubicin, cyclophosphamide, and etoposide (D(T)PACE) is generally reserved as salvage therapy for aggressive MM or plasma cell leukemia (PCL) resistant to conventional therapies. The efficacy and durability of this potentially toxic regimen is unclear. *Methods:* We reviewed all pts who received D(T)PACE for relapsed/refractory MM at 2 tertiary care centres, Princess Margaret Hospital, Toronto, Canada and Mayo Clinic in Arizona. *Results:* 75 pts received D(T)PACE for refractory (84%) or relapsed (16%) MM at median 20 mos from diagnosis (range 1-196): median age 54 (range 33-74); 55% Salmon-Durie stage 3; deletion 13q in 50% of those with cytogenetics; PCL, 16 pts; leptomeningeal disease, 3 pts. Pts were heavily pretreated (median 3 prior regimens, range 1-12; ASCT 33%). Thalidomide was included in 38%. Grade 3-4 neutropenia occurred in 92%; thrombocytopenia, 69%; febrile neutropenia and infections, 50%; re-hospitalization, 28%; TRM 7% (2 sepsis, 1 GI bleed, 2 lung disease). ORR was 49% (37 pts: VGPR 12, PR 25); SD 36%; PD 8%. At median follow-up of 26 mos, median PFS was 5.5 mos (CI 4.3-9.8); OS 14.0 (CI 8.7-19.3) (Fig. 1). 34 pts went onto ASCT, 1 to clinical trial, with median PFS 13.4 mos (CI 7.7-20.1) and OS 20.5 mos (CI 14.8-63.8). *Conclusion:* D(T)PACE is effective salvage therapy for heavily pretreated MM pts. Although the ORR of 49% in this poor prognosis cohort is reasonable, the PFS is short, suggesting the best role for D(T)PACE is in bridging to definitive therapy like transplantation.

Figure 1. Overall survival, whole cohort (n=75)



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HIGH DOSE INTRAVENOUS BUSULFAN (BU) AND MELPHALAN (MEL) FOLLOWED BY BORTEZOMIB (BTZ) AS CONDITIONING WITH AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (ASCT) FOR PATIENTS WITH MULTIPLE MYELOMA (MM)

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Background: Overall survival for MM has improved as a result of novel agents. ASCT has improved minimally due to continued reliance on melphalan (MEL). To improve PFS and OS, better transplant regimens are needed. The combination of BU and MEL delivers better PFS compared to MEL 200 alone (Lahuerta, et al). Bortezomib (BTZ) following MEL 200 appears to increase the VGPR rate when compared to patients receiving MEL 200 alone (Lonial, el al). The IFM trial of MEL 200 plus BTZ also demonstrated superior response rates and PFS compared to historical controls (Roussel, et al). *Rationale:* IV BU and MEL followed by BTZ (BuMelVel) will be an effective preparative regimen with acceptable toxicity for patients with MM. *Methods:* Patients received IV BU 130 mg/kg over 3 hr daily x 4 days on D-6 to D-3. Doses were adjusted to achieve an AUC of 20,000 mMol-min. MEL was administered at 140 mg/ m^2 IV over 15-30 minutes on D-2. BTZ 1.6 mg/m^2 was administered IV push on D-1. *Results:* 24 patients have been enrolled, with 23 evaluable for toxicity and 20 for response at D +100. Median age is 63 (47-69). All patients responded: 85% ≥ VGPR including 55% CR or sCR. The most common grade ≥ 3 toxicities was neutropenic fever (n = 12). No VOD or treatment related deaths were observed. *Conclusions:* BuMelVel is an effective regimen with ≥ VGPR and CR/sCR of 85% and 55% respectively, as compared to responses reported with MEL/BTZ (70% and 32%, respectively); and to MEL 200 alone (43% and 11%, respectively) by Roussel et al. The regimen of BuMelVel may lead to improvements in PFS and OS.

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AML AND MDS ARISING AFTER ASCT AND LENALIDOMIDE MAINTENANCE FOR MYELOMA (MM)

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Increasing use of lenalidomide maintenance therapy after high dose melphalan and ASCT for MM has occurred after the preliminary reports of 2 large phase 3 placebo controlled randomized trials (IFM 2005-02 and CALGB/ECOG/BMT-CTN 100104). These trials showed improved progression-free survival for patients receiving long-term lenalidomide maintenance therapy (10 to 15 mg orally) with acceptable short term toxicity when compared to placebo. A number of second cancers have been observed in the subjects of these studies. We have recently observed 2 cases of AML and 1 case of MDS at our center in patients with MM after MEL 200 ASCT and lenalidomide maintenance therapy. To further assess the prevalence of these events, we performed a retrospective chart review of all patients undergoing single MEL 200 ASCT as part of the first line of therapy at our institution between July 2007 and June 2010 to allow for 6 months to 3.5 years of follow-up. During that time 161 transplants were performed. Post-transplant maintenance approaches included observation (obs) 96, lenalidomide 10-15 mg (len) 58, and thalidomide 50-100 mg (thl) 7. The median follow-up for the obs and thl groups is 27 months, and rev group is 16 months. No AML or MDS has been reported in the thl group. One patient (1/96) has developed AML in the obs group. She had a history of prior breast cancer. Two patients have developed AML in the len group and 1 MDS (5q-, 7q-) (3/58) without prior cancer history. Second AML/MDS remains an uncommon event after ASCT for MM but continued close monitoring and follow-up is warranted.

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BORTEZOMIB (VELCADE®)-BASED MOBILIZATION REGIMEN IN MULTIPLE MYELOMA (MM) PATIENTS (PTS) IS ASSOCIATED WITH SPECIFIC GENOMIC EXPRESSION PATTERNS - DECREASE IN SDF-1 PLASMA LEVELS AND UP-REGULATION OF CXCR4 - AND HIGH-YIELD OF CD34+ CELLS

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Mobilization of stem cells in MM is achieved with G-CSF alone or in combination with plerixafor or high-dose cyclophosphamide (CTX). Bortezomib (Vel) and alkylating agents synergize in vitro and in vivo, implying potential concurrent cytoreduction by adding Vel to mobilization regimens. 25 pts with symptomatic, Durie-Salmon stage II/III MM were mobilized with Vel-mob regimen: Vel 1.3 mg/m2 (day 1, 4, 8, 11), CTX 3 g/m2 (day 8), and Filgrastim 10 ug/kg (day 9-18). Prior to mob, pts were induced with six 21-day cycles of Vel/dex ± liposomal doxorubicin. Compared to G-CSF alone, median CD34+ collection was significantly higher (22.6ŧ106/kg vs 10.6 106/kg), and in 23/25 pts (92%) the number collected far exceeded the study goal (10X106cells/kg) typical of CTX and/or GCSF alone [Table 1]. The mechanism of enhanced mobilization was sought in a pilot study of gene expression on CD34+ cells purified from frozen control apheresis samples. Vel-mob (n=7) compared to G-CSF + CTX (n=8) revealed a significant change in expression of genes associated with docosahexaenoic acid and angiopoietin signaling. CXCR4 mRNA isolated from CD34+ cells in Vel-mob pts was 1.44x higher ($p < 0.05$) than in G-CSF + CTX. AKT upregulation was universal in Vel-mob pts. Mean SDF-1 blood plasma level in Vel-mob was significantly lower (p=0.0012) compared to G-CSF alone (1319 pg/ml vs 2225 pg/ml). Vel-mob, a novel mobilization regimen, produces very high and predictable CD34+ yields. Modulation of SDF-1 protein levels and CXCR4 gene expression in CD34+ cells are important pathways in improved mobilization.

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THE ADDITION OF CYCLOPHOSPHAMIDE TO BORTEZOMIB IMPROVES RESPONSE RATE BUT NOT TIME TO NEXT TREATMENT: FIVE YEAR EXPERIENCE OF BORTEZOMIB FOR MYELOMA IN A REGIONAL CANCER NETWORK

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Bortezomib is now a standard of care for relapsed myeloma within the UK. We analysed 118 patients who received bortezomib in our cancer network between April 2005 and July 2010. Median age at diagnosis 61.6 (range 41.2-88.4). Median time from diagnosis to receiving bortezomib 31.3 months (range 1.5-143.2). No patients received bortezomib as initial therapy. 68% received bortezomib at first relapse. 65.3% (n=77) received bortezomib in combination with steroid. 34.7% (n=41) received cyclophosphamide in addition to bortezomib-steroid at clinician discretion. Both groups received a median of 4 cycles (range 1-8). Overall response rate (ORR=CR+PR) was 56.1% (n=24) in patients receiving additional cyclophosphamide compared to 39% (n=29) in the non-cyclophosphamide cohort (p=0.03) - CR 12.2%, PR 43.9% vs CR 10.4% and PR 31.2% respectively. Fewer patients receiving bortezomib with cyclophosphamide (2.4% vs 11.7%) ceased treatment due to loss of initial treatment response, although this did not reach statistical significance (p=0.16). There was also no statistical significant difference in the median time to next treatment: 11.1 months for the bortezomib-steroid group, compared to 9.9 months for the additional cyclophosphamide cohort (p=0.502). In this observational study although the addition of cyclophosphamide to bortezomib produced a higher ORR, it did not result in an increase in the time to next treatment. The strategy of choosing myeloma treatments by best ORR needs to be tested in prospective randomized trials which include response duration.

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EVALUATION OF A NOVEL CLASS OF SULFONANILIDES FOR TARGETED TREATMENT OF MULTIPLE MYELOMA: DUAL-MECHANISM COMPOUNDS INHIBITING HIF1A-SIGNALING AND INDUCING APOPTOSIS

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Background: We have recently shown HIF1A to be expressed in 95.4% of CD138-purified myeloma cell samples from previously untreated patients (n=329). This makes HIF1A an interesting target in myeloma treatment. *Methods:* The effect of the novel sulfonanilides ELR510444 and ELR510552 on the proliferation of myeloma cell lines, the survival of primary myeloma cells, and the efficacy in two mouse models (RPMI8226-xenograft- and 5T33-model) was tested. The mechanism of action was investigated using a variety of in vitro assays. *Results:* 1) ELR510444 and ELR510552 completely inhibit proliferation of 20/20 myeloma cell lines at low nM concentrations, induce apoptosis in 6/6 primary myeloma cell-samples without major effect on the bone marrow microenvironment, and significantly inhibit tumor growth and bone marrow infiltration in two mouse models. 2) The compounds show a potent inhibition of HIF1A signaling, lead to caspase-3/7 activation and subsequent apoptosis, and induce an initial cellular arrest in G2/M, coinciding with a significant tubulin depolymerizing effect, followed by an increase in a sub-G1 population. 3) Both compounds are well-tolerated at levels that are significantly above the in vitro EC50 in all myeloma cell lines and primary samples. *Conclusion:* ELR510444 and ELR510552 are very active members of a novel class of compounds entering a clinical phase I/II trial in summer 2011.

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TARGETING BRUTON'S TYROSINE KINASE WITH PCI-32765 BLOCKS OSTEOCLASTOGENESIS AND INHIBITS IN VIVO HUMAN MULTIPLE MYELOMA GROWTH VIA SIGNIFICANT DOWNREGULATION OF CYTOKINES AND CHEMOKINES IN THE BONE MARROW MICROENVIRONMENT

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Bruton's tyrosine kinase (Btk) is selectively expressed in osteoclasts (OCs) but not osteoblasts, indicating its role in regulating osteoclastogenesis. We here defined molecular targets of Btk signaling in OCs and MM in the bone marrow (BM) milieu. RANKL and M-CSF stimulate a time-dependent phosphorylation of Btk in CD14+ OC precursor cells (OPCs); conversely, PCI-32765, a potent and selective Btk inhibitor with promising clinical activity in B-cell malignancies, blocks RANKL/M-CSF-induced phosphorylation of Btk and downstream PLCγ2, as well as NFATc1. PCI-32765 inhibits RANKL/M-CSF-induced OC formation from OPCs, as evidenced by decreased tartrate-resistant acid phosphatase 5b (ED50=17 nM). It induces abnormal morphology and impaired actin organization, with resultant defective osteoclastic activity. PCI-32765 strongly blocks cytokine and chemokine secretion from OC cultures, including downregulation of MIP-1α, IL-8, MIP-1β, TGFβ1, RANTES, APRIL, SDF-1 , activin A (ED_{50} = 0.1-0.48 nM); as well as inhibition of IL-6 and RANKL in culture supernatants from human BM osteoprogenitor cells. Importantly, it sensitizes MM cells to Dexamethasone in coculture with mCherry-HS5 stromal cells, induces cytotoxicity against patient MM cells and MM cell lines cocultured with OCs. Continuous PCI-32765 treatment (12 mg/kg) in vivo significantly inhibits MM cell growth ($p< 0.03$) and MM cell-induced osteolysis on implanted human bone chips in a humanized myeloma model. Together, these results provide the rationale for clinical trials targeting Btk with PCI-32765 in MM.

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NKT CELLS IN MULTIPLE MYELOMA: A USEFUL THERAPEUTIC TOOL?

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Natural killer T (NKT) cells are T cells with features of both NK and T cells. They recognize glycolipid antigens such as αgalactosylceramide (αGalCer) presented by CD1d. NKT cells can produce either a Th1 (IFNγ) or a Th2 (IL4) cytokine response. Studies of the in vivo role of NKT cells in MM are limited due to a lack of a suitable mouse model. Here we investigated the functionality of NKT cells in the immune competent 5T33MM model. NKT cell numbers were similar in different organs of healthy and diseased mice. Their activity was tested in vitro by culturing liver and splenic NKT cells with DCs, pulsed with αGalCer. After 72h of co-culture, an increase in IFNγ production could be measured in the supernatant of NKT cells derived from healthy and non-terminally diseased mice but not from terminally diseased mice. To confirm that the NKTs were the producers of IFNγ, an intracellular cytokine staining was performed showing an increase in IFNγ production in the NKTs. To analyze serum cytokine production in vivo, αGalCer alone or αGalCer-pulsed DCs were injected into healthy and diseased mice 16h prior isolation. Secretion of IFNγ could be detected in healthy mice; but only a limited level was measured in diseased mice. Finally, an in vitro cytotoxicity assay using calcein-AM was performed where we found that αGalCer stimulated NKTs could induce lysis of 5T33MM cells. We can conclude that NKT cells can be activated in a myeloma setting and that they represent a promising cell type for immune based therapies.

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THE CAP-TRANSLATION INHIBITOR 4EGI-1 INDUCES APOPTOSIS IN MULTIPLE MYELOMA THROUGH NOXA INDUCTION

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Cancer cells are frequently addicted to deregulated oncogenic protein translation. The interaction between the initiation factors eIF4E and eIF4G is tightly regulated and limiting for the formation of the translation initiating complex eIF4F. Therefore, targeting the translation initiation pathway is emerging as a new potential therapy for cancer. 4EG-I is a small molecule that selectively inhibits the mRNA cap-dependent translation by competing for eIF4E/eIF4G interaction. Multiple myeloma is an incurable disease and new therapeutic approaches are needed. Here, we demonstrated that 25μM 4EGI-1 treatment induces a total inhibition of cell proliferation in 6 myeloma cell lines. As expected, 4EGI-I decreases the expression of eIF4E-regulated proteins cyclinD1 and c-myc. Furthermore, we demonstrated that 50μM 4EGI-1 treatment induces strong apoptosis in 5 out of 6 myeloma cell lines. Apoptosis is associated with Bax activation and caspase-9 and 3 cleavage indicating the activation of the intrinsic mitochondrial pathway. Bak and/or Bax knockdown is not sufficient to inhibit 4EGI-1-induced apoptosis, suggesting that they play a redundant role in this process. Surprisingly, 4EGI-1 triggers a rapid induction of Noxa, which was only found in cells undergoing apoptosis upon 4EGI-1 treatment. Finally, Noxa silencing prevents myeloma cells from 4EGI-1-induced apoptosis, demonstrating that Noxa induction is required in this process. Our results suggest that the use of inhibitors that directly target eIF4F complex may represent a potential novel approach for myeloma therapeutics.

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COMBINATION OF DECITABINE AND JNJ-26481585 TRIGGERS SYNERGISTIC ANTI-MYELOMA ACTIVITY

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DNA methylation and histone acetylation are currently the best understood epigenetic modifications. In multiple myeloma (MM), the epigenetic landscape is often disturbed as evidenced by changes in the above modifications. Interfering with them using a DNA methylation inhibitor (DNMTi) or a histone deacetylation inhibitor (HDACi) has anti-myeloma effects. We hypothesized that combining both drugs enhances their anti-MM effects. Here, we investigated the potential of combining the DNMTi decitabine (DAC) and the HDACi JNJ-26481585 (JNJ) in MM. Potential synergistic effects of DAC and JNJ in vitro were assessed using the MM cell lines OPM-2 and 5T33MMvt. Proliferation and cell viability was time- and dose-dependently decreased upon treatment with DAC or JNJ. Moreover, treatment of cells with a combination of both agents synergistically decreased viability. For 5T33MMvt cells, JNJ alone or in combination with DAC induced a temporal G1-phase arrest followed by an increase of cells in subG1-phase in the combination group compared to single agent groups, while in the OPM-2 cells treatment resulted in a more prolonged G1-phase arrest. In agreement, the number of apoptotic MM cells was synergistically increased in the combination groups for both cell lines. Next, we tested also the potential anti-MM effect of DAC and JNJ in vivo, using the murine 5T33MM model. Combinatory treatment significantly decreased tumor burden and microvessel-density compared to single agent treatment. We conclude that combination of the HDACi JNJ and the DNMTi DAC synergistically induce an anti-MM response.

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SYNERGISTIC CYTOTOXICITY EFFECTS OF TH-302 IN COMBINATION WITH BORTEZOMIB IN MULTIPLE MYELOMA

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As previously demonstrated by us and other groups, hypoxia is a critical microenvironment factor in multiple myeloma (MM). Treatment with the hypoxia-activated prodrug TH-302 has showed promising effectiveness in MM (1). In this study, we investigated the combination effects of TH-302 and Bortezomib on MM. Our in vitro results show that the combination of TH-302 and Bortezomib synergistically induced apoptosis, evidenced by induced cleavage of poly (ADP-ribose) polymerase and caspase-3/8/9. To further determine the mechanism of induction of apoptosis by this combination, we investigated the effect of TH-302, Bortezomib and the combination on Bcl-2 family proteins using immunoblotting. The results show that Bortezomib and TH-302 can trigger different anti-apoptotic and pro-apoptotic responses (see table 1). Importantly, TH-302 can abrogate the accumulation of anti-apoptotic Mcl-1L induced by Bortezomib. The mechanism of abrogating Mcl-1L by TH-302 is tightly related to its influence on decreasing HIF1α/HIF2α and ATF4 expression. In addition, the combination of TH-302 and Bortezomib conducted in the 5T33MMvv mouse model in vivo showed impressive improvements in multiple disease parameters: induced significant decreased tumor burden, paraprotein secretion and microvessel density (MVD), compared to TH-302 or Bortezomib alone treated 5T33MMvv mice ($p<0.01$). *Conclusion:* These studies provide the basis for clinical evaluation of the combination of TH302 and Bortezomib for multiple myeloma patients. Reference: (1) Hu J. et al. Blood. 2010;116(9):1524-7.

Table 1. The changes of Bcl-2 family protein expression following Bortezomib or TH-302 treatment in MM cells

Bcl-2 family protein	anti- or pro-apoptotic	change(by Bortezomib)	change(by TH-302)
Bcl-2	antiapoptotic	downregulation or no change	downregulation
Bcl-xL	antiapoptotic	no change	downregulation
Mcl-1	antiapoptotic	upregulation	downregulation
		cleavage	cleavage
BAX	proapoptotic	no change	no change
BAD	proapoptotic	cleavage	cleavage
BID	proapoptotic	cleavage	cleavage
BIK	proapoptotic	upregulation	no change
BIM	proapoptotic	upregulation	no change
PUMA	proapoptotic	no change	upregulation
NOXA	proapoptotic	upregulation	upregulation

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CARFILZOMIB-DEPENDENT INHIBITION OF CHYMOTRYPSIN-LIKE ACTIVITY OF THE PROTEASOME EXERTS ANTI-TUMOR EFFECT IN WALDENSTROM MACROGLOBULINEMIA (WM), BOTH IN VITRO AND IN VIVO

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Introduction: We evaluated the anti-tumor activity of carfilzomib, a new selective chymotrypsin-like (CT-L) proteasome inhibitor in WM, both in vitro and in vivo. *Materials:* Primary CD19+ WM cells were obtained from bone marrow (BM) of WM patients. Level of imunoproteasome (i20S) and constitutive proteasome (c20S) subunits were detected by an ELISA-based assay. Cytotoxicity, DNA synthesis, adhesion, migration and drug synergism have been performed. In vivo studies were perfomed using BCWM.1-GFP+/Luc+ cells injected into SCID mice. *Results:* Carfilzomib inhibited the CT-L activity of both i20S and c20S in primary WM cells, leading to inhibition of proliferation and induction of cytotoxicity; increased PARP, caspases cleavage, as well as induced activation of JNK and ER-stress in a dose-dependent manner. Carfilzomib targeted WM cells even in the context of BM milieu, where inhibition of adhesion and migration were observed, together with inhibition of WM growth even in presence of BMSCs. Combination of carfilzomib and bortezomib induced synergistic cytotoxicity in WM cells, as shown by enhanced PARP-, caspase-9- and -3-cleavage; and synergy in inhibiting the CT-L activity of the i20S and c20S. Anti-tumor activity of carfilzomib has been validated in vivo: carfilzomib-treated mice presented with a significant reduced tumor burden; increased percentage of apoptotic WM cells; and reduced serum IgM levels ($P<.05$), as compared to control mice. *Conclusion:* These findings suggest that targeting i20S and c20S CT-L activity by carfilzomib represents a valid anti-tumor strategy in WM.

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APOPTOSIS INDUCTION BY HSP90 INHIBITOR SYNERGISES WITH AZACYTIDINE AND BORTEZOMIB IN MULTIPLE MYELOMA

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Heat shock protein 90 (HSP90) is the most abundant HSP in the cytosol and has been found to be elevated in various types of cancers. Furthermore, HSP inhibitors (HSP90i) have been shown to inhibit cell growth and induce apoptosis of some cancer cells. We have evaluated a novel, orally bioavailable, HSP90i on a panel of human myeloma cell lines (HMCLs) (both IL6 dependent and independent), primary myeloma (MM) cells from patients with advanced MM and in combination with azacytidine (AZA) and bortezomib. We found that HSP90i demonstrated IC50s of 50-100 nM against HMCLs at 72 hours in concert with cell cycle arrest. Apotosis induction was confirmed by PARP cleavage within 24 hours. Client proteins of HSP90 including IL6R-alpha, AKT, p-MEK, MEK, p-p65, p65, p-Stat3 and Stat3 were down regulated confirming the pleiotropic inhibitory effects of HSP90i against critical signalling pathways (JAK/STAT, PI3K, NF-kappaB and MAPK). Importantly, the addition of exogenous IL-6 did not prevent HSPi induced-apoptosis or reactivation of p-Stat3. Moreover, HSP90i induced p-MEK translocation from the cytosol to the plasma/mitochondrial/Golgi membrane fraction within 8 hours of treatment potentially blocking ERK nuclear translocation with subsequent inhibition of nuclear substrate activation. Combination studies with AZA or bortezomib against both HMCLs and primary MM were found to be synergistic. We conclude that HSP90 represents a valid target for future MM therapy and that HSP90 inhibition warrants further investigation.

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THE HDAC INHIBITOR LBH589 ENHANCES THE ANTI-MYELOMA EFFECT OF THE IGF-1 RTK INHIBITOR PICROPODOPHYLLIN (PPP)

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Our previous studies have shown that inhibition of the IGF-1R pathway by the IGF-1RTK inhibitor picropodophyllin (PPP) is an effective strategy against multiple myeloma (MM) in vitro and in vivo. Here we performed a combinatorial drug screen (HTS) to select the most efficient combination with PPP. The HDAC inhibitor LBH589 was shown to act in synergy with PPP to reduce survival in MM cells. We analyzed the combinatorial effects on apoptosis, cell cycle distribution and the impact on downstream gene and protein expression in human and mouse MM models in vitro. In the human MM cell line RPMI 8226 treatment either drug alone induced a 3-fold increase of apoptotic and late apoptotic/necrotic cells, as compared to controls, while the combination caused a

5-fold increase. With both drugs we observed an additive effect on the cleavage of the active forms of caspase-8 compared to the single drugs. Also combination resulted in an accumulation of cells in the G2/M phase, and subsequent down-regulation of cell cycle regulated proteins cyclin B1, -E and -D2. These data were also confirmed in the mouse 5T33MM cells in vitro. Confirming the potential of this drug combination, gene expression arrays were performed showing regulated genes mainly in the categories of apoptosis and cell adhesion. Combined treatment in vivo resulted in a significant prolonged survival of 5T33MM inoculated mice when compared to the control and to single drug treatment. In conclusion, the results indicate an improved MM treatment opportunity in using a combination of PPP and LBH589.

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TARGETED INHIBITION OF JAK-STAT SIGNALLING IS EFFECTIVE AGAINST PRIMARY MULTIPLE MYELOMA TUMOURS

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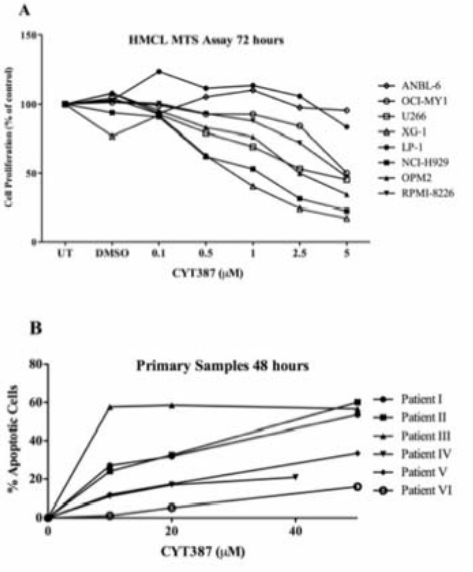
The IL-6/JAK/STAT pathway is important in myeloma cell survival and drug resistance. JAK kinases are signal transduction molecules involved in many cytokine signalling pathways. We evaluated CYT387, an orally bioavailable JAK1/2 inhibitor, against a panel of human myeloma cells lines (HMCL) and primary MM tumours. We studied the effect of CYT387 on IL-6/JAK/STAT3 signalling and found CYT387 completely abolished or dramatically reduced IL-6 induced pSTAT3 in 3/3 HMCL even when cultured with bone marrow (BM) stromal cells. CYT387 (0.1-5μM) inhibited cell proliferation of 6/8 HMCL in a time and dose dependent manner by MTS at 24, 48 and 72 hours (Figure A). Proliferation also decreased by 43-99% in 5/5 HMCL even when cultured with IL-6 as determined by viable cell number. NCI-H929 (IC50 1.5μM), OCI-MY1 (IC50 5μM) and U266 (IC50 5μM) which represent the 'IL-6' and 'IGF-1' phenotype' HMCL demonstrated CYT387 induced apoptosis as determined by Annexin-V/Propidium Iodide at 24 and 72 hours. In addition a two-fold accumulation of cells in the G2/M phase (NCI-H929) was observed. CYT387 showed promising efficacy against primary tumours cultured with autologous BM. A CYT387 time and dose dependant effect was seen to induce apoptosis in up to 37% and 60% of primary MM cells after 24 and 48 hours treatment respectively (Figure B). CYT387 and melphalan synergised in 3/3 HMCL (CI<1) and 2/3 primary samples (CI<1). In conclusion, CYT387 demonstrates considerable anti-myeloma activity in both HMCL and primary tumour cells and synergises with conventional therapy.



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POTENTIAL THERAPEUTIC RELEVANCE OF SIMULTANEOUS MEK/ERK AND MEVALONATE PATHWAY INHIBITION IN MULTIPLE MYELOMA

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The statin family has been shown to trigger apoptosis in cancer cells by regulating several signalling pathways. The MEK/ERK module is overexpressed in hematologic malignancies where it promotes proliferation and survival of the neoplastic cells. Here, we analyzed, in multiple myeloma (MM) cells, proliferative and apoptotic changes induced by the simultaneous inhibition of these pathways. We first exposed different MM cell lines to scalar concentrations of the MEK inhibitor PD0325901 (PD) (1-1000nM) demonstrating its potent growth-inhibitory action, mostly related to the cell cycle arrest. Then, we evaluated the effect of PD in combination with Mevinolin (Mev) (1-100microM). While Mev, as single compound, shows minimal cytotoxic effects, exposure to both molecules resulted in a striking increase of mitochondrial dysfunction and apoptosis induction. An increase of the subG1 was demonstrated in the KMS27 cells, at 72 hours, from 8.8±8.0% (control) to 10.9±8.7% (10nM of PD), 27.4±11.3% (10microM of Mev) and to 66.6±17.0% (PD/Mev) (p=0.004). Preliminary results on purified CD138+ primary cells confirmed that the PD/Mev co-exposure enhanced the cytotoxic effects of the single compounds with a net apoptosis induction, at 72 hours, of 51.5±40% (18.7±2.8% with 10nM of PD and 34.2±25.9% with 10microM of Mev). These results demonstrate that the simultaneous disruption of MEK/ERK and Mev signaling induce a markedly pro-apoptotic activity in MM cells and provide pre-clinical data supporting further evaluation of this combination as potential anti-myeloma strategy.

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TWO VK*MYC MODELS OF MYELOMA TO IDENTIFY CLINICAL ACTIVITY IN EARLY STAGE OR RELAPSED/REFRACTORY MM

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We have demonstrated a high concordance between pharmacologic antitumor activity in our Vk*MYC model and clinical evidence of effective therapeutics. Vk*MYC mice showed dramatic reduction of their M-spike after treatment with known active drugs, while did not respond to inactive drugs, despite the fact that they have been showed to be very active in other pre-clinical studies of MM, including xenografts. Next, we assessed the single agent activity of a panel of 24 drugs that have passed extensive pre-clinical testing and are of putative value against MM. The only active drugs identified are vorinostat, panobinostat, TACI-Ig (but not BAFFR-Ig), PR-047, SNS-032, abraxane (ABR) and perifosine. Although a phase I trial of TACI-Ig in relapsed and refractory MM was not promising, we predict activity of this safe drug in early disease MM patients. Partly based on these studies we have treated a MM patient with no further treatment options with ABR and identified a promising response. We have also derived a syngeneic transplant model that develops a much more aggressive, proliferative and extra-medullary MM and represent a good model for a late stage MM. Interestingly one of these mouse lines retained sensitivity to alkylating agents, while another one is multidrug resistant. Both lines tough showed full response to the combination of BOR + HDACi, while were insensitive to the single agents. Finally, we found that some drugs, like doxorubicin, were inactive in the de-novo Vk*MYC mice, but showed activity in the transplant model, perhaps by targeting the more proliferative MM cells.

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PARATHYROID HORMONE PATHWAY ON CARFILZOMIB ANTIMYELOMA EFFECT

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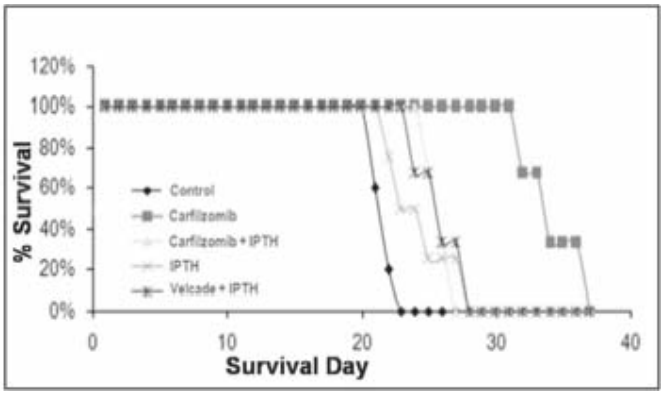
Clinical studies have reported a positive effect of proteasome inhibition on bone health in MM patients, and recently we have reported specific serum PTH variations associated with clinical responses to bortezomib. In this study we have tested the role of PTH/PTHR1 pathway on Carfilzomib activity in vitro and in a mouse model. Carfilzomib PTH and {[TYR34]bPTH-(7-34)} compound (a specific PTH-(7–34) antagonist) were first evaluated at various concentrations in vitro and in the C57BL/KaLwRij mouse model. Cell viability, mice survival and myeloma response by serum IgG level were recorded. The 5TGM1 cell line was initially derived from a 5T33 myeloma cell line that arose spontaneously in aged C57BL/KaLwRij mice. The inoculation of 5TGM1 cells in the inbred C57BL/KaLwRij is able to induce a disease with similar features to human disease. The 5TGM1cell line was found sensitive to carfilzomib inhibition as well as PTH infusion in a dose-dependent fashion. Specific inhibition of PTHR1 by {[TYR34]bPTH-(7-34)} peptide did not affect 5TGM1 cells viability. C57BL/KaLwRij mice treated with Carfilzomib showed a significantly longer survival compared to controls (P= 0.01) (Figure 1). However, when mice were concomitantly treated with Carfilzomib and the PTHR1 inhibitor, the survival benefit was completely abrogated and IgG2b changes supported these findings. These experiments confirm that the proteasome inhibitory effect on myeloma growth in vitro and in mouse model is dependent on the PTH/PTHR1 pathway function.

Figure 1



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GALECTIN-1 EXPRESSION IN MULTIPLE MYELOMA IN RELATION WITH T REGULATORY CELLS AND AUTOLOGOUS BONE MARROW TRANSPLANTATION

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Introduction: regulatory T cells (Tregs) control immune responses to self-and foreign-antigens and maintain the balance between immunity and tolerance. Immunosuppressive CD4+CD25+FOXP3+ Tregs shows high levels in MM. Galectin-1 (Gal-1) induces the inhibition of cell growth and promotes the apoptosis of activated, not resting, immune cells and it is overexpressed in Tregs after activation. The aim of this study is to determine Gal-1 expression in MM and its relation with CD4+CD25+Foxp3+ Tregs levels and autologous bone marrow transplantation (ABMT). *Method:* peripheral blood samples were collected from 19 MM patients and sub-classified into two groups according to presence of ABMT. Peripheral blood mononuclear cells (PBMCs) were isolated by dansity gradient centifugation on Biocoll solution and underwent immediate analysis by flow cytometry for Tregs. Gal-1 was determined by immunocytochemistry on imprints of PBMCs and scored as low, medium and high expression semiquantitatively. *Results:* this study included 19 MM patients and 7 of them were ABMT(+). CD4+CD25+Foxp3+ Tregs were shown to be higher in ABMT(+). Tregs percentage and Gal-1 expression did not show statistical significance (p>0.05). In addition Gal-1 expression did not show statistical significance with ABMT presence or prognosis (p>0.05). Discussion: in this study Gal-1 expression in PBMCs was the first time questioned for the relation of Tregs, ABMT and prognosis in MM. Because of limited number of cases Gal-1 may not show statistical relation with any. Our next step could be determining its expression in bone marrow.

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BORTEZOMIB-MEDIATED DOWN REGULATION OF FANCD2 FACILITATES SYNERGISM IN COMBINATION WITH PARP INHIBITION (OLAPARIB).

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Utilization of bortezomib has lead to landmark improvements in myeloma therapy; however, even with this success relapse remains inevitable. Therefore, preclinical development of new targeted-therapy combinations based on strong biological rationale is critical for control, if not cure, of myeloma. We demonstrated that expression of the Fanconi Anemia (FA)/BRCA DNA repair pathway modulated melphalan-resistance myeloma cell lines.(Yarde et al.) Sensitivity to melphalan was causally linked to the bortezomib-sensitive down regulation of FANCD2 and DNA repair. From these data, we anticipated that bortezomib treatment would sensitize cells to PARP (Poly ADP-ribose polymerase) inhibition in a manner consistent with synthetic lethality elicited in BRCA1 or FANCD1/BRCA2 mutant tumors. Consistent with this rationale, pretreatment of the RPMI8226 myeloma cells with bortezomib (VC or 3.0nM) for 6 hours greatly enhanced sensitivity to olaparib. The inhibitory concentration(IC)-50 was decreased by 17.7-fold (n=3; IC50 Olaparib alone: 62.7 µM (39.0-84.0) and pretreated with 3nM bortezomib 3.54µM (2.4-4.6)). Combination Index (CI) demonstrated a mean of 0.41 in 8226 cells, consistent with a synergistic relationship (U266 cells, CI=0.43; NCIH929 cells, CI=0.83 (moderate synergism)). Further analysis confirmed that synergism correlated with decreased expression of FANCD2 mRNA and protein. Lastly, specific targeting of FANCD2 with siRNA also sensitizes cells to olaparib. These results show that bortezomib and olaparib may represent an exciting new combination for myeloma.

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PTEROSTILBENE IS A NOVEL HISTONE DEACETYLASE 1 INHIBITOR (HDACI1) DEMONSTRATING EFFICACY IN MULTIPLE MYELOMA

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We have shown that pterostilbene inhibits MM tumor cell proliferation and also increases tumor cell apoptosis. We examined H4 acetylation of lysine residues in primary tumor cells from MM patients and MM cell lines following treatment with pterostilbene using. Increases in histone acetylation in MM cells following exposure to this drug occurred in a concentration dependent manner. Specifically, 1 to 10 uM of the pterostilbene markedly induced histone acetylation in MM cells within 24 hrs. To characterize the specific HDAC(s) inhibited by pterostilbene, we determined the HDAC-binding ability of pterostilbene in RPMI 8226 MM and 293 HEK cells with a novel HDAC screening assay. Briefly, tumor cells were lysed with M-PER supplemented with protease and phosphatase inhibitors. The lysates were diluted and incubated with pterostilbene in concentrations ranging from 1 to 200 uM or without the drug. Proteolysis was performed using thermolysin and aliquots were removed every 5 minutes and Western blot analysis completed using antibodies against different HDACs or GAPDH. The results showed that pterostilbene prevents digestion of HDAC1 with thermolysin in tumor cells but did not affect HDAC2, HDAC3 and HDAC5. Notably, the combination of pterostilbene and bortezomib or melphalan showed markedly increased inhibition of tumor cell proliferation and increased

apoptosis compared to these drugs alone. Currently, we are evaluating pterostilbene alone and in combination treatments using our SCID-hu murine models of human MM.

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LENALIDOMIDE ENHANCES ??T CELL EXPANSION AND ITS ANTI-MYELOMA ACTIVITY IN COMBINATION WITH ZOLEDRONIC ACID

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Lenalidomide (LEN) shows pivotal anti-MM activity including activation of immune cells surrounding MM cells in the bone marrow microenvironment. The present study was undertaken to clarify the effects of LEN on γδT cell expansion and its anti-MM activity in the bone marrow. LEN (0.1-10 microM) and zoledronic acid (Zol) in combination substantially expanded γδT cells from peripheral blood mononuclear cells, and up-regulated their surface expression of CD25, CD26, LFA-1 and the costimulatory molecules associated with NK cell-mediated cytotoxicity including NKG2D and DNAX accessory molecule-1 (DNAM-1). However, LEN alone did not show any significant effects on γδT cell expansion, suggesting a costimulatory role of LEN on Zol-primed γδT cells. Although γδT cells showed potent cytotoxic effects on MM cells, bone marrow stromal cells (BMSCs) but not osteoclasts blunted anti-MM effects of γδT cells along with a decrease in DNAM-1 expression on their surface. Interestingly, clinically relevant doses of LEN (1 microM) and Zol (0.1-1 microM) antagonized the suppressive effects of BMSCs on DNAM-1 expression and anti-MM effects of γδT cells. In addition, LEN enhanced CD80 expression on MM cells without affecting their expression of poliovirus receptor (PVR; CD155), a DNAM-1 ligand, and ICAM-1. In contrast, bortezomib impaired γδT cell activity and down-regulated the PVR expression on MM cells. In conclusion, LEN and Zol in combination are able to expand and activate γδT cells and suggested to restore and maintain γδT cell activity and its anti-MM effects in vivo.

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MICROENVIRONMENT SHIELDS MYELOMA FROM ADOPTIVE IMMUNOTHERAPY BY CELL ADHESION MEDIATED IMMUNE RESISTANCE (CAM-IR)

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To date, Multiple Myeloma (MM) is considered an incurable disease. Despite the introduction of powerful novel agents, chemotherapy often fails to eliminate MM due to a microenvironment-induced, cell adhesion-mediated drug resistance (CAM-DR). While cellular immunotherapy, especially allogeneic stem cell transplantation, has been shown to induce long term remissions through a cytotoxic T cell (CTL) mediated anti-myeloma effect, not all patients benefit from immunotherapy. To evaluate whether the tumor microenvironment also plays a role in compromising CTL mediated immunotherapy, we used a compartment specific bioluminescence assay, in which luciferase transduced MM cell lines were co-cultured with MM-reactive CTLs in presence and absence of accessory cells. We found that bone marrow stromal cells, in particular patient derived stromal cells, endothelial cells and fibroblasts, significantly inhibit the lysis of MM cells by CD4+ and CD8+ CTLs. This inhibition was due to both immunosuppressive mechanisms as well as induction of a cell-cell contact dependent myeloma resistance against CTL killing, proving the existence of a cell adhesion mediated immune resistance (CAM-IR). Currently, we are characterizing the molecular sequela of this potentially detrimental phenomenon. In conclusion, we report for the first time that microenvironment may hamper immunotherapy by the induction of CAM-IR. Our results suggest that immunotherapy may be significantly improved by modulation of the interaction of MM with its microenvironment.

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BENDAMUSTINE IS A WELL TOLERATED AND EFFECTIVE THERAPY FOR MULTIPLE MYELOMA PATIENTS WITH RENAL IMPAIRMENT

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Less than 20% of Bendamustine is eliminated by the kidney within 24 hours of dosing, making this drug an important option for patients with renal impairment. We conducted an audit of a fixed dose of bendamustine at 120mg iv day 1 in combination with thalidomide 100mg daily and low dose dexamethasone 20mg days 1, 8, 15, and 22 of a 28 day cycle in myeloma patients to assess toxicity and efficacy. Nine patients were treated for a planned duration of 6 cycles. Patient characteristics are listed in table 1. Two patients required dose reduction; one required dexamethasone to be reduced to 10mg due to cushingoid facies, another stopped thalidomide and dexamethasone after cycle 1 due to constipation and behavioural changes. Toxicities are detailed in table 2. 4 patients received a total of 6 cycles. One patient received four cycles with good response and went on to transplant. Four patients received only 2 or less cycles due to early death or progressive disease. Four patients were dialysis dependent at start of treatment, and 3/4 (75%) became independent with treatment. Of the 7 patients with renal impairment, 5 achieved a disease response of PR or better, and 4 had significant improvement in renal function (Table 3). There were 4 deaths; 2 due to progressive disease, one due to sepsis with renal failure and one due to pre-existing lung carcinoma.

This study shows that bendamustine is an effective, well tolerated treatment for patients with significant renal impairment. A larger national randomised phase 2 trial in relapsed myeloma has opened in the UK to explore its use further.

Table 1

Patient Demographics and Baseline Characteristics

Median Age, (range) years	59 (42 - 76)
Median Prior Therapies (range)	1 (1 – 4)
Median eGFR pre-treatment	18 (10 - 86)
Patients refractory to last therapy	2
ISS Stage	
I	0
II	0
III	9
IgG Myeloma	2
IgA Myeloma	3
Light Chain Myeloma	4

Table 2

Number of patients reporting new haematological and non-haematological adverse events during BTD treatment

	Grade 1-2	Grade 3-4
Haematological		
Anaemia	7	1
Thrombocytopenia	0	2
Neutropenia	4	1
Febrile Neutropenia	0	0
Non-Haematological		
Somnolence	3	0
Nausea	2	0
Parasthesia	2	0
Infection	1	1
Tremors	2	0
Epistaxis	0	1
Dyspnea	1	0
Cramp	1	0
Aggression	1	0
Low Mood	1	0

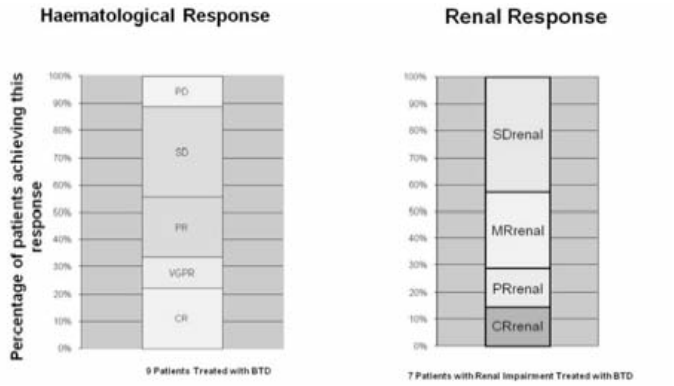

Figure 1

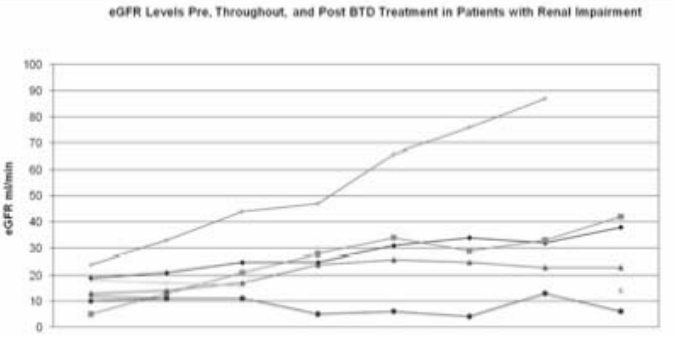

Figure 2

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DISCOVERY OF A NEW IMMUNOTHERAPEUTIC MINOR HISTOCOMPATIBILITY ANTIGEN FOR MULTIPLE MYELOMA.

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Allogeneic stem cell transplantation, alone or followed by donor lymphocyte infusion (DLI) is a treatment with curative potential for multiple myeloma (MM) patients. The therapeutic effect is mainly mediated by donor T cells directed at minor histocompatibility antigens (mHags). The mHags expressed solely on hematopoietic cells are ideal targets for immunotherapy, since they evoke graft-versus-myeloma responses without detrimental graft-versus-host effects. Yet, the number of fully identified hematopoietic mHags is too low to enable broad application of mHag-based immunotherapy. To rapidly identify more mHags, we recently developed a powerful genetic approach: the zygosity-genotype correlation analysis. Applying this technique we now discovered a new mHag with evident clinical relevance. To this end, we generated several mHag-specific T cell clones from a MM patient who after DLI from an HLA-matched sibling achieved a complete remission that at present persists for 8 years. For the most relevant T cell clone, which was isolated at the peak of the clinical response and effectively lyses MM cells, we identified the mHag within 3 months. This new mHag, which we designated as UTA2-1, is encoded by the hematopoietic-specific gene C12orf35. UTA2-1 has a phenotype frequency of 35-40% and is presented by the most common HLA molecule HLA-A2*0101, with a frequency of 47% in the Caucasoid population, indicating that this mHag is applicable in immunotherapy in around 12% of all MM patients. With these properties, UTA2-1 is one of the most clinically relevant mHags identified so far.

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SIRT1 HAS ANTI-MYELOMA ACTIVITY THAT IS CORRELATED WITH DOWN-REGULATION OF MTOR AND NF-KB

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Introduction: SIRT1 is one of the 7 members of the class III HDACs (sirtuins). SIRT1 activators have been shown to have anti-inflammatory property mediated by TNF-alpha. We have investigated the anti-myeloma activity of SIRT1 in MM cell lines and primary MM cells. *Methods:* We evaluated RPMI8226, KMS18, OPM2 and U226 MM cell lines and plasmacells from 6 MM patients. SIRT1 activator 3 was used at increasing doses. Apoptosis has been assayed by flow cytometry evaluating annexin V marker. Western blot analysis was performed to assess the effect of SIRT1 activator 3 agent on NF-kB activity (localization of p65 subunit), AKT, p-AKT, mTOR, p-mTOR, Raptor, Rictor, p70, p-p70. *Results:* The highest level of poptosis was observed in RPMI8226 and U226 cells with SIRT1 activator 3 agent at the dosage of 250- 500 μM at 24 h. (annexin V positivity ranging from 41 to 53%). KMS18 and OPM2 cells resulted less sensitive in the same conditions. SIRT1 activator 3 (100 μM) induced significant apoptosis at 24h (range 39-53%) in plasmacells of 3out 6 patients. Western blot analysis demonstrated strong reduction of p-AKT, p-mTOR and NF-kB in all cell lines. In detail, we observed a strong reduction of Rictor protein (TORC2) and a slight reduction of Raptor protein (TORC1) and p-p70. *Conclusion:* SIRT1 activator 3 induces significant cell death in MM cell lines and primary human myeloma cells. The mechanisms of SIRT1 activator 3 cytotoxicity are related to down-regulation of NK-kB and mTOR phosphorylation. Together, these findings may give useful insights into a novel anti-myeloma therapy.

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ABT-737 IS HIGHLY EFFECTIVE IN A SUB-GROUP OF MULTIPLE MYELOMA DEPENDING ON BCL-2/MCL-1 EXPRESSION PROFILE

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Multiple myeloma is a plasma cell malignancy that is heterogeneous with respect to its causative molecular abnormalities and the treatment response of patients. ABT-737 is a cell-permeant compound that binds to Bcl-2, Bcl-xL but not to Mcl-1. Using 25 cell lines representative of different molecular translocations, we showed that ABT-737 effectively kills 6 cell lines with a LD50 ranging from 7±0.4 nM to 150±7.5 nM. Of interest, all sensitive cell lines harbored a t(11;14). We demonstrated that ABT-737-sensitive and ABT-737-resistant cell lines could be differentiated by the Bcl-2/Mcl-1 gene expression ratio. A screen of a public expression database of myeloma patients indicates that the Bcl-2/Mcl-1 ratio of t(11;14) and hyperdiploid patients was significantly higher than in all other groups (p<.001). ABT-737 first induced the disruption of Bcl-2/Bax and Bcl-2/Bik complexes, followed by the disruption of Bcl-2 heterodimers with Bak, Puma and Bim. Altogether, the identification of a subset of cell lines and primary cells effectively killed by ABT-737 alone supported the evaluation of ABT-263, an orally active counterpart to ABT-737, for the treatment of t(11;14) and hyperdiploid groups of myeloma harboring a Bcl-2high/ Mcl-1low profile.

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HIV-1 PROTEASE INHIBITOR NELFINAVIR IMPAIRS PROTEASOME ACTIVITY AND INDUCES APOPTOSIS IN MULTIPLE MYELOMA CELLS THROUGH AKT DEPHOSPHORYLATION AND CHOP INDUCTION.

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Multiple Myeloma (MM) is characterized by the accumulation of tumor plasma cells in the bone marrow. Recently, the development of new drugs has improved the survival of patients but MM still remains an incurable disease. Proteasome inhibitors such as Bortezomib are

effective drugs in MM, but their use is limited by their toxicity and the occurrence of resistance. Human Immunodeficiency Virus (HIV) Protease Inhibitors (HIPs) have demonstrated anti-tumor activity in solid tumors and may inhibit the 26S proteasome activity. We have tested the effect of the HIP Nelfinavir on MM plasma cells and have investigated the mechanisms of this effect. HIP inhibited the 26S chymotrypsin like proteasomal activity, impaired proliferation and triggered apoptosis of MM cell lines and fresh plasma cells from patients. Nelfinavir induced apoptosis by a decrease phosphorylation of AKT and a phosphorylation of c-jun. In addition, Nelfinavir induced the activation of the pro-apoptotic pathway of the UPR system, including a phosphorylation of PERK and an up-regulation of CHOP. In addition , a synergistic or additive cytotoxicity of Nelfinavir on MM cell lines was demonstrated in combination with Dexamethasone and Histone Deacetylase inhibitors. Moreover, Nelfinavir was able to delay tumor growth in vivo in a MM model.These results suggest that HIPs used at pharmacological dosage, alone or in combination, may be useful in the treatment of MM. Our data provide a preclinical basis for clinical trials using HIPs in patients with MM.

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STUDIES OF BP-1-102, A NOVEL DIRECT SMALL-MOLECULE INHIBITOR OF STAT3 DEMONSTRATES SUBSTANTIAL ANTI-MYELOMA PRE-CLINICAL ACTIVITY

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We describe here a novel, specific and potent small molecule inhibitor of Stat3, derived by modeling of the phosphotyrosine (pY)-SH2 domain interactions in Stat3:Stat3 dimerization, combined with in silico structural analysis of the Stat3 dimerization disruptor, S31-201. We present evidence that BP-1-102 directly interacts with Stat3's SH2 domain, with high affinity and disrupts the binding of Stat3 to the pY-peptide, GpYLPQTV-NH2, with an IC50 of 9 mM making BP-1-102 one of the most effective disruptors of Stat3 protein-phosphopeptide complexation events. BP-1-102 demonstrated broad activity against a panel of 14 genetically diverse multiple myeloma (MM) cell lines inhibiting cell proliferation in the low mM range. BP-1-102 revealed selective activity against primary CD138+ MM cells (>50% decrease in viable cells) in 4/6 samples treated with 10 mM BP-1-102, while minimal toxicity was observed against the CD138- cells. We confirmed activity of BP-1-102 against Stat3 in MM cells observing dose-dependent inhibition of IL-6 induced 1) Stat3 phosphophorylation, 2) Stat3 nuclear translocation and 3) Stat3 transcriptional activity. Treatment of JJN3 cells that harbor aberrant Stat3 activity, induced marked apoptosis (annexin V+/PI-) that coincided with inhibition of constitutive Stat3 phosphorylation. Finally, we observed repression of Stat3 target genes including the critical anti-apoptotic factors, Mcl-1, c-Myc, Survivin and Bcl-x. Together the data are consistent with the targeted activity of BP-1-102 against Stat3 and suggest that Stat3 is a viable therapeutic target in MM.

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THE POTENTIAL OF THE HUMAN CD38-SPECIFIC ANTIBODY DARATUMUMAB TO IMPROVE THE ANTI-MYELOMA EFFECT OF NOVEL MULTI-DRUG THERAPIES INCLUDING PATIENTS REFRACTORY TO LENALIDOMIDE OR BORTEZOMIB

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Over the past decade significant progress has been made in multiple myeloma (MM) treatment using novel immunomodulating agents such as lenalidomide (LEN) and bortezomib (BORT). Daratumumab (DARA) is a first-in-class human therapeutic CD38-specific antibody with a broad mechanism of action. DARA mediates MM cell death primarily via antibody dependent cellular cytotoxicity, complement dependent cytotoxicity and apoptosis. We are currently exploring the possibility to further improve MM therapy by combining novel MM therapeutics with DARA. In ex vivo assays, which allow us to address killing of MM cells in bone marrow aspirates isolated from MM patients, we have already shown significantly improved MM cell killing by combining DARA with LEN. We now demonstrate that the addition of DARA to the LEN+BORT combination significantly exceeds the effectiveness of LEN+BORT treatment ($P<0.001$). Strikingly, killing was most improved in patient samples which showed poor responses to the LEN+BORT. Notably, 5 of these samples were derived from patients who were refractory to BORT and/or LEN. In these samples DARA increased MM cell killing up to 50%. In additional experiments, DARA was combined with two recently introduced triple combination therapies: LEN, BORT, dexamethasone (RVD) and melphalan, prednisone, BORT (MPV). Especially in the low-dose range, addition of DARA to RDV as well as the MPV cocktail almost doubled MM cell killing. These results illustrate that treatment of MM with DARA in combination with novel multidrug therapies bears great promise.

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BONE MARROW NEUTRALIZES HUMAN REGULATORY T-CELLS TO PERMIT GRAFT-VERSUS-TUMOR IMMUNITY IN HUMANIZED MICE

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While application of regulatory T-cells (Tregs) has become a promising tool to prevent Graft-versus-Host Disease (GvHD), Tregs pose the risk of suppressing the curative Graft-versus-Tumor (GvT) effect induced by donor lymphocyte infusion (DLI) in treatment for multiple myeloma. Towards clinical application of Tregs, we explored the in vivo impact of human Tregs on GvT and GvHD in a humanized GvMM model. Herein, we have previously shown that infusion of human PBMC into tumor-bearing Rag2-/-gc-/- mice induces a profound GvT effect but also results in lethal xenogeneic(x) GvHD. We observed that co-infusion of human CD4+CD25+ Tregs suppressed x-GvHD, but allowed effective GvT against human MM localized within the bone marrow, despite efficient Treg-homing to bone marrow. Remarkably, however, Tregs abrogated the GvT effect against the same MM when located outside the bone marrow, suggesting that permittance of the GvT effect in the bone marrow was due to inactivation of Tregs in the bone marrow environment. In detailed exploration of this remarkable phenomenon, we discovered that stromal cells derived from bone marrow neutralize the suppressive phenotype and promote IL-17 in human Tregs through secretion of IL-6/IL-1β. In conclusion, this study for the first time provides a novel in vivo mechanism of how human Tregs can control undesired inflammation while permitting GvT against hematological tumors residing in bone marrow. This mechanism involves the bone marrow stroma, which creates a tumor micro-environment that drives conversion of Tregs into non-suppressor T-cells.

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THE EFFECT OF MGN-3 ARABINOXYLAN ON NATURAL KILLER AND DENDRITIC CELLS IN MULTIPLE MYELOMA PATIENTS

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The oligosaccharides derived from dietary fibers have aroused scientific interest due to their immunomodulatory and antitumor properties.

MGN-3 is a modified arabinoxylan obtained by hydrolyzing rice bran with enzymes from the Japanese medicinal mushroom Shiitake. A considerable number of multiple myeloma (MM) patients display immunodeficiencies that affect both humoral and cellular compartments. Therefore, our aim was to evaluate the effects of daily MGN-3 administration besides the established anti-MM treatment on immunological parameters of 45 MM patients, such as natural killer (NK) cell activity, peripheral blood cell phenotype and plasma cytokine levels in randomized placebo-controlled study. We confirmed that MGN-3 is a potent enhancer of NK cell activity, as we observed the significant increase in NK-mediated cytolysis. MGN-3 also increased the percentage of myeloid dendritic cells (mDC) in the periphery and the mDC/plasmacytoid DC ratio was steadily upregulated during 3 months of treatment. We analyzed the levels of Th1 and Th2 cytokines involved in cell-mediated and humoral immunity, respectively. The prevalence of Th2 immunity in MM patients manifested by the decreased Th1/Th2 ratio was detected in comparison to healthy donors. The levels of crucial Th1 cytokines IL-12 and IFN-γ, among others, significantly increased after MGN-3 treatment. Our study shows that MGN-3 intake can be beneficial for cancer patients because of its ability to tune innate immunity by increasing activity of key effectors - NK cells and maintaining professional antigen-presenting DC.

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TARGETING THE CD138 ANTIGEN FOR THE TREATMENT OF MULTIPLE MYELOMA WITH BISMUTH-213

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Alpha-RIT has been shown to be effective in vivo in different tumor models and seems particularly suited for disseminated tumor cells or small clusters of tumor cells. CD138 was shown to be expressed by most human and mouse myeloma cells. The aim of the study was to evaluate biodistribution, toxicity and efficacy of a 213Bi-anti-mouse CD138 antibody in a syngeneic mouse myeloma model. C57BL/KaLwRij mice were grafted with 106 5T33 murine myeloma cells. 5T33 transfected with luciferase were used for in vivo detection of cells during the course of the disease and showed that inoculated tumor cells invaded bone marrow with evidence of tumor in large bones 12 days after inoculation. Biodistribution showed that tumor uptake of 125I-anti-mCD138 was 5 fold higher with the specific antibody than with a control one. Uptake was also observed in liver and spleen. Toxicity and RIT efficacy were studied in mice injected with 0, 1.85, 3.7, 7.4, and 11.1 MBq of 213Bi-anti-mCD138, 10 days after tumor engraftment. Fifty percent of untreated mice died by 58 d after tumor engraftment. The highest dose (11.1 MBq) induced very high toxicity and mice died within 7 days after treatment. The 7.4 MBq dose was still above the maximum tolerated dose, but, with 3.7 MBq, only slight and reversible haematological radiotoxicity was observed. Long term survival was obtained with the 3.7 MBq dose (more than 60 % of mice still alive 300 days after RIT and was significantly higher compared to controls ($p < 0.0001$). These results show that RIT of MM using alpha emitters is effective with CD138 immunotargeting.

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THE POTENT STAT3/5 INHIBITOR, BP-1-102 DEMONSTRATES SIGNIFICANT ANTI-TUMOR ACTIVITY AGAINST WALDENSTROM MACROGLOBULINEMIA

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Aberrant STAT signaling is prevalent in hematologic malignancies and is recognized as a master regulator of direct and indirect tumor processes including proliferation, apoptosis, invasion, angiogenesis and cancer inflammation. We report the anti-tumor activity of a highly specific and potent small molecule Stat3/5 inhibitor, BP-1-102, on two Waldenstrom Macroglobulinemia (WM) cell lines, Mec-1 and RL. BP-1-102 directly interacts with Stat3's SH2 domain and is one of the most effective disruptors of Stat3 protein-phosphopeptide complexes described to date. Treatment of Mec-1 and RL with low μM doses of BP-1-102 induced dose-dependent decreases in constitutive Stat3/5 phosphorylation, respectively, and inhibited pStat3 nuclear localization in Mec-1 cells. Using a Stat3 dependent luciferase reporter, we confirmed repression of IL6-mediated Stat3 transcriptional activity. Inhibition of Stat3 phosphorylation resulted in decreased cell viability and induction of apoptosis. Analysis of Stat3 target genes revealed that BP-1-102 repressed expression of critical anti-apoptotic factors including Bcl-2, Bcl-XL, Survivin and Myc that correlated with induction of caspase-3 and PARP cleavage. WM cells co-cultured on stroma were more resistant to apoptosis than cells grown in suspension, however, these experiments still confirmed that even stroma-dependent survival could be inhibited with BP-1-102. Collectively, these findings suggest that BP-1-102 blocks cell proliferation and induces apoptosis of WM cells, supporting further exploration of Stat3/5 inhibitors for the treatment of WM.

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IMPACT OF ELOTUZUMAB ON CIRCULATING LYMPHOCYTES, CHEMOKINES, AND CYTOKINES IN PATIENTS WITH MULTIPLE MYELOMA

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Elotuzumab is a humanized monoclonal IgG1 antibody targeting human CS1, a cell surface glycoprotein. CS1 is highly and uniformly expressed on multiple myeloma (MM) cells, with reduced expression on natural killer (NK) cells and little to no expression on normal tissues. In preclinical studies, elotuzumab induced NK cell-mediated antibody-dependent cytotoxicity. Elotuzumab is being studied in three phase 1 clinical trials in relapsed and/or refractory MM: a monotherapy dose-escalation study; a combination study with bortezomib; and a combination study with lenalidomide and low-dose dexamethasone. Objectives for these trials included evaluation of the effect of elotuzumab on lymphocyte counts, including NK cells, and on serum levels of chemokines and cytokines. Peripheral blood samples were analyzed using the TruCOUNT™ flow cytometry assay to determine absolute lymphocyte counts and the Luminex® multiplex bead-based assay to measure serum levels of chemokines and cytokines. In all 3 studies, a transient decrease in the absolute number of circulating total lymphocytes, including NK cells (approximately 75%-90% reduction from baseline), occurred upon first elotuzumab dose, followed by a trend of recovery towards baseline levels as dosing cycles continued. This transient decrease was associated with increases in chemokines and cytokines, and is hypothesized to be related to lymphocyte trafficking rather than lymphocyte depletion.

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GF-15, A NOVEL INHIBITOR OF CENTROSOMAL CLUSTERING, SUPPRESSES MULTIPLE MYELOMA GROWTH IN VITRO AND IN VIVO

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In contrast to normal cells, malignant plasma cells frequently contain multiple centrosomes. To allow for bipolar mitotic division, supernumerary centrosomes are clustered into two functional spindle poles in many cancer cells. We describe the characterization of the novel small molecule GF-15, a derivative of griseofulvin, as a potent inhibitor of centrosomal clustering in multiple myeloma, forcing tumor cells with supernumerary centrosomes to undergo multipolar mitoses resulting in apoptotic cell death. In a wide array of multiple myeloma cell lines its mean inhibitory concentrations (IC50) for proliferation and survival were in

the range of 1-5 μM, associated with activation of caspases 8, 9, and 3. In contrast, non-malignant bone marrow stromal cells were not sensitive to GF-15 up to 30 μM. GF-15 overcomes the growth advantage conferred in bone marrow stromal and endothelial cell-myeloma co-culture systems. Treatment with GF-15 was associated with inhibition of VEGF- and IGF1-triggered myeloma cell migration. In vivo, PK studies revealed rapid clearance by renal excretion of ^{125}I-labelled GF-15. Importantly, treatment of murine xenograft models of human myeloma resulted in tumor growth inhibition and significantly prolonged survival. Immunohistochemistry showed significant induction of mitotic aberrations in GF-15 treated tumors compared to controls. These results demonstrate specific in vitro and in vivo anti-tumor efficacy of a prototype small molecule inhibitor of centrosomal clustering and strongly support the further evaluation of this new class of molecules.

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PROGNOSTIC AND THERAPEUTIC RELEVANCE OF SURVIVIN EXPRESSION IN MULTIPLE MYELOMA

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The IAP-family member survivin inhibits apoptosis and regulates both mitosis and cytokinesis. We examined the expression of survivin in CD138-purified myeloma cells from previously untreated patients at our centers (n=246), trial group (TG), and the UAMS Arkansas (n=345), validation group (VG). Using the PANP-algorithm, survivin was aberrantly expressed in 27% (TG) of myeloma cell samples. It was not expressed in normal bone marrow plasma cell samples (n=7) while expression increased significantly from MM stage I-III (P<.001). Survivin expression correlated with proliferation as assessed by gene expression- (r=.8 P<.001) or propidium iodine (r=.7, P<.001). Presence of survivin correlated with inferior event-free and overall survival in patients undergoing high-dose chemotherapy in the TG (22.6 vs. 35.4 months, P<.001, 52.9 vs. n.r., P=.002) and the VG (12.3 vs. 54.1 months, P<.001, and 17.4 vs. n.r., respectively). Knock-down of survivin by RNAi induced aberrant metaphases and apoptosis in the myeloma cell lines (MCL) U266 and OPM2. The small molecule survivin inhibitor YM155 inhibited proliferation and induces apoptosis in 10 MCLs at an IC50 of 4-50nM. In contrast, primary bone marrow stromal cells tolerated concentrations of up to 500nM. Growth inhibition by YM155 correlated with inhibition of intracellular survivin protein expression. Transient ectopic survivin expression partially rescued MCLs from induction of apoptosis by YM155. In conclusion, we here demonstrate the prognostic significance of survivin expression and a potential therapeutic role for YM155 in MM.

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THE NOVEL, ORALLY BIOAVAILABLE HSP90 INHIBITOR NVP-HSP990 INDUCES GROWTH INHIBITION, APOPTOSIS, CELL CYCLE ARREST AND A DECREASE OF PRO-SURVIVAL KINASES P-AKT AND P-ERK1/2 IN MULTIPLE MYELOMA

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Heat shock protein 90 (HSP90) has recently been identified as a novel therapeutic target in multiple myeloma (MM). We tested the novel, orally bioavailable HSP90 inhibitor NVP-HSP990 for its effect on MM. Exposure of MM cell lines OPM-2, U-266, MM1.S, NCI-H929 and RPMI-9226 for 48 – 72 hours with NVP-HSP990 at concentrations ranging from 0.5 – 500 nM led to a significant time- and concentration-dependent decrease in viability, with IC50 values ranging from 22 nM to 157 nM at 72 h for MM1.S and RPMI-8226, respectively. We determined a significant increase in apoptotic, Annexin-V positive cells after incubation with NVP-HSP990. There was an increase in cleaved caspase-8 after exposure to NVP-HSP990, followed by subsequent activation of caspase-3. Levels of activated kinases p-Akt and p-ERK1/2 were decreased significantly in a time-dependent fashion. Intracellular levels of HSP70 increased significantly after HSP90 inhibition. Combined incubation of NVP-HSP990 with melphalan or with histone deacetylase inhibitors NVP-LBH589 or SAHA resulted in synergistic inhibition of viability (CI<1.0) and increased cleavage of caspases. In this study we could for the first time demonstrate that the orally bioavailable compound NVP-HSP990 inhibits viability, induces cell cycle arrest and apoptosis in MM and disrupts pro-survival signaling pathways via degradation of p-Akt and p-ERK1/2. Together with the demonstration of synergistic effects with chemotherapeutics or novel agents, our data provide a rationale for the design of clinical studies with NVP-HSP990 in multiple myeloma.

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LONG TERM EFFICACY AND DURATION OF RESPONSE OF POMALIDAMIDE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA

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Background: Pomalidomide (pom) is a new immunomodulatory (IMid) drug with a high response rate in relapsed MM including patients (pts) failing thalidomide and lenalidomide. The duration of this response is unknown. We evaluated the long term outcomes of pts treated with Pom. *Methods:* Pom 2 mg/d was given orally continuously on 28 day cycle with weekly dexamethasone 40 mg. All pts received DVT prophylaxis with aspirin, heparin or warfarin. *Results:* 60 pts with relapsed MM were enrolled from Nov 2007-Aug 2008. Median age was 65.5; 36 were male. Median time from diagnosis was 45.6 mo (9.1-192.5). Nineteen (32%) were high-risk according to mSMART (msmart.org), and 78% were ISS Stage 2/3. Prior therapies included transplant (65%), bortezomib (53%), thalidomide (47%), lenalidomide (35%) [previous IMiD 60%] and radiation (38%). Toxicities at least possibly attributed to Pom included G3/4 anemia (5/0%), leukopenia (17/3%) and thrombocytopenia (3/0%). G3 non-hematological toxicities occurred in 29 (48%) and included fatigue (18%), pneumonia (5%), hyperglycemia (5%) and constipation (5%). Only one pt had grade 3 neuropathy. Grade 4/5 pneumonia occurred in 2 pts. With median follow up of 27.2 mo, overall response was seen in 39 (65%) [4 sCR, 5 CR, 14 PR, 16 VGPR] in a median of 1.4 mo (0.8-14.8). Median duration of response was 21.3 mo. Two year survival was 76%. *Conclusions:* Pom/dex is highly effective and well tolerated in relapsed MM, with a response rate of 65% even in pts with prior IMiD use. It provides a durable response of over 21 months and 2 year survival rate of 76%.

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PARP INHIBITOR AS NOVEL THERAPEUTIC APPROACH IN MULTIPLE MYELOMA (MM)

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PARP inhibitors are cytotoxic to tumor cells with impaired DNA damage repair machinery (DRR). While genomic instability of MM cells is well described their sensitivity to PARP inhibitors (PARPi) is not yet reported. We first demonstrated that ABT-888, a selective PARP1-2 inhibitor, significantly reduced PAR polymers in MM cells. Tracking the occurrence of DNA double stranded breaks (DSBs), treatment with ABT-888 only resulted in transient H2AX foci formation and had no effect on the viability of MM cells. Since the ubiquitin-proteasome system plays a key role in DDR, we have postulated that Bortezomib alters the homologous recombination (HR)-mediated repair of DSBs and sensitizes MM cells to PARPi. We first confirmed that Bortezomib did impair HR at the

transcriptional and post-transcriptional levels in MM cells. We then showed that Bortezomib sensitizes plasma cells to ABT-888 and that the cytotoxic effect of this combination was superior to either drug alone. Mechanistically, DSBs were significantly enhanced by this combination with marked increase in apoptotic cell death. No such effects were observed in CD34+ stem cells collected from healthy volunteers. Finally, in a murine xenograft model of human MM, ABT-888 potentiated Bortezomib activity in vivo, with significant reduction in tumor growth and improved survival. Our studies indicate that Bortezomib impairs HR in MM and results in a contextual synthetic lethality when combined with PARPi. A phase I clinical trial of PARPi in combination with Bortezomib in relapsed refractory MM patients is now planned.

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THE NOVEL JAK2 INHIBITOR NVP-BSK805 KILLS MALIGNANT PLASMA CELLS AND SYNERGIZES WITH INHIBITORS OF IGF-1R, MTOR AND MEK

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Janus kinases (JAK) play a central role in multiple myeloma (MM) primarily through their association with cytokine receptors, in particular those of the interleukin (IL)-6/gp130 family. Disruption of JAK activity and downstream signaling pathways may inhibit malignant plasma cell growth and survival. JAK inhibitor NVP-BSK805 (Novartis) displays more than 20-fold selectivity for JAK2 over other JAK family members and more than 100-fold selectivity over a panel of additional kinases (Baffert et al., Mol Cancer Ther 9:1945, 2010). Growth of IL-6 dependent INA-6 cells was inhibited with an IC50 concentration of 1 µmol/L, leading to pronounced decrease of IL-6 induced STAT3 phosphorylation followed by apoptosis. In five different myeloma cell lines, fifty percent growth inhibition was achieved by NVP-BSK805 at 2.6 µmol/L to 6.8 µmol/L. Plasma cell-enriched tumor samples from extramedullary disease showing enhanced proliferation in response to IL-6, were highly sensitive to this compound at IC50 concentrations of 1 µmol/L or less. Using INA-6 cells as a model system, JAK inhibition was combined with other signaling inhibitors. Here, rapamycin, NVP-AEW541 and PD98059 and U0126 yielded the most promising results. The observed synergistic activities are consistent with the important role of IGF-1 as well as the presence of constitutively activated Ras in MM. These studies encourage the use of JAK inhibitors as a therapeutic strategy for patients with MM, although selection of signal inhibitors has to consider different pathway alterations in individual myeloma patients.

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THE EPITOPE TARGETED BY CELL DEATH-INDUCING ICAM-1 ANTIBODY BI-505 IS HIGHLY EXPRESSED IN MULTIPLE MYELOMA

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Complex adhesive and non-cognate interactions participate in multiple myeloma disease progression, resistance to apoptosis, and development of drug resistance. In spite of significant recent attempts to develop new drug classes targeting both myeloma and its microenvironment, multiple myeloma remains an incurable disease warranting development of more effective therapies. Applying novel combined target and drug discovery methodology we have isolated a human tumor cell death-inducing antibody BI-505, targeting ICAM-1. Furthermore, we here show that BI-505 antibody has significant in vivo anti-myeloma activity and that this anti-myeloma activity is antibody Fc:effector cell FcγR-dependent. In addition ICAM-1 is a cell adhesion molecule that is strongly implicated in myeloma pathophysiology, cell adhesion mediated drug resistance and in bone marrow stromal cell mediated disease progression in multiple myeloma. To characterize BI-505 epitope expression in multiple myeloma we have performed multi-color flow cytometry analysis in 40 patients investigated for multiple myeloma referred to the Department of Hematology, Skåne University Hospital, Sweden. The BI-505 epitope was highly expressed on the plasma cell surface in 40 of 40 patients. A clinical phase I trial with BI-505 is proceeding in Sweden and U.S according to the protocol (NCT01025206, www.clinicaltrials.gov).

P-304

EXPRESSION OF TRAFFICKING RECEPTORS ON CD4+CD25BRIGHTFOXP3+ REGULATORY T CELLS AFTER MOBILISATION OF HAEMATOPOETIC STEM CELLS

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The phenotype & number of regulatory T cells (T_{Reg} cells) in mobilised stem cell collections (PBSC) could have important consequences for the outcome of autograft procedures. The ability of TReg cells to traffic to specific tissue sites, including secondary lymphoid tissue and bone marrow, could influence the outcome of residual disease. We have compared the proportion of T_{Reg} cells ($CD4+CD25^{Bright}FoxP3+$ T-cells) in PBSC samples mobilised by cyclophosphamide (CY) or by plerixafor (PL; both administered with G-CSF) with the steady state TReg cells seen in peripheral blood (PB) samples of healthy controls (CON) & MM patients. The expression of trafficking receptors, CXCR4 & CCR7, along with the level of expression (Mean Fluorescent Intensity) of CD25 & FoxP3 was determined by FACS. T_{Reg} cells were significantly increased in proportion & absolute numbers in PBSC-CY but not in PBSC-PL compared to CON and MM. The FoxP3 expression was higher in PBSC compared to CON and MM. Although the number of T_{Reg} cells that expressed CXCR4 was similar between CON/MM and PBSC, the level of CXCR4 expression was significantly higher in PBSC-CY and PBSC-PL. In contrast, CCR7+ T_{Reg} cells were reduced in both PBSC-CY & PBSC-PL with a reduced level of CCR7 expression. The proportion of CCR7-CXCR4-TReg cells was significantly greater in PBSC-PL than PBSC-CY, CON and MM. The observed increased number of T_{Reg} cells in PBSC mobilised with cyclophosphamide may have a detrimental effect on immunomodulation post-autograft including anti-tumour responses.

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ANTI-TUMOR AND ANTI-ANGIOGENIC EFFECTS OF MITHRAMYCIN IN A SYNGENEIC MOUSE MODEL OF MULTIPLE MYELOMA, MOPC-315.BM

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Mithramycin (MTM) is a metabolite produced by Streptomyces, with strong anticancer activity. Clinical use of MTM was limited by the drugs side effects, but there was a renewed interest in MTM because of its anti-angiogenic capacities and the development of analogues with improved pharmacological properties.. In the current study, we analyzed the anti-myeloma effects of this compound in the syngeneic MOPC315 BM mouse MM model. MTM inhibited MOPC315BM DNA-synthesis with an IC50 of 50 nM. On cell cycle progression, the drug induced an arrest in G1. For the in vivo experiment, 20 Balb/c mice (injected with luciferase-transfected MOPC315.BM cells) were treated twice weekly with vehicle or MTM (0.750 mg). Tumor development was followed by in vivo imaging of tumor size. When mice showed signs of paraplegia, they were sacrificed and infiltration of myeloma cells in spleen and bone marrow was determined. In vivo, chronic i.p. treatment with MTM was well tolerated and resulted in a decrease in mean bio-luminescence from 4.2 10x7 U to 1.22 10x7 U ($p = 0.03$), in a decrease in BM invasion by monoclonal plasma cells from 26.4% to 14.8% ($p = 0.02$), and in a normalization of splenic masses from 0.32 g to 0.12 g ($p < 0.001$). Next to having direct effects on myeloma cells, MTM also reduced the myeloma associated neo-vascularization in vivo (by determination of microvessel density on bone marrow sections) and

in vitro in a rat aortic ring assay. These data suggest that MTM has in vivo anti-myeloma and anti-angiogenic effects and support the further research and development of MTM.

P-306

COMBINED BLOCKADE OF AKT AND MEK/MAPK ENHANCES CELL DEATH IN AKT-DEPENDENT MULTIPLE MYELOMA (MM)

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Introduction: Oncogenic pathways are implicated in MM cell growth and survival. We have shown that the Akt pathway has a prominent role for survival in about half of primary MM samples ('Akt-dependent MM'), whereas the MAPK pathway appears less important. Both pathways may crosstalk and mediate mutual escape mechanisms. We analyzed the effects of combined Akt and MEK/MAPK blockade by pharmacological inhibition in MM cell lines (n=6) and primary MM samples (n=21), and by shRNA-mediated knockdown in MM cell lines. *Methods:* MM cells were treated with Akt-inhibitor Akti-1,2 and MEK-inhibitor PD325901 alone or in combination and cell survival was assessed. MM cell lines were transiently transfected with shRNA expression constructs against Akt1, Erk1 & Erk2 alone or in combination and cell survival was determined. Apoptotic cells were analyzed by flow cytometry with Annexin V/PI. *Results:* Akt-dependent MM cell lines showed strongly enhanced apoptosis upon combined inhibition of Akt and MEK compared to single treatments. Cell lines resistant to Akt-blockade remained unaffected by the combination. Similar effects could be observed with single or combined blockade via shRNA-mediated knockdown of Akt1 and Erk1&2. In primary MM samples, pharmacological inhibition of Akt and MEK mirrored the enhanced cell death in Akt-dependent cells. Cells resistant to Akt-blockade responded to combination blockade in half of the cases. *Conclusion:* Combined inhibition of Akt and MEK/MAPK could prove effective in Akt-dependent MM and in a subset of Akt-independent MM, yielding enhanced anti-MM effects.

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MICRORNA PROFILING IN MULTIPLE MYELOMA

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Background: MicroRNAs (miRNAs) play a critical role in biological processes including cellular growth and cancer. *Aim:* We aimed to analyze miRNA expression patterns in multiple myeloma (MM), and to compare miRNA expression to mRNA expression. *Methods:* MiRNA expression profiles were determined in 45 newly diagnosed MM patients included in the HOVON-65/GMMG-HD4 trial; of these, mRNA expression was available in 39 cases (Broyl et al., Blood,116,2543-2553;2010). Normal bone marrow miRNA profiles were obtained from Gutierrez et al. (Leukemia,24,629-637,2010;GSE16558). *Results:* Distinctive miRNA clusters were found, consisting of 4 MM clusters and 1 normal bone marrow cluster. The MM clusters were characterized by up- and down-regulation of distinctive miRNAs. One of the cluster signatures was dominated by the miRNA clusters miRNA-17~92 and miRNA-106~25. Upregulation of let-7f, miR-194 and miR-296 expression was borderline associated with better overall survival (OS; p=0.06). A significant inverse correlation was found between miR-21 expression and gene expression of two of its validated targets, PDCD4 (p=1.6 x10-4) and RECK (p=7.7 x10-4). Other significant inverse correlations were found between let-7c and CDC34 and SLC35D2 and between miR-148b and RAB34. CDC34 has previously been validated as a let-7 target. *Conclusion:* MiRNA profiling defined distinctive miRNA signatures in MM patients. Expression of let-7f, miR-194 and miR-296 is borderline associated with OS. Analysis of miRNA-mRNA expression suggest a role for miRNA-21, let-7c and possibly miR-148b in MM.

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CANCER TESTIS ANTIGENS IN NEWLY DIAGNOSED AND RELAPSE MULTIPLE MYELOMA: PROGNOSTIC MARKERS AND POTENTIAL TARGETS FOR IMMUNOTHERAPY

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Background: In multiple myeloma (MM), cancer testis antigens (CTAs) are of interest for immunotherapy and as prognostic markers. For effective immunotherapy using CTAs, expression of CTAs after treatment is required. *Methods:* A systematic evaluation of CTA expression was performed in newly diagnosed MM cases (HOVON-65/GMMG-HD4 trial (n=320)) and in relapse cases (APEX, SUMMIT and CREST trials (n=264)). Presence of expression using Affymetrix GeneChips was determined for 123 CTAs. The expression restriction in normal tissue was known for 84 CTAs, i.e. expression restricted to testis (TR), to testis and brain (TBR) and not restricted but selectively expressed in testis (TS). *Results:* Out of 84 CTAs, 58 have a frequency of more than 5% in one of the study populations. A significantly lower frequency of presence calls in relapse cases compared to newly diagnosed cases was found for 3 out of 13 TR genes, 2 out of 7 TBR genes and 17 out of 38 TS genes. MAGEC1, MAGEB2 and SSX1 were the most frequent TR CTAs in both data sets, present in 71%, 47% and 30% in newly diagnosed patients, respectively and present in 61%, 47% and 30% in relapse patients, respectively. Both in newly diagnosed and in relapse patients, SSX1 was found to be prognostic for both progression free survival and overall survival. In addition, results of protein expression of CTA genes SPAG9 and MAGEC1 on a limited set of patients will be presented. *Conclusion:* Especially TR and TBR CTAs are present to a similar degree in relapse patients compared to newly diagnosed patients, offering potential for immunotherapy.

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DIFFERENTIAL EFFECTS OF BONE MARROW CELLULAR COMPONENTS ON MYELOMA CELL SURVIVAL

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While great strides in the treatment of myeloma have been made in recent years it is still incurable, with the primary cause of treatment failure being drug resistance. The complex interactions between the cellular, extracellular matrix and soluble components of the bone marrow (BM) microenvironment are believed to play a pivotal role in both the growth and survival of malignant plasma cells. To test the effects of cellular components of the bone marrow on the survival of myeloma cells, we determined the effects of culturing myeloma cells in the presence and absence of additional cellular components on the response to loss of survival signaling. We determined that in freshly isolated, purified myeloma cells, Bim is associated with Bcl-xL and predicted that this would result in sensitivity to the Bcl-2/xL inhibitor ABT-737. We then determined 24 h survival of CD138+ cells in the presence or absence of other cellular components as well as ABT-737. We found that in all patient samples tested the cellular components provided protection of untreated myeloma cells. Surprisingly, upon treatment with ABT-737, the cellular component only provided protection to the myeloma cells in 57% of the samples. In the remaining 43% the IC50 values were the same for the isolated and intact populations. Taken together these data suggest that cellular components of the microenvironment may support myeloma survival, however this does not necessarily translate to alterations in drug resistance.

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CASPASE 8 IS THE FIRST CASPASE ACTIVATED MULTIPLE MYELOMA CELL LINES IN RESPONSE TO BORTEZOMIB TREATMENT

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The proteasome inhibitor Bortezomib has revolutionized the treatment of newly diagnosed and relapsed/refractory multiple. The cytotoxicity of bortezomib has been attributed to disruption of several cellular programs including cell cycle regulation, protein catabolism, DNA damage response, as well as the stabilization or up-regulation of pro-apoptotic proteins. However, the exact mechanism by which bortezomib induces apoptosis is still not completely understood. Numerous studies have suggested a role for both caspase 8 and caspase 9 in bortezomib-induced apoptosis, however the methods used are incapable of determining which initiator caspase is activated first. Therefore we utilized the biotinylated caspase bVAD-fmk to "trap" and then precipitate the initial caspase activated in response to bortezomib. Using this method we identified caspase 8 as the primary initiator caspase involved in bortezomib-induced apoptosis. Furthermore, we localized caspase 8 to the heavy membrane fraction of the myeloma cell, suggesting a possible mechanism of mitochondrial-localized caspase 8 activation in response to bortezomib treatment. siRNA silencing of BH3-only proteins also suggests a role for Bim and Noxa in the execution of bortezomib-induced cell death. We will present data on the mechanism by which these proteins work together to induce apoptosis in response to bortezomib treatment as well as identify the initiator caspase responsible for apoptosis induction in response to other therapeutic drugs, to better understand synergy in apoptotic signaling in myeloma.

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SAFE AND EFFECTIVE TARGETING OF THERAPY-RESISTANT MULTIPLE MYELOMA WITH SUICIDE GENE-MODIFIED CD44V6-REDIRECTED T CELLS

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Standard therapy for multiple myeloma (MM) significantly prolongs survival but is not curative. Conversely, adoptive T-cell therapy in the form of allogeneic hematopoietic stem cell transplantation seems to do so, but is highly toxic. With the aim of targeting therapy-resistant MM, we redirected T cells with a chimeric antigen receptor (CAR) specific for the isoform variant 6 of the hyaluronate receptor CD44 (CD44v6). Recent evidence indeed suggests that CD44v6 is crucial for MM-cell lodging to the bone-marrow niche and resistance to therapy. Genetic redirection was achieved with a second-generation CAR, encompassing the single-chain fragment of a CD44v6-specific mAb fused with the zeta chain of the TCR and a CD28 endodomain (CD44v6-CAR.28z). After retroviral transduction with the CD44v6-CAR.28z, primary T cells acquired specific cytotoxicity against CD44v6-positive MM cells. Interestingly, MM cells up-regulated CD44v6 upon culture with bone-marrow stromal cells resulting in higher recognition by CD44v6-redirected T cells. As a result, in co-culture experiments, CD44v6-redirected T cells specifically proliferated in response to CD44v6-positive MM cells and completely cleared them. Since healthy tissues may express low levels of CD44v6, for safety purposes we next co-transferred the thymidine kinase (tk) suicide gene, which enabled the selective elimination of CD44v6-redirected T cells upon ganciclovir administration. These results warrant the clinical investigation of suicide gene-modified CD44v6-redirected T cells as a safe and effective therapeutic modality for MM.

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A POSSIBLE ROLE FOR NAB-PACLITAXEL IN MULTIPLE MYELOMA (MM) THERAPY

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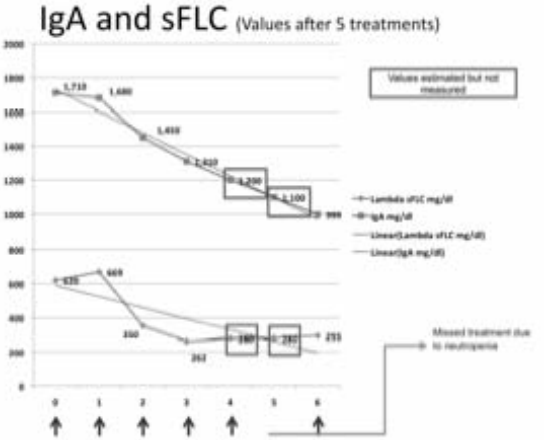
Phase II trials in the 1990s reported that while docetaxel was inactive in MM, paclitaxel had single agent activity: 4/14 (29%) and 5/33 (15%) patients responded– ORR 19%. 4 of 11 MGUS patients treated with paclitaxel or docetaxel in combination with a platinum analogue for concurrent cancers had >50% reduction in M protein. 4 of 8 MM patients treated with paclitaxel plus gemcitabine had a partial response. Taxanes were not further developed in MM due to toxicity concerns. We thus explored the use of nab-paclitaxel (paclitaxel albumin-bound particles) in 6 de-novo Vkappa*myc transgenic MM mice treated for two weeks by intra-cardiac injection with suboptimal dose nab-paclitaxel (50mg/kg). All mice responded with a median reduction of M-spike=33%. Highest reduction 54.7%. Given the prior literature and pre clinical findings we treated with nab-paclitaxel (off label) a MM patient who had failed all major lines of therapy against MM, had renal insufficiency, hypercalcemia, and was dependent on platelet transfusions. A weekly schedule of 100mg/m^2 (3 weeks on one week off) was used. Due to febrile neutropenia during the first cycle he received filgrastim and tolerated treatment well. A high quality partial response in both IgA (1710 to 893 mg/dl) and free light chain levels (669 to 189 mg/dl), became transfusion independent and had normalization of his renal function and hypercalcemia (after 3 cycles his response was maintained). We conclude that nab-paclitaxel should be further studied in MM.



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G-PROTEIN COUPLED RECEPTOR KINASE 6 IS A SELECTIVE THERAPEUTIC TARGET IN HUMAN MULTIPLE MYELOMA THAT REGULATES CXCR4-SRC-STAT3-MCL1 AND PLASMA CELL SURVIVAL

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To promote rational drug development for multiple myeloma (MM) we have previously conducted genome-scale RNA interference (RNAi) studies in human MM cells to functionally identify critical molecular vulnerabilities. From ~7000 genes, 15 kinases and >40 non kinases were consistently vulnerable in MM. While the majority proved equally vulnerable in other cell types, some and particularly G-protein coupled receptor kinase, GRK6, appeared selectively vulnerable in MM. GRK6 is ubiquitously expressed in primary MM, but is absent in most human somatic tissues. Consistent with this, GRK6 silencing via RNAi is selectively cytotoxic to MM cells but is tolerated in non-myeloma cells ($P<0.01$). Notably, mice that lack GRK6 are healthy, though show reduced immunoglobulins (Ig), supporting tolerability and anti-plasma cell efficacy of GRK6 inhibition in vivo. Furthermore, RNAi-induced GRK6 silencing in plasmacytoma in mice causes marked tumor reduction (>1000x), improved survival (>90d) and synergistic tumor suppression in combination with low-dose bortezomib. From various experiments: GRK6 inhibition prevents phosphorylation of GPCR CXCR4, blocks secondary Src and STAT3 phosphorylation and causes sustained reductions in phospho- and total MCL1, providing a potent mechanism for cytotoxicity observed in MM tumor cells. As humans that lack GRK6-CXCR4 interaction (WHIM syndrome) show reduced late-stage B cell differentiation and Ig levels, inhibition of GRK6 represents a

uniquely validated novel therapeutic strategy for MM. Efforts to identify pharmaceutical GRK6 inhibitors are in progress.

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CD80/IL2 EXPRESSING MYELOMA CELLS FOR IMMUNE GENE THERAPY OF MULTIPLE MYELOMA

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Improved experimental therapies are needed for Multiple Myeloma (MM) because, despite major progress in treatment and initial induction of remission, myeloma remains an incurable disease. Although immunotherapy and, in particular, the employment of NK cells offers an approach of interest, recent studies have shown that tumour cells utilise a number different mechanisms to selectively impair NK cell function. We have found that in healthy individuals the peripheral blood mononuclear cell (PBMC) environment is important for optimal activation of NK cells in response to MM targets. Moreover, it has been shown that in MM patients PBMCs as well as MM cells show a compromised antigen presenting ability as they lack the expression of the co-stimulatory molecule CD80. Therefore, following previous results obtained in AML patients, we are examining the possibility of rescuing NK cell anti-myeloma activity by stimulating healthy donor PBMCs with allogeneic MM cell lines genetically modified to express CD80 and IL2. Our results show that allogeneic co-coltures of PBMCs with CD80/IL2 MM cell lines promote NK cells activation. Taken together our data indicate that a cross-talk between NK cells and PBMCs is important in driving NK cell responses again multiple myeloma. However, due to the immuno-suppressive nature of the tumour microenvironment, this cross-talk is frequently impaired leading to abnormal NK cell function. Here we show the therapeutic potential of NK cell activation by CD80/IL2 expressing MM cells.

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HOST-DERIVED ADIPONECTIN IS TUMOR-SUPPRESSIVE AND A NOVEL THERAPEUTIC TARGET FOR MULTIPLE MYELOMA AND THE ASSOCIATED BONE DISEASE

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The contributions of the host microenviroronment to the pathogenesis of multiple myeloma, including progression from the non-malignant disorder monoclonal gammopathy of undetermined significance (MGUS), are poorly understood. In the present study, microarray analysis of a murine model requiring a unique host microenvironment for myeloma development identified decreased host–derived adiponectin, as compared to normal mice. In support, clinical analysis revealed decreased serum adiponectin concentrations in MGUS patients that subsequently progressed to myeloma. We investigated the role of adiponectin in myeloma pathogenesis and as a treatment approach, using both mice deficient in adiponectin and pharmacologic enhancement of circulating adiponectin. Increased tumor burden and bone disease was observed in myeloma-bearing adiponectin-deficient mice, and adiponectin was found to induce myeloma cell apoptosis. Pharmacologic enhancement of adiponectin, using the apolipoprotein peptide mimetic, L-4F, reduced tumor burden and increased survival of myeloma-bearing mice, in addition to preventing myeloma bone disease. Collectively, our studies have identified a novel mechanism, whereby decreased host-derived adiponectin promotes myeloma tumor growth and osteolysis. Furthermore, we have established the potential therapeutic benefit of increasing adiponectin for the treatment of myeloma and the associated bone disease.

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FLEXIBLE MODELING OF NOVEL MYELOMA MUTATIONS: DISSECTING THE RAS-RAF PATHWAY IN VIVO

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Progress at definition of the genetic makeup of myeloma creates new hope for a cure. Despite this essential roadmap, the functional validation of individual mutations remains arduous. Expression of mutant alleles in cell lines and xenografts is plagued by lack of microenvironmental cues (including a functional immune system) as well as possible functional redundancy of the allele under study. Genetically-engineered animal models offer a credible alternative; however, the creation of an animal model for each novel mutation is limited by cost and time considerations. We have developed a novel approach that addresses the need for rapid functional testing of novel myeloma mutations in vivo. We created an animal model (Prdm1 (Blimp-1):TVB-mRFP) that permits the flexible, tissue-specific delivery of genes into plasma cells through ectopic expression of an avian-derived retroviral receptor. These mice have been crossed to the genetically-engineered, preclinically validated Vk*MYC model. Mutant alleles can be rapidly cloned into avian-pseudotyped lentiviral vectors and transduced into Vk*MYC myelomas. We have begun to dissect the function of mutant alleles of the RAS-RAF pathway in this system. Both classical (V600E) and novel (G469A) BRAF mutations as well as KRAS (G12D) have been cloned. Initial endpoints include the impact of each mutant allele on survival, analysis of the transcriptome as well as profiling of cooperating mutations by array CGH. Subsequently, we shall determine the relative susceptibility of each genotype to first- and second-generation BRAF inhibitors in vivo.

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INHIBITION OF BROMODOMAIN 4 CONFERS IN VITRO AND IN VIVO ANTI-MYELOMA ACTIVITY

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Many transcription factors involved in multiple myeloma (MM) establishment or progression are currently viewed as therapeutically intractable. We thus studied proteins with bromodomain (BRD) modules, which recognize acetyl-lysine on histones to regulate transcription factor complexes. BRD4 regulates transcriptional elongation by recruiting positive transcription elongation factor complex (P-TEFb) to transcriptional start sites of growth promoting genes and regulates CDK9/myc-dependent transcription. Oligonucleotide microarrays show higher BRD4 expression in plasma cell leukemia vs. MM ($p=0.0123$); smoldering MM (or MGUS) vs. normal plasma cells ($p<0.001$); and in some MM cell lines after coculture with bone marrow stromal cells (BMSCs) vs. culture in isolation. The thieno-triazolo-1,4-diazepine JQ1 inhibits BRD4. Racemic JQ1 caused dose-dependent decrease in MM cell viability in vitro (IC50 values 250-750 nM at 72hrs for most MM cell lines vs. >750 nM for PHA-stimulated PBMCs). In compartment-specific bioluminescence imaging (CS-BLI) studies, 6/8 MM cell lines had similar or increased JQ1 sensitivity in presence of BMSCs, while 2/8 lines had modest stroma-induced increase of IC50. Isogenic cell line models of decreased bortezomib sensitivity had similar JQ-1 response as parental cells. Racemic JQ1 (50 mg/kg ip qd) significantly decreased tumor burden and prolonged overall survival of mice with orthotopic diffuse MM bone lesions and subcutaneous tumors. BRDs may emerge as attractive targets for MM cells dependent on currently intractable oncogenic transcription factors.

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MTOR AND PI3K INHIBITORS ACT SYNERGISTICALLY IN BLOCKING MYELOMA CELL GROWTH AND INDUCING APOPTOSIS

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The phosphoinositide-3-kinase (PI3K)-AKT pathway and its nutrient-dependent downstream target, the mToR (mammalian target of rapamycin) kinase, are essential for the growth and survival of malignant plasma cells. mToR inhibitors show moderate activity in myeloma patients, although the clinical activity may be limited by the fact that after inhibition of the rapamycin-sensitive Raptor complex, AKT is activated in tumor cells by feedback loops. Selective PI3K inhibitors (Ly294002, NVP-BKM120) as well as dual PI3K-mToR inhibitors (NVP-BEZ235) are available for in vitro studies and in clinical development. In five myeloma cell lines, rapamycin, everolimus, Ly294002, NVP-BKM120 and NVP-BEZ235 (Novartis) induced a dose-dependent growth inhibition. Despite the observed strong anti-myeloma activity of the mToR inhibitors, the AKT pathway was activated in vitro as well as in explanted tumors of INA-6 xenografted SCID mice upon treatment with rapamycin. Therefore, combining mToR and PI3K inhibitors could be an effective strategy to overcome rapamycin-induced AKT activation. Rapamycin together with Ly294002 or NVP-BKM120 led to synergistic growth inhibition in plasma cell lines, accompanied by the abrogation of AKT activation. Combined treatment enhanced apoptosis in cell lines and in patient myeloma cells. Interestingly, the activity of the dual inhibitor NVP-BEZ235 was enforced by rapamycin. Our data suggest that a combination of mToR inhibitors with PI3K targeting compounds or the use of dual inhibitors may lead to additive therapeutic chances and should be further explored.

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SP1 ADDICTION AND ITS ROLE IN MM

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Aberrant activation of transcription factors has been frequently associated with tumor development and progression, making them important targets for cancer therapy. We here provide evidence that Sp1, a transcription factor that controls number of cellular processes, plays an important regulatory role in multiple myeloma (MM) and other B-cell malignancies including waldestrom's macroglobulinemia (WM). We have observed high nuclear expression and activity of Sp1 in MM and WM cells by demonstrating increased DNA binding as well as increased Sp1-responsive promoter activity. Moreover, we have observed ERK-mediated further induction of Sp1 activity after adhesion to BMSC. Using both SiRNA and ShRNA-mediated Sp1 knock-down, we have confirmed the growth and survival effects of Sp1 in MM cells. We have further investigated the anti-MM activity of Terameprocol (TMP), a small molecule which specifically competes with Sp1 for DNA binding domains. TMP inhibits MM cell growth in vitro and in vivo, even in the context of the BM milieu, via activation of the mitochondrial apoptotic pathway and downregulation of the Sp1-related genes survivin and cdc2. Lenalidomide and dexamethasone upregulate Sp1 activity, and their combination with TMP provided synergistic anti-MM activity. Finally, using gene expression profile of MM cells from 172 uniformly treated patients, we correlated overexpression of Sp1-responsive genes with poor clinical outcome in MM. In conclusion, our results demonstrate Sp1 as an important transcription factor in MM pathobiology and its potential as therapeutic target.

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HIGH-THROUGHPUT EVALUATION OF ANTI-TUMOR IMMUNE ACTIVITY AGAINST MYELOMA

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We adapted our recently developed high-throughput Compartment-Specific Bioluminescence Imaging (CS-BLI) co-culture assay to measure the anti-tumor activity of immune effector cells. We studied compounds (N=800) from various libraries for their impact on innate anti-tumor activity against myeloma (e.g. MM1S) and lymphoma (e.g. HT) cells. We observed heterogeneous drug-induced immunosuppression/stimulation depending on the tumor target, across neoplasias and within the same disease. For example, in a comparative screen against MM1S and HT cells, we observed that 288 (36%) compounds were immunosuppressive and 76 (9.5%) immunostimulatory against MM1S cells, while 182 (22.8%) were immunosuppressive and 143 (17.9%) immunostimulatory against HT cells. Among compounds with any statistically significant effect (lack of overlap of 95% CIs) on immune function, 105 had concordant effect on both lines, but 60 had opposite effect (e.g suppressive in MM1S and stimulatory against HT or vice versa). Select compounds screened against more MM, lymphoma and leukemia cell lines reproduced this pattern, a subset of hits having opposing activity against different target cells even within the same disease. This suggests that screening compounds against only one cell line model may miss or overestimate immunomodulatory activity and compromise the clinical translation of these compounds. The high-throughput scalability of CS-BLI is essential for testing multiple variables and conditions and provides a framework to identify and prioritize novel immunomodulatory agents for clinical development.

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HIGH RESOLUTION STUDIES OF MYELOMA DESTRUCTION BY AN ONCOLYTIC VESICULAR STOMATITIS VIRUS

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Oncolytic Vesicular Stomatitis Virus (VSV) is a potent experimental therapeutic that we engineered to incorporate interferon-β (IFN) and sodium iodide symporter (NIS) transgenes, to improve toxicity profile and allow noninvasive imaging of viral bio-distribution. A single intravenous (IV) dose of VSV-IFN-NIS induced complete regression of subcutaneous 5TGM1 myeloma tumors in immune competent syngeneic mice. SPECT-CT monitoring revealed rapid tumor specific viral NIS expression. Detailed analysis of treated tumors show rapidly expanding foci of infection scattered throughout the tumor parenchyma that expand and coalesce destroying tumor cells by 72h post treatment. Preliminary experiments yielded similar results following IV VSV-IFN-NIS administration in Balb/c mice bearing MPC-11 myeloma tumors. Myeloma relapse was more frequent in VSV-hIFN-NIS treated mice versus VSV-mIFN-NIS, suggesting murine IFN enhances clearance of residual tumor cells. Further studies revealed relapse rates after VSV-mIFN-NIS therapy was higher following T-cell depletion. In mice bearing orthotopic 5TGM1 myeloma, IV VSV-IFN treatment significantly delayed disease progression and prolonged survival. VSV-mIFN was more potent that VSV-hIFN, suggesting a critical role for IFN in promoting complete long-term tumor control. Overall, the data establish a paradigm for successful systemic viral therapy where tumor debulking is achieved through intratumoral spread of the systemically administered oncolytic virus, while control of minimal residual disease is achieved through activation of antitumor immunity.

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BORTEZOMIB RESISTANCE IS MEDIATED BY INCREASED SIGNALING THROUGH CANCER OSAKA THYROID (COT) KINASE

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Targeting the proteasome as an anti-cancer therapy was first realized with bortezomib (VELCADE®), now one of the standards used in multiple myeloma. Despite this advance a large number of patients display an innate or acquired resistance to bortezomib. We have created several multiple myeloma cell lines with bortezomib-resistance (BR). Our studies into the changes associated with BR show that cancer Osaka thyroid (Cot) kinase gene expression is up-regulated in BR cell lines. Cot functions to regulate nuclear factor kappa B (NF-κB)-dependent transcription by promoting post-translational processing of inactive p105 to active p50. Consistent with our profiling data we found overexpression of Cot, phospho-Cot, and p50 protein levels in BR cells. Inhibition of Cot decreased activated nuclear p50, which correlated well with a preferential induction of cell death in BR cell lines. Notably, inhibition of Cot kinase activity using shRNA re-sensitized BR cells to bortezomib and the overexpression of Cot in wild-type cells induced a bortezomib-resistant-like phenotype, thus allowing cells to escape bortezomib-induced cell death. Cot kinase inhibitors synergized with a number of drugs, including bortezomib. Finally, inhibition of Cot activity in CD138+ patient samples was a potent inducer of cell death in both bortezomib-naïve and –resistant cells. These data indicate that Cot kinase plays an integral role in bortezomib-resistance in plasma cells and may potentially be a novel therapy for the treatment of patients with bortezomib-resistance.

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NONVIRAL DELIVERY OF A POTENT ONCOLYTIC PICORNAVIRUS FOR MYELOMA THERAPY

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We previously demonstrated that coxsackievirus A21 (CVA21) is a potent antimyeloma agent against established KAS6/1 myeloma xenografts (Kelly et al. Nat. Med. 2008). Because intravenously administered picornavirus particles provoke a robust antiviral antibody response, only the first dose is expected to show any antitumor activity. One possible solution could be to use nonviral vectors to deliver picornavirus genomes as infectious nucleic acid (INA) to initiate a spreading virus infection. Nonviral vectors are easily manufactured, poorly or nonimmunogenic, and have favorable pharmacokinetic properties. Infectious RNA encoding CVA21 was therefore transcribed from plasmid DNA using T7 polymerase. Within 48 hours of injecting this RNA into subcutaneous mouse myeloma xenografts, high titers of infectious CVA21 virions were detected in the bloodstream. Tumors regressed rapidly thereafter and mice developed signs of myositis. At euthanasia, CVA21 was recovered from regressing tumors and from skeletal muscles. Treatment outcomes were comparable following intratumoral injection of naked RNA or fully infectious CVA21 virus. Dose-response studies showed that an oncolytic infection could be established by intratumoral injection of 1 ug of infectious RNA. The oncolytic infection could also be initiated by intravenous RNA injection. Our study demonstrates that INA is a highly promising alternative drug formulation for oncolytic virotherapy. Current efforts are focusing on the use of plasmid DNA as an alternative to infectious RNA.

P-324

HLA CLASS I-SPECIFIC SINGLE-CHAIN FV DIABODY ERADICATES THE SIDE POPULATION FRACTION OF MYELOMA CELLS

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We have reported that HLA class I is highly expressed in multiple myeloma (MM) cells and an engineered single-chain Fv diabody specific to pan-HLA class I (C3B3-DB) can be a potent agent to MM. Recently, cancer stem cells are considered as a target for curative therapy, and side population (SP) cells identified by the ability to efflux Hoechst 33342 dye are thought to be a candidate of cancer stem-like cell fraction. In this study, we characterized SP fraction in MM cells by flow cytometry, and investigated the efficacy of C3B3-DB on SP cells compared with chemotherapeutic agents. MM cell lines and primary MM cells contained SP cells ranging from 0.08% to 0.2% among total cell population. Isolated SP cells exhibited elevated expression of ABCG2 as well as HLA class I. Treatment with melphalan or bortezomib mainly killed non-SP fraction, whereas C3B3-DB caused a significant reduction in both SP and non-SP fractions. In combination with chemotherapeutic agents, prior treatment with C3B3-DB significantly enhanced cytotoxicity of melphalan and bortezomib against MM cells. Moreover, short-term exposure with C3B3-DB suppressed mRNA expression of the pluripotency-associated transcription factors including Sox2, Oct3/4, and Nanog in MM cell lines. Treatment with C3B3-DB inhibited colony formation of SP cells and suppressed tumor development in SCID mice. These results indicate that C3B3-DB has a potent activity to eradicate MM cancer stem-like SP cells, and that the combination therapy will provide a new strategy for improving the efficacy of current therapies in MM.

P-325

A DRUG REDEPLOYMENT STRATEGY IDENTIFIES THE ANTI-HELMINTHIC NICLOSAMIDE AS A POTENT NOVEL ANTI-MYELOMA THERAPY THAT ALSO REDUCES FREE LIGHT CHAIN PRODUCTION

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Multiple myeloma (MM) remains an incurable disease. Many patients will develop renal impairment (RI), predominantly caused by free light chain (FLC) mediated nephrotoxicity. Hence, effective anti-myeloma therapies are required that kill the malignant plasma clone, whilst also reducing FLC production. Importantly, given that most MM patients are >60yrs, therapies must also have limited toxicities. Drug redeployment, the strategy of using drugs licensed for one condition in a new setting, has already shown efficacy in MM with the advent of thalidomide. We utilised this strategy screening 100 off-patent, low-toxicity drugs for anti-MM activity. In the screen, niclosamide, an anti-helminthic, demonstrated potent anti-myeloma activity against both MM cell lines and primary MM cells. Cell death was associated with markers of both apoptosis and autophagy. Niclosamide induced uncoupling of oxidative phosphorylation, as measured by depolarisation of mitochondrial membranes, and a rapid increase in O2 respiration rate. This was coupled to the generation of mitochondrial superoxide, which correlated with loss of cell viability. Furthermore, sub-lethal doses of niclosamide reduced FLC protein secretion from MM cell lines, and some primary MMs. In some, but not all cases, reduced FLC protein secretion was associated with reduction in FLC mRNA transcription most likely through inhibition of NF-kB activity. Hence, this study has identified niclosamide as a promising potential therapy for MM, and other disorders involving excessive immunoglobulin production.

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INHIBITING PROTEIN TRANSLATION WITH SILVESTROL AS A NOVEL STRATEGY TO TARGET MULTIPLE MYELOMA

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Multiple Myeloma (MM) cell survival has been shown to depend on precise control of protein production and degradation. Disruption of protein catabolism through proteosomal or aggresomal blockade results in MM cell death. We hypothesize that inhibiting protein production will have a similarly toxic effect in MM. We explore the consequences of inhibiting of mRNA translation in MM using silvestrol, a powerful inhibitor of ribosomal recruitment, which preferentially disrupts the production of certain cell regulatory and survival proteins. A panel of silvestrol-treated MM cell lines showed profound inhibition of growth and a rapid induction of apoptosis, as seen by MTT viability and AnnexinV expression assays respectively. The average IC50 in MM cells was determined to be 20nM while it was considerably higher in primary cultures of senescent fibroblasts. Apoptosis was observed in primary patient MM samples treated with 20nM of silvestrol. We show that silvestrol inhibits protein translation by inhibiting ribosome binding, decreasing polysome content and increasing 80S ribosomes. Western blot analyses show that silvestrol rapidly decreases the expression of c-Myc and non-canonical NFK-B signaling. Expression of anti-apoptotic proteins such as MCL1 and

BCL-2 decreased while pro-apoptotic proteins, such as BAX, increased with silvestrol treatment. In a novel transgenic mouse model of MM (vk*myc), which has been shown to behave clinically like human MM, silvestrol does not appear to be toxic and is therapeutically effective. Our results warrant clinical evaluation of silvestrol.

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TARGETING NAD+ SALVAGE PATHWAY IS A NOVEL THERAPEUTIC STRATEGY IN MULTIPLE MYELOMA

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Nicotinamide adenine dinucleotide (NAD+) is a coenzyme crucially involved in numerous cellular functions. NAD+-lowering drugs are under investigation for their potential as anticancer therapeutics. Here we investigated the preclinical activity of a selective Nampt (the rate-limiting enzyme in the NAD+salvage pathway)inhibitor APO866 in MM. We found maximal cytotoxicity of APO866 against a panel of 14 MM cell lines, both sensitive as well as resistant to conventional chemotherapy, with an IC50 values ranging from 3-30nM at 96h. A strongly expression of Nampt was revealed by western-blot analysis in all the cell lines analyzed.Remarkably, in healthy leukocytes, APO866 was poorly active and failed to show any cytotoxic effect, indicating an increased reliance on these enzymes'activity by MM cells. The AnnexinV/PI analysis confirmed APO866's ability to induce apoptosis in a dose- and time-dependent fashion.Interestingly Nampt inhibitor showed anti-myeloma activity even in the presence of interleukin-6 and insulin-like growth factor-1, confirming its ability to overcome the proliferative advantage conferred by this cytokines. Mechanistic studies, showed that APO866 cell death occurred in the absence of caspase activation. Accordingly, autophagy but not caspase inhibitors reduced APO866 cytotoxic activity. In conclusion our preliminary data show the efficacy of Nampt inhibitor in MM cell lines,at nanomolar concentrations. Ongoing mechanistic and in vivo studies will delineate the role of Nampt inhibitors in MM and better define its potential for clinical development in MM.

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OVERCOMING RESISTANCE; THE USE OF POMALIDOMIDE (POM) AND DEXAMETHASONE (DEX) IN RE-SENSITIZING LENALIDOMIDE (LEN)-RESISTANT MULTIPLE MYELOMA (MM) CELLS

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The development of Len as an immunomodulatory drug has led to major advances in the treatment of MM in recent years. Unfortunately, all MM patients eventually become refractory to their drug regimes and relapse. Therefore, new compounds and combinatorial treatments are now being sought to re-sensitize refractory tumors. Here we evaluate the use of Len and Pom alone or in combination with DEX in the treatment of Len-resistant MM cell lines. *Results:* Len-resistant or -sensitive H929 cell lines were established and treated with 0-10μM of Len or Pom for 5-days. In Len-sensitive cells, both Len and Pom inhibited cell proliferation (IC50; 6.0μM and 0.2μM respectively). In Len-resistant cells, only Pom treatment affected cell proliferation (IC50; 7.9μM). Len-resistant cells did respond to DEX treatment (IC25; 0.8μM), but no additive effects were seen with the combination of Len and DEX. However, the combination of Pom with DEX was strongly synergistic in Len-sensitive and -resistant cells, inhibiting cell proliferation, as assessed by 7AAD staining, by a further 100-fold in Len-sensitive cells (IC50; Pom alone, 0.2μM, Pom and Dex 0.02μM) and >60-fold in Len-resistant cells (IC50; Pom alone, 8.3μM, Pom and Dex 0.13μM) in addition to inducing apoptosis. *Conclusions:* Len and Pom are strong inhibitors of cell proliferation in MM cells. Len-refractory MM cells are also responsive to Pom alone, however, the combination of Pom and DEX induces a strong synergistic, tumorcidal effects, suggesting that Pom and DEX will be a powerful tool in re-sensitizing Len-resistant cells in the clinic.

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GENE EXPRESSION PROFILING IN MULTIPLE MYELOMA - REPORTING OF ENTITIES, RISK AND TARGETS IN CLINICAL ROUTINE

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Background: Multiple myeloma is characterized by molecular heterogeneity transmitting to survival ranging from several months to over 15 years. Gene expression profiling allows assessment of biological entities, risk, and targets. Its translation into clinical routine alongside conventional prognostic factors has been prevented by lack of tools to report this as clinically digestible information. *Methods:* We present a non-commercial open source software-framework developed in R (GEP-report) using Affymetrix microarray raw-data and a documentation-by-value strategy to directly apply thresholds and grouping-algorithms from a reference cohort of 262 myeloma patients. *Results:* The GEP-report comprises 1) quality control; 2) test of sample identity; 3) biological classifications of multiple myeloma; 4) risk stratification; 5) assessment of target-genes, and 6) integration of expression-based and clinical risk factors within one metascore. This HM-metascore sums over the weighted factors gene-expression based risk-assessment (UAMS-, IFM-score), proliferation, ISS-stage, t(4;14), and expression of prognostic target-genes (AURKA, IGF1R) for which clinical grade inhibitors exist. It delineates three significantly different groups of 13.1, 72.1 and 14.7% of patients with a 6-year survival of 89.3, 60.6 and 18.6%, respectively. *Conclusion:* GEP-reporting allows prospective assessment of target gene expression and integration of current prognostic factors within one risk stratification, being customizable regarding novel parameters or other cancer entities.

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EFFECTIVENESS OF ASPIRIN THROMBOPROPHYLAXIS IN PATIENTS WITH MULTIPLE MYELOMA ON COMBINATION TREATMENT WITH THALIDOMIDE

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Combination treatment with thalidomide is associated with an increased incidence of venous thromboembolism (VTE) in multiple myeloma, but only limited data is available about the risk of arterial thrombosis. 118 consecutive patients with newly diagnosed or relapsed/refractory disease treated with CTD/MPT were included in a single centre, retrospective study, to determine the efficacy of aspirin against both arterial and venous thrombosis. At baseline, 29 patients received anticoagulation with warfarin or LMWH (19 patients with high risk criteria: immobility, previous VTE, nephrotic syndrome and use of erythropoietin), 70 low risk patients received 75mg aspirin, and 2 clopidogrel. 14 patients received no prophylaxis, and multiple agents were used simultaneously in 3 patients. The incidence of thrombosis in the aspirin group was 17.4%, with equal incidence of arterial and venous thrombosis (8.7%). Additional thrombogenic risk factors were identified in this group: previous arterial thrombosis (33.3% incidence of thrombosis), ≥70yrs (23.1%), hyperviscosity (22.6%) and extensive lytic lesions (18.0%). A chi-squared for linear trend demonstrated a near linear association between the number of thrombotic risk factors and the incidence of thrombosis (risk factors and incidence: 0=11.1%, 1=14.3%, 2=10.0%, 3=36.4% >3=40.0%) (p=0.08), supporting a thromboprophylactic strategy according to a risk-assessment model. Our data demonstrates that combination treatment with thalidomide increases the risk of both arterial and venous thrombosis, and identifies additional high risk criteria.

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FEATURES OF EXTRAMEDULLARY MULTIPLE MYELOMA: SOFT TISSUE RELAPSE AS AN EVOLVING CLINICAL DILEMMA IN HEAVILY PRE- TREATED PATIENTS. A RETROSPECTIVE SINGLE-CENTER STUDY OF 24 PATIENTS

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Background: Extramedullary (e) relapse in multiple myeloma (MM) is a serious event for MM patients that is encountered at increasing incidence. When eMM relapse occurs, no validated treatment options exist and most patients eventually die due to uncontrolled disease progression or therapy-related toxicity. We therefore analyzed relapse patterns and individual treatment decisions of 24 eMM cases at our institution to further elucidate this challenging phenomenon. *Results:* Most frequently, eMM occurred at soft tissue sites (67%) followed by parenchymal involvement (25%) and malignant effusion (12.5%). CNS involvement (meningiosis, cerebral masses) was observed in 21% of the patients. At diagnosis of eMM relapse, bone marrow infiltration was absent in 46%, in 21% infiltration was less than 20%. In ten cases, biopsies from eMM lesions were available. The most striking finding was a dramatically increased proliferation index (Ki-67) of about 67% of all myeloma cells. EM relapse was treated with irradiation; dose-intense chemotherapy; novel agent-based therapies; and auto- allogeneic SCT concepts including DLI administration. 13/24 patients responded to therapy (1 CR, 12 PR), 1 patient had stable disease, 9 patients showed progressive disease or mixed response. Progression-free survival was short with a median of 2 months (95% CI: 0.08-3.92). Median overall survival was 7 months (95% CI: 3.56-10.43). *Conclusion:* EMM is not compatible with the characteristics of an indolent lymphoma like MM usually is and, therefore, distinctive therapeutic approaches are urgently needed.

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RAPID PROGRESSION OF ANEMIA IS RELATED WITH TUMOR-LYSIS SYNDROME ASSOCIATED WITH BORTEZOMIB TREATMENT FOR MULTIPLE MYELOMA PATIENTS

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Introduction: Tumor-lysis syndrome (TLS) is very rare complication in patients with indolent hematological neoplasms such as multiple myeloma (MM). However bortezomib (BOR) treatment for MM often causes TLS. We developed an index (Rapid Anemia Progression: RAP index), that was related between duration and progression of anemia, to evaluate risk factor of TLS. *Patients and Methods:* RAP index was obtained by following calculation.

$$\text{RAP index} = \frac{\text{Hb(post) - Hb(pre)}}{\text{Hb(post) - Hb(pre)}} \times 30$$

Day(post) is day when BOR starts. Day(pre) is day that is the nearest from day-30. Hb(post) and Hb(pre) are serum hemoglobin level in Day(post) and Day(pre). We retrospectively reviewed 35 relapsed or refractory MM patients treated with BOR in our institution. We analyzed some parameters when BOR started. We analyzed albumin, lactase dehydrogenase, beta2-MG and M-protein. We evaluated the risk factors of TLS associated with BOR by Cario-Bishop Definition. *Results:* Median age was 64 year (49 - 82 year). TLS appeared in 8 patients totally, and clinical TLS (c-TLS) appeared in 6 patients. TLS appeared during first course of BOR treatment among all the patients. Median date when c-TLS appeared was day 9.5 (day 4 - 12). For a cutoff point of -1.0 g/dl/month of RAP index, high RAP index predicted c-TLS more often significantly (range -3.32 ± 2.57, p= .043). The fluctuation of albumin, lactase dehydrogenase, be-ta2-MG and M-protein was not related with c-TLS significantly. *Conclusion:* RAP index is related with c-TLS associated with BOR treatment for MM patients significantly for a cutoff point of -1.0 g/dl/month.

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ANALYSIS OF SELECTED BIOLOGICAL PARAMETERS SERUM LEVELS IN MGUS AND IN MULTIPLE MYELOMA

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Objective: The aim of the study was to assess the benefit of 18 biological parameters in MGUS and multiple myeloma (MM) in differentiation of both conditions. *Methods:* The analyzed cohort consisted of 59 MGUS and 60 MM individuals. For the evaluation of the selected parameters we used radioenzyme assay, immunoradiometry, enzyme immunoassay, electrochemiluminiscence, quantitative sandwich enzyme immunoassay and Freelite TM system. *Results:* Statistically significant differences between MGUS and MM were found in the case of thymidine kinase (0.0002), ICTP (0.001), MIP-1alpha (0,002), osteopontin (<0,0001), HGF (<0.0001), syndecan-1 (<0,0001) and the κ/λ ratio of serum free light chains (0. 0002). Lower significance was found in the case of angiogenin (0.031) and endostatin (0.011) whilst non-significant differences were within the IGF-1, osteocalcin, b-ALP, PINP, OPG, MIP-1beta, IL-17, VEGF and parathormon serum levels. *Conclusions:* Significant differences between MGUS vs MM were found in 9 of the 18 evaluated parameters, however, due to the overlaping of the measured values, none of the parameters is unabiquously able to distinguish between the compared units. A satisfactory contribution in the discrimination of MGUS from MM was found in markedly increased serum levels of thymidinekinase, MIP-1alpha, osteopontin, HGF and pathology of the κ/λ index. With support of MSM 6198959205.

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ANALYSIS OF FAMILIAL DYSGLOBULINEMIA: A MULTICENTER IFM STUDY

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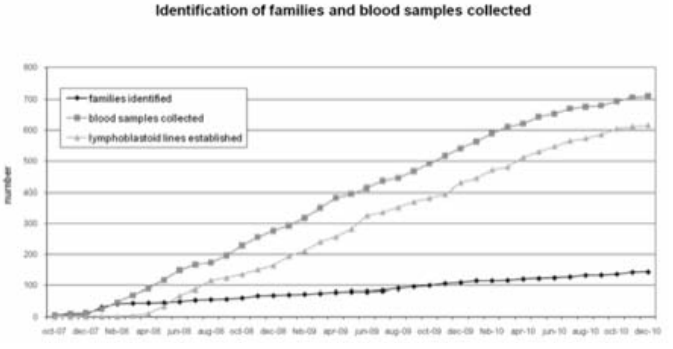
Multiple myeloma is a haematological malignancy which rarely occurs in a familial context. MGUS corresponds to the existence of a clone secreting a monoclonal immunoglobulin and can in some patients evolve to multiple myeloma. Although recurrent cytogenetic abnormalities have been described in tumor cells of sporadic cases of multiple myeloma, there are presently no identified genetic mechanisms which contribute to myelomagenesis, in particular in familial cases. The identification and study of families with several cases of dysglobulinemia should contribute to the determination of genetic factors predisposing to multiple myeloma. The aim of this study is to identify genetic factors predisposing to familial dysglobulinemia, thanks to the establishment of a DNA bank. Peripheral blood samples were collected both from patients with dysglobulinemia and from healthy family members and lymphoblastoid lines established for further studies. For this purpose, we have initiated a multicenter study aiming to identify families with several cases of dysglobulinemia, including both multiple myeloma and/or MGUS. This effort involves the Intergroupe Francophone du Myélome (IFM) and Myeloma Autograft Group (MAG), in coordination with the Réseau des Hémopathies Familiales. This study was activated in Octobrer 2007 and as of January 2011, 141 families with at least two cases of dysglobulinemia have been identified. This study is ongoing in order to collect samples from 200 families and to initiate genetic analysis to identify predisposing factors.



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THE ALBUMIN /MONOCLONAL PROTEIN RATIO AS PROGNOSTIC MARKER FOR MULTIPLE MYELOMA

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Multiple myeloma (MM) is a heterogeneous disease with different prognosis, clinical course, and response to therapeutic interventions. There is a general need for simple, easily available and unexpensive prognostic factor for this disease. In a previous study the A/M ratio containing albumin (A) and monoclonal component (M) emerged as reliable predictor of survival duration in patients treated with conventional chemotherapy (Pathology Oncology Research 2009; 15:383-387). In the current retrospective study authors evaluated the prognostic role of this fraction in the era of novel agents. They assessed the A/M ratio prior treatment in 56 newly diagnosed MM patients from the aspect of the survival time. According their results it turned out that the A/M < 1 heralded poor prognosis while A/M > 1 meant favourable outcome either at 2 year (p=0,01) and at 5 year (p=0,07) survival endpoints as well. These results proved that A/M ratio remained valuable marker for predicting prognosis in patients treated with proteosome inhibitor and antiangiogenic therapy as well. Authors recommend therefore applying this A/M ratio in further studies for the better pre-treatment stratification.

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LEVELS OF NESTIN PROTEIN CORRELATE WITH 1Q21 GAIN AND HYPERDIPLOIDY IN MULTIPLE MYELOMA

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Introduction and Aims: Our previous study showed heterogeneous nestin levels in plasma cells (PC) of multiple myeloma (MM). Nestin is considered to be a suitable diagnostic and prognostic indicator of malignancy and a marker of cancer stem cells in solid tumors. The aim of this study was to correlate nestin levels with cytogenetic abnormalities in PC of MM patients. *Methods:* A total number of 68 MM patients (35M/33F; median age 68 years) were included in this study. Levels of nestin were analyzed by 3-color flow cytometry in CD138+CD38+PC. Nestin expression was assessed as percentage of PC showing positivity for nestin (% Nes+PC) and median fluorescence intensity of nestin (MFI). CD138+PC were analyzed for del(13q14), del(17p53), IgH rearrangement, 1q21 gain and hyperdiploidy (HY) by interphase FISH. Correlation between % Nes+PC or MFI and cytogenetic data was analyzed by non-parametric Mann-Whitney U test. *Results:* The whole group of MM patients had the median percentage of Nes+PC 27.6% (range, 0.0%-98.9%) and median MFI 4.2 (range, 1.8-86.7). Statistical correlation was found between % Nes+PC (p<0,001) or MFI (p<0,001) and 1q21 gain and adverse correlation was confirmed between MFI and HY (p=0.014). *Conclusion:* Our results indicate that high % Nes+PC or nestin levels significantly correlate with 1q21 gain, and low nestin levels correlate with HY. We suppose that nestin could associate with unfavorable prognosis but further studies are required to elucidate its clinical implication for MM. Supported with research program MSM of Czech republic Nr. LC06027 and P304/10/1395.

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THE ASSOCIATION OF KI67 PERCENT POSITIVITY AND CLINICAL OUTCOMES IN THE UPFRONT TREATMENT OF MULTIPLE MYELOMA

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Background: The plasma cell labeling index (PCLI) in Multiple Myeloma (MM) is labor intensive. We explored an immunohistochemical (IHC) stain for the proliferation marker ki67 as an alternative to PCLI. *Methods:* A cohort study of MM patients with bone marrow double IHC staining for CD138 and ki67 was conducted; the percentage of plasma cells (CD138) positive for ki67 was expressed as %ki67. *Results:* %Ki67 was available for 151 patients, with a median %ki67 of 3% (range 0-57%). Median progression-free survival (PFS) for first-line treatment was 232 weeks for %ki67 ≤ 3% vs. 146 weeks for %ki67 > 3% (P = 0.19). Each 1% unit increase in %ki67 was associated with a 3% increase in progression risk (P = 0.02). The %ki67 cutoff of 10% was significant for a difference in PFS, with a median of 232 weeks for %ki67 ≤10% vs. 110 weeks for >10% (P = 0.03). Median survival was not reached; there were 14 deaths (n = 77) for ki67 ≤ 3% (1-yr OS = 93.5%) vs. 21 deaths (n = 74) for %ki67 > 3% (1-yr OS = 90%), (P = 0.44). In regression analysis for factors influencing PFS, only high-risk cytogenetics was significant (P=0.015); %ki67 approached an independent effect (P = 0.07). There was a trend for quicker relapse post autologous stem cell transplant for higher %ki67 (%ki67 > 3% at 108 weeks vs. ki67% ≤ 3% at 177 weeks, P = 0.26). *Conclusion:* %Ki67 is correlated with risk of disease progression in first-line MM therapy and a trend to quicker relapse after autologous stem cell transplant. The relative ease of the ki67 IHC test makes it attractive alternative to PCLI.

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THE FIRST EPIDEMIOLOGIC STUDY OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) : THE EXPERIENCE OF THE NORMANDY REGIONAL REGISTRY OF MALIGNANT HEMOPATHIES

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The plasma cell disorders (PCD) include monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma (MM), primary amyloidosis, plasmacytoma, lymphoproliferative disorders and Waldenström macroglobulinemia (WM). In France, MM and PCD account for 1.4 % of all cancer cases in 2010, with an estimated WSR of 4.9/100000 in men and 2.3 in women. However, epidemiologic studies on MGUS, which in most cases precede MM, are absent. The Regional Registry for Malignant Hemopathies in Basse-Normandie (RRHMBN), belongs to the France Cancer Incidence and Mortality (FRANCIM) network. Between 1997 and 2005, it exhaustively recorded all the new cases of malignant hemopathies occurring in patients residing in Basse-Normandie, France, at diagnosis. The study population consisted of 1858 new cases of PCD with 1009 MGUS (483 male, 526 female), 830 MM (427 male, 403 female) and 19 other PCD. The mean age at MGUS diagnosis was 72 years (18-102) with 382 patients (37.9 %) aged > 75 years. M protein was IgG in 57.2 %, IgM in 25.2 % and IgA in 11.6 %. The world-standardized incidence rates (WSR) of the MGUS were 3.76+/-0.26/100000 increasing with age, from 1.89+/-0.22 in 0-64 years to 42.14+/-7.64 in > 85 years. The median overall survival (OS) is 115.9 months. OS at 5 years is 78.3 %, (decreasing from 95.6 % < 65 to 38 % > 85 years). For the first time, at our best knowledge, we give in this study the WSR for MGUS. We also confirm, in this study, the annual rate progression to MM of 1.41 %.

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N-CADHERIN EXPRESSION IS UPREGULATED IN MULTIPLE MYELOMA

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N-cadherin is a transmembrane or secreted homophilic adhesion molecule that regulates blood vessel morphogenesis and osteoblastogenesis in adult tissues. Increased expression of N-cadherin is associated with disease progression and poor prognosis in epithelial cancers including breast cancer. In this study we investigated the expression of N-cadherin (CDH2) in multiple myeloma (MM) patient samples and cell lines. While appreciable levels of CDH2 expression were not detectable by real time PCR in the B-cell lines ARH-77, Balm and NALM-6, CDH2 expression was detectable in 6 of 8 MM cell lines tested (OPM-2 > NCI-H929 > LP-1 > JIMI > RPMI-8226 > ANBL6). Cell surface expression of N-cadherin in MM lines was confirmed by flow cytometry. Significantly,

CDH2 expression was higher in iliac crest trephines and total bone marrow mononuclear cells isolated from newly-diagnosed MM patients when compared with normal age-matched controls. The levels of secreted N-cadherin in plasma, as assessed by ELISA, were elevated in 16/58 MM and 4/19 MGUS patients, relative to age-matched controls. Furthermore, elevated plasma levels of N-cadherin were associated with poor prognosis in MM patients (high N-cadherin hazard ratio: 4.48). Flow cytometric experiments will confirm whether MM plasma cells are the source of the elevated N-cadherin found in MM patients. These results suggest that N-cadherin may be a viable prognostic marker for MM. Overexpression of CDH2 in MM cell lines will enable the investigation of the role of N-cadherin in mediating the behaviour of MM in vitro and in vivo.

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POST-BORTEZOMIB THERAPY PERIPHERAL NEUROPATHY IN MYELOMA PATIENTS

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Introduction: Bortezomib(BOR) is widely used to treat multiple myeloma. BOR-associated peripheral neuropathy is frequently occurred, especially in the midst of the therapy. In our cases, even after BOR therapy ceased, the neuropathy developed in 3 patients and it got worse in 5 additional patients. Method: We analyzed retrospectively 65 myeloma patients (29 female) who had received BOR therapy at our hospital between Jan 2006 and Dec 2010. The median age was 62 years (range 42-75). Toxicity was scored according to the CTCAE version 4.0. *Results:* Peripheral neuropathy was observed in 37 patients. After BOR therapy was initiated, the onset of neuropathy in 37 of the patients occurred at 1-62 weeks (median 8 weeks). In 3 patients, the neuropathy newly appeared 2-6 weeks (mean 4 weeks) after cessation of therapy. The worst grade of each patient is 1, 2, and 3. One patient became symptom-free at 4th month, the other improved at 3rd month, one remain unchanged within one year. In 5 additional patients, the neuropathy aggravated 2-8 weeks (mean 5 weeks) after cessation of therapy. The change of grade from cessation of BOR therapy to the highest grade is grade 2 to 3 in 1 patient, and 1 to 2 in the other 4 patients. One patient became symptom-free at 11th month. Two improved at 3rd month, one improved at 17th month, and the other one died of myeloma at 10th month without neuropathy improvement. *Conclusion:* BOR-associated peripheral neuropathy can develop or get worse even after treatment ceased. Careful attention should be paid to peripheral neuropathy during and after BOR therapy.

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GENETIC POLYMORPHISMS ASSOCIATED WITH RISK OF VENOUS THROMBOEMBOLISM IN MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE

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Introduction and Aims: Venous thromboembolism (VTE) is a common side-effect of thalidomide treatment in patients with multiple myeloma (MM). In our retrospective study, we analyzed candidate single-nucleotide polymorphisms (SNPs) - CINP (rs7011), CETP (rs289747), ALDH1A1 (rs610529), CDKN1A (rs3829963), GAN (rs2608555), VEGF (rs699947) and ALDH1A1 (rs168351), previously identified in a large association study based on hypothesis-driven candidate gene approach nominated by the International Myeloma Foundation, Bank On A Cure' (3404 SNPs). In that study, the authors built a classification tree enabling prediction of individual risk of VTE in MM patients. *Methods:* A total number of 111 MM patients (53M/58F) were included in our study. All patients underwent thalidomide based treatment and received thromboprophylaxis. Genotypes of these SNPs were determined through TaqMan real-time PCR allelic discrimination. *Results:* We did not confirm the ability of this classification tree to predict VTE risk in MM patients - of these patients, 19 % (21/111) developed high-grade VTE. However, in patients with VTE, we found higher frequency of AC genotype in the CDKN1A gene (42.9 % vs. 16.7%; ORs=3.64) in comparison to CC genotype (p=0.015). SNPs of other genes as well as age and sex of patients had no statistically significant influence on risk of VTE. *Conclusion:* Further studies are needed to confirm the initial analysis that provided predictive information of genetic variations in myeloma patients that may influence risk of VTE. Supported by grants: LC06027, MSM0021622434.

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HOSPITAL-BASED VERSUS POPULATION-BASED MULTIPLE MYELOMA REGISTRY

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A population-based cancer registry includes all new cases of multiple myeloma (MM) in a geographically defined area. A hospital-based cancer registry records all cases at a given hospital. The Granada Cancer Registry covers Granada's province population, i.e., 905.285 inhabitants. Our hospital covers 442.523 of them. To compare the quality and characteristics of hospital-based (HBR) and population-based (PBR) MM registries, during a seven-year period (1999-2005). Only cases that belonged to our hospital catchment area (hca) were included in the PBR. In HBR, 132 cases were recorded, 59 males / 73 females, median age 68,5 (40-91); in PBR, 189 cases, 85 males / 104 females, median age 69,9 (31-90). 10% of cases in PBR had a second malignant tumor. When HBR and PBR databases were compared, we found that 107 MM cases were included in both registries, while 82 and 25 cases were only registered in PBR and HBR, respectively. The group of 25 cases not included in PBR had a permanent address outside the hca. Concordance for basic variables was as follows: name 91,6 %, sex 100 %, ID number 99%, date of birth 93,5%, date of diagnosis 66,4%. On average, 4,4 patients/100.000/year were attended at our centre. New cases per year are shown below Information collected in MM registries must fulfill excellence criteria. PBR is essential to monitoring MM incidence while HBR is mostly involved in patient care. Common efforts should be made to increase the thoroughness of both registries.

	1999	2000	2001	2002	2003	2004	2005
PBR	33	28	30	31	22	25	20
HBR	16	16	19	23	15	23	20
Ratio	2.06	1.75	1.57	1.34	1.46	1.08	1

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HYPOCHOLESTEROLEMIA IN PATIENS WITH MULTIPLE MYELOMA

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Introduction: Lower levels of serum lipids have been noticed in patients with some malignancies. This fact may be due to an increased utilisation of cholesterol by malignant cells for the formation of the cell wall and for the DNA-replication. *Aim:* Comparison of the levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) in the groups of patiens with multiple myeloma (MM) and healthy controls, and assessing the relationship between these levels and types and stages of MM. *Methods:* 91 patients (55 males and 36 females) of mean age 64,2 ± 10,2 years with MM were enrolled to this retrospective study. In the control group, there were 25 healthy persons. Levels of serum lipids were acquired in studied persons and data were statistically analyzed using Student´s t- test and ANOVA-analysis. *Results:* The levels of TC, HDL-C and LDL-C in patiens with MM were significantly lower than in the controls (p<0,001). The levels of TC, HDL-C and LDL-C in advanced stages of MM (st. III by Durie-Salmon (D-S) and IPI) were significantly

lower, too (p <0,05). There was no diference for the levels of TG between controls and patiens with MM, and in the various stages of MM. Lipid parameters were not different between Ig types. *Conclusion:* We confirmed that the levels of TC, LDL-C and HDL-C were significantly lower in pacients with multiple myeloma than in controls. In patiens with MM, there was a correlation between the levels of TC, LDL and HDL-C and stages of the disease (by D-S and IPI).

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TUMOR LYSIS SYNDROME AFTER BORTEZOMIB THEATMENT IN REFACTORY/RELAPSED MYELOMA PATIENTS

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Aim: Tumor lysis syndrome (TLS) has been rare in multiple myeloma (MM) before the era of high dose therapy (HDT) and novel agents. Risk factors for TLS after bortezomib (Bor) therapy have not fully studied. The aim of this study is to extract risk factors for TLS of Bor therapy for refractory or relapsed MM patients. *Materials & Methods:* Between January 2007 and August 2010, 59 patients (male/female=32/27, median age=61ys, HDT/non-HDT=40/19, secondary PCL/plasmacytoma/unfavorable cytogenetic abnormalities=5/13/23) with relapsed/refractory MM were treated with Bor therapy at the National Center for Global Health and Medicine. Diagnosis of TLS was defined according to the criteria of Cairo-Bishop (JCO 2008). *Results:* bor related TLS developed in 17 patients. PCL, extra-medullary plasmacytoma, unfavorable cytogenetic abnormalities, and elevated levels of LDH were risk factors for TLS (12.6-hold, 6.6-hold, 12-hold, and 22.4 in Odds ratio for TLS). The overall response rate, better than VGPR rate, and the estimated median TTP were not significantly different between TLS group and non-TLS group, but median OS of TLS group was shorter than non-TLS group (8 months vs 20 months, p<0.001). *Conclusion:* Risk factors for Bor related TLS in MM patients was extracted. In addition, these factors were poor prognostic factors for response and survival in MM patients. Bor was effective in both TLS group and non-TLS group. However, overall survival was much worse in TLS group than non-TLS group. In TLS group, MM showed rapid progression soon after TLS, which might affect shorter survival.

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RELATION OF IL-18 POLYMORPHISM TO THE SUSCEPTIBILITY AND CLINICAL FEATURE OF MULTIPLE MYELOMA IN JAPANESE PATIENTS

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Introduction: interleukin-18 (IL-18) plays a role in the host's response to tumors and angiogenesis. Several mouse model experiments have shown that IL-18 may have anti-tumor effect in multiple myeloma (MM). However, it is unclear whether IL-18 polymorphisms alter the susceptibility and clinical outcome of MM. We examined -137(G/C) and -607(C/A) of IL-18 genes in Japanese patients with MM. *Methods:* ninety three patients with MM [age range, 35-83 years; IgA (n=15), IgG (n=55), IgD (n=2), non-secretory (n=3), Bence Jones (n=18)], and 153 healthy controls were included. Genotyping was determined by the allelic PCR based technique. *Results:* patients with MM had a significantly higher frequency of the IL-18-137 CC or GC genotype compared to the control group (34% vs. 22%, P<0.05). The number of IL-18-137 C alleles among the patients with MM was also higher than in the control group (19% vs. 12%, p<0.05). Furthermore, IL-18-137 CC or GC genotype was significantly associated with advanced international staging system (ISS) (P<0.05) and lower hemoglobin level (p<0.05). No significant differences in the genotype or allele frequencies of IL-18-607(C/A) were observed between MM patients and the control group. In the clinical characteristics including sex, Ig type, and ISS, there was also no difference between patients with IL-18-607 GG genotype and non IL-18-607 GG genotype. *Conclusion:* these results suggested that the IL-18-137(G/C) may be associated with the susceptibility and the clinical feature of MM in Japanese patients.

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ASSOCIATION BETWEEN EXPOSURE TO NOVEL AGENTS AND EXTRAMEDULLARY INVOLVEMENT OF MULTIPLE MYELOMA : A CLINICOPATHOLOGIC STUDY OF 84 CONSECUTIVE AUTOPSY CASES

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Background: extramedullary involvement of myeloma is frequently associated with poor outcome. However, few data exist about its incidence and predictive factors through analyzing autopsy cases. *Methods:* we reviewed autopsy reports of 84 multiple myeloma cases between 1979 and 2009. Factors associated with extramedullary involvement of myeloma were statistically analyzed. *Results:* extramedullary involvement of myeloma cells was observed in 60 patients (71.4%). The spleen (46.4%), liver (38.1%), kidney (31.0%), lymph nodes (28.6%), lung (25.0%), pancreas (20.2%), and adrenal gland (13.1%) were frequent sites of involvement. Eleven patients were treated with novel agents; 9 were treated with bortezomib, 4 with thalidomide, and 2 with both. Eighteen patients were treated with stem cell transplantation. No significant difference was observed in age, sex, illness duration, type of M protein and frequency of high-risk chromosomal abnormalities in cases with extramedullary involvement, when compared to those without. Patients administered bortezomib and/or thalidomide showed a significantly positive association with extramedullary involvement (p = 0.024), while those undergoing stem cell transplantation showed insignificant association. Furthermore, difference in the incidence of extramedullary involvement was insignificant across three decades (1979–1989, 1990–1999, 2000–2009). *Conclusion:* multi-organ involvement of myeloma is not rare in autopsy cases of the disease. Exposure to novel agents may contribute to extramedullary spread of myeloma cells.

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A POWERFUL TRANSCRIPTOME-BASED RISK SCORE IN MULTIPLE MYELOMA EASILY COMPUTABLE THROUGH AN OPEN ACCESS WEBSITE

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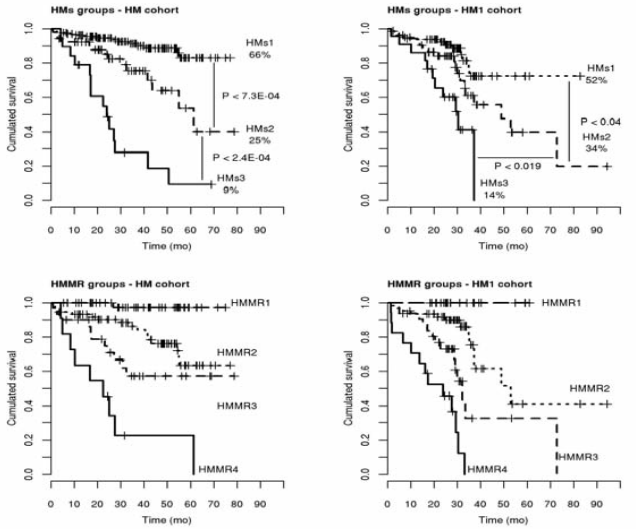
Multiple myeloma (MM) is an incurable malignant plasma cell disease. Using Affymetrix gene expression profiling (GEP), we have defined a "HMs" risk score predicting for overall survival (OS) subsequently integrating conventional prognostic factors into a metascore. Gene expression profiling was performed in purified myeloma cells of independent cohorts of 206 (HM) and 156 (HM1) patients undergoing induction therapy, high-dose chemotherapy and stem cell transplantation. HM patients ranked according to their score were separated by a selectable number of cut-offs maximizing the global log-rank test. HMs methodology presents 2 major improvements compared to previous GEP-based risk scores: i) the number of patients' risk groups is not defined a priori but calculated according to training cohort information; ii) for patients of the validation cohort, HMs score is computed using information of the training cohort only. Nineteen probe sets were identified classifying patients of the training and validation cohorts into 3 groups with significantly different survival. In the validation cohort, they comprised 52, 34 and 14% of patients with a median OS not reached, 49 and 30 months respectively. Only 1 gene was common with previous risk scores. Using multivariate Cox analysis, HMs, HRS and IFM were not independent and only

HMs remained prognostic in the validation cohort. HMs was independent of ISS and poor genetics, allowing designing a metascore combining all information (1).



A easy-to-use open web site is provided to compute HMs and metascore of any new single patient (2).

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PROSPECTIVE COHORT STUDY OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) IN A SPANISH URBAN POPULATION

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MGUS is a highly prevalent premalignant entity occurring in approximately 3% of USA caucasian population over the age of 50 years. Aims: To known the prevalence and incidence of MGUS in a Spanish population. To study the clinical-biological characteristics and evolution risk factors in this cohort of patients. *Materials and Methods:* prospective cohort study in population over 50 years old (Segovia city, Spain). The three-years lasting project will be made on a total of 19673 people. Monoclonal protein (MC) in serum was detected by electrophoresis and confirmed by immunofixation. Participants recruitment was done through health centers or by personal letter. *Results:* in the first 26 months, we contacted 16161 people, which 6681 accepted. The median age of tested volunteers was 66 years old (50 / 98), 57% were women. Table 1 shows the number and type of Monoclonal Gammopathies (MG) diagnosed by screening in the study or by our department daily routine in the same period. Total, 122 MGUS were detected (101 screening (incidence: 1,51%); 21 routine). MC type of MGUS was 62% IgG, 22,3% IgA, 12,4% IgM, 2,5% biclonal and 0,8% light chains. Major risk factors for malignant progression of MGUS patients are shown in Table 2. Thus, 76% of MGUS patients belongs to low-risk group (score 0 or 1 at the Mayo Clinic), 23% to intermediate risk group (score 2) and 1% to high risk (score 3). *Conclusions:* The incidence of MGUS in our population is slightly lower than other previously described, althought these results will be updated in the meeting.

	Screening, n (%)	Routine, n (%)	Total, n (%)
Symptomatic MM	1 (0.7)	6 (4.1)	7 (4.8)
Smoldering MM	4 (2.7)	0	4 (2.7)
Waldenström	3 (2)	1 (0.7)	4 (2.7)
MGUS	101 (69.1)	22	123 (84.2)
MG SLP-associated	0	4 (2.7)	4 (2.7)
Transitory MG	4 (2.7)	1 (0.7)	5 (3.4)

MC, median (min-max)	MC = 1.5 g/dL (%)	Serum Free Light Chain % ratio K/L abnormal	Aneuploidy (%)	Pts. With >95% abnormal plasma cell (%)
0.4 (0.1-2.55)	5.1	29.2	16.1	6.7

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WHICH IS THE BEST BISPHOSPHONATE IN THE MANAGEMENT OF PATIENTS WITH MULTIPLE MYELOMA?

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Background: bisphosphonates are used as supportive therapy to prevent bone destruction for patients with multiple myeloma (MM). We performed indirect meta analysis of various bisphohsphonates to find the superiority of any particular bisphosphonate over others. *Methods:* superiority of bisphosphonates was assessed by the mixed treatment comparison (MTC). *Results:* eighteen RCTs were included enrolling 4,970 patients. For the outcome of overall survival (OS) the pooled analysis demonstrated superiority of zoledronate in comparison with clodronate [HR = 0.83, 95% CI: 0.73 - 0.94] and etidronate [HR = 0.49, 95% CI: 0.32-0.72]. However, zoledronate was not superior to pamidronate [HR = 0.85, 95% CI: 0.61 - 1.14] for the outcome of OS. Zoledronate was superior to clodronate [HR = 0.88, 95% CI: 0.78 - 0.99] for the outcome progression free survival (PFS). Zoledronate was also superior to clodronate [HR = 0.74, 95% CI: 0.63 - 0.87], pamidronate [HR = 0.64, 95% CI: 0.42 - 0.94], and ibandronate [HR = 0.44, 95% CI: 0.25 - 0.71] for the outcome of skeletal related events (SREs). *Conclusion:* the results demonstrate a beneficial effect of zoledronate on OS compared to clodronate and etidronate. Zoledronate is superior to clodronate in improving PFS. Zoledronate is superior to clodronate, pamidronate and ibandronate in preventing SREs. In MTC uncertainty analysis, zoledronate ranked as the best treatment followed by clodronate and pamidronate. These findings underscore the need for RCT comparing zoledronate and pamidronate for their benefits and harms in MM patients.

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C-REACTIVE PROTEIN (CRP) LEVEL IS AN INDEPENDENT PROGNOSTIC MARKER IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB

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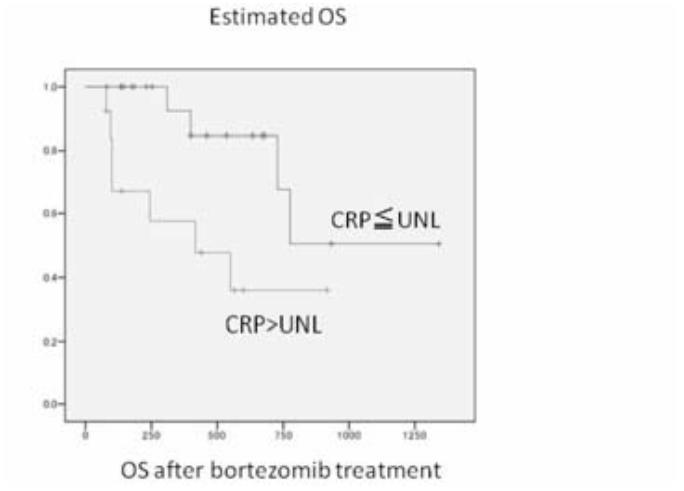
Background: Although bortezomib has improved the overall survival (OS) in patients with myeloma, there are still non-responders. The purpose of this study is to define factors that affect the response to borte-

zomib-combined chemotherapy before treatment. *Patients and Methods:* Relapsed and refractory multiple myeloma patients treated with bortezomib alone or bortezomib-dexamethasone regimen at the Cancer Institute Hospital of JFCR between December 2006 and August 2010 were analyzed retrospectively. *Results:* The response rates (complete or partial response) to bortezomib were 70.6% (24/34), SD 2.9% (1/34), and PD 26.5% (9/34) in 34 eligible patients. By logistic regression analysis, the only factor associated with no response was elevated serum CRP level before treatment (p=0.03): odds ratio was 21 (95%CI: 2.8~156). Other factors of age over 65, sex, performance status, chromosome 13 deletion, number of previous therapies, bone scale, and concentration of albumin or ß2-microglobulin were not associated with response. Serum CRP level before treatment up to week 20 was continuously elevated in non-responders compared with that in responders. Furthermore, elevated CRP level before treatment affected long-term survival (log-rank test: p=0.02, median estimated survival was 417 days vs. not reached). *Conclusions:* CRP is an independent and the most powerful predictor of non-response to bortezomib treatment. Further study is needed to clarify the effect of CRP for bortezomib-combined multi-agent regimens.



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STEM CELL TRANSPLANTATION (SCT) IN MULTIPLE MYELOMA (MM) - FOURTEEN YEAR EXPERIENCE OF THE KING CHULALONGKORN MEMORIAL HOSPITAL (KCMH), THAILAND

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Background: Improvement in response rates using novel agents or combinations in MM has led to questions about the therapeutic necessity of SCT. Method: In this study, we evaluated the outcome of SCT patients in the context of novel agent therapy. Outcomes were analyzed for MM patients (N=83) who had undergone SCT at KCMH from 1997-2010. *Results:* Median patient follow-up was 35.8 months (range, 6.4-123.8). Most patients had previously received conventional chemotherapy or a 2-drug combination including either bortezomib (44.6%) or thalidomide (25.3%). Transplant-related mortality was 3.6% and grade 3-4 complications (mostly brief mucositis) were found in 39.7% of patients. CR rates before stem cell collection, before SCT, and after SCT were 18.5%, 24.7% and 39.2%, respectively (p<0.001). CR rate increased to 45.1% (p<0.001) in 10 cases receiving thalidomide maintenance. The 3-year-overall survival (OS) was 73% and was associated with the depth of response before stem collection (p<0.001). Median time to progression (TTP) was 28.0 months (95% CI, 23.2-32.8). Importantly, TTP was longer for first transplant compare to patients receiving a second (p=0.027, HR 3.44 (1.15-10.28)) and patients achieving CR after completing treatment had longer TTP than those who did not (p=0.021, HR 2.86 (1.18-7.14)). *Conclusion:* In limited resource settings, SCT still has significant benefits and acceptable toxicity. Depth of response for SCT is associated with OS and TTP. These data provide the evidence for continued use of SCT in settings where novel drugs or drug combinations are unobtainable.

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UPFRONT USE OF NOVEL AGENTS IMPROVES SURVIVAL OF UNSELECTED MYELOMA PATIENTS

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Randomized studies have shown that upfront treatment with novel agents (NA) is associated with improved responses, PFS and OS. However, these studies included selected patients that may not be representative of the general population. We analyzed 1203 consecutive unselected patients who initiated treatment after 1/1/1995 and received similar supportive care: 812 received frontline conventional therapy (CT) and 390 received upfront therapy with NA (thalidomide, lenalidomide or bortezomib). Patients who received upfront NA were older (>75 years 27% vs 16%, p<0.001), and had more often ISS-3 disease (40% vs 33%, p=0.002), however, they had superior OS than those who received upfront CT(53 vs 39.5 months, p=0.003). Factors independently associated with superior OS in multivariate analysis were: initial therapy with NA (HR 0.6, p<0.001); age≤75 years (p<0.001), ECOG PS<2(p<0.001), LDH<300 IU/L(p=0.006), platelets≥130.000x109/L(p=0.002) and ISS stage(p65-≤75 years(OS was 43 months with upfront NA vs 30 months for upfront CT, p=0.007). In patients older than 75 years OS was 31.5 months vs 22 months respectively (p=0.338). In conclusion, the upfront use of NA is associated with a significant improvement in the survival of unselected patients with MM, especially in patients younger than 75 years. The benefit in patients older than 75 years is less pronounced.

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NOVEL AGENTS ARE ABLE TO OVERCOME THE ADVERSE PROGNOSTIC IMPACT OF RENAL IMPAIRMENT (RI).

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RI is a common complication of MM and is associated with inferior outcome. Novel agents have significantly improved the prognosis of patients with MM and may abrogate the effect of some adverse features. We evaluated the effect of novel agents on the outcome of patients with RI and the prognostic significance of RI in the era of novel agents in 1654 patients treated within the context of the GMSG. Patients were divided in those who started treatment before 31/12/1999 and those who started after 1/1/2000, after which thalidomide and other novel agents became available. Renal function was estimated by calculating GFR, using the modified MDRD formula and the degree of RI was staged according to the National Kidney Foundation-KDOQI/CKD classification. The median OS of patients who started treatment before 1/1/2000 was 37 months vs. 50 months for those who started after 1/1/2000(p<0.001). For patients treated in the earlier time period, those with CKD 1-2 had a median OS of 47 months and those with CKD 3-5 of 26 months (p<0.001). In the latter time period these figures were 66.5 and 40.5 months respectively, indicating a significant improvement in the survival of patients with and without RI. In order to evaluate the importance of RI in the two time periods we performed a multivariate analysis independently in each cohort. RI was an independent prognostic factor only in patients treated before 1/1/2000 but not in the most recent patients. Our data indicate that novel agents are able to overcome the adverse prognostic impact of RI.

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INCREASED COPY NUMBER OF THE INTERLEUKIN-6 RECEPTOR GENE IS ASSOCIATED WITH ADVERSE SURVIVAL IN MULTIPLE MYELOMA PATIENTS TREATED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

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Interleukin-6 (IL-6) is a potent pleiotropic cytokine that regulates plasma cell (PC) growth via the IL-6 receptor (IL-6R). We hypothesized that upregulation of IL-6R in myeloma cells might confer the growth privilege to myeloma cells over bone marrow (BM) hematopoietic cells. We investigated the frequency and prognostic implication of increased copy number of the IL-6R gene by fluorescence in situ hybridization (FISH) in patients with newly diagnosed multiple myeloma (MM). For 102 MM patients, FISH study was performed using a homemade BAC probe for IL6R at chromosome 1q21. FISH signals were counted among PCs sorted by cytoplasmic immunoglobulin light chain staining. The amplification of IL-6R was detected in 53/102 patients (52.0%). The 5-year overall survival (OS) rate of patients with IL-6R gene amplification was 41.3% versus 44.8% for those with a normal IL-6R ($P = 0.425$). In 44 patients treated with high-dose chemotherapy and autologous stem cell transplantation (ASCT), patients with ≥ 3.1 copy number of IL-6R per PC showed adverse 5-year OS compared to those with < 2.1 copy of IL-6R gene (44.4% versus 78.0%, $P = 0.024$). In multivariate analysis, the increase of IL-6R copy numbers (mean copy/PC ≥ 3.1) could be considered as an independent prognostic factor for MM patients who underwent ASCT. The gain of the IL-6R gene was frequent in myeloma, showing an association with adverse prognosis in myeloma patients treated with ASCT. These findings suggest the potential role of IL-6R in myeloma cell growth and therapeutic implications of the IL-6R blocker in the future.

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RETROSPECTIVE ANALYSIS OF CYTOGENETIC AND CLINICAL CHARACTERISTICS ON 61 PATIENTS WITH MULTIPLE MYELOMA

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To further clarify the correlation between cytogenetic and clinical features on multiple myeloma patients, we described the cytogenetic characterization of myeloma cells from 61 patients via conventional cytogenetics analysis with R-banding technique and interphase fluorescence in situ hybridization (FISH). 14.8% of patients (9/61) showed abnormal cytogenetic aberrations including 88.9% (8/9) cases with ultra complex aberration and complex aberration via conventional cytogenetics. In addition, there were aberrations in 52.5% (32/61) of patients by FISH analysis. Abnormalities of 13q14, 13q14.3, 1q21, 14q32 and 17p13 were detected in 26.2%, 29.5%, 11.5%, 16.4% and 29.5% of evaluable cases. Patients with 1q21 deletion demonstrated significant correlation with 13q deletion. There was no significant difference in gender, age, isotype, levels of albumin, β2-microglobulin, plasma cells in the bone marrow, Durie-Salmon (DS) and International Staging System (ISS) staging between patients with deletion 13q14.3, 14q32, 17p13 and patients not detected. 1q21 deletion is associated with low levels of albumin and patients with 13q14 deletion frequently had poor clinical staging at diagnosis. 17p13 deletion coexist with 13q14, 1q21 and 14q32 frequently correlate with elevated level of β2-microglobulin and poor clinical staging. This study validates myeloma cells are prone to show complex aberration. 1q21 had significant correlation to 13q abnormality. 1q21, 13q14 deletion and 17p13 coexist with 13q14, 1q21 and 14q32 were used to associate with poor prognosis for multiple myeloma.

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EXPLORATORY SURVEY ON MULTIPLE MYELOMA PATIENTS' PERCEPTION

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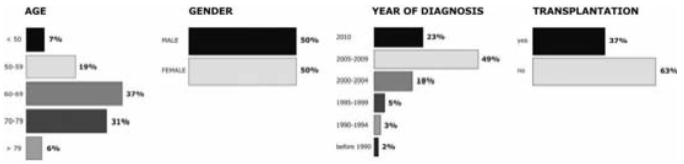
Aims/Methods: The aim of this survey was to deepen the knowledge on multiple myeloma patients'perception of their disease and treatments they receive. A questionnaire built on the results of a previous study (Pelagalli et al. 2010) surveyed the following: relationship with physicians; level of satisfaction with treatments; quality of life; sources of information on the disease; experience of the disease and reaction to it; relationship with other patients and the family; life styles. Between the 20Oct and the 30Nov 2010, 482 questionnaires were gathered (Fig.1) in 11 Italian Hematological Centers. The questionnaire was anonymous and self-reported by the patient. *Results:* In this abstract we highlight results regarding the relationship with the physician. 68% of patients were "very satisfied" with their relationship with their physician.54% of patients thought that their physician was able to make them understand the treatment process while 27% of the sample thought that physicians should give more explanations on treatments. To obtain information on the disease and drugs patients sought information from the hematologist (93%); the General Practitioner (34%); Internet (12%) and family members or friends (6%). *Conclusions:* Initial results confirm the high satisfaction that patients demonstrate towards their physicians. Information on treatments could be considered possible areas of improvement. Moreover, patients show different perceptions toward anti-myeloma therapies they are currently taking. We will further analyze these evidences using multivariate statistics.



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CHROMOSOMAL ABNORMALITIES AMP(1Q21) AND DEL(13Q14) PREDICT SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH CTD REGIMEN

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Chromosomal abnormalities are frequently found in multiple myeloma(MM) and play a major role in patient outcome and management of the disease. In the era of novel agents such as thalidomide, lenalidomide, and bortezomib, risk stratification by chromosomal abnormalities may enable rational therapeutic approach in patients with MM. We analyzed the prognostic value of del(13q14) and amp(1q21) by fluorescence in situ hybridization (FISH) and hyper- (H-MM) or hypodiploidia (NH-MM) by conventional cytogenetic methods in a series of 71 patients with newly diagnosed MM treated with CTD (thalidomide, cyclophosphamide and dexamethasone) between 2007 and 2010. We found del(13q14) in 35 patients, amp(1q21) in 37 patients and combination of del(13q14) and amp(1q21) in 25 patients. Response rate assessed according to EBMT criteria, was 90% for NH-MM patients without addition-

al aberrations (≥VGPR in 60%), 63% for H-MM patients without additional aberrations (≥VGPR in 45%) and 50% for patients with combination of del(13q14) and amp(1q21). The median overall survival (OS) for H-MM patients reached 31 months and was significantly longer than in NH-MM group (17 months, p<0.001). The combination of del(13q14) and amp(1q21) was adverse cytogenetic signature resulting in shortened OS in both groups (13 for H-MM and 6 months for NH-MM, p<0.01). The results of the study suggest that combination of del(13q14) and amp(1q21) is adverse prognostic factor that affects OS by patients H-MM and NH-MM. Improved therapeutic strategies including bortezomib and lenalidomid are required for these patients.

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GLOBAL REAL-TIME QUANTIFICATION/REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (RQ/RT-PCR) DETECTING PROTO-ONCOGENES ASSOCIATED WITH 14Q32 CHROMOSOMAL TRANSLOCATION AS A VALUABLE MARKER FOR PREDICTING SURVIVAL IN MULTIPLE MYELOMA (MM).

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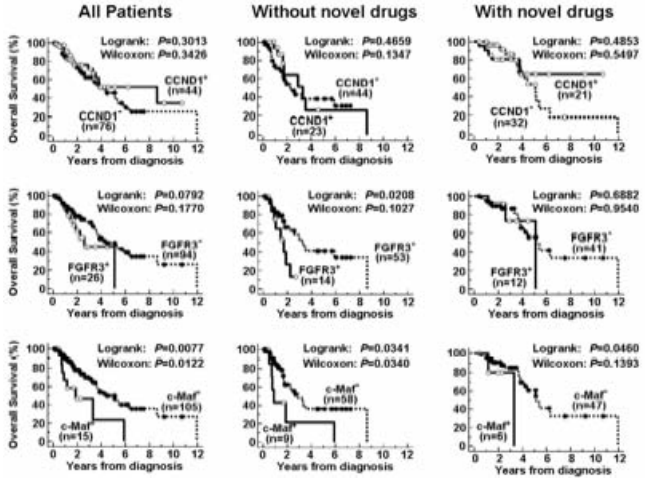
14q32 chromosomal translocation-associated proto-oncogenes, such as CCND1, FGFR3 and c-MAF are involved in the development of MM, and are crucial prognostic parameters for MM patients. We prospectively studied 120 consecutive symptomatic MM patients including 35 undergoing autologous stem cell trensplantaion (ASCT) and 53 treated with novel drugs such as bortezomib, thalidomide and lenalidomide, to clarify the clinical significance of CCND1, FGFR3 and c-MAF mRNA expression in MM patients. Purified MM cells obtained from patients' bone marrow were analyzed for their mRNA expression by global RQ/RT-PCR technique. CCND1, FGFR3 and c-MAF were positive in 44(37%), 26(22%) and 15(13%) of the 120 patients. Forty one (34%) patients were triple-negative. In 6 patients, both FGFR3 and c-MAF were positive. Expression of CCND1 and that of FGFR3 or c-MAF was mutually exclusive in the same patients. FGFR3-positive patients showed shorter overall survival (OS) than negative ones without the use of novel drugs, but not with novel drugs. c-MAF-positive patients showed worse OS than negative ones even with or without novel drugs. Positivity for c-MAF, γ-type light chain, presence of cytogenetic abnormality by G-banded karyotype, no use of novel drugs were independently unfavorable prognostic factors for OS. ASCT improved progression free survival of CCND1-positive patients, and novel drugs extended OS of FGFR3-positive patients. The global RQ/RT-PCR detecting CCND1, FGFR3 and c-MAF is able to predict OS and is useful for planning the stratified treatment strategy of MM patients.



P-359

NUTRITIONAL STATUS AND QUALITY OF LIFE IN OUTPATIENTS WITH SYSTEMIC IMMUNOGLOBULIN LIGHT-CHAIN AMYLOIDOSIS (AL) AT DIAGNOSIS

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Purpose: Nutritional status is a prognostic factor in immunoglobulin light-chain amyloidosis (AL), but its influence on quality of life (QoL) is unknown. The aim of this cross-sectional study was to investigate the association between nutritional status and QoL in AL outpatients at diagnosis. *Patients and Methods:* 150 patients with biopsy-proven AL were assessed for nutritional status by anthropometry (body mass index [BMI], unintentional weight loss [WL] in the previous 6 months and mid-arm muscle circumference [MAMC]), biochemistry (serum prealbumin) and semiquantitative food intake at referral. QoL was assessed by the Medical Outcomes Study 36-item Short Form General Health Survey (SF-36). *Results:* The composite physical component summary (PCS) and the mental component summary (MCS) for AL outpatients were 36.2±10.1 and 44.9±11.3, respectively (P <.001 for both versus the population norms of 50). In multivariate linear regression models adjusted for gender, age, Eastern Cooperative Oncology Group performance status, the number of organs involved, NT-proBNP, energy intake and WL, PCS was significantly lower for serum prealbumin <200 mg/L and MAMC <10th percentile (adjusted difference 4.1; 95% CI, 0.7-7.5; P= .017 and 5.3; 95% CI, 1.9-8.7; P=.002 respectively). MCS was decreased by 0.4 (95% CI, 0.1-0.7; P=.001) for each kg of body weight lost in the previous 6 months. *Conclusion:* Nutritional status significantly affects QoL in AL outpatients since diagnosis. Nutritional evaluation should be an integral part of the clinical assessment of AL patients since the onset of disease.

P-360

CYP2C8 GENE POLYMORPHISM AND BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE JAW IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Osteonecrosis of the jaw (ONJ) is a known complication of bisphosphonate therapy (BP) in multiple myeloma (MM). Single nucleotide polymorphism (SNP) variations have been suggested as potential mechanisms to explain drug effect sensitivity or biologic functions. Previous studies in MM patients with BP showed that the presence of one or two minor alleles of the SNP rs1934951 of CYP2C8 gene was an independent prognostic marker associated with development of ONJ. *Aim:* To validate the impact of SNP rs1934951 in the development of ONJ in 79 patients with MM treated with zoledronic acid. *Patients and Methods:* We studied two homogeneous independent series of patients diagnosed with MM with (n=42) and without ONJ (n=37). All 79 patients received zoledronic acid. Forty-two developed ONJ. Besides, 45 healthy volunteers were analyzed to know the distribution in the Spanish population. The rs1934951 polymorphism was analysed by a TaqMan® SNP genotyping Assay (Applied Biosystems) and was read on a LightCycler 480 Endpoint Genotyping Software. *Results:* In 9 (22%) patients developing ONJ an heterozygous CYP2C8^{CT} genotype was found, in contrast with those who did not (n=4, 11%) or healthy individuals (n=6, 13%), but these differences did not reach statistical significance (P=.131). Besides, 3-year cumulative incidence for patients with CYP2C8^{CT} genotype was 35% and 53% for those with CYP2C8^{CC} (Figure 1, P= .74). *Conclusion:* We could not find a higher risk of development of ONJ in this independent series of patients with MM with the rs1934951 polymorphism on CYP2C8 gene.

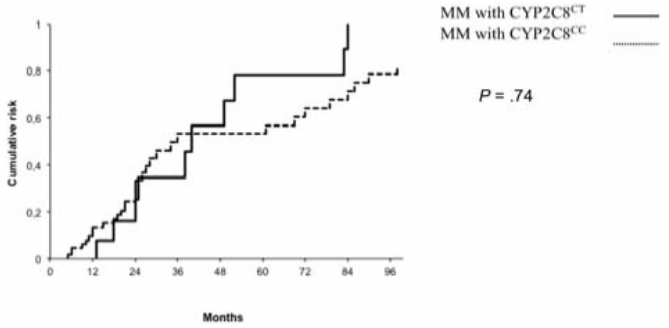

Table 1. Allenic and genotypic distribution of the SNP rs1934951

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EFFECT OF THALIDOMIDE WITH MELPHALAN AND PREDNISONE ON HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: A PROSPECTIVE ANALYSIS IN A RANDOMIZED TRIAL (HOVON 49)

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Thalidomide (T) with melphalan/prednisone (MPT) was defined as standard treatment in elderly multiple myeloma patients based on 5 randomized trials. In one of these trials, HOVON 49, a prospective Health related Quality-of-life (HRQoL) was initiated to asses the impact of T on QoL. Newly diagnosed symptomatic MM patients were randomized to MP or MPT, followed by T-maintenance in MPT-arm. 284 patients were included in this HRQoL study (MP:n=149,MPT:n=135). HRQoL was assessed with the EORTC-core-QoL-Questionnaire (QLQ-C30) and the myeloma-specific-module (QLQ-MY24) at baseline and during treatment. The QLQ-C30 subscales Physical Function (p=0.044) and Constipation (p<.001) showed a treatment related improvement during induction in favour of the MP-arm. During T-maintenance, the scores for the QLQ-MY24-Paraesthesia became significantly higher in the MPT-arm (p<.001). The QLQ-C30-subscales Insomnia (p=0.068), Appetite loss (p=0.074) and the QLQ-MY24-item Sick (p=0.086) scored marginally better during T-maintenance.

Table. QoL dimensions by arm of randomization (MPvs. MPT) and time point in treatment trajectory. Shown are estimated scores (and 95%-CI) at baseline (t0), at the end of induction treatment (i.> 8 mo.), at the start and end of post-induction (p.< 12 mo. and p.18 mo.). P values given: P value time point; P value arm at baseline; P value interaction arm X time: induction and post-induction.

		Introduction		Post-introduction		P-values
QoL dimension	Arm	i0 (baseline)	i.> 8 mo.	p.< 12 mo.	p.18 mo.	
QLQ-C30 Global health	MP	49 (45-52)	65(57-73)	62 (58-66)	64 (57-70)	P time < 0,001 P arm= 0,05
	MPT	54 (50-58)	66 (59-73)	63 (58-67)	70 (65-75)	P arm X time = 0,78; 0,51
QLQ-C30 Emotional Function	MP	65 (61-69)	79 (71-87)	73 (68-77)	73 (67-79)	P time < 0,001 P arm = 0,018
	MPT	72 (68-76)	84 (77-91)	78 (73-83)	85 (80-91)	P arm X time = 0,85; 0,53
QLQ-C30 Pain	MP	50 (45-55)	27 (16-38)	35 (29-41)	36 (27-44)	P time < 0,001 P arm= 0, 71
	MPT	49 (43-54)	19 (10-29)	24 (17-31)	23 (15-30)	P arm X time = 0,66; 0,12
QLQ-MY24 Future perspectives	MP	43 (39-48)	63 (54-71)	55 (50-60)	57 (50-64)	P time < 0,001 P arm = 0,045
	MPT	50 (45-54)	70 (62-77)	62 (56-67)	70 (64-76)	P arm X time = 0,88; 0,42
QLQ-MY24 Sick	MP	20 (18-21)	14 (11-18)	16 (14-18)	17 (14-20)	P time < 0,001 P arm = 0,61
	MPT	20 (19-22)	13 (10-16)	16 (14-18)	14 (11-16)	P arm X time = 0,66; 0,086
QLQ-MY24 Paraesthesia	MP	13 (12-15)	15 (12-18)	15 (13-16)	16 (13-18)	P time = 0,001 P arm= 0,69
	MPT	13 (11-15)	18 (15-21)	20 (18-22)	23 (21-25)	P arm X time = 0,66; < 0,001
QLQ-C30 Constipation %mild/%severe	MP	23,1%/15,4%	162%77.9%	18,6%/10,0%	18,4%/9,8%	P time= 0,002 P arm= 0, 43
	MPT	24,9%/18,5%	24,9%/18,6%	23,3%/15,7%	20,6%/12,2%	P arm X time = < 0,001; 0, 72

The overall QoL-scale showed a significant time trend towards more favourable mean values during protocol treatment but did not reveal differences between MP and MPT. For the QLQ-C30-subscales Emotional function and Future perspectives, difference in favour of the MPT-arm from the start of treatment was observed (p=0.018 and p=0.045 respectively) with no significant 'time x arm' interaction. This study shows that the higher frequency of adverse effects associated with MPT does not translate into a negative effect on HRQoL.

P-362

THE IMPORTANCE OF EDUCATION AND SUPPORT FOR MULTIPLE MYELOMA PATIENTS THROUGH A HOSPITAL BASED EFFORT.

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Background: Multiple myeloma accounts for 3% of cancers diagnosed in the US yearly. Although currently incurable, treatment advances have resulted in making it a chronic disease underscoring the need for providing patients with resources for education and support. *Methods:*We created an educational forum/support group for patients and family/friends at the MGH cancer center. Our group meets every 2 months for 1.5 hours, with 45 min. dedicated to education,questions,support and networking. Each member of the myeloma team (MDs,NP, SW) has presented, along with the pain team, IR, BMT, ortho/onc and complementary medicine. The NP and SW are available to maintain group structure answer questions and offer support. Feedback is obtained via email/paper survey. Educational topics are based on patient feedback. Results: We have had 10 such meetings with an average attendance of 25-30 people. Average age is 58 years, with equal male/female representation. Patients are in active treatment /follow-up(60%) or family/friends (40%) are in attendance. Based on an 88% survey return rate, patients and their family/friends have expressed that they enjoy this forum for education and support. It allows them to stay informed on current trends and provides them with a venue to express anxiety and concerns dealing with a chronic illness. Concern about how data of clinical trials was presented was also voiced. *Conclusion:* Formation of this group has provided support/education to pts. and empowered them to live with myeloma. This group has allowed the healthcare providers to be better clinicians.

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NEUROLOGICAL MONITORING ASSESSED BY NCI-CTC AND TNS REDUCES THE DEVELOPMENT OF SEVERE PERIPHERAL NEUROPATHY IN MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE, BORTEZOMIB OR BOTH DRUGS

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Objective: Dose-limiting peripheral neuropathy (PN) is a disabling complication frequently reported with the use of thalidomide and bortezomib in multiple myeloma (MM) patients. The objective of this study was the monitoring of patients using the National Cancer Institute's Common Toxicity Criteria (NCI-CTC) and the Modified Total Neuropathy Score (TNS) in early detection of PN in newly diagnosed MM patients treated with bortezomib, thalidomide or their combination. Patients: Thirty-five patients (17 men and 18 women), 11/35 treated with thalidomide (group 1), 11/35 treated with bortezomib (group 2) and 13/35 treated with both drugs (group 3), underwent neurological examination and electromyography both at baseline and at the end of treatment. *Results:* Cumulative incidence of PN was 71% using NCI-CTC and 74% using TNS; no grade >2 toxicities were observed. In 23/35 patients the scales agreed in terms of PN grade and their correlation was statistically significant (p<0,001). There was no significant correlation between PN and cumulative dose or dose intensity of the two drugs, treatment duration or median follow-up, while the combination of bortezomib and thalidomide (group 3) was found to be predictive of PN development (p=0.004). *Conclusions:* Our study showed that clinical and electrophysiological examination are both useful in the early diagnosis of PN. Careful monitoring is required to guide the clinician in dose adjusting, especially when the two drugs are used in combination, in order to avoid the development of grade 3-4 PN.

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CLINICAL AND BIOLOGICAL CHARACTERISTICS OF KEY MODULATORY IMMUNE CELLS IN MULTIPLE MYELOMA PATIENTS

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In multiple myeloma (MM) abnormalities in function and number of T regulatory cells (Treg) and dendritic cells (DC) might be responsible for the immunosuppression status. The current study aimed to characterize the frequency of Treg, DC as well as subpopulations of T cells bearing regulatory properties like $CD4^+GITR^+$, $CD4^+CD62L^+$, $CD3^+TCR\gamma\delta^+$ (by flow cytometry) along with the concentration of IL-10, TGFβ, IL-6 (by ELISA) in 66 MM patients. Subsequently the longitudinal analysis of these components of immune system during therapy was assessed. Patients eligible for autoSCT (n=26) were treated with CTD regimen while older patients with MPT chemotherapy. Forty nine patients were followed for three and thirty three for six cycles of therapy. We found lower percentages of DC both myeloid and plasmacytoid as well as $CD3^+TCR\gamma\delta^+$ in MM compared to control group. The frequency of Treg and the percentages of $CD4^+GITR^+$, $CD4^+CD62L^+$ were increased compared to healthy volunteers. Patients with higher percentages of Treg have shorter overall survival (median 21 months vs not-reached, p=0.013). We observed increased frequencies of Treg (median = 13.31%) in patients who progressed and did not responded to induction therapy. Serum levels of cytokines IL-10, IL-6, TGFβ were increased in MM patients and decreased during therapy. In conclusion the identified dysfunction of immune system (decreased antigen presentation along with increased frequencies of suppressive cells and cytokines) might facilitate progression of the disease and infectious complications.

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CONTRIBUTION OF NOVEL M-COMPONENT BASED BIOMARKERS IN DETECTION OF RELAPSE IN MULTIPLE MYELOMA (MM)

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Background: Serum free light chain (sFLC) and ratio (sFLCR), are routinely used in MM. They were the background for developing specific antibodies, that bind the junction of the heavy and light chains on individual Ig isotypes (Hevylite™), making possible to quantify the IgGκ, IgGλ, IgAκ and IgAλ separately. *Aim:* To investigate if the long term monitoring (11yrs) of sFLC, sFLCR, monoclonal IgGκ, polyclonal IgGλ and IgGκ/IgGλ ratio, in an IgGκ-MM pt could give additional information about disease behavior. *Method:* Sera (n=35) of the pt were analyzed from diagnosis to last follow up. Analysis of sFLC was performed by immunoassay (Freelite™) and of IgGκ and IgGλ with Hevylite™ antibody, nephelometrically. File data were reviewed. *Results:*The pt relapsed 10 times during disease course (4 paraprotein rise, 1 acute renal failure, 5 with new plasmacytomas).Total IgG, monoclonal IgGκ, polyclonal IgGλ, IgGκ/IgGλ ratio, sFLC levels and sFLCR follow disease pattern. In 2 occasions sFLC escape was observed with gradual increase of κ-sFLC and sFLCR while shortly after the patient relapsed.The polyclonal IgGλ decreased gradually and IgGκ/IgGλ ratio increased and became very low and very high respectively at relapse. The rest of the parameters(IgG, IgA, IgM , IgGκ and λ-sFLC) remained stable. *Conclusion:* Depression of the polyclonal isotype of the same Ig class(e.g. IgGλ in an IgGκ-MM) and sFLC clonal escape may predict relapse in MM.

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INFLUENCE OF ALBUMIN AND BETA2MICROGLOBULIN ASSAYS IN ISS DETERMINATION: RETROSPECTIVE STUDY ON IFM 2007 02 TRIAL

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The International Staging System is the prognostic score recommended for multiple myeloma patients, using beta2microglobulin (B) and albumin (A) cut off values. It stratifies patients in 3 groups: ISS 1, 2 and 3, with medians of survival are 62 months, 44 months and 29 months, respectively. The aim of this study was to assess the variability of ISS using 8 different analytical combinations (4 A assays and 2 B assays). The presentation samples of 192 patients enrolled in the IFM 2007/02 trial were analyzed according to the following techniques: for A : Nephelometry (AN), Turbidimetry (AT), Bromocresol green (AG), Capillary electrophoresis (AC), and for B: Nephelometry (BN) and Turbidimetry (BT) . For each combination, we have compared the % of well classified patients setting as the historic nephelometric combination BN-AN. The BT-AN combination correctly classifies 92.7 % of patients while BT-AT, BT-AG and BT-AC combinations reclassify up to 18.2 % of the patients ($p < 0.001$). Moreover ISS calculated by BT-AC combination led to a decreased score for 34/192 (17.7%) patients (27 patients from ISS2 to ISS1 and 7 patients from ISS3 to ISS2). WhereasThe BN-AT combination provides the highest pejorative ISS: 14 from ISS1 to ISS2, no combination has changed an ISS1 in ISS3 and vice versa. The ISS score is dependent on combination assays used. For clinical research protocol it seems necessary to ensure that these scores are homogeneously determined. The study of patient survival according to ISS score will determine the clinical relevance of these analytical discrepancies.

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ELECTROACUPUNCTURE IS AN EFFECTIVE THERAPEUTIC MODALITY FOR BORTEZOMIB- OR THALIDOMIDE-INDUCED PERIPHERAL NEUROPATHY IN PATIENTS WITH MULTIPLE MYELOMA: A PILOT STUDY

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Peripheral neuropathy (PN) could be a severe adverse event in patients with multiple myeloma (MM) treated with bortezomib or thalidomide. To investigate the effectiveness of electroacupuncture in its treatment, 20 patients with MM and 1 patient with mantle cell lymphoma were enrolled in this pilot study. All study patients had bortezomib- or thalidomide-induced PN. Acupuncture was over 9 weeks using 23 acupuncture points. Electrostimulation was added to enhance the effects of acupuncture. Objective and subjective study tests were evaluated at baseline, during treatment and after the end of study. There were no toxicities except for minor local irritation, and specifically there were no infections or bleeding or nerve injury. There was progressive improvement in neurotoxicity as reflected by decreased FACT/GOG-neurotoxicity scores before study (21.2), at week 4 (15.1), at week 9 (10.8) and one month after study (11.4, P=0.02). There was progressive and significant reduction of pain as reflected by BPI-pain severity before study 20.3, at week 4 (15.3), at week 9 (10) and one month after study (10.6, P=0.03). Progressive improvements were also observed for physical well-being (PWB), with scores 9.8 before study, 7.4 at week 4, 4.6 at week 9 and 4.8 at one month after study (P=0.02). Coin test (the speed of picking up coins) before study was 101.7 and was much better at 53.0 at week 13 (P=0.01). Acupuncture is an effective therapeutic modality for bortezomib- or thalidomide-induced peripheral neuropathy. Randomized clinical trials using a placebo control group are needed.

P-368

LUNG FUNCTION IN POST-TRANSPLANT MULTIPLE MYELOMA PATIENTS

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Introduction: Chemotherapy followed by autologous stem cell transplant (SCT) is the standard of care for myeloma patients. High dose treatment increases susceptibility to pulmonary morbidity and mortality. Routine pulmonary function tests (PFTs) can identify early decline in respiratory function post-SCT. We report a retrospective analysis of PFTs in post-SCT myeloma patients to assess the impact of transplantation on lung function. *Method/Results:* PFTs were assessed pre and post-SCT (3 monthly). 34 patients undergoing SCT for myeloma (median

age 50, range 36-69) were analysed. Conditioning utilised melphalan 200 (14) and TBI (18). Median follow-up was 12 months (1.5-102). 29/34 had >80% predicted PFT pre-SCT. At 3 months a fall in all parameters with more marked decline in TLCO/KCO was noted, 13/26 showed >20% decline compared to pre-SCT. This continued to worsen up to 6 months, 11/20 with lower TLCO and KCO. 9/13 patients with >20% reduction in TLCO at 3 or 6 months from pre-SCT died within 24 months of transplant. No significant difference observed between conditioning regimens. *Conclusion:* early reductions in FEV1/FVC were observed in a minority but majority had a reduction in TLCO/KCO – a significant proportion experiencing >20% fall. Early fall in TLCO that does not improve in first 6 months was associated with increased mortality. These changes may reflect pulmonary toxicity associated with conditioning therapy, post transplant lung injury or a combination and illustrates the necessity for regular prospective monitoring of PFTs following SCT for myeloma.

Table 1 . Clinical data and FISH results of MM-patients with extramedullary organ manifestation

Material	Gender/Age	t(4;14)	Del(13q14)	Del(17p13)	Myc-Gains	Therapy	Occurrence	Date Of Occurrence	Outcome
Pleura	F/68	-	-	-	+	multiple conventional dosed chemotherapy regimens, tandem autologous PBSCT	relapse after PBSCT	16 months after PBSCT	died after 2 months
Subcutaneous tissue	M/80	+	-	+	+	conventional dosed chemotherapy	relapse	2/2010	alive
Lymph node	M/68	-	-	-	-	autologous PBSCT	at diagnosis	7/2010	alive
Skin	M/45	-	-	-	+	multiple conventional dosed chemotherapy regimens, autologous PBSCT	relapse after PBSCT	n.a	n.a
Pleural effusion	F/67	-	+	+	-	multiple conventional dosed chemotherapy regimens, tandem autologous PBSCT	relapse after PBSCT	2/2010	died after 2 months
Pleural effusion	M/55	+	n.a.	-	n.a.	multiple conventional dosed chemotherapy regimens, tandem autologous PBSCT	relapse after PBSCT	12/2009	alive
Uterus	F/44	+	-	-	-	multiple conventional dosed chemotherapy regimens, tandem autologous PBSCT	at diagnosis	n.a	alive
Skin	M/63	+	-	-	-	n.a	n.a	n.a	n.a
Thyroid gland	M/74	+	-	-	-	n.a	n.a	n.a	n.a
N.a	.	-	-	-	-	n.a	n.a	n.a	n.a
N.a	M/72	-	+	-	-	n.a	at diagnosis	n.a	n.a
Cns	n.a	-	-	-	+	multiple conventional dosed chemotherapy regimens	relapse after conventional chemotherapy	n.a	alive
Liver	M/58	-	-	+	-	autologous PBSCT 11/2002 and allogene PBSCT 3/2003	relapse after PBSCT	n.a	died after 7 months
Ovary	F/41	-	+	+	-				
Liver	M/56	-	-	+	-	tandem autologous PBSCT	at diagnosis	n.a	alive
Lung	F/58	+	-	-	-	conventional dosed chemotherapy	at diagnosis	1/2010	died after 5 months
Omentum majus	M/81	+	-	-	-	n.a	n.a	n.a	n.a

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EXTRAMEDULLARY MYELOMA ORGAN IMPAIRMENT SHOWS SIMILAR INCIDENCE OF PROGNOSTIC GENETIC ABERRATIONS COMPARED TO SOFT TISSUE INVOLVEMENT GROWING PER CONTINUITATEM FROM BONE OSTEOLYSES

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Extramedullary (EM) organ impairment is a rare event in course of the multiple myeloma (MM) disease. It is associated with a very rapid clinical course and unfavourable outcome, e.g. when appearing after high-dose chemotherapy with autologous or allogeneic stem cell transplantation. Recent studies suggest a correlation between the clinical course of patients with EM MM and specific genetic aberrations, e.g. deletion of the tumor suppressor gene TP53 on 17p13. We herein report the first cytogenetic investigation of diverse EM-organ manifestations with a new developed technique of cIg-FISH on paraffin sections. We analysed 17 MM patients with EM-organ impairment and 14 MM patients with bone or soft tissue involvement originating from bone lesions (control group) and compared the incidences of aberrations in the most important prognostic chromosomal regions in MM (Table 1,2). Results showed a deletion of TP53 in 29% vs. 21%, MYC-overrepresentation (Figure 1) in 25% vs. 29%, deletion of 13q14 in 25% vs. 29% and translocation t(4;14) in 41% vs. 23%. Of note, the two groups did not show significant differences in the incidence of the analyzed genetic aberrations, except for t(4;14), which was detected more frequently in the EM-organ group. Beyond that, our results are in contrast to findings of a higher incidence of TP53 deletions in MM patients with CNS-involvement. Further investigations and larger patient samples are needed to proof, if these or other factors are responsible for the aggressive course and worse outcome of EM relapses in MM.

Table 2. Clinical data and FISH results of patients with soft tissue or bone involvement originating from a bone lesion.

Material	Gender/Age	t(4;14)	Del(13q14)	Del(17p13)	Myc-Gains	Therapy	Occurrence	Date Of Occurrence	Outcome
Soft tissue	F/70	-	+	-	-	conventional dosed chemotherapy	at diagnosis	5/2009	alive
Bone	F/65	-	-	-	-	conventional dosed chemotherapy	relapse after conventionl chemoteraphy	8/2009	alive
Bone	M/80	-	-	-	+	radiotherapy	at diagnosis	1990	dead
Chest wall	F/78	-	-	-	-	rocal radiotherapy, multiple chemotherapy regimen	at diagnosis	8/2008	alive
M.iliopsoas	M/65	-	-	-	+	autologous PBSCT	relapse after PBSCT	6/2010	dead
Spine	M/51	n.a.	-	-	+	multiple conventional dosed chemotherapy regimens, autologous PBSCT	relapse after PBSCT	2 years after PBSCT	alive
Spine	M/70	-	+	+	-	multiple conventional dosed chemotherapy regimens,local irradiation	relapse	n.a	dead
Spine	M/59	-	-	-	-	multiple conventional dosed chemotherapy regimens, autologous PBSCT, local irradiation	relapse after PBSCT	n.a	dead
Chest wall	M/73	+	+	+	-	n.a	n.a.	n.a	n.a
Orbita	F/73	+	-	-	-	tandem autologous PBSCT	relapse after PBSCT	4/2009	n.a
M.psoas	F/55	+	+	-	+	multiple conventional dosed chemotherapy regimens, autologous PBSCT	relapse after PBSCT	12/2000	died 2 months later
Soft tissue	M/71	-	-	+	-	n.a	n.a	n.a	n.a
Forehead	F/73	-	-	-	-	n.a	n.a	n.a	n.a
Iliac crest	M/45	-	-	-	-	n.a	n.a	n.a	n.a

F: Female, M: Male, PBSCT: peripheral blood stem cell transplantation, n.a: not available

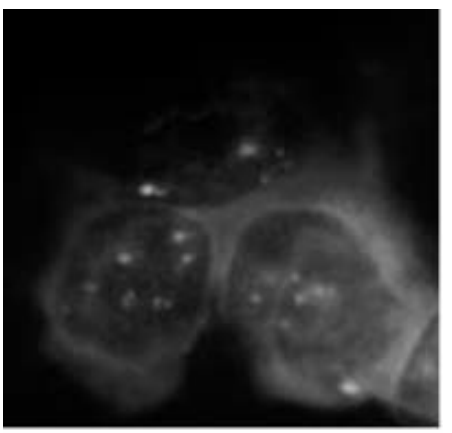

Figure 1. Myc-overrepresentation.

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BRIGHAM' AND WOMEN'S HOSPITAL AND HARVARD MEDICAL SCHOOL

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Risk of MM, a highly fatal cancer, is increased in older age, men, African Americans, and persons with monoclonal gammopathy of undetermined significance. Modifiable risk factors for MM remain obscure. Obesity appears to increase risk; tobacco and alcohol use findings are inconsistent. To further assess the etiologic role of these lifestyle factors, the IMMC pooled individual-level questionnaire data from 10 case-control studies. An analysis of adult height and usual weight and body mass index (BMI) included 2,323 MM cases and 9,685 controls from eight studies. Smoking and alcohol analyses are in progress. A pooled multivariable logistic regression analysis showed an increase in MM risk with increasing BMI (p-trend=0.007), with cases more likely than controls to

have a usual BMI ≥35 kg/m^2 vs. BMI 18.5-<25 kg/m2 [odds ratio 1.4, 95% confidence interval (CI) 1.1-1.8]. We observed a significant positive association of BMI with MM in population-based (2,028 cases, 7,793 controls) (p-trend=0.0004) but not hospital-based (295 cases, 1,892 controls) studies (p-trend=0.66, p-heterogeneity=0.05). No interactions with gender, age, or race were apparent. In two population-based studies (1,262 cases, 3,811 controls) with data on young adult weight at age 25 and 30, young adult BMI was positively associated with MM risk (p-trend=0.0001); persons obese in young adulthood (BMI ≥30 kg/m^2) had a 2.2 fold greater risk (95% CI 1.1-4.5) than those with a BMI 18.5-<25 kg/m^2. These findings provide additional evidence that obesity, particularly in young adulthood, increases the risk of MM.

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INTERIM ANALYSIS OF A STUDY TO INVESTIGATE SAFETY, QUALITY OF LIFE (QOL), PATIENT SATISFACTION & PREFERENCE WITH DOMICILIARY VERSUS DAY WARD ADMINISTRATION OF BORTEZOMIB

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Methods: Relapsed myeloma patients were randomised to bortezomib with cycles 1, 2 at home (Group A) or on the day ward (Group B). The groups then crossed over. Bortezomib 1.3 mg/m2 iv day 1, 4, 8, 11 or a weekly regime day 1,4 were used. All patients were asked to complete QOL questionnaires (EORTC QLQ-C30 &-MY20) pre-treatment, post cycle 2 & 4. Patient satisfaction questionnaires for each administration site (EORTC IN-PATSAT32) and a patient preference questionnaire were also completed. Following 4 cycles, patients were allowed to choose the administration site. *Results:* So far, 16 patients have been recruited and completed the appropriate questionnaires. Patients were given 1-8 cycles of bortezomib (mean 4.5). 126 bortezomib doses were administered in patients' homes out of a total of 239 doses. At trial entry, 11 patients had stage III disease (68%), 3 stage II and 2 stage I. Their ages ranged from 49-91 years (average 76), ECOG performance status 0-3. No infusion reactions were reported and the incidence of adverse events was similar in both groups. Prior to treatment, 12 patients reported a preference for home treatment, 1 patient for day ward treatment and 3 had no preference. Following 4 cycles of bortezomib all patients chose home as the site of administration. The influencing factors related to travelling, convenience, waiting times and concerns about hospital acquired infections. *Conclusion:* Home administration of bortezomib is safe even for elderly patients with advanced myeloma. All patients in this study preferred domiciliary over day ward administration of bortezomib.

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BODY MASS INDEX, OCCUPATION, MEDICAL HISTORY AND MEDICATION USE AND RISK OF MULTIPLE MYELOMA

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Beyond a few established risk factors (age, male gender, African American race, and MGUS), some suspected factors include, obesity, immune-stimulating conditions, infections, and exposure to ionizing radiation, engine exhaust, and farming. To further explore the epidemiology of myeloma, we examined lifestyle, occupation, medical history and medication use in a case-control study of 481 patients and 351 spouse controls. Patients were identified through the International Multiple Myeloma Foundation's Bank On A Cure® project, diagnosed from 1978 to 2008, and U.S. residents. Participants completed a questionnaire. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated using logistic regression. Compared to controls, cases were more likely to have smoked cigarettes but less likely to have consumed alcohol. Non-significant increases in risk with higher education and BMI were observed. Nurse/health practitioners (OR=2.8, 1.3-6.2) and production workers (OR=3.7, 1.0-13.7) had increased risks. Non-significant elevated risks (OR=1.3-1.7) were seen for some occupations linked to diesel exhaust: gas station attendants, railroad workers, machinery and vehicle mechanics, but not for taxi/bus/truck drivers. History of shingles (OR=1.7, 1.2-2.7) and sexually transmitted infections (OR=1.9, 1.0-3.7) were associated with higher risks. Regular use of angiotensin-converting enzyme inhibitors (OR=0.4, 0.2-0.7), anti-convulsants (OR=0.3, 0.1-0.9), antidepressants (OR=0.5, 0.3-0.8), statins (OR=0.4, 0.3-0.8), and diuretics (OR=0.4, 0.2-0.7) were associated with reduced risks.

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ASSESSMENT OF IGM HEVYLITE ASSAY IN A ROUTINE MONOCLONAL PROTEIN LABORATORY

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Serum protein electrophoresis and immunofixation are conventionally used in the assessment of monoclonal proteins. Quantification of IgM heavy/light chain (HLC) pairs may offer a useful adjunct to these techniques. Here we describe the use of IgMκ/IgMλ nephelometric assays in a routine diagnostic laboratory. *Methods:* Consecutive serum samples identified as having an IgM monoclonal protein by capillary electrophoresis immunofixation (IFE) and directly quantified by ultraviolet absorption (PPQ) were analysed using the paired IgMκ/IgMλ HevyLite immunoassays and results compared. *Results:* 14 serum samples were identified as having monoclonal IgM by IFE (κ=7, λ=7). Samples originated from 11 patients with MGUS (median monoclonal protein 4g/l; range 0.5-19), 2 patients with lymphoplasmacytic lymphoma (monoclonal protein: 15, 23 g/l) and 1 myeloma patient (monoclonal protein 22g/l). Median monoclonal protein was 4.5g/l (range 0.5–23g/l). There was good agreement between PPQ measurement and the appropriate HLC assay (r=0.84, p=0.0002) and between total IgM and summated IgMκ + IgMλ (r=0.80, p=0.0026). Appropriate HLC values were elevated in all patients identified by IFE. The alternate HLC pair (i.e. IgMκ in an IgMλ patient) was suppressed in 3/14 patients and 11/14 patients had an abnormal IgMκ/IgMλ ratio. *Conclusions:* HLC data correlated well to PPQ and total IgM. Abnormal ratios were seen in the majority of patients and the assay may aid in identification of difficult to interpret SPE and IFE results.

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INTERMITTENT G-CSF FOR THE MANAGEMENT OF LENALIDOMIDE (LEN)-ASSOCIATED NEUTROPENIA IN RELAPSED/REFRACTORY (REL/REF) MULTIPLE MYELOMA (MM)

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Len-associated neutropenia is a major dose-limiting toxicity in the treatment of rel/ref MM. Although GCSF may decrease the need for Len dose reductions and optimize efficacy, the optimal dosing schedule of GCSF is unclear. In order to economize on GCSF use, we devised an intermittent GCSF regimen (300mcg SC 2-3x/week during weeks 3-4 of a 28day cycle) initiated on 1st episode of grade 3-4 neutropenia and maintained thereon. Efficacy of intermittent GCSF in reducing recurrent neutropenia, infections, and Len dose reduction was retrospectively reviewed in 216 rel/ref MM pts treated at our center with Len 25mg daily x 21 days of a 28 day cycle with dexamethasone as used in the pivotal phase III trials (Weber 2007; Dimopoulos 2007). At a median follow-up of 1.2 yrs (median 6 cycles, range 1-50), grade 3-4 neutropenia occurred in 131 pts (61%) with a median onset in cycle 2 and 106 (54%) pts received intermittent GCSF. Though 55% of pts with grade 3-4 neutropenia developed recurrent episodes, most (69%) were limited to 1-2 events. Len dose reductions were ultimately required in 63 (29%) pts but most due to thrombocytopenia (30%) and multi-lineage cytopenias (21%), rather than for neutropenia alone (17%). Incidence of grade 3-4 infection (37%) and hospitalization due to infection (33%) was high despite GCSF use. Intermittent GCSF, therefore, may be a cost-effective approach to the management of Len-induced neutropenia but ability to prevent Len dose reductions may be limited by other cytopenias. This intermittent GCSF approach should be further evaluated in prospective trials.

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LONG-TERM CARE GUIDELINES FOR PATIENTS WITH MYELOMA

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Introduction: Novel therapies for myeloma have significantly improved survival in patients with newly diagnosed or relapsed myeloma. The International Myeloma Foundation Nurse Leadership Board (NLB) recognizes that nurses play a key role in educating, advocating for, and supporting patients throughout the continuum of care beginning at the time of diagnosis. The NLB identified the importance of a survivorship care plan for patients to minimize treatment-related side effects and co-morbidities. The purpose of the plan is to preserve the health and wellness of patients with myeloma, while acknowledging the effects of the disease, treatment, age, gender, and lifestyle. *Methods:* Based on a group survey and face-to-face discussion, the NLB identified patient-specific needs for those living with a diagnosis of multiple myeloma, including renal disease, bone health, sexual dysfunction, mobility and safety, and health maintenance. *Results:* The Survivorship Care Plan provides recommendations and evidenced-based interventions for each of the identified areas. The goal of this endeavor is to lessen the impact of unmanaged co-morbid conditions on the length and quality of life of patients living with multiple myeloma. Recommendations for prevention, prompt detection, and intervention through education were addressed including plans for broad dissemination to health care providers, patients, and their caregivers.

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IMPACT OF NOVEL AGENTS ON SURVIVAL OUTCOME OF YOUNG PATIENTS WITH MULTIPLE MYELOMA (MM)

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Background: MM is uncommon in the young. Compared to the elderly, young MM patients have longer (~5 years) overall survival (OS) after conventional chemotherapy and stem cell transplantation (SCT). Whether the use of novel agents has made an appreciable difference in their OS is unknown. We studied the outcome of young patients diagnosed over last decade. *Methods:* Records of patients upto 45 years of age, presenting between 01/99 to12/08 were reviewed. Descriptive statistics were used to analyze patient demographics and therapeutic interventions. The Kaplan-Meier method was used for survival outcome estimates. *Results:* Of 1545 patients, 100 (6.5%) were ≤45 years of age at diagnosis (median age 41 years, range: 22-45) 50% of patients were staged as ISS 1. The performance status was <2 in 90% cases. 15 patients had concurrent AL-amyloidosis. The median follow-up was 86 months. 85% received high dose therapy and 15 underwent allogeneic SCT. The median number of regimens used was 5 (range 1-14). 45% of initial regimens were novel agent based. Novel agents were used both in the frontline and relapsed setting (bortezomib 49%, lenalidomide 60%, thalidomide 56% and pomalidomide 9%). The median OS was 93 months from diagnosis (5 and 7-year OS was 69% and 59%, respectively). *Conclusion:* With the use of novel agents, OS of young MM patients is longer than that observed in the series of patients of similar age groups from the chemotherapy era. Good performance status, low ISS at diagnosis may partly account for longer OS in the young population compared to the elderly.

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IMPACT OF IMMUNOFIXATION (IF) AND CLONALITY ASSESSMENTS IN PATIENTS OBTAINING COMPLETE RESPONSE (CR) IN MULTIPLE MYELOMA (MM)

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Background: Currently used response criteria in MM requires <5% bone marrow plasma cells (PCs) to define CR and within this group serum or urine IF positivity defines near CR (nCR or IF+ CR). In the IMWG criteria, patients attaining nCR are now placed under the category of very good partial response (VGPR) whereas those attaining true CR are divided into 2 categories: standard CR and stringent CR (sCR). The latter also requires normalization of the free light chain ratio (FLCR) and absence of clonality by immunohistochemistry. Given the subjectivity involved in IF readout, it is not clear whether IF contributes to response categorization in patients with persistent clonal PCs or abnormal FLCR. *Results:* 659 prospectively followed MM patients who underwent autologous stem cell transplantation (SCT) between 09/2002 and 06/2009 were assessed for the best response. 235 patients (36%) had attained varying degrees of CR. The median estimated follow-up was 67 months from diagnosis and 53 months from SCT. The median overall survival (OS) from SCT was 48 months for patients achieving nCR (n=57) and not reached (NR) for patients achieving CR (n=36) (p=NS). In contrast, those achieving sCR (n=144) had a significantly longer OS (median NR, 4-yr OS, 82 months vs. 59 months for CR). The median time to progression from SCT for patients attaining nCR, CR was 37 and 48 months respectively (p=0.01); and was 63 months for sCR (p<.001). *Conclusion:* No difference in OS is noted between patients achieving nCR and CR when those attaining sCR were categorized separately as per the current IMWG criteria.

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ASSOCIATION OF HEALTH-RELATED QUALITY OF LIFE (HRQOL) WITH BONE DISEASE IN MULTIPLE MYELOMA (MM)

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Objective: Little published HRQOL data exist on patients (pts) with MM in the United States (US). Baseline HRQOL of pts with active, symptomatic MM was assessed by bone disease status. *Methods:* Connect MM® is an ongoing prospective US disease registry of pts with MM. Clinicians reported pt demographics and clinical characteristics. Pts reported HRQOL within 2 months of diagnosis by completing Brief Pain Inventory (BPI), EQ-5D, and Functional Assessment of Cancer Therapy-Multiple Myeloma (FACT-MM). Mean (Standard Deviation [SD]) BPI, EQ-5D and FACT-MM scores were analyzed by bone involvement. Statistical significance was assessed by ANOVA using SAS 9.1. *Results:* 566 pts (189 centers) provided baseline data. Pts were mostly male (56.7%) and white (81.1%) with mean age 66.7 yrs (SD 11.9). Pts were characterized by no bone lesions (n=154), presence of bone lesions (n=290), or severe osteopenia and/or fracture (n=122). EQ-5D pain/discomfort and usual activities scores were most compromised. Increasing bone involvement was associated with worse EQ-5D scores (except anxiety/depression), FACT-MM physical and functional scores, and total FACT-G and FACT-

MM scores, Table. *Conclusions:* Connect MM® baseline findings indicate that bone involvement is associated with greater HRQOL degradation overall, and specifically in physical and functional areas, but not in psychological areas. Data should be assessed as pts undergo treatment to observe effects of disease and treatment on HRQOL over time.

HRQOL Scores Mean(SD)	No bone lesions (n=154)	Presence of bone lesions (n=290)	Severe osteopenia and/or fractures (n=122)	P Value
BPI-Average Pain	2.8(2.9)	3.5(2.8)	4.2(2.9)	0.0006
EQ-5D				
Mobility	1.5(0.5)	1.6(0.5)	1.7(0.4)	0.0003
Self Care	1.2(0.4)	1.3(0.5)	1.5(0.6)	<.0001
Usual Activities	1.6(0.6)	1.8(0.6)	2.0(0.6)	<.0001
Pain/discomfort	1.6(0.6)	1.8(0.6)	2.0(0.5)	<.0001
Anxiety/Depression	1.4(0.5)	1.4(0.5)	1.5(0.5)	0.3176
FACT-MM				
Physical	21.0(5.7)	19.8(5.5)	18.4(5.8)	0.0009
Social/Family	23.4(4.6)	23.5(4.9)	23.0(5.0)	0.7030
Emotional	18.0(4.1)	18.3(4.2)	17.9(4.4)	0.5475
Functional	17.6(6.5)	16.1(6.6)	13.1(6.3)	<.0001
MM subscale Score	37.7(10.8)	36.2(10.6)	33.8(10.8)	0.0151
FACT-G Total	80.1(15.0)	77.7(15.8)	72.3(15.7)	0.0002
FACT-MM Total	117.8(24.0)	113.9(24.4)	106.0(24.8)	0.0005

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TREATMENT COST COMPARISON IN RELAPSED MULTIPLE MYELOMA

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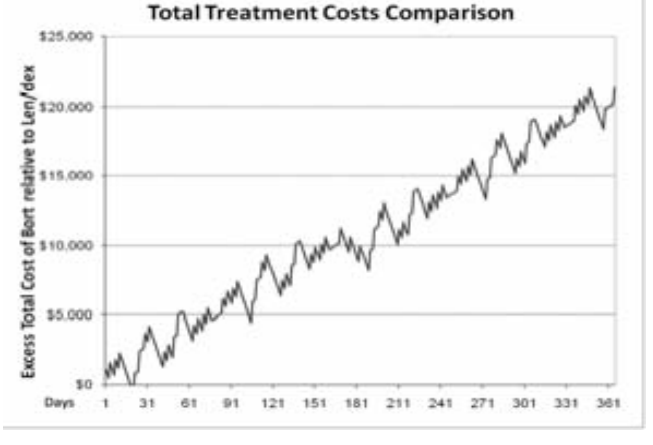
Background: Two therapies, bortezomib (bort) and combination lenalidomide / dexamethasone (len/dex) have been studied for relapsed myeloma patients based on effectiveness at extending time to progression (TTP). At progression/relapse, patients typically receive subsequent treatment. Such patients typically have a worsened prognosis. This study evaluated the economic costs of the two FDA-approved regimens. *Methods:* A managed care payer perspective was used. Drug costs and other direct medical costs (administration, lab tests, treatment of adverse events [AE]) were modeled over one year for a typical patient, with inputs taken from the FDA label. TTP was the primary endpoint for each therapy. The median TTP was 6.2 months for bort and was 13.4 months for len/dex. The model calculated utilization levels using the treatment regimens specified in the clinical trials (APEX and MM009/010); at the median relapse/progression, patients were modeled to re-initiate therapy. Costs were calculated based on: bort at the CMS-published 4Q2010 reimbursement rate; and len/dex at 4Q2010 average wholesale price (AWP)-16%. Costs for medical services and for managing major AE were obtained from the published literature (Fullerton, JMCP 2007). *Results:* Drug costs were comparable, differing by approximately $10 per day. Medical services and costs of treating AEs were higher for bort, amounting to bort having an annual excess total cost of over $20,000 relative to len/dex. *Conclusion:* Total costs for single-agent bort were substantially more than for len/dex, throughout the 12 month period.



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TWO CASES OF NON HEMATOLOGICAL CANCER UNDER CONTINUOUS TREATMENT BY LENALIDOMIDE (LEN) FOR MULTIPLE MYELOMA (MM)

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The IFM2005-02 trial shows a benefit for patients with MM who received a maintenance regimen of LEN after autologous stem cell transplantation (auto-SCT). However, little is known about long-term adverse reactions. We report 2 cases of poorly differentiated carcinoma (PDC) arisen during LEN treatment. Case 1: a 73-year-old woman is followed for a MM diagnosed in 2002, initially treated by ADRIAMYCINE/ONCOVIN/DEXAMETHASONE (VAD), then by two successive auto-SCT after high-dose MELPHALAN (HDM), with a complete response (CR). In 2005, she experiences a relapse of MM and is treated by THALIDOMIDE (THAL) during 24 months, then LEN 15 mg/day, with a very good partial response. In October 2009, a pancreatic PDC is diagnosed, 22 months after the beginning of the LEN treatment. The patient quickly dies. Case 2: a 68-year-old man is followed for a MM diagnosed in 2000, initially treated by VAD, then by two successive auto-SCT after HDM, with a CR. In 2002, he experiences a relapse of MM and is treated by VAD and auto-SCT. A maintenance treatment by THAL is given during 48 months, switched by LEN 25mg/day, with persistent CR. In May 2010, we diagnose a multi-metastatic PDC (pancreas, lung, kidney and brain), 30 months after the beginning of the LEN treatment. The patient dies in 8 months. These two cases of PDC, after 22 and 30 months of LEN treatment, ask the question of the optimum duration of the LEN maintenance treatment. Moreover, it seems very important that physicians report all secondary cancer occurring in MM patient treated with LEN, to better assess this potential risk.

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HUMAN HERPES VIRUS-6 - A NEW RAISING INFECTION IN MULTIPLE MYELOMA (MM) PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) AFTER BEING TREATED WITH BORTEZOMIB

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HHV-6 infection may present with fever, skin eruption, encephalitis and pneumonia. The current study investigated incidence, predisposing factors, clinical characteristics and outcome of HHV-6 infection in MM patients undergoing ASCT. Data on 126 consecutive MM patients (median age 57.3 years), undergoing ASCT between 01.2005 and 09.2010, were reviewed, focusing on pre-transplant induction and transplant-related complications, particularly HHV-6 infection. Diagnosis of HHV-6 infection required a positive PCR in presence of otherwise unexplained fever. Pre-transplant therapies were VAD (n=78, 62%), thalidomide-based (n=17, 13.5%), bortezomib-based (n=27, 21%) and bortezomib-thalidomide combination (n=4, 3.2%). Accumulative dexamethasone dosage in patients receiving VAD, thalidomide or bortezomib approached 1950 mg, 640-2560 mg and 640 mg, respectively. 13 patients (10.3%) were diagnosed with HHV-6 infection; 8 (61.5%) post bortezomib, accounting for a 30% incidence of HHV-6 infection in those receiving bortezomib. HHV-6 was found to be responsible for 66% of cases of "unexplained" fever in patients receiving bortezomib. The most common clinical feature was prolonged fever (100%) accompanied with non-specific lung infiltrations in 6(46%). Infection self-resolved in 11 patients. 2 remaining ones received Gancyclovir due to a high viral load. All patients recovered without sequels. In conclusion, HHV-6 infection is relatively common post ASCT, especially in bortezomib-treated patients. Bortezomib, rather than steroids, appears to be responsible for this complication.

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MULTIPLE MYELOMA OUTCOME IS ASSOCIATED WITH POLYCLONAL IMMUNOGLOBULIN SUPPRESSION OF THE SAME ISOTYPE AS THE TUMOR

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Background: Analysis of heavy/light chain ratios (HLCr) is a quantitative alternative to immunofixation. Here we present data on the prognostic value of HLCr analysis in a retrospective study of 339 multiple myeloma (MM) patients. *Methods:* Nephelometric Ig'κ/Ig'λ (IgG and IgA) values were obtained using archived presentation sera from 339 patients enrolled on the IFM 2005-01 trial. *Results:* At presentation all patients had either IgG or IgA HLCr outside the 95% confidence limits of the normal range. For IgG patients there was a strong negative correlation between the tumour produced immunoglobulin and the alternative HLC pair (e.g. polyclonal IgGλ in a patient with IgGκ MM; Pearson's -0.456, p<0.0001; IgGκ in a patient with IgGl MM; -0.310, p=0.005). Cox regression analysis identified abnormal HLCr as being associated with reduced progression free survival (PFS; p<0.001); the study was too immature for overall survival analysis. The association was independent of other serum markers including Beta 2 microglobulin (β2-M), albumin and markers of genetic aberrations including Del_13, t4_14 and Del_17p. A risk stratification model using the upper tertile HLCr (outside 0.01-200) and β2-M predicted PFS more accurately than the international staging system (p=0.00002 v p=0.017). *Conclusion:* Increasingly abnormal Ig'κ/ Ig'λ ratios correlate with shorter PFS in patients with intact immunoglobulin MM. A risk stratification model with β2-M may aid in identifying patients with a poor prognosis.

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THROMBOSIS IS ASSOCIATED WITH AN INFERIOR SURVIVAL IN MULTIPLE MYELOMA: A POPULATION-BASED STUDY

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Background: Patients with multiple myeloma (MM) are at an increased risk of venous and arterial thrombosis, especially when treated with thalidomide and lenalidomide. Two smaller studies have observed similar survival among MM patients with compared to without venous thrombosis. *Patients and Methods:* We assessed the impact of venous and arterial thrombosis on survival in a population-based study on 9,399 MM patients diagnosed in Sweden 1987 to 2005 using Cox proportional hazards models. *Results:* MM patients with any thrombosis had a higher mortality compared to those without, after 1-, 5-, and 10 years of follow-up, hazard ratio (HR)=3.4 (95% confidence interval (CI) 3.0-3.8), HR= 2.1 (2.0-2.2), and HR=2.0 (1.9-2.2), respectively. Risk of death was significantly higher among MM patients with any venous thrombosis at 1-, 5-, and 10 years, and was 2.9 (2.4-3.5), 1.6 (1.5-1.8), and 1.6 (1.4-1.7). The risks for death among patients with arterial thrombosis was also significantly elevated, with HR=3.4 (3.0-3.8), 2.2 (2.0-2.3), and 2.1 (1.9-2.1), respectively. There was no statistical difference in mortality pattern in MM patients diagnosed before compared to after year 2000. *Conclusions:* In this large population-based study we found, in contrast to previously published studies, that the occurrence of thrombosis was associated with a significantly poorer survival in MM. Our findings confirm that thrombosis in MM patients is a serious complication, increasing mortality, also in the era of novel agents. The prevention of thrombosis in MM is an important goal in the management of MM.

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THE ROLE OF DNA DAMAGE TOLERANCE IN THE DEVELOPMENT OF DRUG RESISTANCE TO ALKYLATING AGENTS IN MULTIPLE MYELOMA

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Drug resistance is a major obstacle in the treatment of multiple myeloma (MM). Tolerance to DNA damage lesions by overexpression of specialised polymerases has been linked to drug resistance in solid but not haematological malignancies. We have investigated damage tolerance in drug resistance in three human MM cell lines (RPMI8226, U266 and JIM-3) by gradually increasing the dose of melphalan (currently cultured in 3, 5 and 3μM melphalan resp.). Resistance has been demonstrated by comparing LD50 data (untreated vs treated for RPMI = 28.09 vs 64.21μM; U266 = 35.16 vs 70.21μM; Jim3 = 49.05 vs 61.97μM) and was confirmed by the MTT assay. Furthermore, assessment of cell doubling time noted up to ten-fold longer initially for treated cells, but as resistance established, the doubling time resembles that of the untreated cells, showing that cell cycle and replication proceed relatively normally. Assessment of polymerase β expression, (suggested to tolerate alkyl lesions), by western blot suggested that treated cells over-express Polβ in U266 and Jim3, but not RPMI8226. Baseline expression of Polβ in untreated cells was low and similar for all cell lines. This observation is being confirmed by flow cytometry and real-time PCR. Some resistance to mechlorethamine suggested cross-resistance to other alkylating agents, which would agree with tolerance playing a role. Our focus in exploring resistance to alkylating agents in MM includes excessive DNA repair and lesion tolerance. Our data will be discussed in the context of DNA damage measurements in the treated vs untreated cells.

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SURVIVAL OF NEWLY DIAGNOSED MULTIPLE MYELOMA CASES IN THE HOVON65/GMMG-HD4 STUDY: RELATION TO THE MOLECULAR CLASSIFICATION AND THE DEVELOPMENT OF A ROBUST AND NOVEL SURVIVAL CLASSIFIER

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Background: Survival of patients with multiple myeloma (MM) is variable, and current prognostic markers are not adequate. Previously we published a gene expression profile (GEP) based MM classification using the HOVON65/GMMG-HD4 study (Broyl et al., Blood,116,2543-2553,2010). Aims: We aimed to evaluate the HOVON-65 classification in relation to survival and to generate a GEP-based classifier of survival in MM. *Methods and results:* Significant differences were found in overall survival (OS) between clusters following conventional treatment with vincristine based treatment (VAD): the MF cluster showed the lowest median OS (3.6 months; p<1x10-4). In contrast, no difference in OS between clusters was observed in bortezomib treated cases (p=0.3). The survival classifier was generated by supervised principal components analysis using the HOVON65/GMMG-HD4 data as training set. Validation GEP sets were UAMS TT2/TT3, APEX and MRCIX sets. The classifier is highly discriminative for high risk versus standard risk MM and is independent of treatment. High risk group size was set to 15% based on the HOVON65/GMMG-HD4, generating a hazard ratio (HR) of 3.8 (p=1.8x10-9) for TT2, HR=3.2 (p=2.5x10-2) for TT3, HR=2.8 (p=9.0x10-8) for the MRCIX trial and HR=4.2 (p=6.4x10-13) for the APEX trial (Figure). *Conclusion:* The HOVON-65 classification identifies clusters with poor survival, such as MF cluster in VAD treated patients. The survival classifier is novel, robust and may result in identifying patients for which a more intensive combination therapy or a higher risk treatment approach is valid.

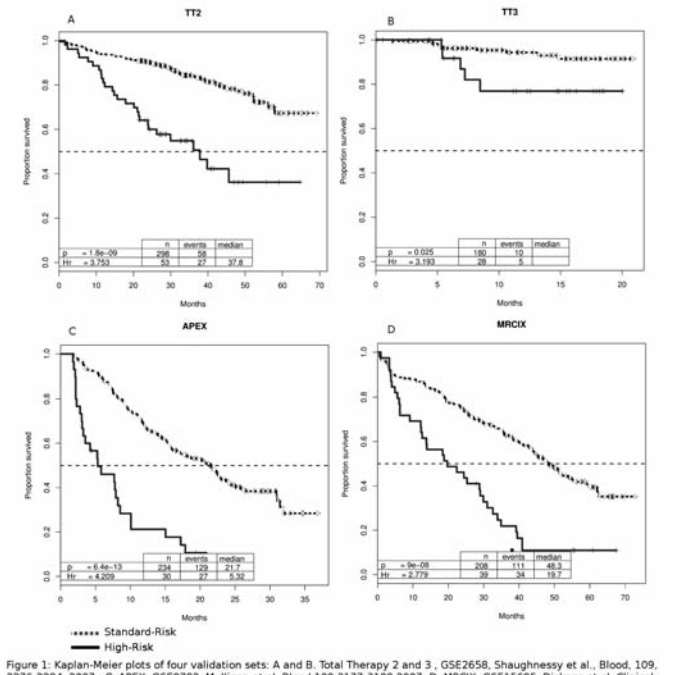

Figure 1: Kaplan-Meier plots of four validation sets: A and B. Total Therapy 2 and 3 , GSE2658, Shaughnessy et al., Blood, 109, 2276-2284, 2007 ; C. APEX, GSE9782, Mulligan et al.,Blood,109,3177-3188,2007; D. MRCIX, GSE15695, Dickens et al.,Clinical Cancer Research,16,1856-1864,2010.

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IMMUNOGLOBULIN HEAVY / LIGHT CHAIN RATIOS EFFECTIVELY MONITOR DISEASE AND PROVIDE PROGNOSTIC INFORMATION IN MULTIPLE MYELOMA PATIENTS

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Introduction: Specific heavy/light chain (HLC) antibodies were used for measuring the involved monoclonal and the respective polyclonal immunoglobulin, for establishing a ratio between both (HLC ratio; HLCr), for response assessment, for monitoring the course of the disease and for prognostication. *Patients and Methods:* HLC analysis was performed on serial serum samples from 106 newly diagnosed multiple myeloma (MM) patients (50 IgG, 56 IgA). Values were compared with serum protein electrophoresis (SPE), immunofixation electrophoresis (IFE), and total immunoglobulin. *Results:* HLCr were abnormal in all patient sera at presentation; 14/56 IgA monoclonal proteins were not quantifiable by SPE. Median overall survival (OS) of the entire group was 46.4 months. 11/106 patients achieved a complete response; in 4/11 patients HLCr indicated residual disease. In 4/7 remaining patients, increasingly abnormal HLCr indicated disease relapse before IFE (median 155 days; range 28-354 days). Multivariate Cox regression analysis identified β2-M (p=0.01), LDH (p=0.0004) and HLCr (p=0.049) as independent prognostic markers. A risk stratification model based on β2M>3.5mg/L and abnormal HLCr (<0.025 or >40) identified patients with 0, 1, or 2 risk factors and was associated with OS (median survival 131.2 v 53.6 v 29.2 months respectively; p=0.01). *Conclusion:* HLCr can detect hard to quantify IgA and low levels of both IgA and IgG paraproteins, indicate persistent disease in IF negative patients, depict relapse earlier than IFE, and provide prognostic information.

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RISK FACTORS AND CHARACTERISTICS OF BLOOD STREAM INFECTIONS IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Despite the advance of novel anti-myeloma therapy, infection remains an important complication contributing to early mortality in multiple myeloma (MM) patients. This study aimed to characterize bloodstream infections (BSIs) and to identify risk factors of BSIs in patients with newly diagnosed MM. *Methods:* We retrospectively enrolled 222 patients with newly diagnosed MM from 2003 to 2008. BSIs occurring within 3 months of MM diagnosis were reviewed. Risk factors for BSI occurrence were analyzed by logistic regression. *Results:* A total of 26 (11.7%) patients developed BSIs, among which 69.2% were nosocomial BSIs. Patients with BSIs had a worse 100-day survival, comparing to those without. Patients with BSIs tended to have advanced stage, poor performance status (ECOG score > 2), anemia (hemoglobin < 10g/dL), hypercalcemia (corrected calcium > 12mg/dL), and renal dysfunction (creatinine > 2 mg/dL) (Table 1).

Table 1. Characteristics of 222 patients with multiple myeloma according to BSI

	No. of patients (%)		P value*
	Non-BSI (n=196)	BSI (n=26)	
Gender			0.64
Male	141 (71)	17 (62)	
Female	55 (29)	9 (34)	
Age (years)			0.54
≥65	127 (65)	19 (73)	
<65	69 (35)	7 (27)	
Myeloma subgroup			0.74
IgG	95 (48)	13 (50)	
IgA	63 (32)	9 (35)	
Light chain disease	29 (15)	4 (15)	
Other types	9 (5)	0 (0)	
Immunoglobulin status			0.733
Severe deficiency**	172 (90)	23 (88)	
Others	19 (10)	3 (12)	
ISS stage			0.04
I	53 (27)	3 (11.5)	
II	44 (22)	3 (11.5)	
III	99 (51)	20 (77)	
ECOG PS			<.001
0-2	137 (71)	9 (37)	
>2	59 (29)	17 (63)	
Hemoglobin (g/dL)			0.025
<10	107 (55)	21 (81)	
≥10	86 (44)	5 (19)	
Calcium (mg/dL)			0.044
≥12.0	178 (91)	20 (77)	
< 12.0	16 (8)	6 (23)	
Creatinine (mg/dL)			0.012
≥2.0	6 (31)	16 (58)	
<2.0	136 (29)	11 (42)	

*Chi-square or Fisher exact test was used for statistics analysis as appropriate
**Severe immunoglobulin deficiency: both of the non-myeloma immunoglobulin levels less than one-fourth of lower limits of normal
Abbreviations: BSI, blood stream infection; IgG, immunoglobulin G; IgA, immunoglobulin A; ISS, International Staging System; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Among blood culture isolates, Gram negative pathogens predominated (53.6%), with Escherichia coli and Klebsiella pneumoniae being the most common pathogens, which were also common nosocomial pathogens in our institute. Multivariate analysis showed risk factors for BSI occurrence were placement of vascular catheter and an admission from emergent department during diagnosis of MM (Table 2). *Conclusion:* BSIs substantially contribute to early mortality in MM patients and are associated with intravascular device and exposure to emergency department. Our findings implicate the importance of hospital infection control in reducing BSIs in patients with MM.

Table 2. Univariate and multivariate analysis for risk factors of blood stream infection (BSI)

	Univariate			Multivariate (d)		
	Odds ratio	95% CI	P value*	Odds ratio	95% CI	P value*
Gender (female vs. male)	1.36	0.571 – 3.266	0.49			
Age (≥65 vs. <65)	1.48	0.591 – 3.681	0.40			
Myeloma subgroup						
IgG vs Non-IgG	1.06	0.469 – 2.410	0.88			
Immunoglobulin status						
Severe Ig deficiency vs Others	1.18	0.324 – 4.303	0.80			
ISS stage						
3 vs. 1 + 2	3.27	1.258 – 8.481	0.02	–	–	–
ECOG PS						
PS > 2 vs PS 0-2	4.39	1.849-10.403	<0.01	–	–	–
Placement of vascular catheter**						
yes vs no	18.60	6.857 – 50.454	<0.01	17.731	6.336 – 49.618	<0.001
Admission from ED						
ED vs non-ED	3.091	1.341 – 7.126	0.01	2.866	1.109 – 7.411	0.030
Hb (<10 g/dL vs. ≥10 g/dL)	3.376	1.222 – 9.322	0.02	–	–	–
Ca (≥12 mg/dL vs. <12 mg/dL)	3.262	1.146 – 9.288	0.03	–	–	–
Cr (≥2.0 mg/dL vs. <2.0 mg/dL)	3.091	1.341 – 7.126	0.01	–	–	–

* P value less than 0.05 as significant
** Placement of catheter prior or during infection
Abbreviations: CI, confidence interval; IgG, immunoglobulin G; Ig, immunoglobulin; ISS, International Staging System; ECOG PS, Eastern Cooperative Oncology Group performance status; ED, emergency department; Hb, hemoglobin; Ca, calcium; Cr, creatinine

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POLYMORPHISMS IN XENOBIOTIC TRANSPORTERS ABCB1, ABCG2, ABCC2, ABCC1, ABCC3 AND MULTIPLE MYELOMA RISK: A CASE-CONTROL STUDY IN THE CONTEXT OF THE IMMENSE (INTERNATIONAL MULTIPLE MYELOMA RESEARCH) CONSORTIUM

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Among several players of the detoxifying/elimination process, ATP-Binding Cassette (ABC) superfamily members B1 (ABCB1), G2 (ABCG2), C2 (ABCC2), C1 (ABCC1) and C3 (ABCC3) play a key role in protecting cells in various tissues, including the blood. To investigate the impact of genetic variation within these genes on Multiple Myeloma (MM) susceptibility, we selected and genotyped 57 tagging and/or functional Single Nucleotide Polymorphisms (SNPs) in ABCB1, ABCG2, ABCC2, ABCC1 and ABCC3 in 523 MM cases and 677 controls from different European populations. Of the 57 SNPs genotyped, three in ABCB1 (rs2214102, rs2235074, rs10276499) and one in ABCG2 (rs2725248) showed a positive association at a conventional $p<0.05$ with MM risk. When correcting for multiple testing, none of the positive hits found reached the threshold p-value ($p<0.001$), even if the two ABCB1 SNPs rs2235074 and rs10276499 showed trends close to significance. With the aim to further investigate these associations, we replicated the 4 SNPs in an independent population of 588 MM cases and 1509 controls of German origin. We did not find any statistically significant association if considering the replication population alone. However, analyzing the two sets jointly, we found that ABCB1 SNP rs2235074 showed a statistically significant associations for the trend test with a decreased risk of MM ($p=0.018$). In conclusion we cannot exclude a role, albeit minor, of ABCB1 polymorphisms in MM risk.

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MULTIPLE MYELOMA PROFILE IN LATIN AMERICA: A WEB-BASED CLINICAL AND EPIDEMIOLOGICAL OBSERVATIONAL STUDY (PRELIMINARY RESULTS)

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Background: Little is known about the incidence and clinical features of MM in Latin America (LA). A clinical registry of LA patients with MM represents an opportunity to gain insight into the prevalence of this disease in this world region, the patterns of care and the current treatment status in different LA countries. *Patients and Methods:* This is a prospective observational study which collected demographic and clinical data from institutional charts, entering them in a central database for analyses. Treatment and patient evaluation were left to the discretion of the investigators. A total of 21 centers participated and the inclusion period for the diagnosis of patients being between January 2005 and December 2007. The follow-up period will extend for 5 years through December 2012. Presently, we have the demographics for the 876 patients included. *Results:* The median age of the patients was 64 years; 53% male; 93% white/non-white and 7% black; M-component IgG 63%, IgA 20% and light chains 11%; creatinine>2mg/dL 25%; 88% with bone lesions; DSSIII:70% and ISS3:32%. Related to the treatment, 25% received high-dose chemotherapy and the others, conventional chemotherapy. *Conclusion:* This preliminary data shows that the majority of the MM patients in LA presented with an advanced stage of the disease upon diagnosis. Even though the median age was less than 65 years, only 25% of the patients received high-dose chemotherapy. In the future, we will be able to show the follow-up of this cohort to evaluate the outcome. on behalf of International Myeloma Working Group Latin America.

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QUANTIFICATION OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH MULTIPLE MYELOMA: FLOW CYTOMETRY VERSUS PCR

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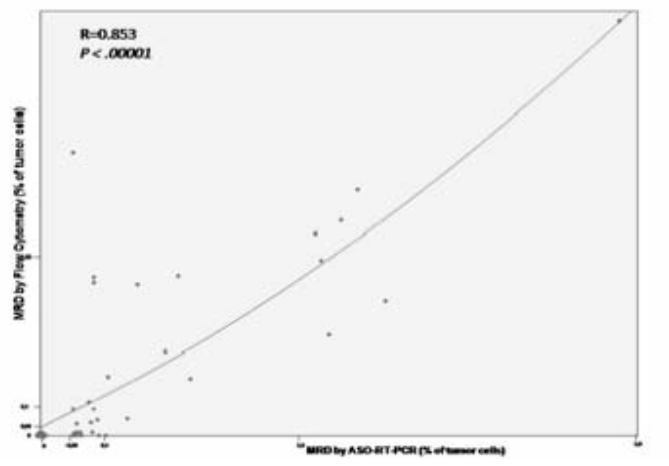
Background: In patients with multiple myeloma (MM), the depth of response is an important prognostic factor. We used allelic specific oligonucleotide real-time quantitative PCR (ASO-RQ-PCR) and multiparameter flow cytometry (MFC) to quantify minimal residual disease

(MRD) and compared their prognostic significance. *Design and Methods:* Bone marrow samples from 56 patients with MM included in GEM (Grupo Español de Mieloma) protocols who had achieved at least a very good partial response were evaluated in parallel by ASO-RQ-PCR (Taqman® approach) and MFC. *Results:* The median [range] number of residual tumor cells identified by both techniques was similar (PCR: 0.2% [0-2.87], MFC: 0.48% [0-4]) with a good correlation between them (R=0.853, p<0.0001), although PCR appeared to be slightly more sensitive. Progression free survival of patients with undetectable MRD by either technique was significantly longer than that of patients in whom malignant cells could be identified (PCR: 54 vs. 29 months, p=0.014; MFC: 54 vs. 26 months, p=0.020). *Conclusions:* MRD assessment in patients with MM by PCR and MFC gives similar prognostic information. Discrepant results between both techniques are attributable to their respective and different sensitivities.



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EXTRAMEDULLARY PLASMOCYTOMAS. EXPERIENCE FROM HOSPITAL ITALIANO DE BUENOS AIRES ARGENTINA

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Extramedullary plasmacytomas (EMP) are plasma cells tumors that arise outside the bone marrow. Epidemiological data of EMP are sparse, especially in Latin America. *Objective:* Investigate the clinical and biological features of patients with EMP. *Methods:* We performed a systematic review of medical records of patients with multiple myeloma (MM) treated at a University Hospital in Argentina. Cases with EMP were compared with MM without EMP diagnosed over the same period and matched by age and ISS stage (case-control 1 to 3). *Results:* 421 patients with plasma cells neoplasms were attended from January 2000 to December 2010. The median age was 70.5 years and 28% ISS stage 3. We identified 16 patients with EMP (3,8%). The median age of EMP was 55 years (p=0,02) , 42,9% stage 3 (p=0,13) and higher LDH (200 vs 162 UI/dl; p=0.003). The sites involved were: orbit (3), liver (3), pleura (2), scalp (3), heart (1), kidney and pancreas (1), ovary (1), stomach (1), muscles (1), larynx (1), peritoneum (1). Most patients received thalidomide, bortezomib or lenalidomide. The overall response rate was 81%. The median overall survival of the 44 controls was 59 months (17-98) and 40 months (17-62) for EMP (p=0,58). We inmunostained paired bone marrow and extramedullary biopsies from 16 cases of EMP and 16 controls for p53, CD56 and Ki67. *Conclusion:* EMP patients were younger and had a higher LDH. Most patients respond to the treatment with the currently available new drugs and the overall survival wasn't significantly lower than that of patients with the same ISS stage.

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SIGNIFICANCE OF ABNORMAL SERUM IMMUNOGLOBULIN HEAVY/LIGHT CHAIN RATIOS (HEVYLITE) IN 294 PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS

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Serum free light chain (sFLC) ratios are prognostic in AL amyloidosis but 5-10% of patients do not have abnormal sFLC ratios. Specific heavy/light chain assays (HLC) that quantify Ig'κ/Ig'λ in serum are of prognostic value in multiple myeloma. We report preliminary data on the importance of HLC measurement in AL amyloidosis in 294 patients with AL amyloidosis seen at the UK National Amyloidosis Centre. HLC (IgG, IgA and IgM) analysis was performed retrospectively on stored sera at presentation. The first 147 patients were selected cases including cases with normal sFLC and the next 147 patients were serial unselected cases. sFLC ratios were abnormal in 142 / 294 patients (41/147 from first and 101/147 from second cohort), and HLC ratios were abnormal in 180 / 294 (92 /147 and 88 /147 respectively) patients. 57 / 180 patients with abnormal HLC ratios had normal sFLC ratios. Median overall survival (OS) of all 294 patients was 43.1 months. Abnormal sFLC ratios were associated with poorer OS (median OS 51.3 vs. 25.3 mos for normal vs. abnormal sFLC p = 0.001). In patients with normal sFLC ratios, an HLC ratio outside the 90 percentile range of all patients for IgG, IgA or IgM was associated with poorer OS (median survival within 90%ile range = 61.6 months, outside 90%ile = 17.3 months p = 0.031) - multivariate analysis will be presented. In conclusion, HLC ratio can be abnormal in AL patients with normal sFLC. Extreme HLC ratios appear to be associated with poor outcomes in AL amyloidosis with normal sFLC.

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NEPHELOMETRIC ASSAYS IGKAPPA AND IGLAMBDA? USED FOR DIAGNOSING AND MONITORING MULTIPLE MYELOMA

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Background: Serum protein electrophoresis (SPE) and immunofixation (IFE) are recommended methods of identifying, characterising and quantifying monoclonal proteins. SPE measurements can be inaccurate depending on the migration of the band. IFE is a more sensitive method but it is not quantitative. Heavy/light chain (HLC) assays can be used to quantify Ig'κ and Ig'λ. Here we compared the use of these assays to SPE and IFE for monitoring multiple myeloma (MM) patients. *Methods:* Sequential archived sera from 156 patients enrolled onto the 2007-01 IFM trial were retrospectively analysed. Ig'κ and Ig'λ (IgG and IgA) levels were measured at presentation, post cycle 2 and 4 of therapy and post autologous translation (ASCT). Comparisons made at complete response (CR) *Results:* At presentation HLC ratio (HLCr) was abnormal in 43/43 IgA and 112/112 IgG MM patients. Post-ASCT 92% of IgA patients were negative (-ve) by SPE with 63% of patients achieving a CR; 55% of patients had a normal IgA HLCr. For IgG patients post-ASCT, 45% were -ve by SPE with 26% of patients achieving a CR. 39% of patients had a normal IgG HLCr. *Conclusion:* MM patients can be monitored using HLCr as an alternative to SPE and IFE. HLCr have a greater sensitivity than IFE for detection of minimal residual disease in IgA MM, but are slightly less sensitive in IgG MM. Further clinical studies are required to assess the impact of these differences.

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MOLECULAR SUBSTUDIES IN THE EMN-02 TRIAL: A PROSPECTIVE RANDOMISED PHASE III INTERGROUP MULTICENTER TRIAL COMPARING BORTEZOMIB, MELPHALAN, PREDNISONE (VMP) WITH HIGH DOSE MELPHALAN FOLLOWED BY BORTEZOMIB, LENALIDOMIDE, DEXAMETHASONE (VRD) CONSOLIDATION AND LENALIDOMIDE MAINTENANCE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM)

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Background: MM is both clinically and genetically a complex disease covering a broad range of chromosomal and molecular abnormalities and treatment outcomes. Recently, the application of novel techniques has led to an increased insight into the molecular characteristics of MM, potentiating further subclassification of this heterogeneous disease. We have previously been able to distinguish 10 different MM clusters as well as risk factors for adverse effects of treatment using gene-expression profiling (GEP), single nucleotide polymorphism (SNP) and fluorescent in situ hybridisation (FISH) data of newly diagnosed MM patients included in the HOVON-65/GMMG-HD4 trial (Broyl et al., Blood 2010;116(14):2543-2553 & Lancet Oncol. 2010;11:1057-65). *Methods:* The EMN-02 trial is a prospective randomised phase III intergroup multicenter study comparing VMP with high dose treatment (HDT), followed by VRD consolidation and lenalidomide maintenance therapy in 1500 newly diagnosed MM patients (see Figure). We aim to perform the following substudies: 1) GEP at diagnosis and at relapse, 2) FISH analysis, 3) SNP analysis on CD138+ cells and peripheral blood, 4) microRNA (miRNA) profiling and 5) multiparametric flow cytometry (MFC) to determine minimal residual disease (MRD). *Conclusion:* Substudies will be performed in the EMN-02 trial to further define MM clusters as well as molecular factors related to treatment outcome and adverse effects and to identify biological pathways involved in drug resistance.

This trial is supported by the HOVON Foundation, Celgene and Janssen.

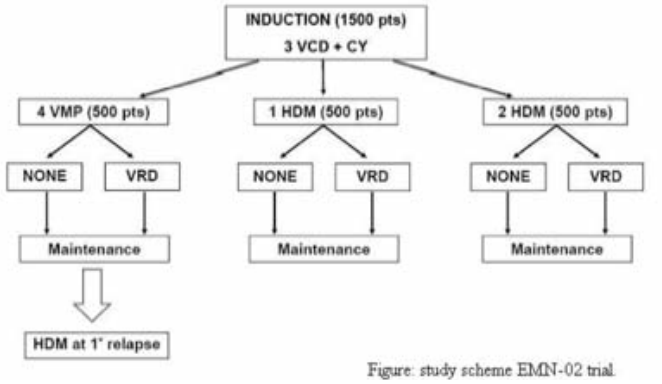

Figure: study scheme EMN-02 trial.

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EXPRESSION OF MYELOID CELL LEUKEMIA-1 (MCL-1) DEMONSTRATED BY USING IMMUNOHISTOCHEMISTRY ON THE BONE MARROW PLASMA CELLS FROM 40 PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA ASSOCIATED WITH LESS RESPONSE RATES TO VELCADE-CONTAINING INDUCTION REGIMENS

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Expression of myeloid cell leukemia-1 (MCL1) has been demonstrated to confer resistance of myeloma cells (MCs) to bortezomib (Velcade®) in vitro. However, its role in the clinical setting is still unclear. We therefore examined the expression of MCL1 by using immunohistochemistry (IHC) staining on the bone marrow (BM) sections from 40 newly diagnosed myeloma patients who all received Velcade-containing induction regimens, including Velcade+Melphalan+Dexa (VMD) and Velcade+Thalidomide+Dexa (VTD) in half the patients each. The positive MCL1 staining on IHC was determined by three reviewers. There were 25 patients (63%) whose MCs were stained positive for MCL1. The clinical characteristics between the patients whose MCs stained positive (MCL1) and those stained negative were not different. However, the overall response rates (ORR) to the Velcade-containing regimens, terms of partial response or better, were 68% and 100% in the MCL1 positive and negative patients respectively, which was statistically significant (p=0.016). Interestingly, on the patients who had VMD (N=20), the ORR was 69% and 100% in the MCL1 positive and negative patients, respectively (p=0.249); and similarly, for patients who had VTD (N=20), the ORR was 67% and 100% in the MCL1 positive and negative patients respectively (p=0.117). From the preliminary results, we suggest that expression of MCL1 in MCs associated with less response to Velcade-containing regimens no matter combination of either melphalan or thalidomide. More patients enrolled are required to validate the observation.

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MYELOMATOUS PLEURAL EFFUSION OF MULTIPLE MYELOMA: CHARACTERISTICS AND OUTCOME

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Introduction: Myelomatous pleural effusion (MPE) in multiple myeloma (MM) is rare and the characteristics of MPE are unclear. We report 45 MM patients presenting with MPE. *Methods:* Multicenter retrospective data were analyzed. Diagnosis of MPE was based on positive cytology or biopsy. *Results:* The median age was 59 (range 21-87). Twenty-six patients were male. The MM isotype were IgG in 14, IgA in 11, light chain in 10, IgD in 7, and non-secretory in 3 patients. Eighty percent of patients had initial D-S stage III. MPE developed at a median of 8.7 months (0 - 77) from diagnosis of MM and was the first clinical manifestation in 12 cases. After onset of MPE, 26 patients were treated with the regimens based on vincristine, adriamycin & dexamethasone in 11, thalidomide in 7, bortezomib in 6 and other regimens in 11. Among them, 5 got autologous stem cell transplantation and one had both autologous and allogeneic transplantation. Pleurodesis was done in 2 patients. MPE disappeared in 12 patients and

median response duration was 123 days (95% CI: 0 - 319). Median survival was 57 days (95% CI: 20 - 94) from onset of MPE. It was significantly longer in patients with disappeared MPE than with persistent MPE (205±75 vs 22±5, p<0.0001). Causes of death were progression of disease, progression with concomitant infection or infection (40, 21 and 16%). *Conclusions:* Base on this analysis, MPE is a poor prognostic indicator. Further evaluation of the utility of various novel agents or stem cell transplantation in the setting of MPE is needed to improve treatment outcome.

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CD28- A MOLECULAR BRIDGE BETWEEN MYELOMA CELLS AND THE MICROENVIRONMENT

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Interactions between myeloma cells (MM) and bone marrow (BM) stroma promote MM survival directly via prosurvival signals within MM and indirectly via growth factors that are subsequently elicited from the microenvironment. Very little is known about the specific BM cell type or the molecular elements that mediate MM survival. Clinical studies have shown that CD28 expression correlates with disease progression in myeloma patients. Flow cytometric analysis of MNC in BM aspirates reveal higher CD11b population (20-37%) in MM vs. healthy (12-15%). Co-cultures of MM cells with CD11b+ dendritic cells (DC) protect MM against cell death, which was lost when CD28-CD80/CD86(B7) interactions were blocked. Moreover a larger fraction of myeloma BM expressed CD28 (11-47%) compared to healthy control (3-7%). Analysis of gene expression datasets of BM plasma cells revealed increasing CD28 expression from healthy→MGUS and significantly from healthy→SM→MM (p<0.05). Within the MM group, CD28 expression peaked in the MF subgroup (p<0.0001) which is typically associated with poor survival in myeloma patients. MM cells also induced DCs to make IL-6 or the immunosuppressive factor IDO (indoleamine 2,3 Dioxygenase), which was significantly reduced upon blocking CD28-B7 interactions. Neutralizing IL-6 in cocultures reduced IL-6 mediated MM survival, while inhibiting IDO reduced IDO mediated suppression of T-cell proliferation. Our data shows that BM-DC is vital for myeloma survival and targeting CD28-B7 pathway might have potential in future strategies in the treatment of myeloma.

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HAVING A GOOD RESPONSE IN FRONT LINE IMPROVES THE LIKELIHOOD OF HAVING A GOOD RESPONSE IN SECOND LINE IN REAL LIFE MULTIPLE MYELOMA PATIENTS

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Aim: Evaluate real life responses and there correlations. All treatment demanding patients, n=321, median age 67 years 54% male, with multiple myeloma between Jan 2000 and Jul 2010 at our centre were included. Near complete response (nCR) was defined as an immeasurable M-protein. Very good partial response (VGPR), partial response (PR) and no response (NR) were defined according to IMW criteria. *Results:* Of the total population 46% were given high dose treatment (HDT). Then median number of treatment lines was 2 independent of HDT.64% of the patients were given at least 2 treatment lines and only 36% 3 or more. For HDT patients the most common 1st line treatment was VAD (57%) and for non-HDT MP (58%). The response distribution for the entire population nCR/VGPR/PR/NR was 25/20/25/30 in 1st line, 14/11/22/53 in 2nd line. In the HDT population the same response distribution was 46/26/18/9 in 1st line and 24/14/20/42 in 2nd line. In the non-HDT population the response distribution was 6/16/31/47 in 1st line and 7/8/23/62 in 2nd line. Responses were significantly better in the HDT population in the first two lines. nCR in the 1st line implied a 40% probability to receive a ≥VGPR and 27% probability to receive an nCR in 2nd line. NR in the 1st line implied a 57% probability to receive a NR in the 2nd line. *Discussion:* Patients receiving 2nd and 3rd line treatment were declining rapidly. To get a good response in 1st line increases the likelihood of having a good response in 2nd line showing the importance of trying to get as good response as possible from the beginning.

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PERIPHERAL NEUROPATHY CLINICAL COURSE DURING LENALIDOMIDE THERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: A SINGLE-CENTRE PROSPECTIVE NON INTERVENTIONAL STUDY.

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Lenalidomide has been claimed to have low toxicity on peripheral nervous system. In a single-centre prospective study, we evaluated the natural history of peripheral neuropathy (PN) in 58 consecutive patients previously treated with bortezomib and/or thalidomide, who shifted to lenalidomide for relapsed or refractory multiple myeloma (MM). The aim was to evaluate the clinical course of PN during lenalidomide therapy. All patients were treated with lenalidomide 25 mg daily for 21 day cycle, alone or associated with low dose dexamethasone. Neurological evaluation was planned at baseline, after 6 and 12 months from the beginning of lenalidomide therapy. Patients were assessed with the Total Neuropathy Score clinical version (TNSc); Nerve conduction studies were performed at the beginning of the treatment and regularly during follow up. Of the 58 patients, 31 (mean age 66 yrs ± 8) were evaluated with at least six months follow up, 10 of whom at one year.

Although an improvement in the TNSc scale could be demonstrated at 6 months (4.13±3.4 vs 3.64±3.1) differences were not statistically relevant. The same pattern was shown at 1 year. At six months, hematological response was documented in 27/31 patients; at one year five patients maintained hematological response. All patients with TNSc score 0 at baseline remained unchanged after 6 months and 1 year of lenalidomide therapy. In conclusion, using validated clinical tools, the results of our prospective study suggest that lenalidomide therapy does not worsen and in selected cases may improve PN, regardless of MM response.

P-400

THE BEST RESPONSE DURING THE 1ST LINE OF TREATMENT IS STRONGLY CORRELATED TO LONGER TIME TO PROGRESSION IN REAL LIFE MULTIPLE MYELOMA PATIENTS

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Aim: Evaluate the time to progression (TTP) and time to next treatment (TTNT) in real life and their correlation. All treatment demanding patients, n=321 with multiple myeloma between Jan 2000 and Jul 2010 at our centre were included. Median age was 67 years, 54% male and 46% got a high dose treatment (HDT). Near complete response (nCR) was defined as an immeasurable M-protein. Very good partial response (VGPR), partial response (PR), no response (NR) and TTP were defined according to IMW criteria. *Results:* The median TTP/TTNT was 407/514 days in the 1st line 203/221 in 2nd line and 194/218 in 3rd line. In the HDT population the TTP/TTNT was 610/736 days in 1st line and 209/239 in 2nd line and 186/220 in 3rd line. In the non-HDT population the TTP/TTNT was 331/363 days in

1st line and 202/214 in 2nd line and 215/215 in 3rd line. In the HDT population both the TTP and the TTNT in the 1st line were significantly better than in the non-HDT with no difference in 2nd or 3rd line. There was a significant trend of increasing TTP/TTNT in 1st line depending on the increased depth of the response with TTP/TTNT for nCR of 730/893 days, VGPR 511/563 days, PR 364/523 days and NR 169/177. The same pattern was seen in 2nd line. *Discussion:* A significantly decreasing trend in TTP/TTNT from 1st line to 2nd line was observed. However, the TTP/TTNT stabilizes around 200 days in later lines independent of HDT in 1st line. Better response translates into longer TTP/TTNT in both 1st and 2nd line showing the importance of trying to get as good response as possible for the real life patients.

P-401

BETTER RESPONSES AND LONGER TTP TRANSLATES INTO LONGER OVERALL SURVIVAL IN REAL LIFE MULTIPLE MYELOMA PATIENTS

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Aim: Evaluate overall survival (OS) in real life patients and its correlation to response, time to progression (TTP) and time to next therapy (TTNT). All treatment demanding patients, n=321 median age 67 years 54% male and 46% got a high dose treatment (HDT), with multiple myeloma between Jan 2000-jul 2010 at our centre were included. Near complete response (nCR) was defined as an immeasurable M-protein. Very good partial response (VGPR), partial response (PR), no response (NR) and time to progression (TTP) were defined according to IMW criteria. *Results:* Median OS was 4.7 years 95% CI [4.0;5.3] with 48% censored. HDT patients had a significant longer median OS 6.1 years 95% CI [5.1;8.5] than the non-HDT patients OS 3.2 years 95% CI [2.3;4.0]. The survival differences in the HDT patients comes from the time within this line with no difference in survival starting from the 2nd line comparing HDT and non-HDT patients OS 2.04 95% CI [1.47;3.49] compared to 2.99 CI [2.5; 3.98]. The median OS for patients with 1st line best response nCR was 6.9 years, VGPR 3.9 years, PR 4.6 years and NR 3.0 years. There was a significantly decreasing OS except for the patients with best response PR in 1st line. There is a strong correlation between TTP in the 1st line and increased OS for the non-HDT patients and this correlation is even stronger for the HDT patients. The median survival for VAD treated HDT patients was 6.14 years 95% CI [5.1;-]. *Discussion:* Both better responses and TTP/TTNT in the first lines translates into survival benefits showing this importance for real life patients.

P-402

EARLY CHANGES IN THE BONE MARROW OF MULTIPLE MYELOMA PRECURSOR DISEASE PATIENTS, PREDICTIVE OF MALIGNANT PROGRESSION

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Background. The bone marrow microenvironment plays an essential role in multiple myeloma (MM) by providing mechanisms of survival, proliferation, and adhesive interactions. Aim of this study was to define the prognostic effect of early changes in the bone marrow on malignant progression in patients with MM precursor disease. *Methods.* We identified >1000 monoclonal gammopathy of undetermined significance (MGUS) patients diagnosed in Sweden 1989-2006 based on clinical history, monoclonal-(M)-protein concentration, plasma cell counts on aspirate smears and morphologic evaluation of core biopsies. We conducted a nested case-control study including 14 MGUS patients who progressed to MM and 18 non-progressing MGUS patients with follow-up until 2009 (median follow-up 6.7 yrs). *Results.* Using CD56 and CD138 immunohistochemistry stains on bone marrow biopsies, we found plasma cell clustering (>10 plasma cells/cluster by CD138 staining) in 6/18 (33%) of non-progressors and 9/14 (64%) of progressors, respectively. In 3/18 (17%) non-progressors and 9/14 (64%) progressors we found CD56 expressing plasma cells ($p<0.01$). Indeed, CD56 plasma cell expression was more prominent in areas where plasma cells clustered. *Conclusions.* Our findings that plasma cell clustering and CD56 expression on plasma cells was associated with progression to MM and supports the hypothesis that the ability of MM cells to adhere to local environment is implicated through syndecan 1 (CD138) and NCAM (CD56).

P-403

PLASMA CELL ESTIMATES USING CD138 IMMUNOHISTOCHEMICAL STAINING IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) ARE PREDICTIVE OF PROGRESSION TO MULTIPLE MYELOMA

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Background. Recent studies show that multiple myeloma (MM) is consistently preceded by a precursor state, MGUS; however, we lack reliable markers to predict progression from MGUS to MM. The aim of this study was to assess the role plasma cell count using CD138 immunohistochemistry in patients with MGUS diagnosis in relation to disease progression. *Methods.* In a cohort of >1000 MGUS patients diagnosed in Sweden 1989-2006 based on clinical history, M-protein level, plasma cell counts on aspirate smears and morphologic evaluation of core biopsies, we conducted a nested case-control study of 14 MGUS patients who progressed to MM and 18 non-progressing MGUS patients (median follow-up 6.7 yrs). We performed CD138 immunohistochemical staining on bone marrow biopsies of all 32 patients obtained at time of MGUS diagnosis. *Results.* CD138 staining revealed that 10/32 (31%) of marrow biopsies from MGUS patients had >10% plasma cells, which was not apparent without the use of CD138 or on aspirate smears, with 9/10 (90%) of these patients progressing to MM (median 8 yrs from initial diagnosis). Among non-progressors, only 1/18 (6%) had >10% plasma cells in the core biopsy, as opposed to 9/14 (64%) of progressors ($p=0.0006$). Median M-protein concentrations were similar in progressors and non-progressors (1.3 vs 0.8 g/dL; $p=0.65$). *Conclusions.* Using CD138 immunohistochemistry in this nested case-control study, we upstaged 31% of previously diagnosed MGUS patients to smoldering myeloma (SMM). Importantly, among the patients who progressed to MM, 64% were upstaged from MGUS to SMM.

P-404

EPIDEMIOLOGY AND CLINICAL FEATURES OF MULTIPLE MYELOMA IN ALGERIA: REPORT OF THE ALGERIAN MYELOMA STUDY GROUP (GETMA)

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Background: Multiple myeloma (MM) is a plasma cell clonal proliferation, it represents 10% of hematological malignancies (HM) in occidental countries. *Aims:* Algeria is in epidemiological transition and still characterized by a young population (70% have less than 30 years old) so this study try to determine the incidence among HM in our country, the proportion of young patients, the clinical features and prognosis factors. *Methods:* Information was gathered about all cases of MM diagnosed between January 1, 2006, and December; 31, 2009. Only two centersamong 14 centers haven't participated in this work. *Results:* Between 2006 and 2009, 713 cases of MM were diagnosed. These cases represent an incidence of 0, 57 per year. The sex ratio (M/F) is: 1, 24 and the median age is 62, 2 years (29- 91 years). In this population, 57% (403 pts) have less than 65 years, and 16% less than 50 years. The bone pains are the major symptom in 88%, anemia in 86% (612pts). Staging was performed according to Durie and Salmon: stage III: 607 pts or 92% and renal failure in 186 cases (28%). *Conclusion:* Epidemiologic and clinical features of our pts are different with

the occidental ones. This distribution has implication for the population eligible for specific types of therapy in younger of them: 57% in our study and 30 % in occidental series.This study will help us to define a therapy strategy.

P-405

RETROSPECTIVE ANALYSIS ON 274 SMOLDERING MULTIPLE MYELOMA CASES: A PROPOSAL FOR A NEW SCORE SYSTEM TO IDENTIFY SLOWLY AND HIGHLY PROGRESSIVE PATIENTS

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Smoldering multiple myeloma (SMM) is an asymptomatic plasma-cell proliferative disorder associated with a high risk of progression to symptomatic multiple myeloma (MM). Prognostic factors for the progression and outcome of this disease are unclear. We carried out a retrospective multicenter cohort study on 274 patients with SMM (M/F 121/153, median age at diagnosis 62 ys, range 25-89) diagnosed between 1980 and 2010. Biological characteristics at diagnosis was as follows: haemoglobin (Hb)13gr/dl (range,10-16.4), serum monoclonal component level (MC) 2.1gr/dl (range 1.4-5.5), bone marrow plasma-cells (PC) 22% (range,1-70), β2-microglobulin (β2M) 2,4 (range,1-3,5).The isotype of MC was: IgAk 32/274, IgAλ 20/274, IgDλ 1/274, IgGk 144/274, IgGλ 73/274, IgGl+IgAλ 1/274, IgGk+IgMk 1/274, monoclonal k and λ light chain 1/274 and 1/274, respectively. Ninety-seven/274 (35%) developed MM with actuarial progression rate at 15 ys of 64%.

BIOLOGICAL CHARACTERISTICS	SCORE
Hb≥12gr/dl	0
Hb10-12gr/dl	1
Bone marrow PC ≥30%	1
Bone marrow PC =10-30%	0
Serum monoclonal level ≤3gr/dl	0
Serum monoclonal level ≥3gr/dl	1

Table 1. Score system models for SMM

Among the biological characteristics evaluated at diagnosis, the serum level of MC (p=0.004), the Hb level (p=0.04) and the bone marrow PC (p=0.007) were significantly associated with progression. Therefore, using these 3 variables, we create a score system (Table 1) to identify slowly (score 0-1) or highly (score 2-3) SMM progressive patients. The median time to progression was 2.5ys and 10ys for the highly and slowly progressive group, respectively. Our results show that this score system at diagnosis is a valuable tool that could help to distinguish slowly and highly SMM.

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CD28: A NOVEL THERAPEUTIC TARGET FOR THE TREATMENT OF MULTIPLE MYELOMA

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Multiple myeloma (MM) is a plasma cell neoplasm which resides in the bone marrow (BM) and is critically dependent upon the BM microenvironment for its survival. It has been observed that CD28 is expressed on MM cells and is correlated with progressive disease (and worse outcomes). Furthermore, we have observed that ligation of CD28 protects MM from chemotherapy. Taken together, these data imply that CD28 is a critical pro-survival molecule for MM. The ligands for CD28, CD80 and CD86, are expressed on APCs, particularly dendritic cells (DC). We and others have observed that DCs infiltrate myelomatous portions of patient BM biopsies and murine plasmacytomas. Therefore, we hypothesize that it is DCs in the microenvironment which is providing the pro-survival signal to the MM via a CD28:CD80/86 interaction. To test this in vitro, we have co-cultured MM and DC, added melphalan, and blocked either the CD28 side with an antibody (CD28.6) or the CD80/86 side with CTLA4-Ig. Our results show that MM survives if the CD28:CD80/86 interaction is intact; however, the DC confer no protective benefit if this interaction is blocked. These results have been recapitulated in human DC as well as murine DC, demonstrating that this may be a viable target in both human and murine systems. One of the mechanisms by which CD28 may be protecting MM is via regulation of apoptotic factors. We have preliminary data which suggest that CD28 activation decreases Bim, a pro-apoptotic factor (thereby increasing MM survival). This data thus suggests a mechanism by which CD28 protects MM from cell death.

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WEEKLY BORTEZOMIB IS EFFICACIOUS IN RELAPSED/REFRACTORY MYELOMA WITH MINIMAL TREATMENT RELATED TOXICITY: A TWO-CENTRE EXPERIENCE

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Background: Weekly bortezomib regimens are tolerable and effective as initial therapy for MM. In the context of relapsed/refractory MM data is limited, having importance if patients have pre-existing treatment-emergent neuropathy. Here we explore the feasibility of weekly bortezomib regimens this patient group. *Methods:* A retrospective analysis was performed on 35 MM patients who received weekly bortezomib either from treatment initiation or after not more than two bi-weekly cycles. Twenty patients received 1.3mg/m2 weekly for 4 weeks of a 5 week cycle, whilst 15 patients received 1.6 mg/m2 on week 1 & 2 of a 3 week cycle. One patient received monotherapy, 34 (97%) received steroid therapy, 20 (57%) with an alkylator and 2 (6%) also received thalidomide. 7 (20%) had primary refractory disease, and 28 (80%) had relapsed disease. The median number of treatment cycles was 6 (range 3-8). The median total dose received was 21mg/m2 (range 9.6-41.6). Twenty eight (80%) patients responded to treatment (PR or greater), 2 (6%) patients were non-secretory whilst 5(14%) had stable disease. No toxicities >grade 3 were seen, and no patient had>grade 1 neuropathy. Ten (29%) were dose reduced; 6 (17%) with neuropathy, 3 (9%) with gastrointestinal toxicity, 1 (3%) with fatigue. The median PFS was 14 months whilst 71% achieved a time to next treatment of greater than 12 months. *Conclusion:* Weekly bortezomib is efficacious and well tolerated in refractory/relapsed MM patients. Treatment related toxicities are minimal and easily managed with dose modification

P-408

LOSS OF FGFR3 IS ASSOCIATED WITH LESS GENOMIC INSTABILITY AND A MORE FAVOURABLE PROGNOSIS IN T(4;14) MYELOMA PATIENTS TREATED ON MRC MYELOMA IX

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Loss of expression of FGFR3 in 25% of cases and recent data that MMSET is involved in an aberrant response to DNA damage has implicated MMSET as the key gene responsible for the poor prognosis of t(4;14) MM. Previously we identified using SNP and expression arrays that loss of expression of FGFR3 is due to deletion of the derivative 14. In this study using IgH/FGFR3 dual fusion FISH on CD138 selected plasma cells from patients enrolled in MRC Myeloma IX, we identified t(4;14) in 120/1053 cases. 21/118 assessable cases had single fusion consistent with loss of FGFR3. There was no association between FGFR3 deleted t(4;14) cases and other high risk FISH abnor-

malities (del 17p/del 1p/gain 1q). FGFR3 deleted cases had similar OS (37.9 vs. 25.6 months, P=0.127) but improved PFS compared with non-deleted t(4;14) cases (19.4 vs. 12.2 months, P=0.007). This benefit was most apparent in those cases without other high risk FISH abnormalities. Copy number analysis using 500K SNP arrays in 12 t(4;14) cases revealed a higher mean number of subchromosomal copy number losses than in 26 non-t(4;14) cases (7.75 vs. 3.81, P=0.03) consistent with increased genomic instability. 4/12 t(4;14) cases had loss of FGFR3 and had significantly fewer copy number losses per case (mean 2.5 vs. 10.38, P=0.046) in comparison with 8 FGFR3 non-deleted t(4;14) cases. This large series of t(4;14) cases demonstrates that loss of FGFR3 is associated with improved clinical outcome and less genomic instability, suggesting a key role for FGFR3 expression alongside MMSET in influencing the prognosis of t(4;14) MM.

P-409

HLA-DRB1*13 AND *15 ARE ASSOCIATED WITH RESPONSE TO THALIDOMIDE IN PATIENTS WITH MULTIPLE MYELOMA

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Thalidomide(T) has been approved for treatment of myeloma. There is no generally accepted predictor for response. The aim of this study was to analyze the impact of HLA antigens on response to T. *Patients and Methods:* Patients(n=46) who received T either as monotherapy(n=36) or combination therapy with dexamethasone(n=10) were retrospectively analyzed. Median age 50 (31-73), male/female 31/15, IgG/IgA/IgM/light chain 33/6/1/6, ISS-I/II/III 21/10/11. Patients were given T for induction/consolidation/relapse 2/18/26 at doses 50-400 mg for median duration of 10(2-72)months. Response to T was classified as responders ≥partial remission(PR) or non-responders(disease progression on T). *Results:* 16 patients had response, 18 patients had disease progression and 12 patients had stable disease on T. Parameters which may have contributed to response to T were compared between responders and non-responders (Table-1). The differences which were statistically significant between the groups were the number of previous chemotherapy lines and the HLA frequencies. HLA-DRB1*15, HLA-DRB1*13 and HLA-DRB1*11 are observed 7.2%, 18.2% and 21.1% in MM population, respectively. In this study, HLA-DRB1*15 and HLA-DRB1*13 were associated with response to T in 55.5% and 66.6%, respectively. There were 4 patients who were HLA-DRB1*15/*13 and all of them responded to T. On the other hand, HLA-DRB1*11 was associated with refractoriness to T (11% response, p=0.01). *Conclusion:* Host related factors such as HLA may have impact on response to T similar to that observed in aplastic anemia and HLA-DRB1*15.

TABLE 1.

	T-responders	Tnon-responders	P
N=46	16 (35%)	30 (65%)	
Age (median, range)	50 (35-73)	50 (31-64)	0.38
Sex (M/F)	8/8	23/7	0.1
Paraprotein			
IgG	11	22	0.74
Non IgG	5	8	
Prognosis			
ISS-I	6	15	0.34
ISS-II or III	10	11	
No. of previous CT cycles (median, range)	1 (0-3)	2 (1-3)	0.02
Previous ASCT			
Present	9	25	0.07
Absent	7	5	
Del13q			
Positive	7	8	0.06
Negative	0	6	
P53			
Positive	1	7	0.42
Negative	1	2	
HLA-DRB1*15			
Positive	5	4	0.24
Negative	11	26	
HLA-DRB1*13			
Positive	8	4	0.01
Negative	7	25	
HLA-DRB1*11			
Positive	2	16	0.01
Negative	13	13	

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POLYMORPHISM OF THE ERYTHROPOETIN GENE PROMOTOR AND THE DEVELOPMENT OF MYELODYSPLASTIC SYNDROMES SUBSEQUENT TO MULTIPLE MYELOMA

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Background: The occurrence of myeloid malignancies after a diagnosis of multiple myeloma (MM) has been recognized for decades. Alkylating agents have long been considered to be part of the cause; the role of susceptibility genes remains unclear. Recently, we found the G/G genotype of the single-nucleotide polymorphism (SNP) rs1617640 in the erythropoietin (EPO) promoter, which is associated with decreased EPO expression, to be significantly ($p<0.001$) more common in myelodysplastic syndromes (MDS) pts (47/187; 25.1%) vs. healthy controls (6/95; 6.3%) (Ma et al, BMC Medical Genetics 2010;11:163). The aim of this study was to test if the rs1617640 G/G genotype is associated with development of MDS subsequent to MM. *Methods:* We conducted a nested case-control study including 17 MM patients who subsequently developed MDS, and 17 MM patients who did not develop MDS. Matching factors were age (+/- 4 yrs), sex, yr of MM diagnosis (+/- 1 yr), and duration of follow-up (+/- 6 months). DNA samples were available for all pts; however, 2 MM-MDS pts with poor DNA quality were excluded. The remaining 32 pts were tested for the EPO rs1617640 genotype. *Results:* 4/15 (27%) of the MM pts who developed MDS had the G/G genotype; only 2/17 (12%) of the controls showed the G/G genotype. The G/T genotype was found in 47% in both groups; MM pts who did not develop MDS had a higher fraction of T/T genotype (41% vs. 27%). *Conclusions:* Our data support a role for susceptibility genes in the development of second malignancies following a diagnosis of MM, likely in combination with MM therapies.

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REFLECTIVE SERUM PROTEIN ELECTROPHORESIS IS USEFUL IN EARLY DIAGNOSIS OF MYELOMA.

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Serum protein electrophoresis (SPE) is used to identify a paraprotein (PP) in patients suspected of having myeloma (MM) or other plasma cell dyscrasia (PCD). Some biochemistry laboratories perform reflective SPE when there is a raised total protein or globulin. There is limited evidence to support its practice. *Method:* Reflective SPE is performed at the North Middlesex University Hospital when the globulin level is >45 g/L. Clinicians were informed of positive results and patients asked to attend the haematology clinic. Patients were assessed with bone marrow and imaging according to published guidelines. *Results:* Between October 2009 and October 2010, 3361 biochemistry samples were found to have a globulin of >45g/L. Tests were initiated in primary care (12%), A+E (13%) and in hospital (62%), 13% unknown. PP was detected in 97 patients. 9 patients were

excluded due to a known PCD. Of the 88 patients with unknown PCD median age was 73y (35-90); 41% female. Isotypes were: 76% IgG, 10% IgA, 12% IgM, 2% Light chain. 52/88 (59%) patients were seen in a haematology clinic. Diagnoses were: MGUS (24 patients, 46%); smouldering MM (17 patients, 33%); symptomatic MM requiring treatment (6 patients,12%); asymptomatic Waldenstroms macroglobulinaemia (WM, 5 patients, 9%). *Conclusion:* Reflective SPE testing identifies a significant proportion of patients with MM and WM (56%) and, importantly, a small group of patients in whom treatment can be initiated for symptomatic MM thereby potentially avoiding morbidity and potentially mortality. Its routine use should be investigated.

P-412

IMPROVEMENT IN SURVIVAL OF MULTIPLE MYELOMA PATIENTS IN WELLINGTON, NEW ZEALAND BETWEEN 1986 AND 2010 IS RESTRICTED TO PATIENTS UNDER THE AGE OF 65 YEARS

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There have been significant changes in the management of multiple myeloma patients over the last twenty-five years. In order to assess the impact of these changes on the survival of patients with multiple myeloma, we conducted a retrospective audit of all patients diagnosed with multiple myeloma at our centre between 1st January 1986 and 31st October 2010. A total of 358 patients diagnosed with multiple myeloma were identified. The median age at diagnosis was 66.4 years (range 30 to 93 years), with 58% males and 42% females. Median overall survival was 32.8 months by Kaplin meier analysis. Using multivariate Cox Regression analysis of the demographic factors that influenced survival, only age and year of diagnosis were shown to have a significant impact. Prior to the 1st January 2000, median survival for patient diagnosed with multiple myeloma was 23.4 months, and after this date was 42.6 months (p=0.008, log rank score). For patients under the age of 65 years, the median survival improved from 31 months prior to 1st January 2000 to 64.3 months for patients diagnosed after this date (p=0.003), whilst for patients over the age of 65 years, median survival was not significantly different (14.2 months prior to the 1st January 2000, 22.8 months for those diagnosed after this date, p=0.198). Despite significant improvements in the management of multiple myeloma, patients over the age of 65 years have failed to show a significant survival benefit.

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IMPACT OF NOVEL M-COMPONENT BASED BIOMARKERS (HEVYLITE®) ON TO PROGRESSION FREE SURVIVAL (PFS) AFTER TREATMENT IN MULTIPLE MYELOMA (MM) PATIENTS

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Background: The depth of response has been associated with longer PFS in MM. Specific antibodies, have been recently developed, that bind the junction of the heavy and light chains on individual immunoglobulin isotypes (Hevylite™),making possible to quantify the IgGκ, IgGλ, IgAκ, IgAλ and their ratios IgGκ/IgGλ, IgGλ/IgGκ, IgAκ,/IgAλ and IgAλ/IgAκ (HLCRs) separately. *Aim:* To investigate the importance of normalisation of HLCRs at plateau on PFS. *Patients and Methods:* 51 intact immunoglobulin MM patients were studied from diagnosis to last follow up. All patients were symptomatic. Their sera samples (n=312) were analyzed for IgGκ, IgGλ, IgAκ, IgAλ with Hevylite™ antibody, nephelometrically. HLCRs values above the 95%-ile of normal individuals were considered as abnormal. File data were reviewed. *Results:* HLCR was abnormal in all patients at diagnosis. Median lines of therapy were 2 (range 1-11). Median follow up was 28 months (4-135). Retreatment was initiated in all patients according to standard criteria. As expected the quality of response correlated to PFS and patients in sCR, CR and nCR had a longer PFS than the others. HLCRs normalization only was a strong parameter of increased PFS (p=0,016) after treatment at any line. *Conclusion:* HLCRs normalization reflects prolonged responses.

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DUAL FEATURES OF AL AMYLOIDOSIS OCCURING AS RELAPSE OF MULTIPLE MYELOMA (MM) : AN AGRESSIVE AND RAPIDLY FATAL OR A MORE SMOLDERING DISEASE

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AL amyloidosis (ALA) is usually associated with a asymptomatic MM and development. Concomitant overt MM associated with AL amyloidosis is unfrequent. Conversely, ALA may occur in the course of MM and is often asymptomatic. However, ALA related organ disease may represent the main clinical features of MM relapse. We describe a series of 12 patients (pts) with MM (7M, 5F; median age 66 years; ISS2 : 7, ISS3 : 5) in whom ALA was diagnosed as relapse of MM. Immunoglobulin isotype was IgG k , IgA lambda or IgD k in 4, 2 and 1 cases, respectively. Light chain only MM was detected in 5 cases (4 lambda ;1k). No clinical feature of ALA was observed at diagnosis. Three pts had renal failure with light chain cast nephropathy. At diagnosis of ALA, pts had a median of 2 lines of treatment (1-5). MM Relapse was detected by a nephrotic syndrom (n=2), cardiac failure (n=5) or digestive manifestations (n=5). ALA was histologically demonstrated in 8 pts. In 9 pts ALA progress rapidly and death occured with 6 months (1-12). Median time to MM diagnosis was 3 years (1-5). In the 3 remaining cases, ALA occured after a period median of 7 years. ALA may occur as relapse of MM, either as an agressive and fatal disease or more slowly, after a long period of time. ALA may represent a feature of an agressive MM relapse, even in the absence of an overt ALA at diagnosis of MM. Understanding the molecular mechanism of this "acute" form of ALA is needed to prevent such fatal outcome.

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FREE LIGHT CHAIN CLONAL ESCAPE REFLECTS RELAPSE IN INTACT IMMUNOGLOBULIN MULTIPLE MYELOMA (MM)

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Background: Serum free light chains (sFLC) and ratio (sFLCR), are used for the evaluation of sCR in intact immunoglobulin (Ig) MM. Their use is not established for follow up and treatment initiation in intact Ig MM. *Aim:* To investigate the importance of sFLC and sFLCR elevation for the detection of clinical relapse, in the absence of any other clinical and laboratory finding. *Patients and Methods:* 51 intact immunoglobulin MM patients were studied from diagnosis to last follow up. Their sera samples (n=312) were analyzed for sFLC quantification using Freelite™ immunoassay. *Results:* Median lines of therapy were 2 (range 1-11). Median follow up was 28 months (4-135). Retreatment was initiated in all patients according to standard criteria. In 8/51 patients during remission (2 in plateau,1 in MR, 1 in PR, 3 in nCR and 1 in sCR) only sFLC and sFLCR increased gradually (light chain clonal escape) and shortly after they relapsed, (2 acute

renal failure,1 acute renal failure and rise of paraprotein, 2 plasmatocytomas, 1 liver plasmacytoma and rise of paraprotein, 1 rise of paraprotein and 1 plasmacytic leukemia).Median time from onset of light chain clonal escape to clinical relapse was 6 months (2-11months). *Conclusion:* Light chain clonal escape was observed during disease course in 15% of patients with intact Ig MM; shortly after they relapsed. Measurement of sFLC during follow up is useful for early detection of relapse in a subset of patients.

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NON-RANDOM CHROMOSOMAL DELETION CLUSTERING AT 20Q IN WALDENSTRÖM MACROGLOBULINEMIA

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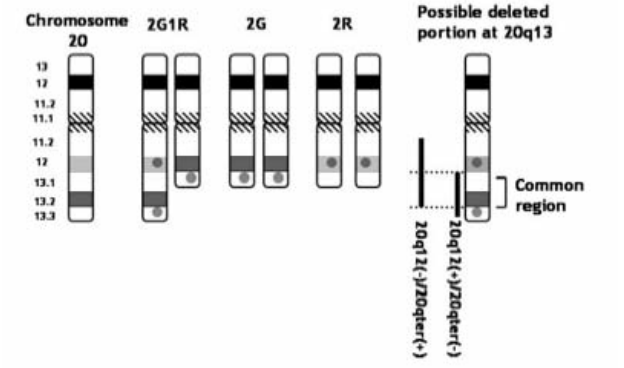
Chromosome change at 20q11-q12, including del(20q), is sometimes reported in plasma cell dyscrasia, but most cases are found during or after chemotherapy. It is therefore still uncertain whether del(20q) is a primary change or therapy-related. We performed cytogenetic studies and fluorescent in situ hybridization (FISH) analysis using 20q12 and 20qter probes to ascertain the possible involvement of 20q in nine patients with Waldenström macroglobulinemia (WM) The FISH study demonstrated deletions of 20q12 and/or 20qter in four of nine patients (44%) with WM at diagnosis, and one of them had the del(20q) chromosome. Moreover, one patient had de novo appearance of the del(20q) chromosome with 20q12 deletion after chemotherapy, although this patient had neither the del(20q) chromosome nor 20q12 deletion at WM diagnosis. Based on the results of this study, we conclude that chromosomal breakage at 20q13 is a non-random genetic change which plays a role in the neoplastic process of WM.



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PRE-STEM CELL TRANSPLANT (SCT) THERAPY DOES NOT AFFECT POST-TRANSPLANT SURVIVAL IN PATIENTS UNDERGOING SCT FOR LIGHT CHAIN (AL) AMYLOIDOSIS

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Objective: To ascertain the impact of pre-SCT therapy on the post-SCT outcomes in AL. Pts and *Methods:* From a prospectively maintained database, we identified 388 AL pts who underwent SCT within 12 months of diagnosis (dx), and within 3 mos of completing pre-SCT therapy, and form the study group. *Results:* The median (range) age of pts at SCT was 57 yrs (26-75); 229 (59%) were males. The median (range) duration from AL dx to SCT was 4 mos (1-12), and 252 (65%) pts were alive at time of analysis. 128 (33%) pts were conditioned with reduced dose melphalan (100-160 mg/m^2). Although pts who received pre-SCT therapy had higher BMPC% and more cardiac involvement, no survival difference was seen within each group: a) cardiac vs. no cardiac involvement; b) BMPC >10% vs. <10%. The median OS with (N=116) and without (N=272) pre-SCT therapy was NR and 94.7 mos, respectively (P=0.6) (Fig 1). Among pts who received pre-SCT therapy and were evaluable for response, the median OS for those with (N=34) and without hematologic response (N=36) was 82.1 and 50 mos (P=0.18), respectively (Fig 2). *Conclusion:* Among patients undergoing SCT for AL, pre-SCT therapy does not affect outcome. Among those who did receive pre-SCT therapy, there was a trend to poorer outcome among those with no hematological response, but this difference was not significant. However, this study does not address the possibility that pre-SCT therapy may allow sufficient improvement to allow SCT in an otherwise ineligible patient.

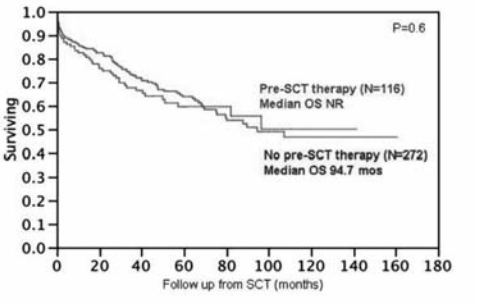

Fig. 1

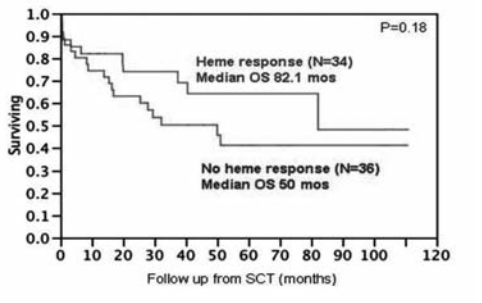

Fig. 2

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ASSESSMENT OF SERUM FREE LIGHT CHAIN ASSAYS BY MININEPH PLUS ANALYSER IN THE DIAGNOSIS OF MONOCLONAL GAMMOPATHY.

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Background: After the development of a reliable method to detect free light chains (FLC) in sera, several surveys have been conducted to evaluate their importance in the detection of plasma cell dyscrasias (PCD). *Aim:* To assess the input of serum FLC kappa and lambda assay in the diagnosis of monoclonal gammopathy (MG), in sera from Moroccan patients. *Methods:* We analyzed sera from 26 patients from the department of Hematology. The FLC assay was made on the Binding Site MININEPHplus nephelometric analyser, using Freelite reagents. The FLC assay results (κ/λ ratio) were interpreted and compared to the results of serum protein electrophoresis (SPE), and the final clinical diagnosis retained for each patient. *Results:* All sera presenting an abnormal κ/λ ratio 31% (8/26) are associated with clinical diagnosis of MG of Multiple Myeloma either complete immunoglobulin or FLC (n = 6), Plasmacytoma kappa light chain (n = 1), and Non-Hodgkin lymphoma (NHL) plasmablastic (n = 1). The sensitivity, specificity, positive and negative predictive values are summarized in the table. An abnormal κ/λ ratio, associated with clinical data can diagnose up to 80% of PCD, particularly multiple myeloma. *Conclusion:* In our series, the FLC assay allowed the diagnosis of all patients presenting with MG clinically confirmed. Thus the κ/λ ratio seems to be a sensitive and specific marker for the detection of monoclonal gammopathy.

Table: Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of FLC alone or combined to SPE, in screening for monoclonal gammopathy.

Parameter	Sensitivity	Specificity	PPV	NPV
SPE Alone	90%	25%	47%	80%
FLC Alone	80%	100%	100%	89%
SPE + FLC	100%	100%	100%	100%

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ENHANCEMENT OF BORTEZOMIB-INDUCED RETICULUM STRESS BY IGF1 IN MULTIPLE MYELOMA

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Multiple Myeloma (MM) is a clonal plasma cell disorder whose growth and proliferation are linked to a variety of growth factors, including insulin-like growth factor type 1 (IGF-1). Bortezomib, the first-in-class proteasome inhibitor, has displayed significant antitumor activity in multiple myeloma and has been suggested to induce apoptotsis by reducing NF-κB signalling and by enhancing reticulum stress resulting in an unfolded protein response (UPR). We analyzed the impact of recombinant IGF-1 combined with the proteasome inhibitor bortezomib on human plasma cell lines and fresh human myeloma samples. In all cases IGF-1 increased the sensitivity of multiple myeloma cells to bortezomib. In a xenograft model the combination of IGF-1 and bortezomib was significantly more potent to delay tumor growth than bortezomib alone. While a monoclonal antibody directed against the IGF-1 receptor was found to suppress the potentiation of bortezomib by IGF1 in vitro. Western blot analysis demonstrated that the combination bortezomib and IGF-1 caused an earlier and stronger induction of GADD153 and XBP-1s than either compoud bortezomib alone. Our results suggest that it is possible to specifically sensitize tumor cells to proteasome inhibitors by increasing the baseline level of ER stress. This can thus be assimilated to a tumor cell "priming" effect specific for cells with a high level of ER stress such as myeloma cells.

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MICRORNA-9* MODULATES HISTONE ACETYLATION IN WALDENSTROM

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Introduction: WM cells present with a reduced expression of miRNA-9* that has been predicted to target histone acetylation regulatory genes: we therefore looked at miRNAs as possible regulators of histone-acetylation in WM. *Material:* miRNA- and gene-expression-profiling have been assessed on primary WM and normal CD19+ cells. Functional assays were performed on WM transfected cells (scramble-, precursor(pre)miRNA-9*-probes). Signaling cascades and apoptosis have been evaluated by immunofluorescence and western-blot. DNA synthesis, cell survival, cell cycle progression were assessed by 3HdT uptake, MTT, PI, flow cytometry analysis, respectively. HDAC activity was tested by a colorimetric HDAC activity assay kit. *Results:* WM cells present with a decreased level of miRNA-9* vs normal cells (P< 0.01). Predicted targets for miRNA-9* included HDAC4, HDAC5 and histone-acetyltransferases (HATs). We found an unbalanced expression of HDACs and HATs in primary WM cells, at gene level; a decreased acetylated-histone-H3, -H4, at protein level, and an increased HDAC activity. miRNA-9* regulated histone-acetylation and HDAC activity in WM cells, leading to increased toxicity in pre-miRNA-9*-transfected cells, as shown by reduced proliferation, cell cycle arrest, induction of apoptosis, PARP- and caspases-cleavage. miRNA-9* induced autophagy in WM cells by modulating Rab7, LC3B. *Conclusion:* Histone-modifying genes and HDAC activity are deregulated in WM cells, driven by a reduced expression of miRNA -9* in the tumor clone, providing the basis for miRNA-targeted therapies in this disease.

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HIGH DOSE MELPHALAN WITH AUTOLOGOUS STEM CELL TRANSPLANTATION OVERCOMES POOR PROGNOSIS OF PRIMARY AMYLOIDOSIS.

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Background: High-dose melphalan followed by autologous hematopoietic stem cell transplant (auto HCT) is associated with improved outcomes in systemic light chain amyloidosis (AL). *Methods:* We identified 46 pts with AL who received auto HCT between 01/1998 to 05/2010 at MDACC. *Results:* Median age at auto HSCT was 56 years (34-74). Disease characteristics are summarized in Table 1. Median time from diagnosis to auto HCT was 6.6 mos (2.2-29.4 months). Median follow up from the time of diagnosis was 22.4 mos. High dose Melphalan dose was 200 mg/m^2 in 24 pts (52%). Out of the 38 evaluable patients, the post-transplant organ responses were: ≥PR 25(66%), >stable disease 35(92%) (Table 2).

Table 1: Disease Characteristics

Variable	N (%)
AMYLOIDOSIS SITE	
Bone Marrow	13 (28.3%)
Kidney	35 (76.1%)
Subcutaneous tissue	11 (23.9%)
Heart	11 (23.9%)
Gastrointestinal tract	10 (21.7%)
Tongue	4 (8.7%)
Lymph node	2 (4.3%)
Peripheral nerve	3 (6.5%)
Liver	4 (8.7%)
Skin	1 (2.1%)
Prostate	1 (2.1%)
BM Plasma cell category	
<10%	26 (56.5%)
≥10%	20 (43.5%)
Hemoglobin, g/dl median (min-max)	13 (7-17)
Hemoglobin <10g/dl	8 (17.4%)
Hemoglobin≥ 10g/dl	38 (82.6%)
Creatinine level	
Creatinine <2 mg/dl	39 (84.8%)
Creatinine ≥ 2 mg/dl	8 (17.4%)
Beta 2 microglobulin	
<3.5 mg/l	23 (50%)
≥3.5 mg/l	23 (50%)

The hematologic responses(HR) were: CR=5(13%), ≥VGPR = 10 (26%), ≥PR=26(68%), ≥SD=37 (97%). Organ response (OR) correlated with hematologic response (X2; p<10-3). Day-100 TRM was 8.7% and 1-yr TRM was 13%. Median PFS and OS from autoHCT was 73.8 mos and not reached. In multivariate analysis, heart involvement (p=0.01), female sex (p=0.011), age ≥60 yrs (p=0.002), BM plasma cells≥10% (p=0.043) and B2M >3.5 mg/L (p=0.02) were associated with poor OS. Improved OS correlated with OR (52.6 vs 11.4 mos; p=0.01) and HR (52.6 vs.6.1 mos; p=0.002). Hemoglobin <10 g/dL (p=0.047), BM plasma cells≥10% (p=0.043) and age≥60 years (p=0.075) were associated with shorter PFS. *Conclusion:* Pts with primary systemic AL amyloidosis have durable responses with auto HCT.

Table 2: Organ Response Distribution

Organ Involvement	Response Distribution
Kidney	ED=5, TE=2, SD=6, PR=13, MR=6, NR=1, NR=1, PD=2
Gastrointestinal Tract	TE=2, PR=4, SD=1, PD=1, ED=1
Heart	SD=1, PR=4, MR=3, TE=1, ED=1, VGPR=1
Liver	PR=4
Lymph Node	PR=1, SD=1
Peripheral Neuropathy	SD=1, NR=1, PR=1
Tongue	SD=1, PR=3
Prostate	PD=1
Skin	PR=1

ED=early death, TE=too early to evaluate, PD=progressive disease, NR=no response, MR=minimal response, SD=stable disease, PR=partial response.

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TRENDS AND OUTCOMES OF MODERN STAGING OF SOLITARY PLASMACYTOMA OF BONE (SPB)

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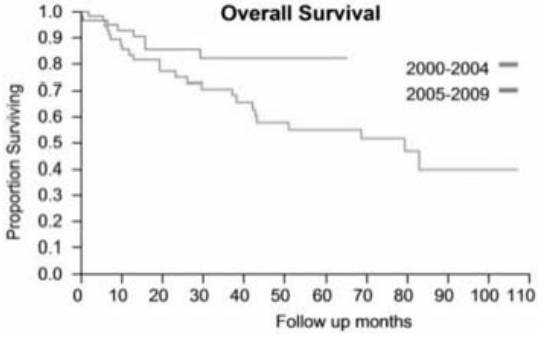
SPB is a rare localized plasma cell dyscrasia (PCD) with no evidence of systemic disease. The majority of patients with SPB progress to multiple myeloma (MM) with a median disease free survival of 5 to 10 years. Our hypothesis was that the low rate of cure of SPB is in part due to inadequate clinical staging, i.e. occult bone or bone marrow involvement. A retrospective analysis of 127 consecutive Mayo Clinic patients diagnosed with SPB between 2000 and 2010 was done. The trends in imaging techniques (magnetic resonance imaging, computed tomography, and nuclear medicine imaging), immunohistochemistry and flow cytometry were evaluated. Inclusion criteria were 1) patients with biopsy proven plasmacytoma; 2) no evidence of systemic disease on skeletal survey; and 3) fewer than 10% plasma cells on bone marrow biopsy. Patients with any form of systemic disease defined as anemia, hypercalcemia or renal injury related to PCD were excluded. Overall survival was calculated using the Kaplan-Meier method. There was a significant increase in the use of CT-PET (p =0.002) and flow cytometry (p =0.005) for initial staging in the latter half of the decade. In addition, overall survival was significantly improved in the latter 5 years, p=0.05 (figure). These data suggest that more optimistic estimates of overall survival may be appropriate for patients with SPB in the modern era when more advanced staging is employed.



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THE ROLE OF HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN PATIENTS WITH POEMS SYNDROME (POLYNEUROPATHY, ORGANOMEGALY, ENDOCRINOPATHY, PARAPROTEINAEMIA AND SKIN CHANGES): A RETROSPECTIVE STUDY OF THE MM SUBCOMMITTEE OF THE CHRONIC LEUKEMIA WORKING PARTY OF THE EBMT.

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POEMS syndrome is a rare syndrome. Effective treatment can control the disease & symptoms. The aim of this study was to describe the clinical outcome of ASCT, determine the impact of key prognostic factors, the incidence of engraftment syndrome & pattern of relapse. Variables were collected according to the data entries in the EBMT database, including tracking incomplete data entries.113 patients underwent an ASCT between 1997-2009 & satisfied the entry criteria. The median age was 50 yrs (range 26-69). The median time to ASCT was 8.2 mns (range 1-346) with 35.4% >12 months from diagnosis. Haematological disease status at ASCT was: 30% CR/PR, 31% SD/MR & 12 in PD. Engraftment was seen in 97.3% of ASCT. Details of the occurrence of engraftment syndrome are currently under analysis. Best clinical disease response will be presented. With a median follow-up of 7.8 mns (range 0.1-132), 94.7% of patients are alive & only 8% of patients have relapsed. The non-relapse mortality was 4.4%. Causes of death:infection(n=4), graft failure(n=1), cardiac toxicity(n=1). The 3-yr PFS & OS are 76% & 93%, respectively. This data demonstrates that ASCT is an effective & safe therapeutic modality in POEMS syndrome.

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A PATIENT-ADAPTED PILOT STUDY OF LENALIDOMIDE IN COMBINATION WITH LOW DOSE DEXAMETHASONE (LD) AS INITIAL THERAPY FOR PRIMARY PLASMA CELL LEUKEMIA (PPCL)

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On March, 2009, we started the first clinical trial aiming to evaluate safety and antitumor activity of Ld in previously untreated PPCL. Newly diagnosed patients received lenalidomide at a dose of 25 mg daily for 21 days and oral dexamethasone at a dose of 40 mg daily on days 1, 8, 15, and 22 for each 28-day cycle. After 4 cycles, responding patients not eligible for stem cell transplantation (SCT) continued until 8 cycles of full-dose Ld, if tolerated, followed by a maintenance dose of single agent lenalidomide equal to 10 mg/day on days 1-21 of each 28-day cycle. Patients responding after 4 Ld cycles and eligible for SCT proceeded according to single Centre transplant policy. So far, 20 out of 22 planned patients have been enrolled (median age 66 years, range 45-81). On intention-to-treat analysis, eleven out of 18 evaluable patients who completed 4 Ld cycles achieved at least PR (61.1%), with 39% of patients achieving at least VGPR. Causes of early interruption were PD (four patients), adverse events (1 acute renal insufficiency, 1 Stevens-Johnson's syndrome), one death in PR due to causes unrelated to treatment or disease. OS and PFS at 12 months were 77% and 50%, respectively. Four out of 6 eligible patients underwent autologous SCT after Ld therapy (one patient refused, one patient failed to collect PBSC): all these patients are alive and in remission phase. This interim analysis suggests that Ld is a promising initial therapy for PPCL, which can rapidly control the disease in the majority of cases, allowing following single patient-adapted therapeutic strategies.

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BASELINE PLASMA VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) LEVELS IN POEMS AND RELATED DISORDERS

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The POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes, sclerotic bony lesions, fluid overload and thrombocytosis) is a high VEGF state. Several studies have compared serum VEGF among diseases, but it is controversial if serum VEGF is reliable and reproducible given variable platelet VEGF release. We therefore evaluated plasma VEGF in patients with POEMS and related disorders. We analyzed plasma VEGF done in our center between 12/5/2005 to 7/31/2010. These were patients with POEMS (70), Castleman's (26), myeloma (17), MGUS (5), amyloidosis (5), peripheral neuropathy/PN (48), connective tissue disease &/or vasculitis (17), capillary leak syndrome/CLS (3) and others (18). Patients with baseline plasma VEGF were separated. Our results are shown in table 1. We show that plasma VEGF can distinguish POEMS from other plasma cell neoplasms and PN. Given that plasma VEGF is more reliable by being unaffected by platelet count and/or activation as opposed to serum VEGF, we propose using plasma VEGF to aid in the diagnosis of POEMS.

Table 1 Baseline plasma VEGFa levels

	POEMS b,c,d	Castleman's ds b,c,d	Myeloma	MGUS	Amyloidosis	PN	CLS	CTD &/or vasculitis	Other
Number (105)	29	9	9	2	4	29	2	9	12
Median	342	412	68	86.5	38.5	50	124.5	142	45.5
Range	48-2112	132-2030	35-271	64-109	31-42	31-180	113-136	35-40	31-134
Inter-Quartile Range	122.5-462.5	195.5-1285.5	57.5-106.5	64-109	32.2-41.7	31-72.5	113-136	53.5-292	38-81.5

[a] Normal 31-86 pg/ml
[b] p<0.0001 POEMS/Castleman's vs other plasma cell dyscrasia
[c] p<0.0001 POEMS/Castleman's vs PN
[d] p 0.0089 POEMS/Castleman's vs CTD/vasculitis

P-425

LENALIDOMIDE IS EFFECTIVE FOR EXTRAMEDULLARY DISEASE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background: our study reports the role of lenalidomide for EP in daily clinical practice. *Methods:* we analyzed the clinical data of 18 patients (eight females, median age 68 years) with refractory or relapsed multiple myeloma (MM) with EP treated with lenalidomide outside of clinical trials in 12 GEM/PETHEMA centers between October 2007 and July 2010. The treatment consisted on lenalidomide ranging from 10 to 25 mg, given on days 1-21 of a 28-days cycle. The response rate of MM was evaluated according to the international criteria and the response of EP by measuring size changes. The median observation time was 12 months (3–24). *Results:* A median of 3 previous lines of therapy (1-6) were given, including autologous stem cell transplantation (4/18), and bortezomib (18/18) and thalidomide (3/18). Median number of lenalidomide cycles administered was 7 (3 - 21) with a maximum response after a median of 3 cycles (2 - 10). The overall response rate (ORR) of MM was 61.1%, (complete response (CR) 16.6%, very good partial response (VGPR) 22.2%). EP disappeared in 8/18; EP size decreased in 3/18. The progression free survival (PFS) and overall survival (OS) were 9.8 and 14.6 months, respectively. Lenalidomide toxicity was predominantly hematologic (8/18) and; the incidence of venous thrombotic events was low (1/18). *Conclusions:* our results suggest lenalidomide could be an effective and manageable drug for advanced myeloma with EP. A randomised trial is needed to assess the role of lenalidomide for EP.

P-426

DISTURBANCES OF CARDIAC RHYTHM IN AL AMYLOIDOSIS

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Many patients with cardiac AL amyloidosis die suddenly. The aim of our study was to determine the spectrum and frequency of arrhythmias in patients with AL amyloidosis. Patients with AL amyloidosis attending our centre between May and October 2010 underwent 24 hour Holter monitoring in addition to their standard clinical assessment. Fifty patients had cardiac amyloidosis and 26 patients did not. Patients with cardiac amyloid had a median IVSd of 15 (range 11 – 28) mm versus 12 (range 9-20) mm in those without. Small complexes were seen in 17 cardiac patients (34%) compared to 5 (19.2%) non-cardiac patients. 5 (10%) cardiac patients were in AF compared to 1 (3.8%) non-cardiac patient. All arrhythmic activity was asymptomatic in both groups. Arrhythmias were more commonly seen in patients with cardiac involvement (figure 1). Atrial ectopics (AEs) and ventricular ectopics (VEs) were common in both groups with significantly more VEs in the cardiac group (figure 2). 4 patients in the cardiac group had asymptomatic non-sustained VT including one who had a previous syncopal episode who was admitted for an ICD. More patients in the cardiac group were already taking anti-arrhythmic medications at the time of the Holter monitor. Holter monitoring revealed frequent asymptomatic arrhythmias. Further work is required to determine whether any particular arrhythmias predict sudden cardiac death and to establish the role of anti-arrhythmic therapy in this disease.

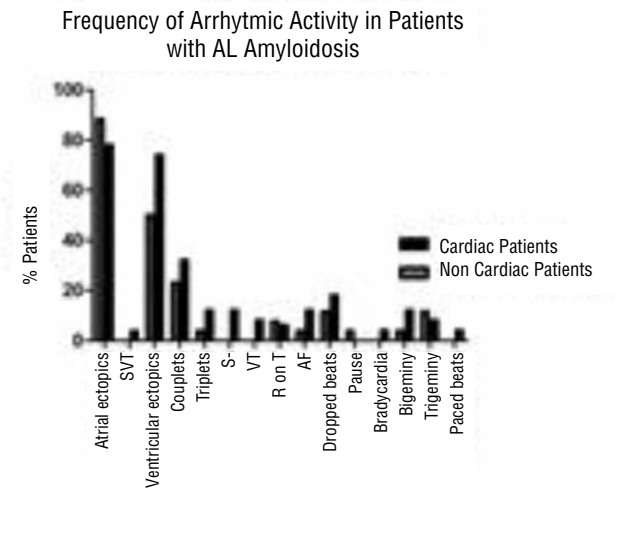
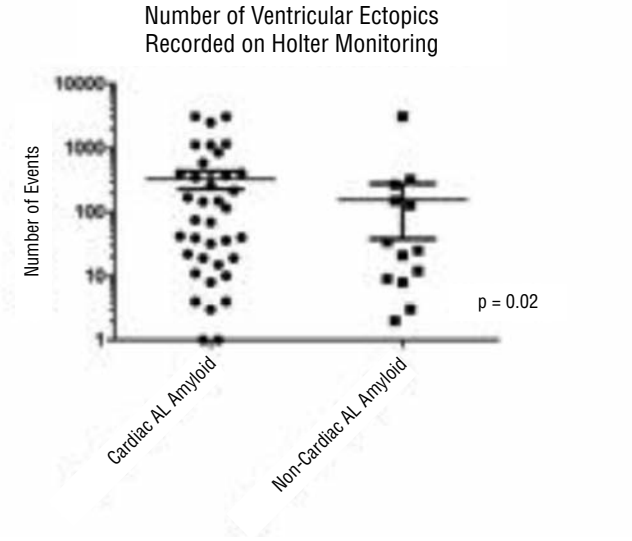


Figure 2



P-427

A PHASE II STUDY OF RISK-ADAPTED INTRAVENOUS MELPHALAN IN PATIENTS WITH AL AMYLOIDOSIS

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Background: High-dose IV melphalan is standard autograft (ASCT) conditioning in patients with AL. In patients ineligible for ASCT, intermediate-dose IV melphalan (IDM) may provide advantages over oral dosing where the already variable intestinal absorption is complicated by gastrointestinal amyloid deposition. *Methods:* Transplant candidates (minimal cardiac disease including BNP<300ng/L, age <65, ECOG<2, <2 organs involved) received melphalan at 140 or 200mg/m^2. All other patients received melphalan 20mg/m^2 IV d1 and dexamethasone 40mg PO d1-4 every 4 weeks for 3-6 cycles. *Results:* 21 patients enrolled with median age 61yrs. Organ involvement was cardiac 48%, renal 81%, liver 14%, neurologic 43%. 6, 10 and 5 were low, intermediate and high cardiac biomarker risk, respectively. 7 underwent ASCT and 14 IDM. The trial closed early due to excessive myelotoxicity in the IDM arm. In this cohort, grade 3/4 neutropenia and thrombocytopenia during the 1st cycle occurred in 54% and 23%, with 15% having neutropenic fever. First cycle severe neutropenia was not predicted by age, cardiac or renal function but was significantly more common with lower body surface area (p=0.01). 8 IDM patients died before the 6 month response assessment, 2 achieved a 50% reduction in baseline involved FLC and 4 failed to respond. All 7 patients receiving ASCT are alive at a median of 33 months. *Conclusions:* IV melphalan at 20mg/m^2 is too myelotoxic for patients with AL. BNP<300ng/L may identify patients suitable for ASCT. This study was supported by the Leukaemia Foundation of Queensland and Amgen Australia.

P-428

BASES OF SENSITIVITY OF LIGHT CHAIN AMYLOIDOSIS TO PROTEASOME INHIBITORS: ASSESSING PROTEOSTASIS AND PROTEASOME STRESS

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Proteasome inhibitors (PI) are established therapeutic agents against multiple myeloma (MM), with the balance between proteasome expression and degradative workload contributing to determine apoptotic sensitivity to PI. Light chain amyloidosis (AL) is a plasma cell dyscrasia caused by a bone marrow (BM) plasma cell clone synthesizing structurally unstable monoclonal Ig light chains, which polymerize into toxic amyloid fibrils. Interestingly, AL patients respond even better than MM patients to PI in clinical trials, raising the question as to whether, and if so why, AL cells are intrinsically more sensitive to PI than MM. Here we tested the hypothesis that AL cells face constitutive proteotoxicity due to misfolded Ig light chain synthesis. In purified patient-derived AL cells, we investigated the intrinsic sensitivity to the PI bortezomib, proteasome activity, and ubiquitinated (Ub) proteins and Ig light chain accumulation. Our studies demonstrate: 1) a significantly higher PI sensitivity of AL cells as compared to primary MM cells; 2) a strong correlation between accumulation of Ub proteins and light chain content; 3) a great inter-individual variability of proteasome activity, similar to MM. Moreover, unlike MM cells, AL cells don't show a clear correlation of proteasome activity with PI sensitivity, supporting the hypothesis that amyloidogenic chains are intrinsically toxic to AL cells. Our technological platform enables to investigate proteostasis and proteotoxic stress in primary AL cells to identify molecular mechanisms of potential prognostic and therapeutic use.

P-429

PROGRESSION OF IDIOPATHIC SYSTEMIC CAPILLARY LEAK SYNDROME (SCLS) TO MULTIPLE MYELOMA (MM): A SINGLE INSTITUTION EXPERIENCE

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Background: Idiopathic SCLS is a rare, difficult to diagnose and often a fatal disorder characterized by recurrent attacks of hypovolemic shock, generalized edema, hemoconcentration and hypoalbuminemia. All four features are required for diagnosis. The M protein, present in majority, is the only pertinent abnormality during quiescent phases. It is unclear whether dysproteinemia is pathogenic or a peripheral phenomenon. Reports of SCLS evolving to MM and other plasma cell disorders exist. However, the rate of transformation to MM is not known. Our goal was to assess the rate of progression of SCLS to MM in the largest well-defined cohort of SCLS to date. *Results:* We reviewed records of all patients presenting to our institution between 1981 and 2008 who were presumed to have SCLS. 25 patients fulfilled all the diagnostic criteria. The median age at diagnosis was 44 years and median follow-up was 4.9 years. The 5-year overall survival was 76%. Monoclonal gammopathy, predominantly of the IgG-κ type, was found in 19 (76%) patients. Recurrence or severity of attacks of SCLS did not correlate with the size of M-spike. One patient progressed to MM after 17 years. This progression was heralded by

an abrupt increase in M protein along with renal insufficiency and anemia. The progression rate to MM was 0.7% per person-year of follow-up for the cohort. *Conclusion:* The rate of transformation of SCLS to MM appears to be comparable to that of 1% per year reported for monoclonal gammopathy of undetermined significance. Surveillance for MM is therefore necessary in patients with SCLS.

P-430

NO IMPACT OF SYSTEMIC HYPOGAMMAGLOBULINEMIA (SH) AND IMMUNOSUPPRESSION OF THE SAME IG CLASS (ISC) ON TIME TO FIRST TREATMENT AND OVERALL SURVIVAL IN WALDENSTROM'S MACROGLOBULINEMIA (WM) PATIENTS

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Background: SH is a very common finding in WM. Low levels of IgG have been associated with disease progression. Specific polyclonal antibodies, that recognise epitopes spanning the junction of the heavy and light chains of the individual immunoglobulin isotypes have been recently developed (Hevylite™). Decreased concentrations of polyclonal isotypes of the same Ig class have been associated with shorter progression free survival in multiple myeloma. *Aim:* The purpose of the study was to evaluate the effect of SH and ISC on time to first treatment (TTT) and overall survival (OVS) in a series of WM patients. *Methods:* Retrospective sera from 70 WM patients at diagnosis were included in the study. Median age was 66yrs (range 44-91), half were males. Analysis of IgG and IgA was performed using standard antibobies, while IgMκ and IgMλ with Hevylite™ antibody, nephelometrically. SH was defined as IgG<700 mg/dl, IgA<70mg/dl or both, ISC as IgMλ<10mg/dl in IgMκ patients or IgMκ<10mg/dl in IgMλ ones. *Results:* 48 out of 70 patients were or became symptomatic during follow up. Median follow up was 37 months. At diagnosis median IgG was 896 mg/dl (206-4750), median IgA 89 mg/dl (22-638), median IgMκ 2000mg/dl (7-17300) and median IgMλ 28mg/dl (1-13000). SH was present in 34/70 patients, ISC in 18/70 and both in 9/70. Sixteen patients died from disease. Neither SH nor ISC or both correlated with TTT (p=0,358, p=0,874 and p=0,718 respectively) or OVS (p=0,159, p=0,817 and p=0,854 respectively).

P-431

VALIDATION OF DFLC METHOD FOR MONITORING CLONAL RESPONSES IN SYSTEMIC AL AMYLOIDOSIS: SUPERIOR OUTCOME OF PATIENTS ACHIEVING >90% RESPONSE (VGPR)

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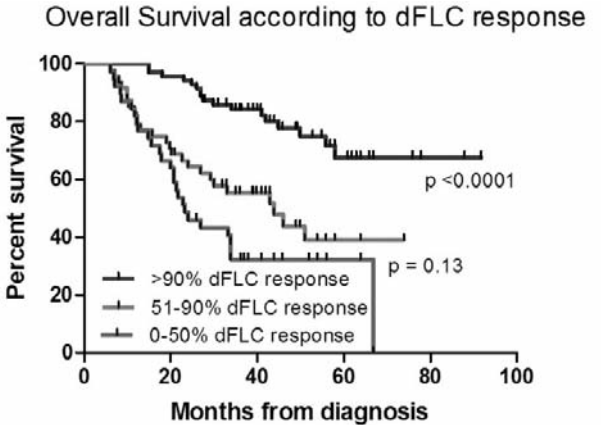
In AL amyloidosis, the criteria for assessing clonal disease response have been adopted from myeloma, although the target for reduction in serum free light chain (FLC) concentration and value of whole paraprotein measurements remain unclear. We analysed the outcomes of 159 AL patients who survived 6 months or greater, treated with cyclophosphamide, thalidomide and dexamethasone (CTD) in whom conventional analyses of clonal response were compared with the dFLC method, in which the difference between the involved amyloidogenic and uninvolved FLC concentrations is monitored. Reductions >50% in dFLC occurred in 120 (75%) patients, including >90% reduction defining a very good partial response (dFLC-VGPR), in 72 (45%) cases. Overall survival (OS) was superior in patients with dFLC-VGPR compared to those with poorer dFLC responses (median not reached vs. 29 months; p<0.0001). OS was not superior among patients who achieved complete response (CR) using conventional criteria compared to those who achieved dFLC-VGPR, even when a whole paraprotein persisted in the latter cases (p = 0.98). Median time to next treatment was 60 months with dFLC-VGPR vs. 16 months with dFLC-PR (p <0.0001), and amyloidotic organ responses were more frequent among the dFLC-VGPR group (58% vs 25%; p = 0.0003). These findings validate the dFLC method of monitoring clonal responses, support the use of dFLC-VGPR rather than dFLC-PR as the primary treatment goal and highlight the limited importance of a whole paraprotein CR in clonal measurements in AL amyloidosis.



P-432

VITAMIN D LEVELS IN AL PATIENTS UNDERGOING PBSCT

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Levels of vitamin D have been shown to be predictive of survival in a variety of neoplasms, including non-Hodgkin's lymphoma, multiple myeloma and chronic lymphocytic leukemia. Levels of 25-hydroxyvitamin D {25(OH)D} and 1,25-dihydroxyvitamin D {$1,25(OH)_2D$} were measured using stored serum from 116 AL patients that underwent peripheral blood stem cell transplant (PBSCT) at the Mayo Clinic. Levels of total 25(OH)D and $1,25(OH)_2D$ were measured on stored serum collected within 30 days of PBSCT. The mean value for 25(OH)D was 14 ng/mL and $1,25(OH)_2D$ was 28 ng/mL . Levels of both 25(OH)D and $1,25(OH)_2D$ correlated positively with renal involvement (p<.0001), but not cardiac, hepatic or neurologic involvement. No overall impact on survival was seen (p<.07). Total vitamin D levels also correlated strongly with both serum albumin levels and renal protein loss. Levels did not predict the likelihood of renal response. Levels of 25(OH)D and $1,25(OH)_2D$ in AL patients undergoing PBSCT were the lowest seen in any cohort examined to date. The strong correlation with renal involvement and urinary protein loss supports previous observations of vitamin D deficiency in patients with nephrotic syndrome, likely as a result of elevated excretion of vitamin D binding protein. Despite the lack of a detectable impact on overall survival in this group of patients, the severity of vitamin D deficiency supports aggressive replacement in AL patients, particularly those with renal involvement.

P-433

ALCHEMY - A PROSPECTIVE STUDY OF CHEMOTHERAPY IN AL AMYLOIDOSIS

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Clinical trials in AL amyloidosis are hampered by rarity, lack of validated endpoints, cost, and, crucially, exclusion of poor prognosis patients. ALchemy was designed to collect comprehensive treatment, outcome and toxicity data in newly diagnosed patients attending a single national referral centre over a 3 year period. Chemotherapy recommendations were made in line with current UK practice, but at

the discretion of treating haematologists. We present here data from 175 patients recruited during the first 16 months. At baseline, 20% of patients were Mayo stage 1, and 40% each had stage 2 and 3 disease. Renal (49%) and cardiac (31%) presentations were most common. CTD, melphalan- and bortezomib-containing chemotherapy regimens were administered first-line to 72%, 10% and 6% of patients respectively; 9 patients (6%) died prior to starting, and a further 9 patients are awaiting chemotherapy. Toxicity, ≥grade 3, occurred in 55%, and there were 84 hospitalisations among 73 patients during first-line treatment. Central review after 3 cycles revealed that 36%, 40% and 24% of 86 evaluable patients had achieved a FLC CR, PR and NR respectively. Inadequate response prompted recommendation of a change in chemotherapy, predominantly to bortezomib (82%), among 34% of cases. After median follow-up from baseline of 6.1 months, 29% of patients had died, with non-responders, those with Mayo stage 3 disease and/or a cardiac presentation at greatest risk. Prognosis remains dismal among a substantial proportion of patients with AL amyloidosis for whom new therapies are urgently required.

P-434

OUTCOME OF AL AMYLOIDOSIS AFTER HIGH-DOSE MELPHALAN AND AUTOLOGOUS STEM CELL TRANSPLANTATION: LONG-TERM RESULTS IN A SERIES OF 421 PATIENTS

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Treatment of AL amyloidosis with high-dose melphalan and autologous stem-cell transplantation (HDM/SCT) results in a median overall survival (OS) of about 5 years. Previous studies have suggested that the greatest benefit is seen in patients achieving hematologic complete response (CR). We analyzed a series of 421 consecutive patients treated with HDM/SCT at a single referral center and compared outcomes for patients with and without CR. Treatment-related mortality was 11.4% overall, decreased to 5.6% in the last 5 years. For the entire group, the median event-free survival (EFS) and OS were 2.6 and 6.3 years, respectively. Of 340 patients evaluable at 1 year beyond HDM/SCT, 43% achieved CR and 78% of them experienced an organ response. For CR patients, median EFS and OS were 8.3 and 13.2 years, respectively. Among the 195 patients who did not obtain CR, 52% reached an organ response, and the median EFS and OS were 2 and 5.9 years, respectively. A subgroup of 26% of the non-CR patients remained clinically stable at 5 years of follow-up. In summary, treatment of selected AL patients with HDM/SCT resulted in a high organ response rate and long OS even for those patients who did not achieve CR.

P-435

ESTABLISHMENT OF SERUM FREE LIGHT CHAIN REFERENCE INTERVALS IN A CHINESE POPULATION

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Introduction: The detection and quantification of monoclonal proteins is important for the management of patients with plasma cell dyscrasia (PCDs). Serum free light chain (sFLC) assays provide increased sensitivity relative to urine protein electrophoresis (UPE), however, the 0.26-1.65 sFLC ratio reference intervals (RI) have not been validated in a Chinese population. *Methods:* sFLC RIs were determined using 326 healthy, Chinese donors, aged 20-86 years (160 female and 166 male). The sensitivity and specificity of the sFLC ratio cutoffs and UPE analyses were determined using 68 pre-treatment, immunofixation positive samples (64 multiple myeloma (MM), 1 smoldering MM and 3 NHL) and 56 immunofixation negative samples from patients without PCD. *Results:* The 100% range for the FLC ratio in our Chinese cohort was 0.32-1.52. By ROC analyses, both the 0.32-1.52 and 0.26-1.65 ratio cutoffs demonstrated higher diagnostic sensitivity and specificity (AUC of 0.97 and 0.98 respectively) than UPE analyses (AUC 0.81). Serum FLC kappa/lambda (or lambda/kappa) ratios in the range of 10-50 were observed in 68% of UPE negative samples. For the test cohort, a MM screening panel of SPE plus sFLC assays achieved 100% diagnostic sensitivity, irrespective of the FLC RI used, compared to 97% for UPE plus SPE. *Conclusions:* The sFLC ratio cutoffs of 0.26-1.65 are both sensitive and specific in a Chinese population and provide significantly improved diagnostic sensitivity relative to UPE analyses. The screening panel of SPE plus sFLCs has a comprehensive diagnostic sensitivity for MM.

P-436

A RETROSPECTIVE AUDIT ON THE DIAGNOSTIC WORKUP OF PATIENTS WITH A IGM PARAPROTEIN REFERRED TO OUR UNIT FOR IMMUNOPHENOTYPING AND CYTOGENETICS.

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IgM MM and WM are both characterised by an IgM paraprotein. We conducted an audit on patient's bone marrow samples to determine whether appropriate investigations had been undertaken to reach the correct diagnosis. We used two key criteria to distinguish between MM and WM. The presence of lytic bone lesions and/ or fusion IGH/CCND1 favoured a diagnosis of MM over WM. If neither were present, a diagnosis of WM was assigned which in conjunction with cytogenetic abnormalities such as 6q deletion, trisomy 3 or trisomy 12, lent weight to the diagnosis. In total there were 20 samples referred. All had a bone marrow examination, of which 16 were referred for cytogenetics. FISH panels identified three positive for 6q deletion, one for trisomy 3 and one for trisomy 12. Two samples were positive for IGH/CCND1 fusion. Five samples failed on FISH and five were negative. All but two patients had either a staging CT scan or skeletal survey of which lytic lesions were found in one. Two were diagnosed with MM, eighteen with WM/LPL. In conclusion, we identified 9/20 patients who had insufficient workup to confidently distinguish between MM and WM. There are a number of novel agents which can be used upfront in IgM paraproteinaemias, for example Bortezomib in patients with WM underscoring the importance of reaching a correct diagnosis on presentation with the use of cytogenetics and appropriate radiological investigations.

P-437

UTILITY OF PLASMA VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) LEVELS IN THE FOLLOW-UP OF PATIENTS WITH POEMS

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The POEMS syndrome is associated with high VEGF, and VEGF levels correlate with disease activity. Serum VEGF levels may be unreliable owing to variable VEGF release from platelets. We evaluated plasma VEGF levels at various time-points after treatment in POEMS. We analyzed plasma VEGF done as part of clinical care in 209 patients from our institution between 12/5/2010-7/31/2010. Of 96 POEMS patients, 25 had serial plasma VEGF measurements at diagnosis and after treatment. We evaluated whether there was a difference in VEGF between non-CR and CR patients at last follow-up and at a uniform time point (day 180) after therapy. Complete Response (CR) was defined according to the IMWG criteria. 72% were male with median age of 52.1 at diagnosis. Table 1 lists primary treatments. All patients who had therapy had improvement in their clinical manifestations, but only 10 (40%) achieved CR. The median follow-up of these patients was 304 days (range 31-947 days) and the median number of VEGF measurement was 3. We found no significant difference in plasma VEGF levels between these two groups (table 2). Plasma VEGF levels correlate with clinical improvement. There was no correlation between VEGF and depth of response.

Table 1 Primary therapy

	ASCT	Radiation	Other
Number	17	5	3
CR	8	1	1

Table 2 Plasma VEGF levels

	POEMS
Number	25
Median VEGFa (Inter-Quartile range)	342 (156-503)
Plasma VEGF on day 180[b]	
CR (n=7)	131
Non-CR (n=7)	75
Median VEGF at last follow-up	
CR (n=10)	59.5
Non-CR (n=15)	69

aNormal 31-86 pg/ml. bRange 114-255 days

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EUROPEAN COLLABORATIVE STUDY OF 153 PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS WITH MAYO STAGE III DISEASE

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N-terminal fragment of BNP (NT-proBNP) and cardiac troponin –T (TnT) or I (TnI) form a useful staging system in AL amyloidosis. Poor outcomes were reported in stage III patients treated before routine use of novel agents. We report the outcomes of 153 patients with Mayo stage III AL amyloidosis seen at amyloid centres in London (UK), Pavia (Italy) and Athens (Greece). Presenting features were [n(%)/median (range)]: cardiac, renal and liver involvement in 145 (95%), 111 (72%) and 22 (14%) respectively, NT-proBNP 8392 ng/L (701-75000); TnI - 0.18 ng/ml (0.1-2); TnT -0.09 ng/ml (0.04-1.83);IVS 15 mm (7-24). 50%/0%, 18%/23%, 12%/25%, 6%/17% and 0%/42% of patients treated with bortezomib, CTDa, MDex, MDex-Thal and CRevDex achieved complete (CR) and partial (PR) responses respectively on intent to treat basis. The median overall survival (OS) was 7.6 mos. Among 94 (61%) treatment response evaluable patients, 24 months estimated OS from assessment was 95%, 62% and 25% for CR, PR and non-responders, respectively. NT-proBNP > 7932 ng/L (HR 1.7; p=0.017) and ECOG performance status ≥3 (HR 1.8; p=0.006) were independent predictors of poorer OS and patients with none (n=59 - stage IIIa) or either NT-proBNP or ECOG above thresholds (n=94 - stage IIIb) had an OS of 29 and 5 mos respectively (p<0.0001) with 73% deaths occurring in stage IIIb cases. Achieving a rapid CR should be the treatment goal in stage III AL amyloidosis. OS has improved for stage IIIa patients but clinical trials using novel low toxicity treatment for stage IIIb patients are urgently needed.

P-439

THE NEW HIGH-SENSITIVITY ASSAY FOR CARDIAC TROPONIN T (HS-CTNT) CAN BE USED FOR CARDIAC STAGING IN PATIENTS WITH AL AMYLOIDOSIS

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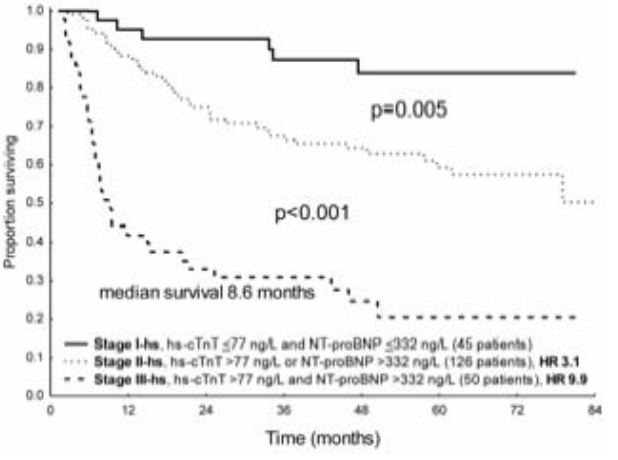
Survival of patients with AL amyloidosis is dictated by the severity of heart involvement, which is best assessed by cardiac troponins (cTn) and N-terminal proBNP (NT-proBNP). A staging system based on standard cTn and NT-proBNP is used to assess prognosis and eligibility to clinical trials. In most European clinical laboratories high-sensitivity (hs) cTn is replacing cTn, and in AL amyloidosis hs-cTnT is the single most powerful prognostic determinant. Thus, there is the need to assess whether an hs-cTn based staging can be used in AL amyloidosis. A total of 221 patients (71% with cardiac involvement) diagnosed between 2004 and 2010 at 2 European referral centers were included. At diagnosis median hs-cTnT was 35 ng/L, NT-proBNP 1794 ng/L, difference between involved and uninvolved free light chains (dFLC) 143 mg/L, and glomerular filtration rate 65 mL/min per 1.73 m^2 (<15 mL/min per 1.73 m^2 in 4%). Median survival was 77 months, median follow-up of living patients 53 months. The hs-cTnT cutoff best predicting survival was 77 ng/L. With this cutoff and the commonly used 332 ng/L NT-proBNP threshold, we identified 3 groups with different outcomes (Fig. 1). Among stage III patients, those with NT-proBNP >6000 ng/L and dFLC >100 mg/L had an extremely poor survival (median 5 vs. 25 months, p=0.006). These patients are unlikely to have time to respond to treatment and might benefit from heart transplant followed by chemotherapy, if cardiac involvement is isolated and age ≤60 years. A staging based on hs-cTnT and NT-proBNP can accurately stratify patients with AL amyloidosis.



P-440

OUTCOME PREDICTION IN SOLITARY PLASMACYTOMA OF BONE (SPB): DEVELOPMENT OF A RISK STRATIFICATION MODEL UTILISING BONE MARROW FLOW CYTOMETRY AND LIGHT CHAIN ANALYSIS

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Local irradiation is the treatment of choice in SPB but approximately 50% will progress and the median time to progression (TTP) is short at <2 years. Given that local relapse rates following irradiation are low it seems likely that progression is due to the presence of occult marrow disease (OMD) which is not demonstrable by standard staging procedures. In this study we have used multiparameter flow cytometry (MFC) to detect OMD in patients with SPB and assessed its value in predicting outcome. 50 patients were included in this analysis. Staging BM aspirates were assessed by MFC and aberrant phenotype plasma cells were demonstrable in 68% and comprised a median of 0.52% of BM leucocytes. We considered these cells to be indicative of OMD and sought to assess their effect on outcome. With a median follow up of 3.5 years, 28/50 patients have progressed with a median TTP of 1.4 years. The presence of OMD predicted progression as this occurred in 72% with OMD and 12.5% without (TTP 26 months v NR, p=003). Similarly, the presence of urinary light chains (ULC) at presentation was also predictive as progression occurred in 91% with ULC and 44% without (TTP 16 v 82 months, p<0.001). Utilising both parameters it was clear that patients lacking both OMD and ULC had an excellent outcome (6% progressed) whereas the presence of OMD and/or ULC identified high-risk patients in which progression was documented in 27/34 (79%) pts (p=0.001). Trials of adjuvant therapy are warranted in these high-risk patients.

P-441

PROADRENOMEDULLIN (MR-PROADM): A NOVEL POWERFUL PREDICTOR OF EARLY DEATH IN AL AMYLOIDOSIS

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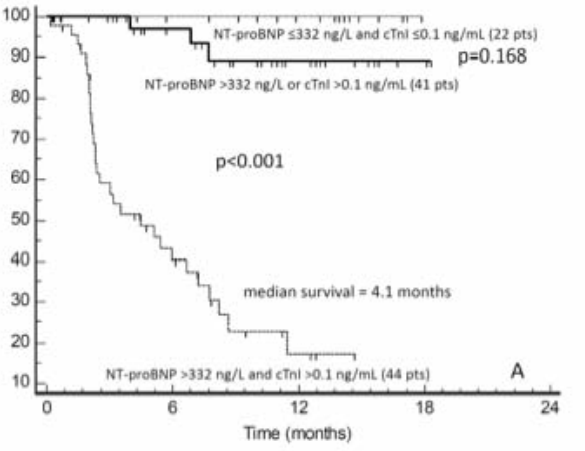
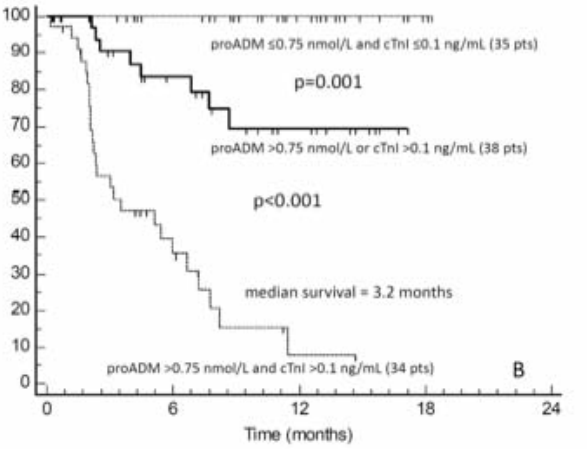
In AL amyloidosis prediction of survival is contingent upon assessment of heart dysfunction, currently based on the cardiac biomarkers N-terminal proBNP (NT-proBNP) and troponins (cTn). The ability to accurately identify patients at risk of early death is crucial in the design of treatment strategy. Midregional proadrenomedullin (MR-proADM) is emerging as a powerful prognostic marker in cardiac diseases. We prospectively assessed the prognostic role of MR-proADM in 107 consecutive newly diagnosed patients enrolled between August 2009 and October 2010. Midregional proADM was measured using a commercial assay (95 centile in normal subjects = 0.52 nmol/L). Seventy-four patients (69%) had heart involvement by standard echocardiographic criteria. Overall median (range) MR-proADM was 0.81 nmol/L (0.12-4.35 nmol/L), NT-proBNP 2578 ng/L (31-77701 ng/L), cTnI 0.08 ng/mL (0-3.8 ng/mL) and serum creatinine 0.94 mg/dL (0.54-6.47 mg/dL). With a 10 month median follow-up of living patients, median survival was not reached and projected 6 month survival was 74%. The MR-proADM cutoff best predicting survival was 0.75 nmol/L (median 7.5 months vs. not reached, p<0.001, HR 6.4). The standard staging system based on NT-proBNP and cTnI was unable to detect an early survival difference between stage I and stage II patients (Fig. 1a). Whereas, substituting NT-proBNP with MR-proADM identified 3 groups with significantly different survivals (Fig. 1b). Midregional proADM improves the short-term prognostic discrimination of patients with AL amyloidosis and can provide guidance in the choice of therapy.





ACCEPTED ABSTRACTS

A-442

CLINICAL VALUE OF SERUM IMMUNOGLOBULIN FREE LIGHT CHAIN QUANTIFICATION IN MULTIPLE MYELOMA

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Diagnostic value of serum immunoglobulin free light chain κ/λ ratios (rFLC) was compared with serum and urine protein electrophoresis and immunofixation (IFE) in 100 multiple myeloma (MM) and 8 monoclonal gammopathy of undetermined significance (MGUS) patients. FLCs were measured on Siemens BNTMII nephelometer, using FreeliteTM Kit , Binding Site, UK. Of 35 light chain (LC) myeloma patients, 19 were positive for LC and 16 for λLC. All of these patients had monoclonal LC detected by IFE in urine and 29 also detected in serum. Although 25 of patients had M spike in urine, only 9 had M spike in serum. All patients in κ subgroup had an increased FLCs in serum and increased rFLC. In κ group all patients had both increased λFLCs and decreased rFLC in serum. In 5 of 8 patients who had previously been deemed to have non-secretory myeloma FLCs were increased. Among 24 patients treated with autologous transplant in 17 normal rFLC documented stringent complete response. Discrepancy between IFE and FLC results were found in 4 patients. In all 25 MM patients with intact immunoglobulin assessed at diagnosis rFLC was abnormal, in 3 patients in CR after bortezomib therapy rFLC was normal and in 2 of 3 patients with PR rFLC was abnormal. In all 4 patients with extramedullary plasmacytoma rFLC was normal. Four of 8 MGUS patients with follow-up from 10 to 26 years had abnormal rFLC. MM progression developed in one patient with abnormal rFLC and in one patient with normal rFLC.

A-443

HIGH SERUM LDH CORRELATES TO AEROBIC GLUCOSE METABOLISM IN MYELOMA CELLS; POSSIBILITY OF NEW THERAPEUTIC STRATEGY TARGETING WARBURG EFFECT RELATED MOLECULES

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Introduction and methods: previous studies showed that high serum lactate dehydrogenase (LDH) is an adverse prognostic factor in multiple myeloma (MM). LDH is a key enzyme for glycolysis converting pyruvate to lactate. It had been reported cancer cells utilize glycolysis pathways even in the presence of adequate oxygen (Warburg effect) to provide energy for cancer cells. In the present study, we examined mRNA expressions of genes associated to Warburg effect in purified myeloma cells. Glucose consumption and cytotoxic effects of LDH-inhibitor were also analyzed in myeloma cell lines. *Results:* In our cohort, high serum LDH correlated to poor survival. A significant correlation between serum LDH levels and expression of LDHA, a gene encoding LDH, was found. LDHA expression was significantly higher in MM cells than in plasma cells from patients with monoclonal gammopathy of undetermined significance (MGUS). LDHA expression correlated to expression of pyruvate dehydrogenase kinase 1 (PDK1), a key enzyme for Warburg effect, c-myc expression which activates most glycolytic enzyme genes, and GLUT1 (glucose transporter 1) expression. More glucose consumption was observed in MM cell lines with higher LDHA expression. An LDHA-inhibitor, oxamate, activated caspase-3 and induced apoptosis to MM cells with high LDHA expression. *Conclusion:* Our results suggested that aerobic glycolysis (Warburg effect) is upregulated in MM cells of patients with high serum LDH and LDH could be a target to improve a significant poor survival of MM patients with high serum LDH.

A-444

ANALYTICAL EVALUATION OF SERUM FREE LIGHT CHAINS (FREELITE,SFLC) ASSAY USING SPA PLUSTM PROTEIN ANALYSER AND COMPARISON AGAINST BN PROSPEC® SYSTEM

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Inter-laboratory variation in sFLC measurements may be due to inherent analyser variations, antigen excess (AgXs) and the polymeric state of the sFLC. We compare the analytical performance of the sFLC assay on the SPAPlus (SPAP) and BN Prospec (BNP). Assays were performed as per manufacturer's instructions. AgXs samples identified on the BNP were analysed: 7κ (Median 550; range 161-11000mg/L) and 5 λ (637; 167-2990mg/L). Analytical performance was assessed using ~ 47 clinical samples. Diagnostic performance was evaluated on 20 samples identified with abnormal sFLC ratio (FLCr; Ref. R 0.26-1.65). All comparisons are made with respect to BNP results. FLCr results compared well between analysers (r^2=0.89). Within assay precision performed on quality control (QC) and patient samples (n=10); <5.4% for κ (range 14.14 - 412.08mg/L) and <1.8% for λ (range 29.41- 63.90 mg/L). Inter assay precision was determined by triplicate analysis of QC samples (x10); <7.1% for (Median=16.0 and 31.5mg/L) and <3.8% for λ (31.7 and 64.0/L). SPAP identified all AgXs samples correctly. Deming regressions were; κ y=1.11X-1.53 (n=45, 0.27–11000 mg/L) and λ y=1.09X-4.71 (n=47, 2.93–2990mg/L). Altman-Bland plot showed a bias of +27% and -27% for κ and λ assays. 16/20 diagnostic samples had abnormal FLCr, 4/20 samples had borderline low FLCr (0.26-0.48). sFLC measurements on the SPAP analyser compared favourably to the BNP. However, it is advisable to establish baseline values for monitoring individual patients across analysers.

A-445

ELEVATED FGFR3 EXPRESSION AS A RESULT OF TRANSLOCATION T(4;14) IN PATIENTS WITH MULTIPLE MYELOMA

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Translocation t(4;14) is adverse cytogenetic prognostic factor in patients with MM. Increased expression of FGFR3 gene as a result of t(4;14) (IgH enhancer juxtaposition) was also reported. We analysed FGFR3 gene expression in patients with/without t(4;14). Translocation t(4;14) was evaluated by FISH in MACS separated CD138+ cells obtained from 32 MM patients. Total RNA was transcribed into cDNA (Ambion WT Sense Target assay), labeled and hybridized to the Affymetrix GeneChip Human Gene ST 1.0 array. Acquisition of Affymetrix array images, RMA normalization algorithm, t-test (with Benjamini-Hochberg FDR) were performed by appropriate software. The t(4;14) was detected in 7 of 32 patients. Patients with t(4;14) [when compared with 25 patients lacking t(4;14)] had significant elevated expression of FGFR3 gene (FC = 5.083; $p < 0.001$). However, only 4 patients with t(4;14) showed highly elevated expression of FGFR3 [mean log2 = 4.275 (min 3.700 – max 5.845)]. Remaining three patients had FGFR3 level similar to patients lacking t(4;14) [mean log2 = -0.232 (min -0.401 - max -0.129)]. In patients lacking t(4;14) mean of normalised log2 = -0.002 [(min -0.534 - max 1.189)]. We anticipate, that the IgH enhancer might be deleted during ilegitimate switch recombination, thus t(4;14) did not always lead into FGFR3 overexpression and patients [with t(4;14) and without FGFR3 overexpression] might represent group of patients with favourable outcome. Our finding should be confirmed by survival analysis in larger cohort. This work was supported by grants IGA NS10207 and IGA NT11154.

A-446

AGGREGATED SERUM FREE LIGHT CHAINS (FLCS) MAY PREVENT ADEQUATE REMOVAL BY HIGH CUT-OFF HAEMODIALYSIS

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Background: Serum free light chains (FLC) are responsible for renal injury in multiple myeloma (MM) patients with cast nephropathy. The removal of FLC by high cut-off haemodialysis (HCO-HD) may enhance chemotherapy. Here we describe two cases were HCO-HD was unable to effectively remove FLCs. *Methods:* Patients' sera were fractionated by size exclusion chromatography (SEC). Fractions were screened for FLC and intact immunoglobulin (Ig) using nephelometric and Western blot methods. *Results:* An IgAλ MM patient had a λFLC concentration of 7510mg/L. Following 6 dialyser sessions with the Gambro HCO-HD 100 membrane, median FLC reduction was 7%. SEC identified λFLCs in 3 separate fractions (50kDa, 150-200kDa and >200kDa). Western blot evaluation of these fractions confirmed the presence of FLC with no Ig. Nephelometric results suggested that λFLC dimers accounted for 9% of the λFLC in the patient sera, the remaining FLC were present in polymers. An IgGκ MM patient had a κFLC concentration of 7280mg/L. Following 5 dialyser sessions with the HCO-HD 100 membrane, median FLC reduction was 10%. SEC identified FLC in 3 separate fractions (25kDa, 50kDa and >100kDa). Western blot confirmed the presence of FLC with no Ig. Nephelometric measurements indicated that κFLC monomers and dimers accounted for 20% of the FLC present, with the remainder being present as polymers. Analysis of the patient's dialysate fluid demonstrated only FLC monomers. *Conclusion:* FLC aggregation can reduce the effectiveness of HCO-HD.

A-447

STUDIES OF SMALL DISCRETE POPULATIONS OF B CELLS BY MICROARRAY TECHNOLOGY

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Multiple myeloma (MM) is an incurable B-cell malignancy characterised by the accumulation of malignant plasma cells in the bone marrow. It is at present unclear whether the malignant transformation occurs solely in the end stage differentiated plasma cell or in some instances can be traced back to an earlier B-cell maturation stage like the memory B-cell or plasmablasts. The hypothesis is that dysregulated and aberrantly spliced genes in B-cell subpopulations can provide clues to the cellular origin of the disease and that subpopulation specific gene expression in patients is correlated to disease outcome. The aim of the project is to establish a protocol for handling small number of cells and performing global gene expression on B-cell subpopulations from blood and bone marrow of MM patients and healthy donors. *Results:* By combining an optimized panel of CD markers for flow sorting and mRNA purification with magnetic beads, successfully amplification has been performed on 5000 memory B-cells and 1600 plasmablasts from healthy individuals for the Affymetrix Exon array 1.0. An important observation is that the mRNA content from different B subpopulations varies from a fixed number of cells. Preliminary data show that the oncogene WHSC1 is expressed at the same levels, however with marked differences in splice pattern between naive, memory and plasmablasts in the blood from healthy donors and a MM patient. The goal is to identify population-specific splice variants in patient samples which could provide novel clues to the understanding of the cellular origin of MM.

A-448

DIFFICULTIES IN IMMUNOFIXATION ANALYSIS : A CONCORDANCE STUDY ON IFM 2007-02 TRIAL

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Immunofixation (IF) interpretation requires an attentive examination of the profile obtained, especially when very thin bands are observed. Because this interpretation is based on a human evaluation, it presents a certain degree of subjectivity which conditions its performances. The purpose of this work is to estimate this subjectivity in order to evaluate the importance of this parameter in IF analysis. Therefore we considered serum IF performed within the IFM 2007-02 trial, in the cases where electrophoresis profile was normalized. 119 IF were selected as difficult to read and were revised by 5 biologists. In 61 % of the cases, patients presented monoclonal immunoglobulins of more anodic migration (on β1- or β2-globulin zone) than the γ-globulin zone. IF were realized in 54% of the assessments after autograft. These two circumstances represent the cases when interpretation is commonly the most difficult. Statistical analysis of the results was made by the calculation of Kappa coefficient and showed a good global inter-operator concordance (K=0.75). We also confirmed a correct homogeneity of the practices by a preliminary international study showing excellent immunochemical response agreement (96%) on 26 profiles defining near CRs (proceeding with the same analytical system). This evaluation is all the more important as it conditions the stratification of the answer type, particularly for nCR and CR. It is also important to measure the variability of these assays in the time of evaluation of professional practices.

A-449

STABLE SELECTION OF MULTIPLE MYELOMA SUBCLASS SIGNATURES

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Many attempts have been made to classify cancers into molecular subclasses with predictive value based on microarray technologies. Such signatures often lack robustness and stability across datasets and in order to ease the discovery of biological processes and new drug targets it is necessary to increase the signature´s stability. Here we present our results from generating stable subclass signatures in multiple myeloma (MM). We suggest using a regularised version of multinomial logistic regression to model polytomous subclasses. To mimic stability across datasets we use stability selection. Stability selection is used to construct stability paths for each gene which is the probability for the gene to be selected at various values of the regularisation parameter when randomly resampling half the data set. The proposed method is evaluated in an MM dataset made public available by the University of Arkansas for Medical Sciences (UAMS). The UAMS data was split into a training set and a validation set. It was possible to identify a classifier with 3-5 genes for each of the 7 subclasses, 24 in total, capable of classifying the validation set with an accuracy of 90% concordance to the previous classification. In conclusion, our study supports that lists of potential subclass marker genes in MM can be markedly reduced by stable selection methods without losing class predictive value. This will allow us to prepare stable gene lists for subclasses of the myeloma disease which can be considered a target panel for extended functional validation studies in cell lines and phase 0 studies.

A-450

MULTIPLE MYELOMA (MM) IN AFRICAN AMERICANS (AA): AN ASSESSMENT OF INCIDENCE, TUMOR GENE EXPRESSION AND OUTCOMES

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Background: Incidence of MM in African American (AA) is twice that of White (W) population. Large population based studies have demonstrated improved survival in MM with ASCT and novel therapies- IMiDs and proteasome inhibitors. Survival improvement among AA was smaller and non-significant, possibly due to unequal access of care or diverse responsiveness to novel therapies. We evaluated differences among two cohorts of pts treated with bortezomib-based therapies. *Methods:* Cohort 1 consisted 59 W and 29 AA pts in bortezomib-based clinical trials at Winship Cancer Institute. Cohort 2 included 251 MM samples (229 W and 22 AA) with Affymetrix gene expression profiling from pts enrolled in bortezomib trials from Millennium. Mean gene expression was compared between AA and W. *Results:* In Cohort 1, mean age of presentation in AA and W is 54 and 58 years respectively. Hgb, Ca, Cr are similar between both groups. 5-year survival rates and overall survival for W/AA were similar (log rank p=0.11). In Cohort 2, AA and W had similar response to bortezomib. Median age of AA is 57 years and W is 61 years. Three genes (RPS26, PSPH, and GDF2) were differentially expressed (RPS26; p= 1.6e-09, PSPH; p= 6.8e-05 and GDF2; p= 1.3e-4). Expression will be tested for correlation with response or survival. *Conclusions:* Pts with similar access to care (AA/W) had similar outcomes. The significant difference in gene expression, including PSPH which was previously linked with higher expression in AA, could be explored as a cause of diverse pathogenesis contributing to the observed racial differences in MM.

A-451

BORTEZOMIB-BASED REGIMEN INDUCED COMPLETE REMISSION OF MULTIPLE MYELOMA WITH REGRESSION OF OSTEOLYTIC LESION IN FEMUR

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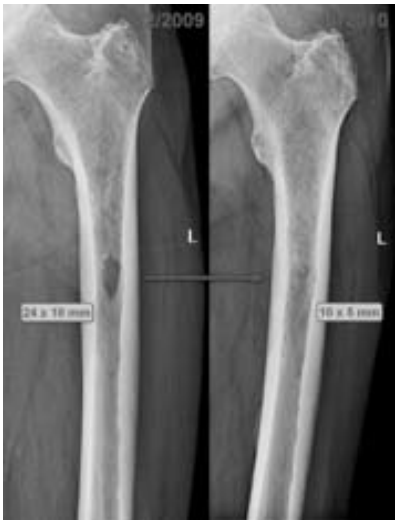
Diffuse or localized bone loss is one of the major clinical characteristics of multiple myeloma. Radiologically apparent recalcification of osteolytic lesions is exceptional. Generally, osteolysis only stops in progression during complete remission of the disease. Herein we report the case of a male diagnosed with IgA multiple myeloma accompanied by extensive bone disease affecting the skull, lumbar spine, humeri and femora. First line anti-myeloma treatment with 4 cycles of VAD chemotherapy (vincristine, adriamycin, dexamethasone) followed by autologous transplantation and maintenance treatment with interferon- put the disease into a very good partial remission. However, in 2009 the disease relapsed with progression of osteolytic lesions seen on conventional radiography. Second line treatment using CVD regimen (cyclophosphamide, bortezomib, dexamethasone, 8 cycles in total) was given and complete remission with rapid decrease of monoclonal immunoglobulin after the 1st cycle and negative immunofixation after 3 cycles was achieved. A follow-up skiagram of the left femur after a year revealed over 50% regression of the osteolytic lesion (from 24 x 10 mm in December 2009 to 10 x 5 mm in November 2010). Concomitant supportive therapy with bisphosphonates (zoledronate, ibandronate and from 2007 clodronate) was administered. In conclusion, in a long-term clodronate treated patient with relapsed multiple myeloma, complete remission of the disease after administering bortezomib-based regimen was accompanied by radiologically apparent reossification of the lytic bone lesion.



A-452

THE EFFICACY OF FDG-PET FOR DETECTING BONE LESIONS OF MULTIPLE MYELOMA

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Purpose: several studies have suggested that fluorodeoxyglucose positron emission tomography (FDG-PET) is superior to plain radiography for the detection of bone lesions in patients with multiple myeloma. However, the comparison of the efficacy of FDG-PET with that of plain radiography for detecting the lesions of each bone is not yet clear. Patients and *Methods:* we analyzed 7 patients with untreated multiple myeloma who underwent FDG-PET and plain radiography from 2006 to 2009 in our hospital. We divided the skeletal bones into 16 segments and evaluated the number of segment with lesions that was detected by FDG-PET or plain radiography. *Results:* in 6 cases, FDG-PET revealed bone lesions that were not revealed by plain radiography. FDG-PET revealed significantly more number of bone lesions than plain radiography ($p < 0.01$). FDG-PET and plain radiography could detect an average of 4.7 and 0.15 sites of bone lesions per patient, respectively. In FDG-PET imaging, several bone lesions were detectable as diffuse uptake patterns even when the SUVmax (maximum standardized uptake value) of the lesions was as low as 3, especially in the spine and the pelvis. On the other hand, in 1 case, plain radiography detected punched-out lesions in the cranial bones, which could not be detected by FDG-PET. *Conclusion:* FDG-PET is superior to plain radiography for detecting the bone lesions in the spine and pelvis of patients with multiple myeloma. However, plain radiography may be useful for detecting lesions in the cranial bones.

A-453

COMPLETE REMISSION FOLLOWING TREATMENT WITH PAD (BORTEZOMIB, DOXORUBICIN, DEXAMETHASONE) AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN IGE MULTIPLE MYELOMA PATIENT

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Since first case of multiple myeloma (MM) with IgE monoclonal protein reported in 1967 approximately 45 cases were published to date (*Wozney et al. J Clin Oncol 2009; 27: 637; Takemura et al. Int J Hematol 2009; 90:402*). Experience with new anti-myeloma therapies in this entity is very limited. We report a new IgE myeloma case treated with bortezomib and autologous stem cell transplantation (ASCT). A 49-year-old patient presented with bone pain, hepatosplenomegaly and allergic skin changes, peripheral blood plasmacytosis $1.62x10^9$/L, anemia, hypercalcemia, proteinuria and osteolysis. IgEk monoclonal protein 4.4 g/dL

was found in serum and Bence- Jones kappa 1.8 g/24 h in urine. Bone marrow was infiltrated by myeloma cells (90%) that immunophenotypically corresponded to CD38++, CD138+, CD44+, CD54+, CD56+, CD117-, CD45-, CD19- plasma cells and CD19+dim, CD38++, CD138+ lymphoplasmacytes. Genetics abnormalities included chromosome 6 long arm deletion, chromosome 13 aberrations and t(11;14). IgE MM was diagnosed. First-line VAD treatment resulted in partial response and bortezomib with doxorubicin and dexamethasone (PAD) was then administered leading to near complete remission. Following high-dose melphalan chemotherapy and ASCT, complete remission was achieved - M-protein no detectable in serum and urine immunofixation, normal ratio of k free/ l free in serum (0.95) and normal bone marrow picture in cytological (plasmocyte rate- 1%) and histological (plasmocytes -3%) examinations. To date, 21 months since diagnosis, the patient remains in a 8 -month complete remission.

A-454

PROLIFERATIVE GLOMERULOPATHY WITH UNUSUAL SUBENDOTHELIAL DEPOSITS OF STRIATED STRUCTURE IN MULTIPLE MYELOMA

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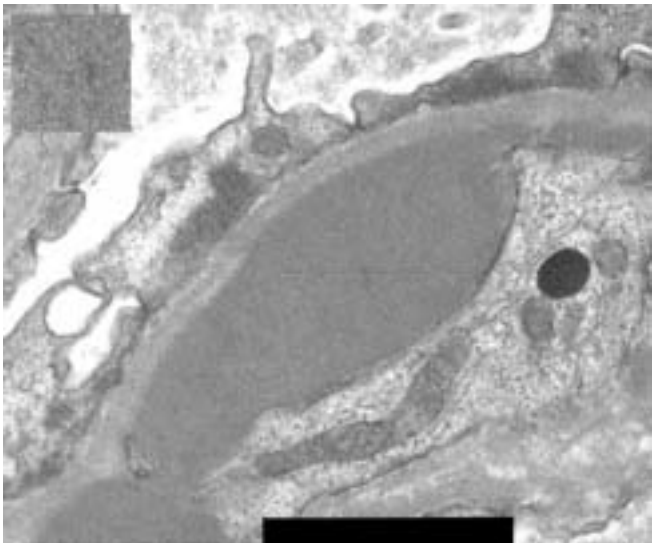
A 50-year-old woman was admitted for the evaluation of proteinuria and renal biopsy. On the basis of the serum monoclonal protein, marrow plasma cell dyscrasia and end organ damage (nephrotic range proteinuria), multiple myeloma was diagnosed. A renal biopsy showed a membranoproliferative glomerulonephritis pattern of injury and unusual organized deposits of striated structure in the subendothelial space, which were identified as non-amyloid non-immunoglobulin-derived deposits. These deposits contained regularly stacked straight electron-dense bands, which have not been described in the setting of paraproteinemia and/or plasma cell dyscrasia.



A-455

LENALIDOMIDE AND DEXAMETHASONE IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A MONOCENTRIC EXPERIENCE

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Background: Lenalidomide is a therapeutic option for unfit pretreated patients with multiple myeloma, especially in association with steroids. *Methods:* Oral daily lenalidomide (10-25 mg) for 21 days every month (median courses 11.6) and weekly dexamethasone were administered until progression in 13 pretreated patients (median age 67.3 yrs) observed between June 2008 and June 2010. Past therapy included alkylants, anthracyclines, IFN-α, thalidomide, bortezomib and autograft. Eleven patients were evaluated after 6 courses of therapy and prospectively followed-up; 2 patients weren't considered because of early death. Treatment response was assessed according to IMWG uniform response criteria. *Results:* ORR was 45.4% (5/11 pts) with a median duration of response of 8.6 months: 5 patients reached PR, while 6 were in SD. Among patients in PR, 1 died for PD after 16 courses administered as fifth line therapy. The median PFS was 13.2 months. All the other patients are still alive (4 PR in continuous therapy, 6 PD in salvage therapy) in good clinical conditions. The reported side effects included pneumonia, mild to moderate neutropenia, diarrhea, peripheral sensitive polyneuropathy, headache, transient liver failure, cutaneous rash, transient aphasia, all resolved with brief therapy discontinuance and specific support therapy without hospitalization. *Conclusions:* This study shows that lenalidomide in combination with steroids is a completely oral effective salvage therapy with a good response rate and manageable side effects for relapsed and refractory multiple myeloma patients.

A-456

CASE REPORT: RESPONSE TO LENALIDOMIDE IN A PATIENT WITH REFRACTORY MM AND EXTRAMEDULLARY PLASMACYTOMAS

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Multiple Myeloma (MM) is currently incurable, progressive and becomes disseminated (90% cases) unless treated. IgG is the most common monoclonal protein in MM(Ganjoo2006). Lenalidomida+Dexamethasone (Len+Dex) has demonstrated excellent efficacy in patients for the treatment of recurrent/refractory MM.(Weber2007,Dimopoulos2007,Palumbo2008). We evaluated Len+Dex response in a patient with refractory MM and extramedullary plasmacytomas. A 62-year-old woman diagnosed with MM (IgG,kappa,IIIA) in September 2003 was treated with VAD (7cycles) and assessed for a transplant but unable to receive high dose treatment due to intolerance.

1) May 2007 Patient displayed poor Performance status and loss of strength

Table 1: Analytical parameters

Date	Hb (g/dl)	Platelets (u/mm3)	WBC (cel/mm3)	Cr (mg/dl)	Total protein	Albumina	IgG	IgM	IgA	PCR	LDH	Plasma cells (%)	ESR (mm/hr)
May 2007	9.4	128000	8250	4.10	NA	NA	NA	NA	NA				
January 2010	10	96000	4160	2.5	N	N	NA	NA	NA			5	
September 2010	9.2	72000	6120	1.92	N	N	N*	N	N	N	N		20
December 2010	11.9	118000	9640	1.9	N	N	N	N	N	N	N		

* serum immunoelectrophoresis: showed only weak IgGkappa band.

Legend: N: normal NA: not available

2) January 2010 A large extramedullary plasmacytoma (extraosseous), approximately 10cm in left pectoral and in right para-umbilical region (10cm). Myelogram: all cell lines without stopping maturation, no major signs of dysplasia, normal Kariotype. CT: myelomatous osseous vertebral bodies' involvement of all the dorsal vertebrae and involvement of the anterior ribs of left rib cage that causes soft tissue mass

Table 2: Responses to drug treatment

Date	Treatment	Response
September 2003	7 VAD cycles	Objective response
May 2007*	6 VAD cycles, 4 units PRBC, G-CSF, antifungals, antibioteraphy and oxygenotherapy	Minimal response after 7 months
December 2007	Len+Dex (40mg/d in 3 pulses of 4 days each) compassionate use	Complete response after 9 months
September 2008	Treatment withdrawal	ND
January 2010**	Len 10mg/day+ Dex 40mg once week	Considerable decrease in both plasmocytomas
September 2010	Len 10mg/day+ Dex 40mg once week	2/3 reduction in the size of plasmacytomas
December 2010	Len 10mg monotherapy	plasmacytomas were undetectable

Legend: ND: Not Applicable

*The patient relapsed

**The patient relapsed with two plasmocytomas

The patient was treated for 11 cycles with Len-Dex in which initial reduction of the plasmacytomas was seen after the third cycle. Currently the patient continues treatment with 10mg/day of lenalidomide. These results show that Len-Dex is effective for the treatment of multiple myeloma and demonstrated considerable decrease in the size of extramedullary plasmacytomas.

A-457

TOXICITY AND SUPPORTIVE TREATMENTS IN PATIENTS WITH MULTIPLE MYELOMA INELIGIBLE FOR TRANSPLANTATION TREATED WITH BORTEZOMIB-MELPHALAN-PREDNISONE (VMP)

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Introduction: Standard treatment in patients not candidates for high-dose therapy has been melphalan-prednisone. The addition of bortezomib to standard therapy has resulted in a higher response rate and increased disease-free survival with acceptable toxicity. Patients and *Methods:* From July 2007 to November 2010, we have treated 62 patients with nine 6-week cycles of VMP: Bortezomib (1.3 mg/m2 days 1, 4, 8, 11, 22, 25, 29 and 32, cycles 1-4 and days 1, 8, 22 and 29, cycles 5-9), with melphalan (9mg/m2) and prednisone (60mg/m2) days 1-4, cycles 1-9.Toxicity was evaluated according to the scale of the NCI CTCAE v3.0. *Results:* After a median of 4.25 cycles administered (range 1-9) and a median follow up of 9.5 months (1-24) observed adverse effects are described in Table 1.

Adverse events	Any grade(%)	Grade 3-4 (%)
Trombocytopenia	29	7
Anemia	35	7
Neutropenia	27	12
Peripheral neuropathy	45	13
Gastrointestinal toxicity	29	10

Dose reduction of bortezomib and melphalan was required in 39% and 24% of patients, respectively. Prednisone was adjusted in 4 patients. Treatment was discontinued in 22 patients (36%), 14 for adverse events. Support therapy was erythropoietin in 26%, colony stimulating factors in 10% and 45% received zoledronic acid. *Conclusions:* The adverse events profile with VMP was predictable and consistent with the known safety profile of each of these agents. Dose adjustment in the early stages of peripheral neuropathy with supportive treatment allowed the continuation of treatment in most patients.

A-458

IGM MULTIPLE MYELOMA : MORE ON A RARE AND HETEROGENEOUS DISEASE

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IgM multiple myeloma (IgM MM) is a very rare and heterogeneous disease sharing clinical and biological manifestations with Waldenstrom's macroglobulinemia (WM). Accurate diagnosis is crucial for both because of their different therapeutic approach and the long-term prognosis. The two recent most large series proposed diagnostic criteria including bone lesions or immunophenotypic profile, both completed by the t(11;14)/CyclinD1(CCND1) overexpression. We present here 3 cases of true IgM multiple myeloma with atypical presentations. Only one patient had bone lesions and CCND1 over-expression at diagnosis. The second one had a rare hyperviscosity-syndrom with cryoglobulinemia and renal amyloïdosis. The last one presented an acute renal failure. Flow cytometry (CMF) of bone marrow cells confirmed the presence of tumoral plasma cells, positive for CD38/CD138 phenotype. The patient with cryoglobulineamia had an atypical CD56+ phenotype. Furthermore, CMF ruled out WM and others B-cells lymphoproliferative disorders. All our three patients received new agents in first-line therapy. The two patients with renal failure had a bortezomib-dexamethason induction, the third one had an investigative lenalidomide-dexamethasone regimen. Despite this treatment 2/3 patients died within 6 months. We suggest that in this rare disease clinical findings, molecular tests for t(11;14)/CCND1 and CMF are complementary tools for the diagnosis. Furthermore, we confirm in this small series the heterogeneity of the disease and the bad prognosis of IgM MM at the era of novel agents.

A-459

ASSOCIATION OF MELPHALAN, PREDNISONE AND THALIDOMIDE (MPT) AS UPFRONT THERAPY IN ELDERLY MULTIPLE MYELOMA PATIENTS: A MONOCENTRIC EXPERIENCE

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Background: Thalidomide, an immunomodulating drug, represents a therapeutic option for elderly patients with multiple myeloma, also for untreated ones unfit for graft procedures. *Methods:* Between April 2009 and May 2010 eight patients (median age 72.6 yrs) with multiple myeloma at presentation were treated with monthly oral melphalan (0.18 mg/kg d 1-4), prednisone (50 mg/sm d 1-4) and thalidomide (100 mg/die) as upfront therapy. One patient prematurely withdrawn thalidomide due to severe bradycardia and constipation, just continuing melphalan and prednisone. Treatment response was assessed according to the IMWG uniform response criteria. *Results:* The median follow-up time was 7.3 months. After 6 MPT courses the ORR was 85.7% (6/7 pts) with a median duration of response of 10.3 months: 3 patients showed CR, 1 VGPR and 2 PR. No response was observed in one patient.During follow-up, among patients in PR either started second line chemotherapy, other received 3 further MPT courses; all patients in CR and VGPR are alive in good clinical conditions on maintenance treatment with daily thalidomide. Non responder patient is still with stable disease and off therapy 4 months after treatment ending. Despite side effects such as bradycardia, peripheral sensitive polyneuropathy and constipation, none required hospitalization and only one patient discontinued therapy. *Conclusions:* This study shows that association of oral melphalan, prednisone and thalidomide is an effective first line therapy with high response rate and manageable side effects for unfit elderly multiple myeloma patients.

A-460

A SINGLE CENTRE EXPERIENCE WITH LENALIDOMIDE FOR THE TREATMENT OF MULTIPLE MYELOMA ELDERLY PATIENTS

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Background: Lenalidomide is a new immunomodulatory drug with a dual mechanism of action: tumoricidal and immunomodulatory effect . It represents an important treatment option for multiple myeloma patients. In this way Lenalidomide increases the available treatment options. *Aims:* In our Department, lenalidomide was administered in resistant/relapsing myeloma patients and as consolidation/maintenance therapy in elderly myeloma patients with stable disease (partial remission) after induction therapy or more lines of chemotherapy. *Methods:* We treated 36 patients (19M and 17F) with median age of 70 years (range 66-80). We have evaluated 29 patients with a median follow up of 21 months. These patients are been treated with lenalidomide at variable doses(5-25 mg/die p.o., according to tolerability of each patient, for 21 days every 28 days), in association of very low doses of dexametasone (10 mg/die p.o. days 1,2,3,4) or alone. We used Enoxaparin for prophylaxis of venous thromboembolisms. Clinical restaging was performed after three, six and twelve months, in course of therapy. *Results:* At the present we didn't observe any progression of disease and in 18 cases we observed a good impact on monoclonal component. In all patients the therapy was well tolerated and were not found significant adverse events. *Conclusions:* Exists a consolidated role for lenalidomide with dexametasone or alone for continous treatment in previously treated elderly myeloma patients. This therapy leads an improvement in prognosis of these patients, without causing severe complications.

A-461

HIGH EFFICACY OF MELPHALAN/DEXAMETHASONE/VELCADE (MDV) AS AN INDUCTION TREATMENT FOR NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS- A MULTI-INSTITUTIONAL PHASE II TRIAL IN TAIWAN (PROTOCOL MA3; HEMATOLOGY SOCIETY, R.O.C.)

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From July 2008 to December 2010, a total of 34 patients with newly diagnosed multiple myeloma (MM) were enrolled and 29 of them were evaluable. They had received MDV regimen consisted of melphalan (9 mg/m2 p.o. once daily on Days 1 to 4 of each 6-week cycle), dexamethasone (40 mg p.o. once daily on Days 1 to 4, 8 to 11 of each 6-week cycle) and velcade (1.3 mg/m2, i.v. twice weekly [weeks 1, 2, 4, and 5] for cycles 1-4 followed by once weekly [weeks 1, 2, 4, and 5] for cycles 5-8) for up to maximal eight cycles as an induction treatment. After a median follow-up of 16 months, these patients had received a median of four cycles treatment and eight patients (28%) had completed the maximal eight cycles. Based on intent-to-treat analysis, three patients (3/29, 10%) achieved complete response, 12 patients (41%) achieved very good partial response, nine patients (31%) achieved partial response; therefore, the overall response rate was 82%. The median time to response was two months and the estimated two-year progression-free survival and overall survival were 79% and 82%, respectively. Twenty-seven patients (93%) had trial drug-related adverse effects (AEs), and the top five AEs by incidence were as follows: diarrhea (14/29, 48%), constipation (9/29, 31%), decreased appetite (8/29, 28%), herpes zoster (7/29, 24%), peripheral sensory neuropathy (6/29, 21%); nearly all the AEs could be managed. We concluded that MDV regimen had high efficacy as an induction treatment for newly diagnosed MM and with acceptable toxicities. Longer follow-up is still ongoing.

A-462

SAFETY AND EFFICACY OF BORTEZOMIB, NON-PEGYLATED LIPOSOMAL DOXORUBICIN TWICE A CYCLE, DEXAMETHASONE AND THALIDOMIDE IN RELAPSED OR POOR-RESPONDING MYELOMA PATIENTS

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Achievement of CR predicts a superior outcome in MM. Bortezomib enhance chemosensitivity to other drugs and overcome drug-resistance. Doxorubicin and bortezomib reciprocally increase their efficacy. We had observed an improvement of response in R/R MM by adding non-pegylated liposomal doxorubicin (Myocet®) to VTD (Ciolli S., BJH 2008). A pharmacodynamic trial with bortezomib plus doxorubicin on day 1 and 8 had a higher antitumor activity compared to bortezomib/weekly antracycline (LoConte NK, Cancer Chemother Pharmacol 2008). Thus, we treated patients relapsed or achieving less than VGPR after induction with a modified My-VTD. Eligibility criteria: age >18 years, no liver/cardiac diseases/ severe PNP. Therapy: bortezomib 1.3 mg/m2 i.v. twice weekly for 2 weeks in a 28-d cycle, Myocet 25 mg/m2 on day 1 and 8, dexamethasone 24 mg with the standard scheduling and thalidomide 100 mg/daily for a maximum of six cycles. HV infection and DVT prophylaxis as standard. Response, EBMT criteria. Adverse events NCI criteria (v 3.0). *Results:* from June 2008 to January 2011, 20 patients were enrolled. Median age 56 yrs, 10 IPSS ≥2. Toxicity: 2 neutropenia (grade 1), 3 PNP grade ≤2. No change of LVEF. Therapy on outpatient basis. 16 (80%) ≥VGPR (9 CR/nCR, 7 VGPR), 2 PR, 2 SD. 10 patients, eligible for transplant, collected PBSC and 7 were transplanted. After a median follow-up of 21 months, all patient are alive and 3 are relapsed (12-30 months). *Summary/Conclusions:* The treatment applied appear safe and efficacious in relapsed/poor-responding myeloma.

A-463

PULMONARY MYCOBACTERIUM TUBERCULOSIS INFECTION IN MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB

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Backgrounds: Activation and proliferation of CD4+ T cells are crucial to the host's defense against Mycobacterium Tuberculosis. Proteasome inhibitor, such as bortezomib, suppresses essential immune functions of human CD4+ T cells. Therefore, suppressing CD4+ T cell immunity by bortezomib might increase the susceptibility of mycobacterial infection. *Methods:* We performed a retrospective study to know the incidence of Mycobacterium Tuberculosis infection in 115 patients with multiple myeloma that treated with bortezomib. We used chest CT scan, sputum culture, tuberculosis PCR for diagnosis of Mycobacterium Tuberculosis infection. *Results:* Total 8 patients (8/115, 7%) were diagnosed as Mycobacterium Tuberculosis infection and all patients showed lung lesion by chest CT scan. Four patients were detected tuberculous infection by sputum culture, one patient by sputum acid-fast bacillus stain and another one patient by tuberculosis PCR test on tissue specimen. Two patients were diagnosed by chest CT scan and clinical symptom without bacteriologic confirmation. All patients were treated with standard regimen (EHRZ) and no one died of uncontrolled tuberculous infection. But, four patients were discontinued the bortezomib treatment due to grade 3 or 4 asthenia and they showed disease progression afterward. *Conclusions:* Bortezomib treatment in multiple myeloma may be increases the pulmonary tuberculosis infection and that will make it harder to perform the consecutive treatment. Key words: bortezomib, multiple myeloma, Mycobacterium Tuberculosis

A-464

A MULTICENTER RETROSPECTIVE ANALYSIS OF RETREATMENT WITH BORTEZOMIB FOR MULTIPLE MYELOMA: RESULTS OF THE KOREAN MULTIPLE MYELOMA WORKING PARTY (KMMWP)

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The aim of this restrospective study was to evaluate the efficacy and toxicity of retreatment with bortezomib in patients with multiple myeloma. Data were retrieved from the database of the Korean Multiple Myeloma Working Party (KMMWP). Thirty-seven patients, all of whom had reliable data for response and toxicity to both initial treatment and retreatment with bortezomib-based chemotherapy, were analyzed. The most common regimen was bortezomib single and in combination with dexamethasone at an initial treatment (81%) and retreatment (78.4%). The median time between initial and retreatment with bortezomib was 12.5 months. In initial bortezomib treatment, the overall response rate (complete response (CR)+very good partial response (VGPR)+ partial response (PR)) was 83.8% and the median time to progression (TTP) was 13.1months. In bortezomib retreatment, 51.4% achieved response (including CR of 5.4%, VGPR of 13.5%, and PR of 32.4%). The median TTP on retreatment was 5.6 months. Among 20 patients with CR/VGPR to initial treatment, CR, VGPR and PR rates

were 10%, 20% and 35%, retrospectively. Toxicity contributed to discontinuation in 24.3% of patients during initial treatment and 16.2% during retreatment. Retreatment with bortezomib-based chemotherapy showed appreciable response with acceptable toxicity profiles in patients with multiple myeloma.

A-465

ACETYL-L-CARNITINE (A) AS PROPHYLAXIS AGAINST BORTEZOMIB (B) INDUCED PERIPHERAL NEUROPATHY (PN) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (M)

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B induced PN is a common problem related to dosing schedule, duration of treatment, other drugs, and pre existing PN. We conducted a trial for pts with relapsed/refractory MM with B 1.3 mg/m2 IV on days (d) 1,4,8,11; Doxorubin (D) 15 mg/m2 on d 1, 8 and dexamethasone 20 mg PO on d 1,4,8,11, up to 8 cycles. CR/PR was 55% in the first 19 pts; incidence of >grade (Gr) 2 PN was 60%, and PN was the leading cause of treatment discontinuation. A is an oral supplement that has shown activity in the prevention of PN. We ad≤ded A 1.5g twice daily to this regimen. In vitro, A did not prevent B or D cell kill in primary MM cells obtained from study pts. Incidence and severity of PN were assessed using questionnaires and peg board testing before treatment and q6w until end of treatment. *Results:* 13 patients were treated on the modified regimen. A was well tolerated. Combined CR/PR response rate was 58%. The incidence of >Gr2 PN was 50%, with 31% incidence of Gr3 neuropathy in the A group vs 60% and 35% in the no A group (P=ns). Median number of cycles in the no A group was 4, vs 6 in the A group. 27% completed all 8 cycles in the A group vs only 11% in the no A group; 17% stopped treatment in the A group due to PN vs 27% in the no A group. Peg board times for patients receiving A did not increase. Of interest, patients receiving A experienced more hematologic toxicity (100% >gr 2) than the no A group. *Conclusions:* the addition of A to a B-containing regimen did not significantly reduce the incidence of Gr3 PN but appeared to delay the onset of PN.

A-466

LIGHT CHAIN ESCAPE IN PATIENTS WITH PARAPROTEINAEMIAS

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Introduction: The term 'light chain escape' (LCE) describes a shift from secretion of intact immunoglobulins to mainly free light chains (FLC) at relapse. We describe two cases of LCE in myeloma and one of lymphoplasmocytic lymphoma (LPL); the first to be described in the literature. Cases: The first case is a patient with IgAλ myeloma, paraprotein of 47g/L at diagnosis. After an initial reduction in paraprotein with first line treatment, he relapsed twice, each time with an increase in paraprotein but a disproportionate and escalating increase in FLC. He developed renal failure and, despite three lines of treatment, died with a paraprotein of 50g/L and λ FLC of 6970mg/L. The second case is a patient with IgGκ myeloma. Presentation paraprotein of 10g/L fell to 2g/L after an autograft and continued to fall subsequently. However, he presented in acute renal failure and despite a sustained undetectable paraprotein, his κ FLC was 3730mg/L and 6.6mg/L. The third case is a patient with LPL confirmed on bone marrow studies and an IgMκ paraprotein of 23g/L. Despite the spontaneous fall of the paraprotein to 10g/L, he presented in acute renal failure and was found to have elevated serum FLC of κ 21.3mg/L and lambda 3330 mg/L. *Conclusion:* These cases highlight the phenomenon of light chain escape and illustrate the importance of monitoring patients with paraproteins using the SFLC assay, especially LCE may be preceded by a drop in the level of intact paraprotein, thus giving false reassurance that the patient remains in plateau phase.

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NATURAL HUMAN IGM ANTIBODIES TARGETING PRIMARY MULTIPLE MYELOMA

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The existence of humoral tumor immunity in cancer patients is established and have opened the avenue of isolating tumor reactive antibodies that can be used for cancer immunotherapy. We therefore analysed a panel of tumor reactive IgM antibodies if they can specifically bind and induce lysis of primary multiple myeloma (MM) cells. Two fully human IgM antibodies- Mab 1 and Mab 2 - were able to specifically target several myeloma cell lines as well as primary MM cells, which were freshly obtained from MM patients. No binding was detected on primary healthy hematopoietic tissue. Antibody treatment of both MM cell lines and primary MM cells (n=6) caused significant cell death, which was mediated by induction of apoptosis. In addition, cell death was increased by adding complement to the cell cultures resulting in significant complement dependent cytotoxicity (CDC). This CDC interaction was observed in primary MM samples (n=8) independent from myeloma subtype and stage of disease. In summary, patient derived IgM antibodies induce cytotoxicity by intrinsic induction of apoptosis and CDC and therefore provide a promising approach for immune therapy of multiple myeloma.

A-468

BENDAMUSTINE BASED-REGIMENS: RETROSPECTIVE CASE SERIES OF THREE PATIENTS WITH REFRACTORY/RELAPSE MULTIPLE MYELOMA (MM)

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Background: Salvage therapies are becoming important in the treatment of MM since relapse is common even after tandem autotransplantation. Phase I/II studies suggest that bendamustine (B) combined with lenalidomide (R) or bortezomib (V) may be an effective option for relapsed/refractory MM. *Methods:* Three patients (median age 50 years), affected by relapsed MM following autotransplantation and after the failure of at least one prior conventional salvage therapy, were treated with B combined with other drugs in a compassionate use program. Two patients received B 150 mg/m2 on days 1-2, V 1,3 mg/m2 on days 1, 4, 8, 11 and dexamethasone (D) 40 mg on days 1-2, 4-5, 8-9, 11-12 (BVD) after 3 salvage therapies. One patient received B 150 mg/m2 on days 1-2, R 25 mg on days 1-21 and D 40 mg on days 1-4, 15-18 (BRD) after only one salvage treatment. Each cycle was repeated every 4 weeks. *Results:* All patients completed 3 cycles of therapy and were evaluable for response. The 2 regimens were well tolerated. The patients treated with BVD after 3 salvage therapies, were non-responders. Complete remission was observed on the patient who received BRD following failure of only one salvage therapy. *Conclusions:* We have examined the anti-MM activity and toxicity profiles of the combinations BRD and BVD. The BRD combination was more effective than BVD combination. Our experience appears to suggest the importance of an early start of salvage regimens and a possible synergic action of the combination B-R.

A-469

RESOLUTION OF ACUTE RENAL FAILURE DUE TO CAST NEPHROPATHY BY HIGH CUT-OFF HAEMODIALYSIS AND BORTEZOMIB, THALIDOMIDE, DEXAMETHASONE (VTD) ALLOWED TO AUTOLOGOUS STEM CELLS TRANSPLANTATION (ASCT) IN MULTIPLE MYELOMA (MM) PATIENT

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Introduction: Acute renal failure in MM mainly due to cast nephropathy is an adverse complication that negative impact on the patient outcome, precluding the eligibility to high dose chemotherapy and autologous stem cells transplantation (ASCT). Haemodialysis with new filters permeable to proteins up to 60 kD demonstrated effectiveness in removing serum free light chains (sFLC) and restoring renal function in combination with bortezomib. Case presentation: We report a case of a 61-year-old man affected by MM IgG kappa with no bone lytic lesions, presenting with acute renal failure, kappa sFLC concentration of 4224 mg/L and serum creatinine 23 mg/dl, haemoglobin 8,5 g/dl, serum protein 9,8 g/dl and monoclonal component 3,91 g/dl. Renal biopsy showed cast nephropathy. The patient underwent high cut-off haemodialysis (Theralite™) three times a week (total 6 procedures) and therapy with bortezomib, thalidomide and dexamethasone (VTD) for 5 cycles. Serum creatinine and kappa sFLC was 2 mg/dl and 622 mg/L respectively at the end of haemodialysis and after the first cycle of VTD. After 4 more cycles of VTD the patient achieved the normalization of renal function and kappa sFLC reduced to 148 mg/L (3,5% of starting level). Afterwards in PR he underwent stem cells mobilization with endoxan plus G-CSF and ASCT with melphalan 200mg/m2. *Conclusion:* The combination of high cut-off technology haemodialysis and treatment with novel agents including bortezomib is highly effective in reducing sFLC and restoring renal function, allowing the ASCT in MM patients with cast nephropathy.

A-470

VISUALIZATION OF MYELOMA HOT REGION BASED UPON PROTEIN-SYNTHESIZING PROPERTY OF MYELOMA CELLS USING C11-METHIONINE PET SCAN - WHAT CAN WE LEARN THROUGH THE DIFFERENCE OR DISCREPANCY OF PET IMAGINGS IN A RELAPSED MYELOMA CASE AFTER AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION?

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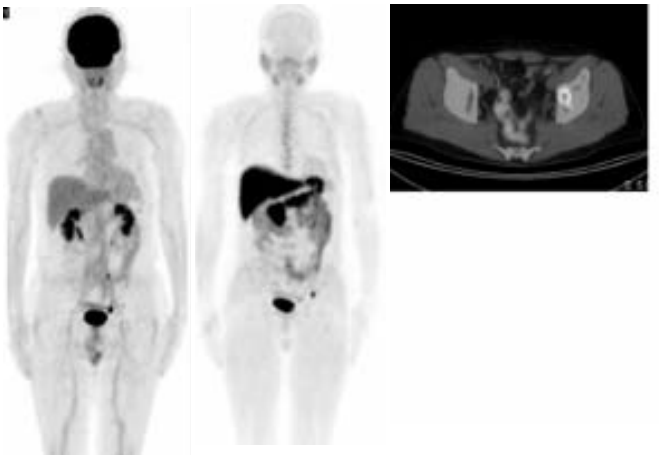
Aim: Multiple myeloma (MM) is frequently associated with extramedullary disease (EMD). FDG-PET is one of the tools to detect EMD as shown in Durie' s PLUS staging system. However, whether myeloma cell nests (MCNs) can be detected as hot or not, is controversial, because hot region by FDG-PET can be principally visualized when glycolysis is an active ATP donor in MCNs. FDG-PET negativity is frequently reported in follicular lymphoma, as well as in some MM. On the contrary, MM cells are protein-synthesizing, implying if radio-labeled amino acid is used as an imaging reagent, it will become a powerful tool to detect MCNs. *Case & Methods:* Sixty-two-year-old male MM patient was transferred to our institute because of left pelvic pain not detected by FDG-PET after a-PBSCT. Minimal elevation of monoclonal IgG was detected, but bone marrow showed negativity of clonal myeloma cells. Being a doctor, he was eager to receive pathophysiology-based detection of MCNs. We prepared radioactive C11-methionine (Met) solution and analyzed the uptake of this material into his pelvis, because this procedure was approved by institutional ethical committee based upon efficient imaging and safety. Figure 1 (1st figure below)showed FDG-PET negativity. Figure 2a&2b(2nd & 3rd figure below) showed prominent Met-PET positivity. *Discussion & Conclusion:* Through this case, it was shown that in some myeloma, Met-PET could become a powerful diagnostic procedure even when MCNs might be obscure by FDG-PET. We conclude that MM pathophysiology-based PET will be widely used in myelomology in near future.



A-471

DISTRIBUTION OF BIM DETERMINES MCL-1 DEPENDENCE OR CO-DEPENDENCE WITH BCL-XL/BCL-2 IN MCL-1-EXPRESSING MYELOMA CELLS.

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Dependence on Bcl-2 proteins is a common feature of cancer cells and provides a therapeutic opportunity. ABT-737 is an antagonist of anti-apoptotic Bcl-2-proteins and therefore is a good predictor of Bcl-xL/Bcl-2 dependence. Surprisingly, analysis of Mcl-1-dependent multiple myeloma cell lines revealed co-dependence on Bcl-2/Bcl-xL in 50% of the cells tested. Co-dependence is not predicted by the expression level of anti-apoptotic proteins, rather through interactions of anti-apoptotic proteins (Mcl-1, Bcl-2 and Bcl-xL) with Bim. Consistent with these findings, acquired resistance to ABT-737 results in loss of co-dependence through redistribution of Bim to Mcl-1 or loss of Bim expression. Interestingly this loss of expression occurs at the transcriptional level and results in the stable acquisition of ABT-737 resistance as cells maintain this phenotype when out of selection for more than 30 days. Overall, these results suggest that complex interactions, and not simply expression patterns of Bcl-2 proteins need to be investigated to understand Bcl-2 dependence and how to better utilize agents like ABT-737.

A-472

PARANEOPLASIC SPINOCEREBELLAR ATAXIA IN MULTIPLE MYELOMA: A CASE REPORT

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Paraneoplasic spinocerebellar ataxia is a disorder presenting with pyramidal and cerebellar symptoms. These forms are reported with gynaecologic, breast, lung or Hodgkin cancer. The association with multiple myeloma (MM) is rarely described. We report the case of a patient suffering of a IgG kappa MM associated with a paraneoplasic spinocerebellar ataxia. Since 2004, this 56-year-old man is followed for a IgG kappa MM. He was considered in remission after chemotherapy (VAD 4 cycles) and transplant. In 2008, a bone relapse in L5 was observed and treated by local irradiation. In 2009, the patient presented a spinocerebellar syndrome. Clinical examination pointed to pyramidal and cerebellar symptoms. A blood analysis showed: recurring IgG kappa monoclonal protein, light kappa chains 176 mg/l (nr: 0,62-3), 2 microglobulin 3,37 mg/L (nr: 0,7-1,8). A Pet Scan showed the apparition of metabolic localisations in the sternum, Th2 and 5th right rib. Cerebral and full spine IRM were normal. CSF analysis showed: protein concentration at 41 mg/dl, with a IgG kappa monoclonal peak at immunoelectrophoresis, and total IgG at 3,87 mg/dl. A diagnosis of MM relapse with a spinocerebellar ataxia was made. After chemotherapy (bortezomib), a com-

plete remission was obtained and the pyramidal and cerebellar symptoms disappeared. There are many types of spinocerebellar ataxia including paraneoplasic forms associated with different cancers. This is the only case of spinoceberellar ataxia associated with MM, the paraprotein of which could be a functionnal auto-antibody against neuronal antigens.

A-473

PERIPHERAL NEUROPATHY MANAGEMENT USED A PATIENTS REPORTED SCORING SYSTEM (FACT/GOG-NTXV4.0) IN MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB

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Bortezomib should be used under appropriate dose adjustment and/or administration schedule modification. Primary symptom of BIPN is related to sensory disturbance and it is difficult to detect the early sign of sensory disturbance by general nursing practice using conventional objective assessment. In 2009, the FACT/GOG-NtxV4.0 (FACT) was incorporated into the revised critical path as a peripheral neuropathy-specific questionnaire for patients. As a result, mean FACT scores in cycle 1 or 2 of treatment showed a significant reduction from baseline ($p = 0.0004$) that enable the medical staff to recognize early sign of sensory disturbance and allowed considering a necessity of dose adjustment and schedule modification at early stage of BIPN. Through the introduction of the FACT-based critical path and the successful operation of the FACT in the team-based medical service system, careful observation of score changes can help early detection of BIPN and allow nursing care deeply contributing to avoid discontinuation of bortezomib treatment.

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EXTRADURAL SPINAL CORD COMPRESSION - CLINICAL PICTURE AT ONSET OF MULTIPLE MYELOMA CASE PRESENTATION

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Objective: Multiple myeloma sometimes presents at the onset extranodale determinations Presence of spinal determination at the onset creates diagnosis difficulties and treatment dilemma. *Material and Methods:* The study of one case of spinal tumors admitted in our hospital in the Neurosurgery Service. Female, 42 years old , was admitted for thoracolumbar column pain and SPE paresis. The CT diagnosis was L4 right herniated disc. Although the appearance of CT was conclusive for HDL is found an epidural tumor that partially infiltrates a L4 vertebral body. This tumor was totally removed. After 5 days we develops strong paraparesis, urinary retention. The emergency thoraco-lumbar junction NMR highlights a giant tumor that infiltrates the T12-L1 vertebrae corpus with epidural extension and dural compression, with intrathoracyc extension of the tumor. Histopathologic diagnosis – extramedullary plasmacytoma. The bone marrow aspirate showed 60% plasmocytic infiltration. The serum protein electrophoresis monoclonal showed protein component in fraction (IgGλ type). The patient was received two lines of chemotherapy, with complete response and partial neurologic recovery. The patient relapsed after two years with pontocerebellous plasmacytoma. *Conclusions:* Clinical pictures of spinal determinations at the onset is nonspecific with pain and muscle contracture. Radiographic appearance of the spine is nonspecific .In the beginning, it made MRI in cases with persistent pain in order to decide the surgical intervention before the onset of cord compression.

A-475

RECOMMENDATIONS FOR STANDARDISED REPORTING OF PROTEIN ELECTROPHORESIS IN AUSTRALIA AND NEW ZEALAND

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Background: Although protein electrophoresis of serum (SPEP) and urine (UPEP) specimens is a well established laboratory technique, the reporting of results using this important method varies considerably between laboratories. The Australasian Association of Clinical Biochemists recognised a need to adopt a standardised approach to reporting SPEP and UPEP by clinical laboratories. *Methods:* A Working Party considered available data including published literature and clinical studies, together with expert opinion in order to establish optimal reporting practices. A position paper was produced which subsequently was revised through a consensus process involving scientists and pathologists with expertise in the field throughout Australia and New Zealand. *Results:* Recommendations for standardised reporting of protein electrophoresis have been produced. These cover analytical requirements: detection systems; serum protein and albumin quantitation; fractionation; paraprotein quantitation; urine Bence Jones protein fractionation and quantitation; paraprotein characterisation; cryoglobulin characterisation; and laboratory expertise and staffing. The recommendations also include general interpretive commenting and commenting for samples with paraproteins and small bands together with. illustrative examples of reports. *Conclusion:* Recommendations are provided for standardised reporting of protein electrophoresis in Australia and New Zealand. It is expected that such standardised reporting formats will reduce both variation between laboratories and the risk of misinterpretation of results.

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GENETIC ABERRATIONS IN NORWEGIAN MULTIPLE MYELOMA PATIENTS

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Detection of cytogenetic abnormalities by fluorescence in situ hybridization (FISH) yields prognostic information in multiple myeloma. In this retrospective study we wanted to examine the prevalence of the most common primary translocations and deletions/amplifications in Norwegian multiple myeloma (MM) patients. We have included 296 Norwegian MM patients between Jan 2005 and Dec 2010 in which 259 patients could be analysed by FISH. FISH was performed on CD138+ separated cells or with cytoplasmatic-immunoglobulin-FISH on mononuclear cells to detect IGH split, del 1p, gain 1q, del 13q, and del 17p. When an IGH split was found, FISH was performed for t(4;14), t(11;14), t(6;14) and t(14;16). An IGH-translocation was found in 45% of the patients, including 17% t(11;14), 14% t(4;14), and less than 1% t(6:14) or t(14;16). 12% had an unknown IGH-translocation. Because of sparse sample material 36% of the patients with an unknown IGH translocation were only analysed for t(4;14) and t(11;14). del13 was found in 35%, del17 in 18% del1p was found in 13% and 1q amplification in 33%. Correlations between cytogenetic findings and clinical parameters and a possible impact on survival will be analyzed and presented at the conference.

A-477

MGUS EVOLVING TO SMOLDERING MULTIPLE MYELOMA WHILE ON IMATINIB THERAPY FOR CML. IS THERE A RELATIONSHIP?

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Chronic myelogenous leukemia (CML) and multiple myeloma (MM) involve distinct cell lineages. Simultaneous CML and MM is rare (13 reports). CML preceeding MM is rarer (4 cases). A 46 y.o. asymptomatic woman, diagnosed with CML, had an initial M-spike (IgG , 0.8 g/dL), but bone marrow (BM) and kariotype were typical of CML without plasmocyte´s (PC) alterations. Imatinib (IM) 400 mg/day lead to major molecular response (MMR) after 7 months. After 12 months, the M-spike persisted (1.1 g/dL), but now the BM showed 15% clonal PC (CD38+,CD56+), without end-organ damage. No therapy was prescribed for MM. FISH analysis now showed del13q. Two years after diagnosis, she keeps MMR for CML with an increasing M-spike of 1.4 g/dL and 15% clonal PC in the marrow. These findings indicate coexistence of CML in chronic phase and monoclonal gammopathy of uncertain significance (MGUS), quickly progressing to smoldering MM (SMM), during IM treatment. It is of note that a young woman, with a low M-spike, and no initial increased number nor clonal PC in the BM, has quickly evolved to SMM with high counts of clonal PC, and increasing M-spike, within few months after starting IM. Association of CML and MM is considered coincidental, but different authors find it possible that IM may induce progression to MM. This is the 14th reported case of concomitant occurrence of CML and MM, the 5th in which CML precedes MM, and it´s characteristics may give further support for the hypothesis already raised in the medical literature of IM influencing the evolution of MGUS to MM.

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IMPACT OF ETHNICITY ON MYELOMA PRESENTATION CHARACTERISTICS

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Introduction: Myeloma is a rare haematological malignancy with reported incidence in the UK of 6.6/100,000 persons. Reports have observed an increased incidence in black patients both in the UK and USA with a lower age at diagnosis. However these reports have not distinguished between black African (BA) patients and naturalised black populations (e.g Black British). We report a retrospective analysis of consecutive patients presenting to a UK myeloma centre and compare baseline characteristics between BA patients and other patients (NBA; excludes black British and Caribbean). *Results:* 25 patients were identified as BA and 38 patients as NBA with a median follow-up of 15 and 10 months respectively. No difference was observed in patient sex or immunoglobulin isotype. BA patients were diagnosed at a significantly younger age than NBA patients (mean 54.9 years v 67.4 years, p=0.0134). No significant difference was observed in mean presentation haemoglobin (10.3 v 10.3g/dl), calcium (2.30 v 2.33mmol/l), albumin (37 v 37g/l), β2M (6.2 v 9mg/l), LDH (508 v 405IU/l) or presence of lytic lesions (44% both groups). There was a trend towards a higher mean creatinine (125 v 242 μmol/l, p=0.07) and higher ISS stage at diagnosis in the NBA group (ISS 3: 25% v 50%, p=0.06). Median overall survival in both groups was 86% at 2 years. *Conclusions:* In this cohort black African patients were diagnosed at a significantly younger age and had a trend toward lower presentation creatinine and lower ISS stage but all other presenting features were similar to non black myeloma patients.

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MONITORING OF SERUM FREE LIGHT CHAINS IN THE TREATMENT OF LIGHT CHAIN DEPOSITION DISEASE WITH HIGH CUT-OFF HAEMODIALYSIS

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Background: Light chain deposition disease (LCDD) is a entity where serum free light chains (sFLCs) are precipitated on the basement membranes of cells in kidney. Severe renal failure is the common presenting feature.This disease does not have a clearly defined treatment and patients have an adverse prognosis.We present the case of a patient with LCDD and severe renal failure, treated by chemotherapy and haemodialysis with High Cut-Off membrane. *Case report:* A 57 year old man was admitted to the hospital due to very intense bone pain. He was diagnosed with LCDD.The κ/λ FLC ratio was abnormal at 43.The serum kappa FLC was 289 mg/l and the serum lambda FLC was 6.74 mg/l.While on analgesics, the bone pain improved but acute renal failure was detected (creatinine was 9,70 mg/dl) in the context of LCDD.The patient began treatment with Bortezomib and haemodialysis (daily six-hour periods over six days) with high cut-off membrane in order to remove FLCs in serum.We measured pre- and post-haemodialysis sFLC, the κ/λ FLC ratio and the creatinine in serum.The percentage of serum kappa FLC removed was 61% and the levels decreased from 90 to 59mg/dl during the treatment.The percentage of κ/λ FLC ratio reduction was 57% (from 11.1 to 6.3 during treatment).This improved the patient's renal function with a decrease of 88% in the creatinine serum levels (from 5.9 to 0.7 mg/dl). *Discussion:* A combination of efficient and direct removal of the toxic excess of serum FLCs using high cut-off membranes with Bortezomid allowed us to reduce the serum FLCs levels.

A-480

PREVALENCE OF MONOCLONAL GAMMOPATHIES AND REFERENCE VALUES OF FLC IN ELDERLY POPULATION (80-101 YEARS)

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Introduction: Free light chain (FLC) evaluation in serum is one of the recommended methods for detection and monitoring of monoclonal gammopathies (MG). It is known that concentration of FLC increases with age. In our work we focused on group of elderly persons above the age of 80 years without hematological disorders and no significant comorbidities. We observed prevalence of MG, concentration of FLC, kappa/lambda index and also heavy/light chain (HLC). *Methods:* electrophoresis and immunofixation (Sebia), ELISA (BioVendor), Freelite (Binding Site) and Hevylite (Binding Site). Studied population: 73 persons (54 females, 19 males), age 80 – 101 years. *Results:* Monoclonal immunoglobulin (MIg) in serum was present in 11 (15%) of persons. FLC reference values were estimated in 62 persons without MIg. Two methods were used. ELISA: kappa (2.7-71.9 mg/l), lambda (6.8-67.6 mg/l), kappa/lambda (0.194-3.815); FreeLite: kappa (11.4-75.7 mg/l), lambda (9.2-58.6 mg/l), kappa/lambda (0.868-2.668). Frequency of positive HLC was evaluated in the set of 37 persons without MIg: IgG kappa 3x, IgG lambda 1x, IgA kappa 7x, IgA lambda 3x, ratio IgG kappa/lambda 2x and ratio IgA kappa/lambda 2x. *Conclusion:* We are able to confirm increased prevalence of MG in elderly population in 11 out of 73 persons. Hevylite is probably able to detect formation of MIg earlier than classic electrophoresis and immunofixation. Reference range of FLC values (kappa, lambda and kappa/lambda ratio) increases with age significantly. Supported by grant IGA MZ CR NS/10387-3, NS/10406-3 and research project MZO00179906.

A-481

A SINGLE-INSTITUTE EXPERIENCE WITH ONCE-WEEKLY BORTEZOMIB IN COMBINATION WITH ORAL CYCLOPHOSPHAMIDE AND DEXAMETHASONE FOR ELDERLY PATIENTS WITH MULTIPLE MYELOMA

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Introduction: Recently, three drugs combination, including bortezomib, demonstrated higher response rate and improved outcome in multiple

myeloma (MM). However, twice-weekly schedule of bortezomib often caused peripheral neuropathy (PN) and it was main dose-limiting toxicity. Under these circumstances, we introduced once-weekly schedule of bortezomib combined with oral cyclophosphamide and dexamethasone (CBD) into elderly patients to reduce the toxicity of bortezomib-associated PN. Methods and Patients: The protocol consisted of bortezomib given intravenously at a dose of 1.3 mg/m2 once a week on days 1, 8, 15, cyclophosphamide orally at a dose of 50 mg daily on days 1-21, and dexamethasone orally or intravenously at a dose of 20 mg daily on days 1,2,8,9,15,16 in 4-week cycles. Total of 10 patients, including two patients with extramedullary lesions, were treated with CBD and evaluated its efficacy and safety. The median age was 74 years. 6 were male and 4 were female. 6 were newly diagnosed and 4 were relapsed. According ISS, 2 patients were classified in stage I, 4 were in II, and 4 were in III. Result: 8 out of 10 patients (80 %) achieved objective response (1 nCR, 3 VGPR and 4 PR), and extramedullary lesions were disappeared. Neither hematological nor non-hematological grade 4 adverse events were observed. Only one patient developed grade 1 PN, and no patient reduced or discontinued bortezomib due to PN. *Conclusion:* Our results suggested that our combination therapy with once-weekly schedule of bortezomib is safe and effective approach for elderly patients with MM.

A-482

SKEWED HEVYLITE RATIOS (HLR) MAY SENSITIVELY PREDICT EARLY RELAPSE: A FIRST CASE REPORT AND OUTLINE FOR FURTHER STUDIES

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Introduction: Hevylite™, a novel immunoassay panel is designed for analysis of immunoglobulin heavy chain/light chain pairs. These assays can identify, separately, the different light chain types of each immunoglobulin class i.e. IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ. The molecules are then measured in pairs e.g. IgG /IgG to produce ratios of monoclonal immunoglobulin/background polyclonal immunoglobulin concentrations. HLR may serve as a parameter for myeloma induced immunoparesis and serve as a new marker for validating remission depth and relapse probabilities. *Case History:* A 54 year old male patient received a 2nd line treatment for relapse of a IgAλ myeloma in the setting of the VANTAGE study (Bortezomib/Vorinostat vs. Bortezomib/Placebo). 1st line treatment had been TD followed by autologous transplantation resulting in CR over 3 years. *Results:* 8 cycles of therapy resulted in a sCR, while the HLR remained constantly heavily skewed all over the observation period. Early, high dynamic, relapse developed in the patient within 3 months of end of therapy.

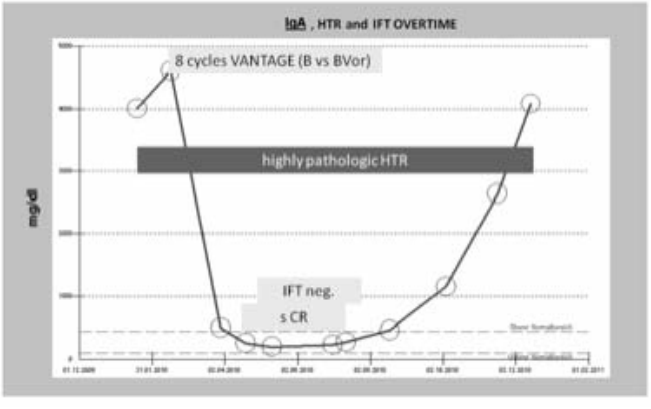

Figure 1. IgA, IFT and HLR overtime.

Conclusions: As this case illustrates HLR may serve as new diagnostic tool for rational treatment allocation, especially with respect to maintenance and consolidation strategies. An update of ~ 30 myeloma pts. will be presented, as well as the outline of a cooperative European project to further validate this putative biomarker will be introduced. HLR should be validated against techniques using sCR, flow-CR and molCR.

A-483

SERUM FREE LIGHT CHAINS IN MONITORING A LAMBDA LIGHT CHAIN MULTIPLE MYELOMA PATIENT´S TREATMENT

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Background: The patients with an excess of free light chains (FLCs) are difficult to diagnose as they frequently show no abnormality when tested by serum protein electrophoresis (SPE). The FLCs are very important markers for monitoring patients with multiple myeloma (MM) and other monoclonal gammopathies. *Case report:* A 66 year old man was diagnosed in May 2008 with lambda light chain MM (LCMM) with plasmacytoma. In June 2008, he began treatment with six cycles of VAD (Doxorubicin, Vincristine and Dexamethasone) and one month later the κ/λ FLC ratio was normal while SPE and serum immunofixation (sIFE) were negative. In October 2008, after the first chemotherapy cycles, the κ/λ FLC ratio was abnormal (0.002) while SPE and sIFE were negative. The value of the ratio predicted a relapse in the patient's condition. Due to partial response, the patient began a treatment of five cycles of Bortezomib and dexamethasone from January to May 2009. It improved the symptoms but the κ/λ FLC ratio remained altered (0,01) until June 2009. Then, he started a new cycle with Lenalidomide and dexamethasone improving the patient's condition. The κ/λ FLC ratio was normal during this treatment and the ratio did not predict a relapse. Currently, the SPE and IFE are negative, the κ/λ FLC ratio is normal (0,73) and the patient is clinically well. *Conclusions:* This case is a good example of the utility κ/λ FLC ratio in the monitoring of MM and it can predict future relapses in the patient.

A-484

UTILITY OF FREE LIGHT CHAINS IN THE DIAGNOSIS AND MONITORING OF MONOCLONAL GAMMOPATHIES

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Introduction: The detection of monoclonal inmunoglobulin free light chains (FLCs) is very important for the diagnosis and monitoring of patients with multiple myeloma and others monoclonal gammopathies. When the serum FLCs are present in low concentrations, they are difficult for the detection by conventional methods as serum protein electrophoresis (SPE) and immunofixation (IF). The detection for serum FLCs by quantitative nephelometric assays are more sensitive for FLCs in serum than conventional electrophoretic techniques. We report here four patients for whom FLCs were either undetectable or barely detectable using the conventional qualitative assays. *Methods:* Sera of the four patients were sent to the protein laboratory for the study of the monoclonal gammopathies. SPE were peformed on CAPILLARYS 2™ (Sebia), the monoclonal component were identified by IF on HYDRASYS™ (Sebia), serum inmunoglobulins (IgA, IgG and IgM) were measured by nephelometry on BNII nephelometer (Dade Behring) and the FLCs were measured by FREELITE™ (The Binding Site) nephelometric assay. *Results:*

Case N°	Age	Sex	SPE	IF	κ(mg/L)	(mg/L)	κ/λ	Diagnosis
1	66	M	Normal	Normal	1,42	524,0	0,002	Relapse of Lambda Multiple Myeloma
2	74	F	Normal	Normal	17,7	1800,0	0,009	Primary Amyloidosis
3	57	M	Weak peak	Kappa	289,9	6,74	43,00	Light Chain Deposition Disease
4	64	M	Weak peak	Weak lambda	6,36	752,0	0,008	Relapse of Nonsecretory

Conclusion: The nephelometric assay of FLCs (FREELITE™) allows us an accurate quantification of serum FLCs in the diagnosis and monitoring of patients with monoclonal gammopathies.

A-485

IGD LAMBDA MULTIPLE MYELOMA WITH VERY AGGRESSIVE DISEASE COURSE

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Introduction: Multiple Myeloma (MM) is a malignancy of B cells characterized by an atypical proliferation of plasm cells. IgD MM has a very low incidence (2%) and it´s characterized by an aggressive course and a worse prognosis than other subtypes. We present the clinical case of IgD lambda MM with a fatal course of the disease. *Case presentation:* A 83 year old woman was admitted to the Hospital due to a bad condition and a loss of appetite and weight. She had a hematocrit of 23,8% and a hemoglobin of 8.3 g/L. She was transfused with red blood cells. She was studied with the following *Results:* 2.770.000 red cells/µl, hemoglobin of 8.6 g/L and a hematocrit of 25.3%. Immunoglobulins were decreased (IgA = 11 mg/dL, IgG = 515 mg/dL, IgM = 32 mg/dL) with an abnormal serum κ/λ free light chain (FLC) ratio (kappa=11.20 mg/l, lambda=1410 mg/l and κ/λ FLC ratio=0.008). The renal function was impaired (creatinine=3.00 mg/dL). The serum protein electrophoresis had a monoclonal peak of high amplitude at beta region and serum immunofixation detected monoclonal bands of IgD and lambda light chains. The bone marrow report revealed a 28% infiltration by plasma cells. The patient was diagnosed with IgD lambda MM with present symptomatology and organ involvement. One month later the patient showed a great injury and presented progressive renal failure. Six days after, she died in the palliative care unit. *Conclusions:* IgD lambda MM in advanced stages is a disease with an aggressive course and it´s associated with a higher rate of renal failure and mortality.

A-486

ASSESSMENT OF MONOCLONAL IGG PROTEINS USING THE HEVYLITE ASSAY

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Introduction: Quantification of IgG heavy chain/light chain (HLC) pairs using the Hevylite nephelometric assays may offer a useful adjunct to conventional techniques for detecting and quantifying serum monoclonal proteins. We describe the use of the IgG Hevylite assays in a routine diagnostic laboratory. *Methods:* Consecutive IgG monoclonal proteins identified by capillary electrophoresis immunofixation (IFE) and quantified directly by ultraviolet absorption (PPQ) were assessed by IgGκ and IgGλ Hevylite immunoassays and results compared. *Results:* 37 serum samples were identified as having a monoclonal IgG by IFE (κ=19, λ=18). The patients comprised 21 Myeloma (MM) patients (median monoclonal protein 15.5g/l; range 1-36g/l), 12 monoclonal gammopathy of undetermined significance (MGUS) patients (Median PPQ 6g/l; 1-12g/l) and 5 other diagnoses. There was good agreement between PPQ and the appropriate HLC assay (r=0.81, p<0.0001) and between total IgG and summated IgGκ+IgGλ (r=0.95, p<0.0001). The appropriate HLC assay was increased in 9/19 IgGκ and 12/18 IgGλ samples and an abnormal IgG HLC ratio was present in 17/33. Importantly the appropriate HLC level was increased in 6/11 IgGκ and 8/11 IgGλ MM patients with an abnormal IgG HLC ratio in 18/21 MM patients. *Conclusion:* HLC data correlated well to PPQ and total IgG. The assay detected most MM patients suggesting utility for monitoring. Further work is required to determine the significance of HLC results in MGUS and other monoclonal protein producing disorders.

A-487

REDUCING TREATMENT TOXICITIES WHILE MAINTAINING RESPONSE BY INDIVIDUALIZING THE DOSING

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Despite a wide variation in the observed efficacy and toxicities for patients who are administered the same dosage regimen for multiple myeloma (MM), individualized dosing is not being practiced today. Reasons include expense and inconvenience for the patient and clinician. These barriers can be overcome by a novel Bayesian strategy, which exploits recent advances in computational capabilities, namely speed and mass storage, to calculate assumption free population distributions for PharmacoKinetically (PK) Sensitive Treatment Agents (PSTA). From one or two strategically and conveniently timed blood draws, the population PK distribution is tailored to an individualized posterior PK parameter distribution for the patient. This information predicts the concentration time profile at any time following drug administration. If a PharmacoDynamic model is available the tool can be used to individualize the dosing strategy to minimize the toxicities while maintaining efficacy. Literature data suggests that Velcade, Thalidomide and Dexamethasone are all PSTA's. Prospective clinical studies with these agents are planned but have not been completed. However the process will be illustrated with gabapentin - an anticonvulsive approved by the FDA for the treatment of neuropathic pain associated with postherpetic neurolgia and used for treatment of peripheral neuropathy in MM patients. In a small 28 patient cohort it was found that over 20% of the patients were receiving ineffective treatment despite a wide therapeutic window.

A-488

IGA HEVYLITE ASSESSMENT IN A ROUTINE MONOCLONAL PROTEIN LABORATORY

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Introduction: Identification of monoclonal IgA proteins by serum protein electrophoresis (SPE) can be hampered by the immunoglobulins comigration with other proteins. Analysis of serum IgA heavy chain/light chain pairs (HLC) may aid their identification and be a useful addition to laboratory assessment. Here we report on the use of IgA HLC nephelometric assays in a routine laboratory. *Methods:* Consecutive serum samples identified by capillary electrophoresis immunofixation (IFE) and quantified by direct ultraviolet absorption (PPQ) were analysed using the paired IgAκ/IgAλ Hevylite immunoassay and results compared. *Results:* 17 serum samples were identified by IFE as having an IgA monoclonal protein (κ=9, λ=8) . The patients comprised of 11 Myeloma (MM) patients (median PPQ 8g/l; range 0.5-19g/l) and 6 MGUS patients (Median PPQ 3g/l; 0.5-9g/l). There was good agreement with PPQ measurements and the appropriate HLC assay (r=0.92, p<0.0001) and between total IgA and summated IgAλ+IgAκ (r=0.94, p<0.0001). The appropriate IgA HLC was increased in 13/17 patients (8/9 IgAκ, 5/8 IgAλ). There was an abnormal HLC ratio in 9/11 MM patients and 4/6 MGUS patients. In 4/17 patients IgA monoclonal protein was <1g/L and HLC ratio was normal (2 MM, 2 MGUS). *Conclusions:* HLC data correlated well to PPQ and total IgA. HLC analysis identified IgA monoclonal proteins irrespective of their migration. All IgA monoclonal proteins >1g/l were correctly identified. In the absence of immunosupression minor IgA bands may be reported with a normal ratio.

A-489

FAMILIAL MULTIPLE MYELOMA - STUDY OF 3 FAMILIES WITH 6 CASES OF MULTIPLE MYELOMA (MM)

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Introduction: The Familial Multiple Myeloma frequency is 3.2 per 1000 cases of MM with an incidence of 0.1 cases per million people per year, which ranks it as a very rare event. However, the risk of relatives developing MM is 1.7 times, and MGUS 2.9 times, greater than that of the general population. Case Studies: We describe 3 unrelated families with 2 cases of MM in each. Family 1: The first proband, male, black, 38 y.o., was identified as MM IgG-Kappa. Eleven years later, his brother, 59 y.o.

was diagnosed as MM IgG-Kappa. Of the remaining 8 siblings, we evaluated 5 with protein electrophoresis and immunofixation and identified one case with the monoclonal protein IgG-Kappa. In the two MM cases the same profile of HLA - (A, B, C, DRB1, DQB1) was found. Family 2: Proband female, 44 y.o., white, diagnosed as MM IgG since October, 2000. In 2009 her mother was diagnosed as MM IgA-Kappa at 74 y.o. Family 3: MM in first degree cousins. Male, white, 67 y.o. with a diagnosis of MM IgG-Lambda since January 2008. Male, white, 65 y.o. with a diagnosis of MM IgA-Kappa in June 2009. *Conclusion:* Different from what is being found in the cohort of familial MM, we found co-occurrence of the monoclonal immunoglobulin isotype in the same family (1). Further analysis with other family members is being made. This study establishes a precedent for the diagnosis of familial MM, considered a risk factor for other members of the same family to develop some kind of gammopathy.

A-490

REACTIVATION OF HEPATITIS B VIRUS IN HBSAG-NEGATIVE PATIENTS WITH MULTIPLE MYELOMA: THREE CASE REPORTS

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Reactivation of hepatitis B virus (HBV) is a well-recognized complication in hepatitis B surface antigen (HBsAg)-positive patients who received hematopoietic stem cell transplantation or chemotherapy. However, it is unclear whether HBsAg-negative patients with multiple myeloma have the risk of HBV reactivation. We encountered three HBsAg-negative patients who developed HBV reactivation during the treatment for myeloma. Case 1: a 58 year-old male was treated with vincristine, doxorubicin and dexamethasone. HBcAb was positive before the treatment. He received high-dose melphalan followed by ASCT. He then received bortezomib and dexamethasone (BD). Five months after ASCT, HBV-DNA was elevated by 3.6 log copies/mL and HBsAg was detected. After the administration of entecavir, liver dysfunction was resolved and HBsAg and HBV-DNA became negative. Case 2: a 70 year-old female was treated with melphalan and predonisone. HBsAb was positive before the treatment. After relapse, thalidomide followed by BD was given. During BD therapy, HBV-DNA was elevated by 2.1 log copies/mL. However, HBV-DNA became negative after interruption of BD. Case 3: a 63 year-old female received thalidomide and dexamethasone (TD) after ASCT. HBsAb was positive before the treatment. During the therapy, HBV-DNA was elevated by 2.1 log copies/mL. However, HBV-DNA became negative after interruption of TD. Thus, it is necessary to pay attention to HBV reactivation not only after ASCT but also during the therapy with novel agents in HBsAg-negative myeloma patients. Serial HBV-DNA monitoring is recommended.

A-491

SURVEY ON THE MANAGEMENT OF MYELOMA BONE LESION BASED ON THE RESPONSE TO QUESTIONNAIRE BY JAPANESE PATIENTS WITH MULTIPLE MYELOMA

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According to recent evidence-based guidelines, appropriate intravenous bisphosphonate (BP) administration and patient education including prevention of adverse events are important in the management of multiple myeloma (MM). However, few studies have been reported about adherence of these guidelines, especially in the viewpoints from the patients. We planned the survey on the management of bone lesions of MM by questionnaire to the patients. The survey was conducted by International Myeloma Foundation (IMF) Japan. The questionnaire was send to 563 members of IMF Japan (including patients themselves and their family members). Valid responses were obtained from 438 members (78%) from October to November, 2008. Diagnosis of MM bone lesion was made in approximately 60% of responders of the questionnaire. BP was most frequently used for the treatment of bone lesion (more than 80%). There are some cases in which BP were not applied appropriately (such as oral BP administration). About half of the responders were satisfied by intravenous BP treatment, regarding as its effects, adverse events and route of administration. However, many of them were not satisfied about the drug cost. On the other hand, only 15% of the responders were satisfied about the effects of BP when received by oral route. To manage bone lesion of MM, collaboration among each departments (hematology, orthopedics, dentistry and so on) are important (related to early diagnosis, and management of complicating disease). Interactive information provision may be necessary among doctors, co-medicals and patients.

A-492

ESTABLISHMENT OF NORMAL RANGES FOR IMMUNOGLOBULIN HEAVY CHAIN/LIGHT CHAIN PAIRS IN THE UNIVERSITY HOSPITAL OF VIRGEN DE LA MACARENA, SEVILLA, SPAIN

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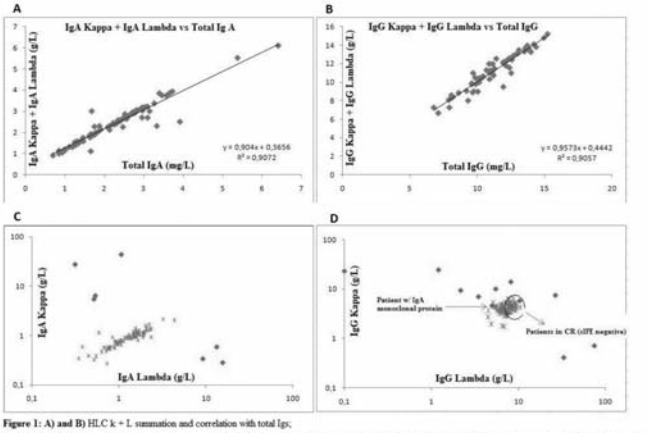

Figure 1: A) and B) HLC k + L summation and correlation with total Igs;
C) and D) Dot Blots showing the IgA k / IgA L and IgG k / IgG L from the donor population and the MM patients (in blue: donor sera sample; red: patients sample).

IgA

	IgA k (g/L)	IgA λ (g/L)	ratio IgA k/λ
N	83	83	83
Mean	1,37	0,92	1,47
Median (95% Range)	1,34(0,38-3,12)	0,92(0,32-2,01)	1,44(0,66-2,47)
Median (95% Range)[1]	1,27(0,43-2,36)	0,87(0,4-1,73)	1,40(0,58-2,52)
Median (95% Range)[2]	1,19(0,48-2,82)	1,00(0,36-1,98)	1,27(0,80-2,04)

IgG

	IgG k (g/l)	IgG λ (g/l)	Ratio IgG k/λ
N	75	75	75
Mean	7,10	4,10	1,81
Median (95% Range)	7,00(4,08-9,56)	4,02(1,76-6,24)	1,75(0,98-3,69)
Median (95% Range)[1]	7,76(4,23-12,08)	4,00(2,37-5,91)	1,96(1,26-3,2)
Median (95% Range)[2]	6,85(4,03-9,78)	4,81(1,97-5,71)	1,87(0,98-2,75)

1 - Clinical Chemistry 55:9, 1646-1655(2009)
2 - Serum free light chains plus Hevylite (2010).6th Edition

Table 1: Normal HLC ranges from a Spanish population and comparison to previously described ranges.

Background: Detection and quantification of monoclonal proteins by serum protein electrophoresis can often be difficult, especially in cases of low amount of paraprotein or when the band is masked. Immunofixation improves sensitivity to the detection protocol but is not quantitative. A new assay is now available that allows the quantification of specific heavy chain/light chain pairs (HLC) (IgAk, IgAL, IgGk, IgGL), and it is our aim to determine normal ranges in healthy individuals considering that the use of ratios help us improve the diagnostic and follow-up of MG. *Methods:* We measured HLC concentrations in blood donor sera by turbidimetry. Immunoglobulins G and A were also measured by turbidimetry in order to evaluate if the summation of both pair of chains are equivalent to total Igs. 20 myeloma samples (7 IgA MM + 13 IgG MM) were also analyzed.

Results: Normal ranges were determined (Table 1) and are in agreement with the ranges previously published. The HLC K + λ summation had a good correlation with the total Igs levels (Figure 1). 20 pathologic samples were also analyzed and compared with the determined normal ranges (figure 1). The HLC ratio was normal for only three patients that also had a negative sIFE. *Discussion:* HLC assay allows the determination (typing and quantification) of individual Ig K and λ concentrations and its ratios. It presents an enormous potential for the identification and the follow-up of patients with very low monoclonal components or in cases where it is difficult to identify a monoclonal protein hidden by other proteins.

A-493

MANAGING BORTEZOMIB RELATED TOXICITIES - INVOLVING THE PATIENT

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Managing bortezomib related toxicities – involving the patient. *Background:* The efficacy of bortezomib in relapsed or refractory multiple myeloma has necessitated accurate assessment of treatment related toxicities, in particular neuropathy. Here we propose a neuropathy questionnaire enabling both the patient and the specialist nurse to rapidly identify toxicities. This encourages timely dose modification and initiation of supportive therapy, thereby maximising treatment duration and minimising deleterious effects on quality of life. *Observations:* Approximately 25 patients a week receive bortezomib as an outpatient at University College London Hospital. The introduction of a single page questionnaire, completed by patients on day 1 and day 8 of therapy, has enabled prompt assessment of bortezomib related toxicities. It has also proven to be a valuable educational tool for patients, encouraging a greater degree of autonomy in assessing their well-being. In addition, nursing staff are alerted early as to complications of treatment, facilitating early medical input. This has enabled appropriate dose modification acting to minimise both acute and chronic treatment related side effects. *Conclusion:* We propose that a bortezomib toxicity questionnaire is an effective tool in educating patients in reporting bortezomib related side effects, thereby maximising the tolerability of bortezomib regimens enabling early intervention for treatment related toxicities.

A-494

SERUM POLYCLONAL IMMUNOGLOBULIN (SPIG) LEVELS AND INFECTIONS IN PATIENTS WITH MULTIPLE MYELOMA (MM) RECEIVING TWO DIFFERENT BORTEZOMIB-BASED CHEMOREGIMENS

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An important feature of MM is the dysregulation of polyclonal immunoglobulin production. Bortezomib is widely used to treat patients (pts) with MM, however SPIgs during treatment have not been systematically observed. *Aims:* The objective of this study was to record SPIg levels and infections in pts with MM receiving bortezomib-based treatment. *Methods:* The study included 25pts (20 /5 ;age range: 44-71y) who were treated in the past 3.5 years. 13pts received bortezomib/dexamethasone(VD) and 12pts received bortezomib/lipos.doxorubicin/dexamethasone(PAD). SPIgs (IgG,IgA,IgM) were assessed before and after treatment. Patients' files were reviewed retrospectively for infectious complications and response to treatment. *Results:* VD group: SPIg levels were low in 12/13pts at diagnosis. After completion of therapy, 9pts exhibited an increase of SPIg levels, although only in 4pts levels rose above the lower limit of normal. In 4pts SPIgs remained stable or slightly decreased. 5 infections were reported in 5pts. 12/13pts showed response to treatment (sCR:3,VGPR:3,PR:6). PAD group: SPIg levels were low in 10/12pts at diagnosis. In most pts (9/12) SPIg levels were stable or decreased during therapy. The 2pts with normal SPIgs at diagnosis eventually developed hypogammaglobulinemia despite achieving CR. 11 infections reported in 9 pts. All pts responded (sCR:2,CR:5,VGPR: 2,PR:3). *Conclusions:* In our group of patients SPIgs were more profoundly affected in those receiving PAD than in those receiving VD. Infections were more frequent in the PAD group despite a very good response profile.

A-495

AN INTRACTABLE COUGH AND A SURPRISING HISTOPATHOLOGICAL FINDING IN A LIGHT CHAIN MULTIPLE MYELOMA PATIENT

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A 72-year-old man was diagnosed 15 years ago, as suffering from solitary plasmocytoma. No monoclonal peak in protein electrophoresis was detected, immunoelectrophoresis was normal, as were calcium, creatinine and hemoglobin, skeletal survey, but a positive lambda Bence Jones in urine and 30% plasma cells in the bone marrow were detected. After radio- and chemotherapy, the urinary Bence Jones disappeared and the patient was in complete remission untill the end of 2009, when Bence Jones reappeared and kappa/lambda free light chain was within abnormal (but very mild) limits. In 09/2010 the patient complained of intractable cough and the CT examination revealed a massive tumor (10 x 12 cm) in the chest wall, at the level of the7th rib, with pressure on the chest wall, right lung and liver. He underwent surgery, the tumor was extirpated with the involved rib. At histopathological examination an amyloidoma was diagnosed. A bone marrow biopsy showed this time less than 5% plasma cells and without amyloid deposition. The skeletal survey continues to be normal, as are the protein and immunoelectrophoresis. Amyloidoma (tumoral amyloidosis) is a solitary localized tumor, formed by a deposit of amyloid; there are no signs of systemic amyloidosis. Amyloidoma is rare in comparison with diffuse deposition of amyloid. Chest wall amyloidoma is extremely rare, only two cases have been reported in the literature. The singular facts of this case are: the extremely long remission (13 years) and the appearance of tumoral amyloid approximately concomitant with lambda light chain reappearence.

A-496

LYMPHOMATOUS MENINGITIS AND SKIN INVOLVEMENT IN A CASE OF WALDENSTROM MACROGLOBULINEMIA

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Background: Infiltration of the meninges is rarely reported in waldenstrom macroglobulinemia (WM).Skin lesions are uncommon in waldenstrom macroglobulinemia. *Case Report:* A 75 y/o male a of waldenstrom macroglobulinemia came with headache and dizziness.The diagnosis of WM had been made according to high serum IgM titer ,cold agglutinin disease and lymphoplasmacytic infiltration in bone marrow aspiration and biopsy.He had been received 3 cycles of cladribine. Physical examination revealed tortuous retinal veins and a large flesh-colored periumbilical plaque in skin of abdomen .Serum IgM level was very high. Initially with the diagnosis of hyperviscosity syndrome we started urgent plasma exchange and chlorambucil and prednisolone but headache did not improved. Brain MRI was reported normally. CSF analysis revealed increased lymphocytes and CSF cytology showed lymphoblasts. Skin biopsy showed lymphomatous infiltration and IHC was positive for CD20 and LCA. We considered intratechal chemotherapy and then brain irradiation for him. *Results:* We found skin involvement and lymphomatous meningitis in this case with waldenstrom macroglobulinemia. *Conclusion:* We should think for meningeal lymphomatosis in patients with WM and headache.Brain imaging and CSF analysis is recommended for patients with WM and headache or cranial nerve palsies.Skin lesions are uncommon but may be seen in WM.Skin biopsy and IHC study is recommended for differentiation between lymphoplasmacytic infiltrartion, deposition og IgM in epidermal basement membrane and skin vasculitic changes which may be seen in WM.

A-497

SCHNITZLER SYNDROME: A REPORT ON TWO PATIENTS WITH CHRONIC URTICARIA AND PARAPROTEINEMIA

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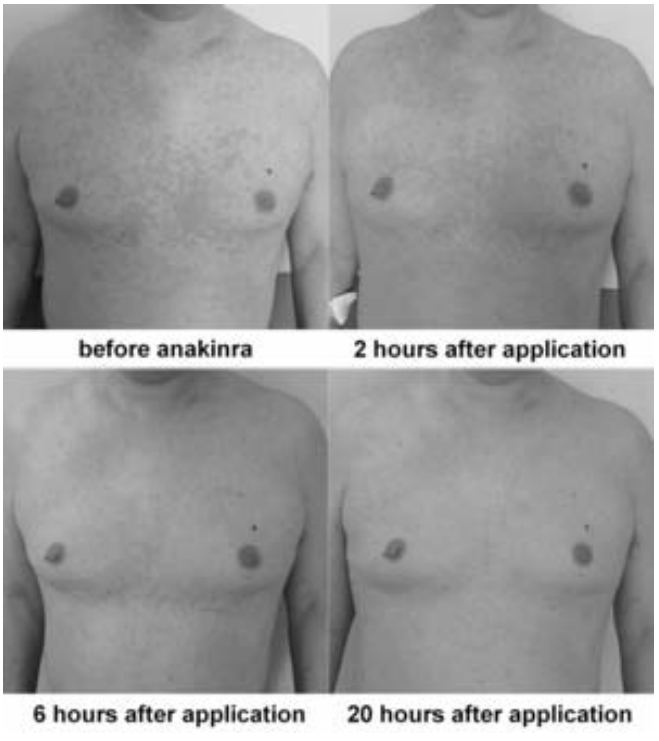
Chronic urticaria and presence of monoclonal IgM are the hallmarks of Schnitzler syndrome, a rare idiopathic disease with potentially life-threatening complications such as development of systemic amyloidosis or transformation into malignant lymphoma (e.g. Waldenström macroglobulinemia or multiple myeloma). We report on our long term experience with follow-up of two male patients. Both presented pruritic and nonpruritic urticarias at the age of 45 and 43 years, respectively. Based on further symptoms (fever, bone pains), laboratory tests (monoclonal IgMκ, leukocytosis, elevated CRP) and radiological findings (osteolytic-osteosclerotic and hyperostotic skeletal changes), they were subsequently diagnosed with Schnitzler syndrome. Follow-up times were 15 years and 1 year, respectively. As regards therapy, we used several medications (antihistamines, bisphosphonates, corticoids, cladribine, interferon-α, cyclosporine, thalidomide, bortezomib) and PUVA treatment, but none of these put the disease into complete remission. Only anakinra (interleukin-1 receptor antagonist) minimized Schnitzler symptoms in both patients with very good drug tolerance. The first patient has been on anakinra therapy for more than 3 years (10/2007-2/2011) at a dosing interval of 24-48 hours without any signs of Schnitzler syndrome. In conclusion, the possibility of life-threatening complications and the fact that patients are often referred to oncology clinics due to monoclonal gammopathy are the main reasons why clinical oncologists and haematologists should be aware of Schnitzler syndrome.



A-498

MULTIPLE MYELOMA ASSOCIATED IGA PEMPHIGUS: COMPLETE REMISSION AFTER THERAPY WITH BORTEZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE REGIMEN

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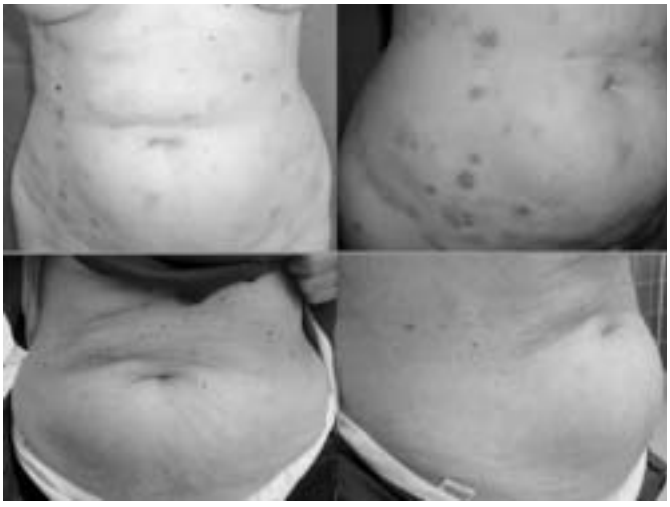
Subcorneal pustular dermatosis type of IgA pemphigus associated with multiple myeloma is a rare autoimmune skin manifestation of monoclonal gammopathy the therapy of which has been very difficult with inconsistent treatment responses. Herein we are the first to report on successful bortezomib therapy in one patient, a female born 1940, who presented vesiculobullous lesions on her trunk and extremities at the age of 50. Due to a finding of monoclonal IgAλ in the serum, the patient was referred to our clinic in 2001. Immunosuppressive treatment (cyclophosphamide + dexamethasone, then rituximab) proved no lasting effect. In 2007 disease transformation into symptomatic multiple myeloma was identified. First line anti-myeloma treatment (cyclophosphamide + adriamycine + dexamethasone, 4 cycles) remained without any treatment response. Second line treatment (cyclophosphamide + thalidomide + dexamethasone, 1 cycle) significantly deteriorated dermal symptoms up to a clinical picture of erythrodermia. Only when third line treatment (cyclophosphamide + bortezomib + dexamethasone, 6 cycles) had been given, rapid decline in monoclonal IgA concentration with negative immunofixation after 5 cycles were achieved. From the 3rd cycle on the patient has been completely without dermal symptoms and complete skin and haematological remission was maintained for 18 months (10/2008-4/2010). Thus, in IgA pemphigus associated with monoclonal gammopathy of unknown significance transformed into multiple myeloma, the combined regimen with included bortezomib has definitely proved effective



A-499

CARDIAC IMPAIRMENT AND AL-AMYLOIDOSIS

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Introduction: The systemic AL-amyloidosis(AL) is a systemic haematological disease belonging to the group of monoclonal gammopathies. Cardiac impairment is usually present by more than a half of the patients(pts) and represents the most significant prognostic indicator of this disease. *Aim:* The study aimed at detecting the presence of cardiac impairment by means of laboratory and non-invasive methods by pts with AL, examined at the time of the diagnosis. *Methods:* The group consisted of 17 pts with the histologically verified AL (12x AL, 5x associated with multiple myeloma), all pts were examined using ECG, echocardiography(ECHO), levels of NT-proBNP and Troponin T were determined, and some of the pts underwent myocardial magnetic resonance (MRI). *Results:* 13 (70%) out of 17 pts met the criteria of the International Society for Amyloidosis (ISA) for cardiac impairment according to ECHO; an increased level of Troponin T was recorded in 12 out of those pts. Levels of NT-proBNP were higher by 15 pts, whereas 13 of the pts exceeded 1000ng/l. MRI of the heart was positive by 8 out of 11 examined pts who, however, had already shown ECHO signs of cardiac impairment. By 3 pts with a negative MRI finding, no ECG or ECHO sings of cardiac impairment were detected. *Conclusion:* Our experience confirms the very positive benefit of the implemented examination algorithm of pts with AL, which lies in a combination of imaging methods, heart biomarkers, and together with the ISA criteria, it seems to be the optimum approach in the diagnostics of amyloid cardiomyopathy. Supported by VVZ MSM 619895205.

A-500

ASSOCIATION OF MELPHALAN, PREDNISONE AND BORTEZOMIB (MPV) AS FIRST LINE THERAPY IN AN ELDERLY PLASMA CELL LEUKAEMIA PATIENT WITH ACUTE RENAL FAILURE

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Background: Plasma cell leukaemia (PCL) is a rare haematological disease with poor prognosis which can be primitive or usually secondary to multiple myeloma as terminal event. Bortezomib represents a therapeutic option, especially in combination with oral melphalan and prednisone (MPV). *Methods:* From March 2009 we treated with 6 upfront MPV courses a 69 yrs old male patient with secondary PCL as multiple myeloma exordium. Peripheral blood haematochemical assays showed increased WBC (25.730/µL with PC > 40% of nucleated cells), LDH 1015 U/L, non oliguric acute renal failure (BUN 90 mg/dL, creatinine 6.6 mg/dL), hypercalcemia (15 mg/dL) and hyperuricemia (17.3 mg/dL). Primary treatment with intravenous fluids, steroids, furosemide and biphosphonate has been administered in order to reduce serum calcemia and uricemia achieving quick renal function improvement before chemotherapy beginning.Treatment response was assessed according to the IMWG uniform response criteria. *Results:* The patient reached a stringent complete response (sCR) with good quality of life and persistent normal renal function. A disappearance of peripheral circulating plasma cells was documented just within the first course of therapy; also serum creatinine became normal within the second course. No adverse event has been reported. The PFS is actually of 11 months while on consolidation treatment with 3 further dose-reduced MPV courses. *Conclusions:* MPV scheme could be an effective upfront therapy in terms of haematological and renal response not only for multiple myeloma, but also for secondary PCL patients.

A-501

RECURRENT SPONTANEOUS LIVER HEMORRHAGE AND HEMOPERITONEUM IN EXTENSIVE AL-AMYLOIDOSIS: CASE REPORT

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Spontaneous hepatic bleeding is a rare but potentionally life-threatening complication of primary systemic amyloidosis. We report the case of a female, born 1950, repeatedly admitted to hospital and surgically revised because of hemoperitoneum. The first disease symptoms, mild and non-specific (subfebriles, nausea, vomitus, diarrhea), appeared in our patient in 2008. After finding a liver infiltration suspected of hemangioma, an embolization complicated by liver rupture and developement of a chronic subhepatal and retroperitoneal hematoma was performed in June 2009. Nevertheless, the diagnosis of lambda-AL amyloidosis was determined from a liver biopsy as late as in April 2010. Based on clinical and radiological evaluations, progressive hepatomegaly and formation of new subcapsular and intraparenchymal hematomas (maximum size 16 x 6 cm) were confirmed. Applied systemic chemotherapy (cyclophosphamide, dexamethazone) didn't lead to any substantial improvement of the disease due to its advanced stage. Two more epizodes of hemoperitoneum had occured in this patient (6/2010 and 10/2010) and the woman died of multiorgan failure in November 2010. Autopsy showed AL-amyloidosis with massive infiltration of the liver (estimated weight 4000 g), spleen, kidneys, intraabdominal lymph nodes and suprarenal glands. The liver was described as extremely fragile, disintegrating at a touch. Herein we present macroscopic pictures of the affected organs, histologic results including congo red staining as well as CT, PET/CT and MRI images of the liver infiltrated with amyloid deposits.

Figure 1. Massive liver infiltration in AL-amyloidosis.

A-502

TREATMENT OF AL AMYLOIDOSIS WITH AUTOLOGOUS STEM CELL TRANSPLANTATION: RESULTS IN A SERIES OF 41 PATIENTS FROM A SINGLE INSTITUTION

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Treatment of immunoglobulin light chain (AL) amyloidosis is a real challenge. Long-term experience on high dose melphalan and autologous stem cell transplant (HDM/SCT) from single referral centers has shown its potential to obtain durable hematologic responses, a high organ response rate, and prolonged overall survival, with a decreased transplant related mortality (TRM) in recent years. We analyzed a series of 41 consecutive patients (median age: 56; range, 34 to 70) treated with HDM/SCT at a single center from November 1997 to December 2009. Median number of involved organs was 2 (range, 1 to 5), with cardiac involvement in 58% and >2 organs involved in 49% of patients. Median time from diagnosis to SCT was 8 months and 16 patients had received previous therapy. A reduced dose of melphalan (140 mg/m2) was administered as conditioning regimen in 13 (32%) patients according to age, heart involvement, renal dysfunction and/or performance status. In an intention to treat analysis, the overall hematologic response rate was 33% (17% complete remissions) and 44% achieved an organ response. Thirteen patients died during the first year following SCT and the TRM was 24.4%, decreasing from 42.8% during the first 5 years to 14.8% for the last 27 patients (p= 0.06). Moreover, none of the last 11 patients transplanted died from TRM. Median time to progression and overall survival were 3.4 and 6 years, respectively. Our results support the efficacy of SCT in AL and the fact that a careful selection of patients and experienced management significantly reduces TRM in this disease.

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REFRACTORY PLASMA CELL LEUKAEMIA TREATED WITH VTD-PACE CHEMOTHERAPY

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Plasma cell leukaemia (PCL) is a very aggressive and rare type of plasma cell disease. The prognosis is usually poor and the leukaemia is resistant to treatment. We report a case of PCL refractory to VTD (bortezomib, thalidomide and dexamethasone) chemotherapy but showed response to VTD-PACE (VTD and cisplatin, adriamycin, cyclophosphamide and etoposide) regimen with sustained remission. *Case history:* This was a 60-year-old man with good pre-morbid presented with fever and malaise for 3 weeks. Complete blood picture (CBP) revealed haemoglobin of 4.5g/dl, platelet of 5x10^9/L and white cell count of 5.0 x10^9/L, plasma cells 54%. Bone marrow exam confirmed plasma cell leukaemia with 46% plasma cells. Serum protein electrophoresis showed paraprotein of 6g/L at IgG lambda. Beta2-microglobulin level was 8.59 ug/ml. Two courses of VTD chemotherapy were given. There was transient control of leukaemia but plasma cells of 22% reappeared

in blood within 2 weeks after chemotherapy. VTD-PACE was given for the refractory leukaemia. There was complication of fungal pneumonia with aspergillus. A course of voriconazole was given and his chest condition improved and patient was discharged later. His CBP was normalized for 5 months and no plasma cells can be seen in blood smear. Patient refused further chemotherapy for personal reason. There was relapse of PCL 5 months after VTD-PACE with plasma cells in peripheral blood. Patient finally died 1 month after relapse of leukaemia.*Conclusion:* VTD-PACE is effective for the treatment of plasma cell leukaemia, especially for refractory disease.

A-504

OUTCOME IMPROVEMENT IN PLASMA CELL LEUKAEMIA PATIENTS TREATED WITH AUTOGRAFT AND OR NOVEL AGENTS: A SINGLE CENTRE EXPERIENCE

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Background: Plasma cell leukaemia (PCL) is a rare haematological disease with poor prognosis which can be primitive without prior evidence of multiple myeloma or secondary as terminal event. The results of conventional therapy are disappointing though autologous graft may improve survival. *Methods:* Between September 2004 and November 2010 six consecutive patients (median age 61.1 yrs, ECOG PS 1-3, median peripheral blood plasmocytosis 38%) with secondary PCL were treated to our Haematology Department with upfront therapeutical approaches including cyclophosphamide, lenalidomide, melphalan and bortezomib in association with steroids. One patient was treated in 2004 with DAV induction protocol followed by double autograft; two patients showed acute renal failure at presentation. Treatment response was assessed according to the IMWG uniform response criteria. *Results:* ORR was 33.3% (2/6 pts) with a 14 months longlasting VGPR achieved after double autograft and a stringent CR obtained with MPV scheme in two patients with acute renal failure at presentation. Among these responder patients the former died 63 months after diagnosis with progressive disease during the 16th lenalidomide course administered as fifth line salvage chemotherapy; the latter is alive in good clinical conditions still on MPV maintenance treatment with a PFS of 11 months. Non responder patients died all due to PD with a median OS of 3 months. *Conclusions:* Novel agents such as bortezomib and lenalidomide, thus as autologous transplantation can improve the outcome of PCL patients whose survival remains poor.

A-505

SHORT TERM FOLLOW UP OF PATIENTS WITH SERUM FREE LIGHT CHAINS OF MORE THAN 500MG/L WITH MGUS AND ASYMPTOMATIC MYELOMA

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Introduction: The serum free light chain (SFLC) assay is an important test in the evaluation of patients with plasma cell dyscrasia in terms of diagnosis, prognosis and monitoring disease activity. However there is a lack of data about the cut off point of this assay in predicting the possibility of renal failure due to cast nephropathy. Also no guidelines exist as to the level which would mandate the start of treatment in patients with high levels of FLC. We present 7 cases of MGUS and two cases of asymptomatic myeloma (AM) who had high levels of free light chains >500mg/L) who have not so far required treatment. *Patients:* Patients underwent the usual screening and staging including SFLC. Of these 6 patients had bone marrow biopsies. The results are presented in the table below. No patients showed evidence of end organ damage. *Discussion and Conclusions:* We present 9 cases of plasma cell dyscrasia with high levels of FLC and no end organ damage or requirement for treatment. This suggests that there is no set level of FLC within the range in this study that mandates treatment or that is nephrotoxic. It also interesting to note that FLC levels do not correlate with BJP and that the light chain component of the M proteins would be missed in many cases if the SFLC assay was not carried out .This study has the limitations of a small number of patients and also follow up period but can form a basis for future studies. These findings suggest that this group of patients should be followed up at close intervals in case of a sudden rise in FLC which may lead to renal failure.

	PP	Amount g/L	BJP g/L	Kappa mg/L	Lambda Mg/L	Ratio	BM Aspirate %	BM trephine %	FU period months
ME	Gκ	14	0.0	2540	14.9	170	ND	ND	6
DB	Gκ	13	0.3	853	0.8	1066	ND	ND	42
OR	Gκ	11	0.0	537	14.9	38.6	2	6	4
NH	Gκ	UQ	0.4	1300	26.1	49.8	1	30	3
IF	Gk	29	0.59	709	17	41.7	11	50	16
PV	Gk	33	UQ	1820	1.2	1516.7	18	50	43
DH	Gλ	20	UQ	6.4	585	0.011	4	15	13
CM	Aλ	14	0.0	9.9	920	0.011	ND	ND	3
JD	Mλ	7	UQ	10.8	579	0.19	16*	35 *	36

UQ= unquantifiable. ND=not done *=Lymphoid cells

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